Fludioxonil

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[1]

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18/1/17	Amended in response to questions from the RMS including addition of study summaries for studies submitted in last EU review which are still relied upon. Data included for the metabolite SYN545245 throughout all relevant data points. All amendments are highlighted in yellow.	CGA173506_11758 11 April 2016 updated 18/1/17
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[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Introduction

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

Fludioxonil was included into Annex I of Council Directive 91/414/EEC (Commission Directive 2007/76/EC; 20 December 2007). 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 fludioxonil under Regulation (EC) 1107/2009. Where appropriate this document refers to the Commission Implementing Regulation (EU) No. 540/2011 for fludioxonil and to the EFSA report for fludioxonil (**EFSA Scientific Report (2007) 110, 1-85**), and in particular the endpoints provided in Appendix I.

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 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 fludioxonil, new data and/or reviews and/or risk assessments have been provided. Where additional and/or new data on fludioxonil are provided, a justification has been included. Also a justification has been given if new data are required but none were provided.

As requested by the Rapporteur Member State (RMS), summaries for previously reviewed studies have been included. The report details for previously reviewed studies are given in *italics* and the original Annex II data point is provided. These reports are not provided in KCA or mentioned in the associated Supplementary LCA reference list.

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 fludioxonil 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

Organism	Test type	Endpoints		Comment	Reference (author, date, Syngenta Ref)
Bobwhite quail (*Colinus virginianus*)	Acute oral	EU	LD_{50} >2000 mg/kg bw	The extrapolated endpoint 3776 mg a.s./kg will be used for risk assessment	***Hakin (1990) CGA173506/0065***
Mallard duck (*Anas platyrhynchos*)			LD_{50} >2000 mg/kg bw	Historically, this study has not been used as most birds in the study regurgitated	***Hakin (1990) CGA173506/0064***
Canary (*Serinus canaria*)	Dietary study	New study	LC_{50} 3283 ppm a.s.	For US compliance. Conducted as a dietary study due to regurgitation issues. It will not be used for EU risk assessment	***Hubbard (2015) CGA173506_51396***
Bobwhite quail	Short-term dietary	EU	LC_{50} >833 mg/kg bw/d	Dietary endpoints are no longer required. This will not be used in risk assessment.	***Hakin (1990)*** [b] ***CGA173506/0062***
Mallard duck		EU	LC_{50} >1191 mg/kg bw/d		***Hakin (1990)*** [b] ***CGA173506/0063***
Bobwhite quail	Sub-chronic and reproductive	EU	NOAEC = 6562.8 [d] mg/kg bw/d	The highest rate tested (equivalent to 700 ppm)	***Johnson et al. (1993)*** [c] ***CGA173506/0355***
		EC_{10}/EC_{20 [a]	Could not be estimated	-	***Taylor & Howells (2015) CGA173506_11843***
Bobwhite quail		New study	NOAEC = 446 mg/kg bw/d	Reproduction range finding study	***Frey et al. (2014) CGA173506_11722***
Bobwhite quail		New study	NOAEC = 389 mg/kg bw/d	To clarify the NOEC for this species	***Bryden et al. ((2015) CGA173506_11828***
Mallard duck		EU	**NOEC = 134126 [d] mg/kg bw/d**	**Proposed avian chronic endpoint**	***Johnson et al. (1993)*** [c] ***CGA173506/0354***
		EC_{10}/EC_{20 [a]	Could not be estimated	-	***Taylor & Allen (2015) CGA173506_11844***

New study refers to studies performed since EU review of fludioxonil

[a] Estimation attempted in accordance with **Commission Regulation (EU) No. 283/2013**

[b] Date in EU DAR erroneously stated as 1993.

[c] Date in EU DAR erroneously stated as 1996.

[c] At RMS request the conversion of the endpoint from ppm to mg/kg bw proposed in the initial submission (DAR 2005) has been revised to exclude the pre-exposure period. The new conversion is based on study results considering the individual body weight for male and female reported for week 0, 2, 4, 6, 8 and 23 and the food consumption reported for each week of exposure (week 1 to 23).

Acute endpoint

Under Point 2.2 of the EFSA Guidance Document[1] it is recommended that short-term avian toxicity studies should only be conducted for those active substances which a have a mode of action (e.g. organochlorines or anticoagulants) or have results from mammalian testing that indicate a potential for the dietary LD_{50} measured by short-term exposure to be lower than the LD_{50} based upon an acute oral study. In such instances where a study exists and the dietary LD_{50} is lower than the acute LD_{50}, then the dietary LD_{50} should be used in the acute risk assessment. The Guidance Document recognises that although a requirement for data on dietary LD_{50s} is no longer triggered for the majority of active substances, pre-existing data may be available and although its routine use is not required it may be used in higher tier assessments.

For fludioxonil, a short term toxicity has been conducted with the bobwhite quail resulting in a dietary LD_{50} of >833 mg/kg bw/day (the highest rate tested). Although the dietary LD_{50} is potentially lower than the acute LD_{50} of >2000 mg/kg bw (also obtained with the bobwhite), since there were no mortalities in the dietary study there is no indication of increased toxicity due to dietary exposure and therefore the acute risk assessment will be conducted using the acute endpoint as recommended. A short term dietary toxicity study was conducted the mallard duck showing no mortalities and a dietary LD_{50} of >1173 mg/kg bw/day (the highest rate tested). Therefore there is no increased toxicity observed in the mallard duck.

Canary study

A study was conducted on the canary (*Serinus canaria*) for USA compliance. During the acute oral range finding phase, where two zebra finches and four canaries were dosed at 2000 mg a.s./kg and all bird regurgitated the dose. One canary per dosage level of 66.6, 245, 904 and 3330 mg/kg was also dosed and the levels of 245, 904 and 3330 mg/kg resulted in regurgitation. No mortalities occurred during the acute range finding phase. As regurgitation was observed during oral dosing, then the test design was changed to a dietary study, in accordance with the test guideline OCSPP Number 850.2200.

Under Point 2.2 of the EFSA Guidance Document it is recommended that short-term avian toxicity studies should only be conducted for those active substances which a have a mode of action (e.g. organochlorines or anticoagulants) or have results from mammalian testing that indicate a potential for the dietary LD_{50} measured by short-term exposure to be lower than the LD_{50} based upon an acute oral study. In such instances where a study exists and the dietary LD_{50} is lower than the acute LD_{50}, then the dietary LD_{50} should be used in the acute risk assessment. The Guidance Document recognises that although a requirement for data on dietary LD_{50s} is no longer triggered for the majority of active substances, pre-existing data may be available and although its routine use is not required it may be used in higher tier assessments.

For fludioxonil, the short term toxicity conducted with the canary resulted in a dietary LC_{50} of 3283 ppm a.s.; this value is calculated as required for EPA submission. However, the birds exposed to fludioxonil at concentrations >1000ppm responded by eating less food and losing bodyweight and condition. This combination of both toxicity and starvation contributed to their death. Given the small size and high metabolic rate of passerines, they are very sensitive to reduced food intake. The maximum tolerated dose was 132 mg/kg bw (mean daily dose) and 660 mg/kg bw (cumulative). Surviving birds recovered by day 8 increasing food consumption and bodyweight (overcompensated compared to controls). The consequence of this in the field is a degree of avoidance of the treated food and likelihood that the birds would forage on another source.

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

As this study produces a result which is confounded by starvation, it will not be used in this risk assessment.

Since this study is not considered appropriate for risk assessment the study report will not be submitted. For completeness the study is mentioned in the unsubmitted section of the **LCA Section 8 Supplement**.

Extrapolation of the bobwhite quail acute toxicity endpoint

In the acute oral toxicity studies conducted with the bobwhite quail (***Hakin, 1990***) no mortalities were observed and therefore the LD_{50} was reported as >2000 mg/kg bw. Under Point 2.1.2 of the **Guidance Document**[2] 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 factors are presented in Table 1 of the guidance document and are dependent upon the number of animals tested and whether no, or a single mortality, was observed in the study. The acute toxicity value for the bobwhite has been extrapolated and is presented in the table below.

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

Study	Test species	Experimental LD_{50} (mg/kg bw)	Number of animals tested	Number of mortalities	Extrapolation factor [a]	Corrected LD_{50} (mg/kg bw)
Hakin (1990)	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)[2]

The extrapolated LD_{50} value of 3776 mg/kg bw (***Hakin, 1990***) will therefore be used in the subsequent risk assessment.

In the acute oral toxicity studies conducted with the mallard duck (***Hakin, 1990***) no mortalities were observed and therefore the LD_{50} was reported as >2000 mg/kg bw. However due to the emetic effect observed at the limit dose, the mallard duck will not be considered further for the acute oral toxicity studies in this risk assessment.

Long-term endpoint

The EFSA Guidance Document indicates that the acute $LD_{50}/10$ should be used as an endpoint in long-term risk assessment where it is lower than the long-term endpoint. The endpoint from the reproduction study with mallard duck of 126 mg / kg bw/ day has been used in calculations of the TERs value since it is lower than the extrapolated $LD_{50}/10$ value of 377.6 mg /kg bw.

The original bobwhite quail study produced a NOEC of 62.8 mg a.s./kg bw from the dose of 700 ppm (Johnson *et al*. 1993; Syngenta file number CGA173506/0355), this was the highest dose tested. Therefore a new reproduction study was conducted to define an actual NOEC to enhance certainty in the risk assessment. The new definitive study is by Bryden *et al* 2015 (Syngenta file number CGA173506_11828); with a preliminary range finding study (Frey *et al* 2014; Syngenta file number CGA173506_11722).

Parental body weight

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

The data for parental body weight from the two definitive studies have been combined in Table 8.1.1-3 The original bobwhite quail study (Johnson *et al*., 1993) showed a statistically significant 4.8% reduction for males at 700 ppm leading the study authors to conclude that the NOEC was 300 ppm and the LOEC 700 ppm based on body weight. The original RMS Denmark considered the NOEC for body weight too conservative as there was <5% effect and concluded the NOEC was 700 ppm. The PRAPeR Expert Meeting agreed with this conclusion and the NOEC was set at 700 ppm (62.8 mg a.s./kg bw/d). The new bobwhite quail definitive study (Bryden *et al* 2015) showed a statistically significant 7% increase in female body weight at termination, but no effects in the control, 2400 or 4800 ppm a.s. treatment groups for males or females. In both cases the changes were minor and the changes were not consistent over time or concentration responsive, therefore the NOEC for parental weight is this 4800 ppm a.s. (389 mg a.s./kg bw/d).

Table 8.1.1-3: Mean adult body weight (g) ~ bobwhite quail combined data (Johnson *et al*. 1993; Syngenta file number CGA173506/0355 & Bryden *et al* 2015; Syngenta file no CGA173506_11828)

Study	Experimental group (ppm a.s.)		Sex	Week -2	Week 0	Week 2	Week 4	Week 6	Week 8	Term Week 22/20[c]
	ppm	mg/kg bw/d								
Johnson *et al* 1993	0	-	Male	190	193	195	197	199	202	205
			Female	190	188	189	192	192	204	235
Bryden *et al* 2015	0	-	Male	-	196	199	200	202	204	215
			Female	-	189	192	193	194	196	230
Johnson *et al* 1993	125	11.1	Male	192	193	196	197	197	197	203
			Female	189	190	192	192	193	207	223
Johnson *et al* 1993	300	26.6	Male	190	192	195	198	199	198	201
			Female	187	189	189	192	191	204	231
Johnson *et al* 1993	700	62.8	Male	188	191	193	195	194	196	195[a]
			Female	187	191	193	192	191	205	225
Bryden *et al* 2015	1200	94.8	Male	-	196	202	202	202	204	216
			Female	-	193	198	198	198	200	246[b]
Bryden *et al* 2015	2400	190	Male	-	195	201	200	201	202	211
			Female	-	190	196	196	196	197	235
Bryden *et al* 2015	4800	389	Male	-	194	195	197	198	199	207
			Female	-	194	193	196	195	196	241

[a] Significantly different from the control ($p<0.05$)
[b] Significantly different from the control ($p<0.05$) (Dunnett's test)
[c] Johnson et al 1993 finished on Week 22, and Bryden et al 2015 finished at Week 20.

Effects on reproduction

In the first bobwhite quail study (Johnson *et al*. 1993; Syngenta file number CGA173506/0355) no statistically significant effects were seen in any reproductive parameters measured (see Table 8.1.1-4). However, in the original report effects on reproduction were considered to be of biological significance and the NOEC and LOEC were reported as 125 and 300 ppm a.s., respectively. In July 1996, this was subsequently revised by report amendment to base the results on statistically significant effects; the NOEC / LOEC were revised respectively to 300 and 700 ppm a.s., based on the parental male weight; therefore the NOEC based on reproductive parameters is 700 ppm a.s.. Table 8.1.1-5 shows that there were also no significant effects on reproduction in the Bryden *et al* 2015 study for any of the treatments. Based on both studies where no significant or dose responsive reproductive effects were seen for any dietary doses, the NOEC for reproduction is therefore 4800 ppm (389 mg/kg bw/d).

Table 8.1.1-4: Summary of effects of fludioxonil on fecundity of bobwhite quail (Johnson *et al* 1993; Syngenta file no CGA173506/0355)

Parameter	Control	125 ppm CGA173506	300 ppm CGA173506	700 ppm CGA173506
Eggs laid per female	62.6	59.9	55.4	66.2
Eggs damaged	44	26	49	43
Eggs damaged of eggs laid (%)	3.8	2.3	5.0	3.3
Mean egg shell thickness (mm)	0.21	0.20	0.21	0.21
Eggs set	1020	1048	858	1180
Viable embryos	927	946	742	1008
Viable embryos of eggs set (%)	91	90	86	85
Live 3-week embryos	915	924	723	991
Live 3-week embryos of viable embryos (%)	99	98	97	98
Normal hatchlings	842	802	626	909
Normal hatchlings of viable embryos (%)	91	85	84	90
Normal hatchlings of live 3-week embryos (%)	92	87	87	92
14-day old survivors	747	705	555	810
14-day old survivors of eggs laid (%)	65	61	57	62
14-day old survivors of normal hatchlings (%)	89	88	89	89
14-day old survivors per female	41.0	36.6	31.5	40.8
Chick bodyweights at hatching (g)	6.9	6.7	6.7	6.7
Chick bodyweights at 14 days (g)	25	25	25	25

The reproductive parameters from the Bryden *et al* 2015 are shown below.

Table 8.1.1-5: Summary of effects of fludioxonil on fecundity of bobwhite quail (Bryden *et al* 2015; Syngenta file no CGA173506_11828)

Parameter	Control	1200 ppm	2400 ppm	4800 ppm
Eggs laid per female	38	46	44	51
Mean egg shell thickness (mm)	0.231	0.234	0.231	0.233
Total Eggs laid	645	825	800	870
Eggs cracked	4	4	12	12
Eggs cracked % of eggs laid	1	1	1	1
Fertile eggs	541	695	674	669
Eggs set	553	736	702	774
Viable embryos	532	687	661	647
Viable embryos of eggs set (%)	96	93	93	85
Live 3-week embryos	529	685	658	645
Live 3-week embryos of viable embryos (%)	99	100	100	100

Parameter	Control	1200 ppm	2400 ppm	4800 ppm
Normal hatchlings	485	611	597	609
Normal hatchlings of eggs set (%)	88	82	84	80
Normal hatchlings of live 3-week embryos (%)	92	89	91	95
14-day old survivors	450	522	531	562
14-day old survivors of eggs set (%)	81	75	76	74
14-day old survivors of normal hatchlings (%)	92	90	90	92
14-day old survivors per female	26	31	30	33
Chick bodyweights at hatching (g)	6.1	6.1	5.9	5.8
Chick bodyweights at 14 days (g)	23	23	23	23

Low number of eggs per female in the 300 ppm dose (Johnson et al. 1993; Syngenta file number CGA173506/0355)

A more detailed investigation of the lower number of eggs laid per female and consequent lower numbers of embryos, survivors and survivors at 14 days has been made from the Johnson *et al* 1993 bobwhite quail study (see Table 8.1.1-6). Twenty pairs were used for each dose. In the 300 ppm group four pairs were lost due to female mortality. The early mortality equates to a combined total loss of 205 potential egg laying days.

There were four mortalities, three birds died / were humanely sacrificed due to bullying and one was a spontaneous mortality with no prior symptoms. In addition, one hen was a poor layer producing only 8 eggs over the 22 weeks. In combination these account for the low number of eggs laid in the 300ppm a.s. treatment. There is no dose response between treatment and parental mortality or poor laying performance. The number of eggs laid has been adjusted to remove eggs laid by these birds and shows the numbers range from 60 to 66 in treatments and are therefore similar indicating no dose response. The last column in the table shows the sum of eggs per female which are calculated per week during the study. This helps take into account deaths during the study, but does not address the effects of poor layers, these numbers range from 55 to 66 in the treatments, although this was not dose responsive. This analysis shows that there are no effects on the number of eggs laid per hen at the 700 ppm a.s. dose, which is further supported in the Bryden *et al*. 2015 at a 4800 ppm a.s. dose.

Table 8.1.1-6: Total number of eggs laid in the bobwhite quail study (Johnson *et al.* 1993; Syngenta file number CGA173506/0355)

Treatment (ppm)	Initial number of pairs	Total number of eggs laid (weeks 11 to 22) [c]	Female mortality before week 22		Poor laying females alive at end of study (number of eggs produced)		Surviving females at week 22 (excluding poor layers)		Adjusted number of eggs per living female (excluding poor layers alive at week 22)	Reported number of eggs per female [d]
			Number of female mortalities	Eggs laid	Number of poor laying females	Eggs laid	Parents	Eggs laid		
0	20	1146	3 [a]	7	None	-	17	1139	67	62.6
125	20	1154	1	20	1	19	18	1115	62	59.9
300	20	981	4 [b]	73	1	8	15	900	60	55.4
700	20	1316	0	0	None	-	20	1316	66	66.2

[a] One died due to spontaneous mortality with no prior symptoms early in study (day 45); two died/ were humanely sacrificed due to injuries from bullying
[b] Two died from bullying, third humanely sacrificed due to injuries from bullying, fourth was a spontaneous mortality with no prior symptoms
[c] From Table 3 of original report.
[d] From Table 3 of original report. The sum of the number of eggs laid per hen per week. This helps ameliorate the effects of hens lost to mortality but does not address losses due to poor laying performance.

Conclusion on avian reproduction:

- Based on all available data for bobwhite quail the NOEC for the new bobwhite quail study is 4800 ppm (equivalent to 389 mg a.s/kg bw/d) for all reproductive and parental weight parameters.

- The proposed EU endpoint for avian long term endpoint for use in risk assessment is the mallard duck NOEC of 700 ppm (equivalent to 134~~126~~ mg a.s/kg bw/d).

To facilitate comparison of the two definitive bobwhite quail studies the following table has been added giving data per surviving hen as requested by the RMS.

Table 8.1.1-7: Comparison of the two definitive bobwhite quail studies for fludioxonil on fecundity (as requested by RMS)

	Bobwhite quail reproduction study (Johnson *et al* 1993; Syngenta file no CGA173506/0355)				Bobwhite quail reproduction study (Bryden *et al* 2015; Syngenta file no CGA173506_11828)			
Parameter	**Control**	**125 ppm**	**300 ppm**	**700 ppm**	**Control**	**1200 ppm**	**2400 ppm**	**4800 ppm**
Number of surviving female	17	19	16	20	17	18	18	17 [a]
Total eggs laid	1146	1154	981	1316	645	825	800	809
Total eggs laid for surviving female	1139	1134	908	1316	645	825	800	809
Eggs laid per surviving female	67	60	57	66	38	46	44	51 [b]
Eggs damaged	44	26	49	43	4	4	12	11
Eggs damaged of eggs laid (%)	3.8	2.3	5.0	3.3	1	1	1	1
Mean egg shell thickness (mm)	0.21	0.20	0.21	0.21	0.23	0.23	0.23	0.23
Eggs set	1020	1048	858	1180	553	736	702	719
Viable embryos	927	946	742	1008	532	687	661	647
Viable embryos of eggs set (%)	91	90	86	85	96	93	93	90
Live 3-week embryos	915	924	723	991	529	685	658	645
Live 3-week embryos of viable embryos (%)	99	98	97	98	99	100	100	100
Normal hatchlings	842	802	626	909	485	611 [b]	597	609 [b]
Normal hatchlings of viable embryos (%)	91	85	84	90	92	89	91	95
Normal hatchlings of live 3-week embryos (%)	92	87	87	92	92	89	91	95
14-day old survivors	747	705	555	810	450	552	531	562
14-day old survivors of eggs laid (%)	65	61	57	62	70	67	66	69
14-day old survivors of normal hatchlings (%)	89	88	89	89	92	90	90	92
14-day old survivors per female	41.0	36.6	31.5	40.8	26	31	30	35
Chick bodyweights at hatching (g)	6.9	6.7	6.7	6.7	6.1	6.1	5.9	5.8

	Bobwhite quail reproduction study (Johnson *et al* 1993; Syngenta file no CGA173506/0355)				Bobwhite quail reproduction study (Bryden *et al* 2015; Syngenta file no CGA173506_11828)			
Parameter	**Control**	**125 ppm**	**300 ppm**	**700 ppm**	**Control**	**1200 ppm**	**2400 ppm**	**4800 ppm**
Chick bodyweights at 14 days (g)	25	25	25	25	23	23	23	23

[a] One euthanased as pen 566 contained two females (identified upon necropsy)
[a] One hatchling found dead before weighing so it was not weighed
[b] Clarified that Table 7 in the original report was wrong, Appendix 12 data was correct. Error due to amendment of numbers following discovery of to females in one pen (566); data from this pen was then excluded from the report.

Metabolites

The metabolism of fludioxonil has been extensively studied in a number of plant species (see MCA Section 6.2.1). Since metabolites are formed at <10% of parent level in edible crop parts and mammalian testing indicates that they are no more toxic than the parent, it can be concluded that the risk to birds will be low and no further risk assessment is conducted **(Fludioxonil; EFSA Scientific Report 110, 2007)**.

CA 8.1.1.1 Acute oral toxicity to birds

Since fludioxonil 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)[3] ~~therefore additional study summaries are not repeated here~~. The endpoints are summarised in Table 8.1.1-1 above. At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.1.1 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.1.1-1 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.1.1 Hakin B., Rodgers M., Anderson A. & Dawe S.I. (1990). The Acute Oral Toxicity (LD_{50}) of CGA 173506 to the Bobwhite Quail. Report Number CBG 497/90471. Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, ENGLAND. (Syngenta File No. CGA173506/0065)*

Guidelines

EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Section 71-1 Avian single-dose oral LD_{50} test (1982)

GLP: Yes

Executive Summary

The 14-day acute oral LD_{50} for northern bobwhite exposed to CGA173506 was determined to be > 2000 mg a.s./kg body weight, the highest concentration tested.

Materials

Test Material	CGA173506
Lot/Batch #:	805002
Purity:	97.5%
Description:	Yellow powder
Stability of test	Stable under test conditions

[3] European Commission EU Review programme: Fludioxonil Volume 3, Annex B9: Ecotoxicology, Draft assessment report (DAR), Rapporteur Member State: Denmark, 2006.

compound:	
Reanalysis/Expiry date:	June 1992

Treatments

Test doses:	Blank control and nominal concentrations: 500, 1000 and 2000 mg CGA1735806/kg b.w.

Test organisms

Species:	Bobwhite quail (*Colinus virginianus*), over 16 weeks of age
Source:	Wise D.R. & Wise R.E., Monkfield, Bourn, Cambridgeshire, England
Acclimatisation period:	At least 14 days
Treatment for disease:	None reported. All birds were in apparent good health at the start of the pre-treatment period.
Weight:	176 - 214 g at test initiation

Test design

Replication:	1 per treatment
No. of birds/treatment:	10 (5 males and 5 females, housed separately)
Duration of test:	14 days

Environmental test conditions

Temperature:	15 - 19 °C
Humidity:	75 %
Photoperiod:	7 hours light/ 17 hours darkness

Study Design and Methods

Experimental dates: 24th January to 21st February 1990

Dose levels were based on the results of a range-finding test at 2000 mg/kg bw in 2 males and 2 females. No mortalities were observed. In the main study, five male and five female northern bobwhite quails, approximately 16 weeks old and in good health, were assigned to each test group on the basis of bodyweight. The birds were housed in cages constructed of steel wire, by test group and sex. Birds were starved for at least 15 hours prior to treatment

The nominal concentrations, administered at test commencement, were 500, 1000 or 2000 mg CGA173506/kg b.w. This was dosed in corn oil via oral intubation using a disposable syringe and Ch10 Nelaton plastic catheter (10 mL/kg). Control birds received a corresponding volume of corn oil only. During the test each bird was fed with a laboratory-formulated diet, and received this and water *ad libitum*.

The birds were observed daily for toxicological responses and abnormal behaviour throughout the test. Individual body weights were measured at Days -14, -7, test initiation and on Day 7 and at test termination (Day 14). Feed consumption was measured for Days -14 to -8, -7 to -1, 1 to 7 and 8 to 14. At termination of the study, post-mortem examination was carried out on all ten birds from the highest dose group.

Results and Discussion

Mortality and mean body weight gains are summarised in the table below.

Table 8.1.1.1-1: Summary of effects of CGA173506 on mortality and growth of northern bobwhite (*Colinus viginianus*) following acute oral exposure

Treatment (mg CGA173506/kg b.w.)	No. of birds	Cumulative mortality	Mean weight gain day 0 to 7 (g/bird)	Mean weight gain day 7 to 14 (g/bird)
Control	5 male 5 female	0 0	+7 +6	0 -1
500	5 male 5 female	0 0	+7 +4	0 0
1000	5 male 5 female	0 0	+6 +3	0 +2
2000	5 male 5 female	0 0	+4 -1	+3 +6

Bodyweight changes were variable and there was no clear evidence of any treatment-related effect

There were no mortalities or overt signs of toxicity in the control or treatment groups and all birds appeared normal throughout the test period. Food consumption was considered to be variable, with no evidence of treatment-related effects. No abnormalities were detected in macroscopic post-mortem examination. There were no mortalities after dosing and therefore it was not possible to calculate the LD_{50} of CGA 173506 to the bobwhite quail.

Validity Criteria

No validity criteria are reported.

Conclusions

The 14-day acute oral LD_{50} for northern bobwhite exposed to CGA173506 was determined to be > 2000 mg CGA173506/kg body weight, the highest concentration tested.

(Hakin *et al*., 1990)

This study complies with the current validity criteria for acute toxicity testing with birds, and thus is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Hakin, Rodgers, Anderson, Dawe, 1990 CGA173506/0065	US EPA Subdivision E, Guideline 71-1	OECD No. 223 (2016) or US EPA OPPTS 850.2100 (2012)	Compliant with both OECD and US EPA guidelines	No deviations and the validity criteria were met: 1. Mortality in the control was ≤10% (0%). 2. No additional control birds were added during the course of the study The study is considered reliable.

Minor differences from OECD 223:

Photoperiod used = 7 hrs light/17 hrs dark (guideline states 8hrs light /16hrs dark)
No light intensity measurement is reported (as indicated it should be in the guideline)
Food consumption was measured on days -14 to -8; -7 to -1; 1 to 7 and 8 to 14 (guideline states that food consumption should be measured daily for 3 days post-dosing)
Mortality was monitored daily, but there were was no indication of continuous monitoring for 2 hrs post-dosing as indicated in the current guidance
Birds were not weighed on Day 3 as indicated in the current guidance (body weights are presented for Days -14, -7, 0, 7 and 14 only.

Minor differences from US EPA OPPTS 850.2100:

Dosing volume of 10 mL/ kg bw was used (2012 guideline states max dosing volume up to 8 mL/kg-bw).
Photoperiod used = 7 hrs light/17 hrs dark (guideline states 10 hrs light /14 hrs dark)
No analysis of contaminants in feed

Birds should be monitored closely for the first 60 to 120 minutes after dosing. Any regurgitation should be noted. Additional observations of test birds should be made a minimum of three times on the day of dosing. There is no indication in the report that these additional assessments were carried out.

The following study was previously submitted in the EU and was summarised in point B.9.1.1 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.1.1-1 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.1.1 Hakin B, Rodgers M, & Anderson A. (1990). The Acute Oral Toxicity (LD_{50}) of CGA 173506 to the Mallard Duck. Report Number CBG 498/90385. Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, ENGLAND. (Syngenta File No. CGA173506/0064)*

Guidelines

EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Section 71-1 Avian single-dose oral LD_{50} test (1982)

GLP: Yes

Executive Summary

The 14-day acute oral LD_{50} for mallard duck (*Anas platyrhynchos*) exposed to CGA173506 was determined to be > 2000 mg CGA173506/kg body weight, the highest concentration tested. The compound was found to be emetic at all dose levels in this study (51.2, 128, 320, 800 and 2000 mg CGA173506/kg b.w.).

Materials

Test Material	CGA173506
Lot/Batch #:	805002
Purity:	97.5%
Description:	Yellow powder

Stability of test compound:	Stable under test conditions.
Reanalysis/Expiry date:	June 1992
Density:	Not applicable

Treatments

Test doses:	Nominal concentrations: 51.2, 128, 320, 800 and 2000 mg CGA173506/kg b.w.

Test organisms

Species:	Mallard duck (*Anas platyrhynchos*), over 6 months of age
Source:	Mr J. Coles, The Country Game Farms, Ashford, Kent, England.
Acclimatisation period:	At least 14 days
Treatment for disease:	None reported. All birds were in apparent good health at the start of the pre-treatment period.
Weight:	925 - 1200 g

Test design

Replication:	1 per treatment, total of 10 birds
No. of birds/pen:	5 (males and females separately)
Duration of test:	14 days

Environmental test conditions

Temperature:	14 - 17 °C
Humidity:	70 %
Photoperiod:	7 hours light/ 17 hours darkness

Study Design and Methods

Experimental dates: 26th January to 23rd February 1990

Dose levels were based on the results of a range-finding study in 9 male and 9 female mallard ducks. In the main study, five male and female mallard ducks, approximately 6 months old and in good health were assigned to each test group on the basis of bodyweight, with ten birds in each. The birds were housed in wire grid and galvanised steel floor pens (1.84 x 1.20 m) by test group and sex. Birds were starved for at least 15 hours prior to treatment.

The nominal concentration, administered at test commencement, were 51.2, 128, 320, 800 and 2000 mg CGA173506/kg bw. This was dosed in corn oil via oral intubation using a disposable syringe and Ch14 Nelaton plastic catheter (5 ml/kg). Control birds received a corresponding volume of corn oil only. During the test each bird was fed with a laboratory-formulated diet, and received this and water ad libitum.

The birds were observed daily for toxicological responses and abnormal behaviour throughout the test. Individual body weights were measured at Days -14, -7, test initiation and on Day 7 and at test termination (Day 14). Feed consumption was measured for Days -14 to -8, -7 to -1, 1 to 7 and 8 to 14. At termination of the study, post-mortem examination was carried out on all ten birds from the highest dose group.

Results and Discussion

Mortality and body weight gains are summarised in the table below.

Table 8.1.1.1-2: Summary of effects of CGA173506 on mortality and growth of mallard ducks (*Anas platyrhynchos*) following acute oral exposure

Treatment (mg CGA173506/kg b.w.)	No. of birds	Cumulative mortality*	Mean weight gain day 0 to 7 (g/bird)	Mean weight gain day 7 to 14 (g/bird)
Control	5 male 5 female	0 0	+32 +105	-9 +55
51.2	5 male 5 female	0 0	+11 +60	+19 +65
128	5 male 5 female	0 0	+47 +74	0 +83
320	5 male 5 female	0 0	+26 +87	+10 +10
800	5 male 5 female	0 0	+24 +69	-10 +24
2000	5 male 5 female	0 0	+42 +76	+25 +77

Bodyweight changes were variable and there was no clear evidence of any treatment-related effect
* On day 9, a male bird was unable to stand and was therefore sacrificed and replaced with a spare bird

There were no mortalities or overt signs of toxicity in the control or treatment groups and all birds appeared normal throughout the test period. However, during the first hour after dosing, vomiting was observed in all treatment groups in a dose-dependent manner. Food consumption was considered to be variable, with no evidence of treatment-related effects. No abnormalities were detected in macroscopic post-mortem examination. There were no mortalities after dosing and therefore it was not possible to calculate the LD_{50} of CGA173506 to the mallard duck.

Validity Criteria

No validity criteria are reported.

Conclusions

The 14-day acute oral LD_{50} for mallard duck (*Anas platyrhynchos*) exposed to CGA173506 was determined to be > 2000 mg CGA173506/kg body weight, the highest concentration tested. The compound was found to be emetic at all dose levels in this study (51.2, 128, 320, 800 and 2000 mg CGA 173506/kg bw).

(Hakin B, Rodgers M, Anderson A, 1990)

This study complies with the current validity criteria for acute toxicity testing with birds. However, as regurgitation occurred at all dose levels, the LD_{50} estimate is unreliable.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Hakin, Rodgers, Anderson, 1990 CGA173506/0064	US EPA Subdivision E, Guideline 71-1	OECD No 223 (2016) or US EPA OPPTS 850.2100 (2012)	Compliant with US EPA guidance Not compliant with OECD guidance due to regurgitation.	The following validity criteria were met: 1. Mortality in the control was ≤10% (0%). 2. No additional control birds were added during the

				course of the study However, the LD_{50} estimate is unreliable due to regurgitation at all dose levels.

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.

A new study was conducted on the canary (*Serinus canaria*) for USA compliance. During the acute oral range finding phase, regurgitation was observed during oral dosing, then the test design was changed to a dietary study, in accordance with the test guideline OCSPP Number 850.2200. As this study produces a result which is confounded by starvation, it will not be used in this risk assessment.

Report:	Submitted for purposes of renewal as conducted on fludioxonil for USA compliance: KCA 8.1.1.2/01 Hubbard P. (2015) Fludioxonil – A Dietary LC_{50} Study with the Canary. Report Number 528-413. Wildlife International, 8598 Commerce Drive, Easton, MD 21601 USA (Syngenta File No. CGA173506_51396)

Guidelines

US EPA Ecological Effects Test Guidelines, OCSPP 850.2200: Avian Dietary Toxicity Test (2012)

GLP: Yes

Executive Summary

A dietary LC_{50} study with canary was conducted at nominal fludioxonil concentrations of 0, 562, 1000, 1780, 3160 and 5620 ppm a.s. The canaries were exposed to the treated feed for a period of five days, with a three-day post-exposure observation period. The dietary LC_{50} value for canary exposed to fludioxonil was estimated to be 3283 ppm a.s. with a 95% confidence interval of 2441 to 4846 ppm a.s. The slope of the dose responsive cure was 3.9 and the Chi-square value was 3.43. The no mortality concentration was 1000 ppm a.s. (132 mg a.s./kg bw/day or a cumulative dose of 660 mg a.s./kg bw).

Materials

Test Material	Fludioxonil
Lot/Batch #:	SMO3J0464
Actual content of active ingredients:	97.7% (w/w)
Description:	Brownish powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	End of October 2017

Treatments

Test concentrations:	0, 562, 1000, 1780, 3160 and 5620 ppm a.i

Test organisms

Species:	Canary (*Serinus canaria*)
Source:	Maryland Exotic Birds of Pasadena, Maryland USA
Acclimatisation period:	Facility: 5 weeks Caging: 15 days
Treatment for disease:	None
Weight:	18.0 to 28.8 g at test initiation

Test design

Replication:	10 birds per treatment and control
No. of birds/cage :	1
Duration of test:	8 days (5-day dietary exposure followed by a 3-day post-exposure observation period)

Environmental test conditions

Temperature:	20.7°C - 21.8°C
Relative Humidity:	15 - 42%
Photoperiod:	8 hours light (approximately 507 lux)/15.5 hours dark 15 minutes of dim light for transition period between light and dark

Study Design and Methods

Experimental dates: 10th to 26th November 2014

The dietary LC_{50} test consisted of five test concentrations (562, 1000, 1780, 3160 and 5620 ppm a.s.) and a control group. The dietary test was conducted based on regurgitation observed during an acute oral range-finding exposure and the maximum dietary test concentration as established based on the results of a dietary probe study. Ten canaries in good health were randomly assigned to each treatment and control group. The birds were housed individually in batteries of pens. Each pen had a floor space of 29 x 26 cm, with a ceiling height of 31 cm.

Test diets were prepared by mixing the test substance with corn oil and stirring the solution directly into the feed. The control group diet was prepared by mixing corn oil (absent test substance) into the diet. Diets were presented daily to the birds during the exposure period. Following the five-day exposure period, all birds were given untreated basal diet for three days during the post-exposure observation period. Feed consumption was determined daily.

Samples of the test diets were collected to verify the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets. Homogeneity of the test substance in the diet was evaluated by collecting six samples from the 562 and 5620 ppm a.s. test diets at preparation on Day 0. The homogeneity samples also served as verification samples. One verification sample was collected from the control diet and two verification samples were collected from the 1000, 1780 and 3160 ppm a.s. test concentrations at preparation on Day 0. Samples were collected from feed troughs of the control group and the treatment group on Day 5 of the test to assess stability of the test substance under actual test conditions.

The birds were observed at least twice daily for any signs of toxicity, abnormal behaviour and regurgitation. Individual body weights were measured at the initiation of the test (Day 0), on Day 1 and Day 5 and at termination of the test on Day 8. Gross necropsies were performed on all mortalities and on three birds from the control group and each treatment group at test termination.

Results and Discussion

The following guideline validity criteria were met:

1. Birds were randomly assigned to control and treatment pens.
2. The mortality in the control group did not exceed 10%.
3. Concentrations of the test substance in the diet were satisfactorily maintained (at least 80% of Day 0 values) throughout the exposure period.
4. The test substance was administered in diet for five consecutive days (five ~ 24 hr. periods).
5. Ten birds were used for the control group and each treatment group.

Based on the diet analysis results, homogeneity and stability of the test substance in the diets was confirmed. Samples analysed for verification of the test substance concentration ranged from 94 to 106% of nominal concentrations.

Feed consumption, calculated dietary dose and effects on mortality and body weight are summarised in the table below.

Table 8.1.1.2-1: Feed consumption, dietary dose and effects of fludioxonil on mortality of canary (*Serinus canaria*) following dietary LC_{50} exposure

Treatment (ppm a.s.)	Feed Consumption (g)	Cumulative Dietary Dose (mg a.s./kg bw)	Cumulative Mortality (%)
Control	3.7 (0.7)	0 (0)	0
562	3.4 (0.5)	460 (51)	0
1000	2.7 (0.4)	660 (77)	0
1780[a]	2.0 (0.5)	908 (205)	10[d]
3160[b]	1.0 (0.4)	725 (366)	70[e]
5620[c]	0.7 (0.2)	895 (201)	70[e]

[a] The clinical signs noted at the 1780 ppm a.s. test concentration were ruffled appearance, lethargy and depression.
[b] The clinical signs noted at the 3160 ppm a.s. test concentration were ruffled appearance and lethargy.
[c] The clinical signs noted at the 5620 ppm a.s. test concentration were ruffled appearance, lethargy, wing droop, loss of coordination, depression and prostrate posture.
[d] One male mortality
[e] Four male and three female mortalities

Table 8.1.1.2-2: Effects of fludioxonil on body weight of canary (*Serinus canaria*) during a dietary LC_{50} exposure

Treatment (ppm a.s.)		Exposure Period					Post-Exposure Period		Total Change
		Day 0	Change[a]	Day 1	Change[a]	Day 5	Change[a]	Day 8	
Control	Mean	21.8	-0.1	21.7	0.2	21.9	-0.2	21.7	-0.1
	SD	1.7	0.3	1.8	0.6	1.5	0.6	1.2	1.0
562	Mean	21.1	-0.5	20.7	0.4	21.1	0.6	21.7	0.5
	SD	1.1	0.3	1.1	0.4	1.3	0.5	1.3	0.8
1000	Mean	21.1	-0.9*	20.2	-0.3	19.9	1.5**	21.4	0.3
	SD	2.0	0.4	1.8	0.6	1.6	0.7	1.9	0.9
1780	Mean	21.4	-1.5**	19.9	-2.1**	18.1**	2.7**	20.8	-0.9
	SD	2.7	0.5	2.4	0.5	2.4	0.9	2.7	1.3

3160	Mean	21.1	-2.4**	18.7**	-4.7**	15.3**	3.3**	19.4	-3.2**
	SD	2.3	1.0	1.5	1.5	1.9	1.4	0.4	3.0
5620	Mean	22.7	-2.3**	20.4	-5.3**	17.0**	4.3**	23.0	-3.6**
	SD	2.9	0.6	2.6	0.6	2.5	1.0	0.4	2.3

[a] Mean change is calculated separately from the mean body weights using individual body weights (See Appendix 6).
* Difference from the control group statistically significant at $p < 0.05$ (Dunnett's t-test).
** Difference from the control group statistically significant at $p < 0.01$ (Dunnett's t-test).

Gross necropsies were performed on all mortalities. The findings that were observed for most of the mortalities included loss of muscle mass, thin with a prominent keel, pale heart, pale spleen, pale liver, pale kidneys and a primarily empty gastro-intestinal tract. These findings were considered to be treatment related.

Gross necropsies were also performed on three birds from the control group and three birds from each treatment group at test termination. Of the three birds in the control group one bird (F37) was noted as thin with a loss of muscle mass and a prominent keel. Of the three birds at the 1780 ppm a.s. test concentration, one bird was noted with slightly pale kidneys and with white plaques on the heart, in the air sacs and covering the liver. Of the three birds at the 3160 ppm a.s. test concentration, one bird was noted as thin with a loss of muscles mass, a prominent keel, a slightly pale heart, a slightly pale liver and slightly pale kidneys. Of the three birds at the 5620 ppm a.s. test concentration, one bird was noted with slightly cloudy air sacs and one bird was noted with a pale liver and a pale spleen.

Conclusions

A dietary LC_{50} study with canary was conducted at nominal fludioxonil concentrations of 0, 562, 1000, 1780, 3160 and 5620 ppm a.s. The canaries were exposed to the treated feed for a period of five days, with a three-day post-exposure observation period. The dietary LC_{50} value for canary exposed to fludioxonil was calculated to be 3283 ppm a.s. with a 95% confidence interval of 2441 to 4846 ppm a.s. The slope of the dose responsive cure was 3.9 and the Chi-square value was 3.43. The no mortality concentration was 1000 ppm a.s. (132 mg a.s./kg bw/day or a cumulative dose of 660 mg a.s./kg bw).

(Hubbard, 2015)

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Since fludioxonil 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 therefore additional study summaries are not repeated here. The endpoints are summarised in Table 8.1.1-1 above.

The RMS requested an analysis of the biological relevance, considering as a trigger for relevance of effect percentages equal of greater than 10%. A benchmark dose could also be proposed following the EFSA Journal (2009) 1150, 1-72 (Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment).

Syngenta considers that the BMD approach is usually used for mammalian studies for use in human risk assessment. It is not considered to be useful in this instance.

The bird reproduction studies conducted for fludioxonil follow a test design with a control + 3 dose groups. This particular test design does not allow a 10% difference to be detected. This is acknowledged

in the EFSA recurring issues document when discussing the calculation of an EC10 is inappropriate (*EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA Supporting Publication 2015; 12(12):EN-924. 62 pp. doi:10.2903/sp.efsa.2015.EN-924*). As the BMD requires the study to be able to detect 10% different then this is not a suitable analysis for this study type. In addition. The use of only three test concentrations would not be enough points to accurately define the curve.

It should also be noted that in the case of both the mallard and bobwhite quail studies there were <10% decreases in effect for the % of damaged eggs laid, % of viable embryos of eggs set, % of live 3-week embryos of viable embryos, % of normal hatchlings of viable embryos, % of normal hatchlings of live 3-week embryos, % of 14-day old survivors of eggs laid and the % of 14-day old survivors of normal hatchlings. Therefore the top dose is the NOEC.

An updated OECD summary has been provided at the request of ANSES during the pre-submission meeting on 14 July 2015. The report has also been included in the dossier for completeness.

Report:	Re-submitted the OECD summary at the request of ANSES for ease of dossier evaluation: K-CA 8.1.1.3/01 Johnson AJ (1993). CGA 173506 – Mallard Duck Dietary Reproduction and Tolerance Studies, Report Number CBG 542/543/930707. Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. (Syngenta File No CGA173506/0354)

Guidelines

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms*, subsection 71-4

GLP: Yes

Executive Summary

The effects of CGA173506 on reproduction in the mallard duck (*Anas platyrhynchos*) were determined in a 23-week toxicity test. Mortality, body weight, food consumption, reproductive parameters, and any other overt signs of toxicity, were assessed at nominal dietary concentrations of 125, 300 and 700 ppm CGA173506, alongside an untreated control group.

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 700 ppm CGA173506, the highest concentration tested. This is equivalent to 126 mg a.s./bw/day.

Materials

Test Material	CGA173506 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
Lot/Batch #:	P910007 (confirmed from raw data; reported erroneously as P91007)
Purity:	96.4 % w/w
Description:	Light brown powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	December 1993

Density:	Not applicable
Treatments	
Test doses:	125, 300 and 700 ppm CGA173506, alongside an untreated control
Food:	Basal diet (26.90 % protein, 7.73 % oil and 3.66 % fibre for adults)
Water:	Domestic quality potable water supplied by Anglian Water
Analysis of test concentrations:	Homogeneity tested day 0 of week 1; stability tested days 0, 9 and 18. Additional samples collected at the time of mixing the diet on weeks 1, 12 and 22 to measure/verify test concentrations. Samples taken from control and treated diets on each occasion.
Test organisms	
Species:	Mallard duck (*Anas platyrhynchos*) 5 months old at test start
Source:	J. Cole, The County Game Farms, Ashford, Kent, England
Acclimatisation period:	Not reported. Pre-treatment period: 14 days.
Treatment for disease:	Oxytetracycline for 4 days post-hatching. No treatment prior to or during the test.
Weight:	887 to 1063 g at test initiation
Test design	
Replication:	6
No. of birds/pen :	Seven (2 males and 5 females)
Duration of test:	23 weeks (11 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 pen 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 : 15 and 18 ºC Egg storage prior to incubation: 16 ºC Egg storage during incubation: 37.7 ºC Egg storage during hatching: 39.0 ºC Chicks, mean daily minimum and maximum temperatures: 24 and 26 ºC
Humidity:	Adult birds, mean daily relative humidity: 67 to 75 % Egg storage during incubation: 55 % (RH) Chicks, mean daily relative humidity: 51 %
Photoperiod:	Adult birds: 7 hours light per day from test initiation to Week 8, increasing to 16 hours light per day until Week 9 and 17 hours light was provided from Week 10 until the end of egg production phase. Mean light intensity was 182 lux with a range of 100 – 240 lux. Chicks: 14 hour light photoperiod

Study Design and Methods

Experimental dates: 8th October 1992 to 27th April 1993

Adult mallard duck (48 males and 120 females), approximately 5 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 125, 300 and 700 ppm CGA173506. The mix for the control groups consisted of basal ration only. The birds were housed in pens measuring 0.73 x 1.48 m. The pens were constructed of galvanised steel wire and had solid sides, wire mesh doors and floors, and during egg production the floors were covered with plastic pillow matting. During the test all birds were given food and water *ad libitum*.

Adult birds were observed daily for signs of toxicity or abnormal behaviour. Offspring were observed daily from hatching until 14 days old. Adult body weights were measured on weeks -2, 0 (immediately before introduction of test diets), 2, 4, 6, 8 and 23 (study completion). Feed consumption per pen was recorded weekly. 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 12 and stored on plastic egg trays in a refrigerator at 16 °C. Following each seven 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 °C and 55 % relative humidity and turned once hourly until incubation day 23, when they were placed in hatching trays.

Eggs laid on the first days of weeks 12, 14, 16, 18, 20 and 22 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

Fecundity is summarised in the table below.

Table 8.1.1.3-1: Summary of effects of CGA173506 on fecundity of mallard duck (*Anas platyrhynchos*) following oral exposure

Parameter	Control	125 ppm CGA173506	300 ppm CGA173506	700 ppm CGA173506
Eggs laid per female	39.4	34.6	44.0	34.1
Eggs damaged	45	23	32	32
Eggs damaged of eggs laid (%)	3.9	2.5	2.4	3.1
Mean egg shell thickness (mm)	0.35	0.34	0.35	0.36
Eggs set	1021	845	1189	921

Parameter	Control	125 ppm CGA173506	300 ppm CGA173506	700 ppm CGA173506
Viable embryos	826	732	1149	887
Viable embryos of eggs set (%)	81	87	97	96
Live 3-week embryos	779	674	1061	847
Live 3-week embryos of viable embryos (%)	94	92	92	95
Normal hatchlings	542	416	750	638
Normal hatchlings of viable embryos (%)	66	57	65	72
Normal hatchlings of live 3-week embryos (%)	70	62	71	75
14-day old survivors	516	405	734	627
14-day old survivors of eggs laid (%)	45	44	56	61
14-day old survivors of normal hatchlings (%)	95	97	98	98
14-day old survivors per female	17.8	15.0	24.5	21.0
Chick bodyweights at hatching (g)	35	34	35	35
Chick bodyweights at 14 days (g)	254	255	240	254

There were no treatment-related signs of toxicity during the experimental period and in the *post mortem* examination. The distribution of mortality was variable over the treatments. During the course of the study 2, 4 and 1 bird/s died in the 0, 125 ppm and 300 ppm doses, respectively. No statistically significant effects from treatment with CGA 173506 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.

The no-observed effect concentration was 700 ppm CGA173506, the highest~~lowest~~ concentration tested.

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 700 ppm CGA173506, the highest concentration tested. This is equivalent to 126 mg a.s./bw/day.

(Johnson AJ, 1993)

The conversion of the endpoint from ppm to mg/kg bw proposed in the initial submission (DAR 2005) has been revised at the request of the RMS. The originally calculated average consumption of 188 g/bird/day included a two week pre-exposure period. The new conversion based on study results on the individual body weight for male and female for weeks 0, 2, 4, 6, 8 and 23 and the food consumption reported for each week of exposure (week 1 to 23). The conversion is shown in the table below.

Table 8.1.1.3-2: Recalculation of conversion from ppm to mg/kg bw/d doses for fludioxonil effects on fecundity of mallard duck (*Anas platyrhynchos*) following oral exposure

Dose	Average body weight 1 to 23 weeks (g)	Average food consumption (g/bird/day)	Nominal concentration of dose (ppm)	Mean actual dose (mg/kg bw/d)

Dose	Average body weight 1 to 23 weeks (g)	Average food consumption (g/bird/day)	Nominal concentration of dose (ppm)	Mean actual dose (mg/kg bw/d)
125 ppm	1055	205	125	24
300 ppm	1047	216	300	62
700 ppm	1031	198	700	134

The recalculated NOEC for the reproductive toxicity test of fludioxonil on the mallard duck is 134 mg/kg bw/d.

In accordance with **Commission Regulation (EU) No 283/2013**, attempts have been made to estimate EC_{10} and EC_{20} values for the avian reproduction study on mallard conducted by Johnson in 1993 (CGA173506/0354). A summary of the 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. & Allen M. (2015). Fludioxonil (CGA173506) - Mallard Duck Dietary Reproduction and Tolerance Study, statistical re-analysis. Report Number CEA.1596, Reference Number CCE2068_009. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11844).

Summary

Huntingdon Research Centre Ltd. report number CBG 542/543/930707 (Johnson, 1993; Syngenta file number CGA173506/0354), for the reproductive toxicity test of CGA173506 on the mallard duck, 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 were intended to be re-analysed in an attempt to provide these values.

The test design consisted of three concentrations of the test substance (nominally 125, 300 and 700 ppm) and a control. There were six replicate pens for each test concentration and the control, each containing an initial population of two males and five females per pen.

Statistical analysis in the original report determined that there were no statistically significant ($P > 0.05$) treatment related effects on any of the parameters at any of the concentrations tested. As a result, it can be reasonably concluded that no EC_{10} or EC_{20} values could be derived from the data.

As a result of these findings, it can be concluded that there was no reliable dose response and therefore EC_x values could not be determined and no additional statistical analysis was performed on any of the parameters from the original study.

Results

Statistical analysis in the original report determined that there were no statistically significant ($P > 0.05$) treatment related effects on any of the parameters at any of the concentrations tested. As a result, it can be reasonably concluded that no EC_{10} or EC_{20} values could be derived from the data.

As a result of these findings, it can be concluded that there was no reliable dose response and therefore EC_x values could not be determined and no additional statistical analysis was performed on any of the parameters from the original study.

Conclusion

Following examination of the data in the original report it can be concluded that no reliable dose response and therefore no ECx values could be determined. Therefore, no additional statistical analysis was performed on any of the parameters from the original study.

(Taylor & Allen 2015)

An updated OECD summary of the following previously evaluated study has been provided at the request of ANSES during the pre-submission meeting on 14 July 2015. The report has also been included in the dossier for completeness.

Report:	Re-submitted the OECD summary at the request of ANSES for ease of dossier evaluation: K-CA 8.1.1.3/03 Johnson A (1993a). CGA 173506 - Bobwhite Quail Dietary Reproduction and Tolerance Studies, Report Number CBG 540/541/903706. Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. (Syngenta File No. CGA173506/0355)

Guidelines

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms*, subsection 71-4

GLP: Yes

Executive Summary

The effects of CGA173506 on the bobwhite quail (*Colinus virginianus*) were determined in a 22-week reproduction toxicity test. Mortality, body weight, food consumption, reproductive parameters, and any other overt signs of toxicity, were assessed at nominal dietary concentrations of 125, 300 and 700 ppm CGA173506, alongside an untreated control group.

A statistically significant reduction in male bodyweight was observed in the 700 ppm CGA173506 treatment group at test termination. Based on the statistics performed, the no effect level was 300 ppm CGA173506.

Materials

Test Material	CGA173506 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
Lot/Batch #:	P910007 (confirmed from raw data; reported erroneously as P91007)
Purity:	96.4 % w/w
Description:	Light brown powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	December 1993

Density:	Not applicable
Treatments	
Test rates:	125, 300 and 700 ppm CGA173506, alongside an untreated control
Food:	Basal diet (26.90 % protein, 7.73 % oil and 3.66 % fibre for adults)
Water:	Domestic quality potable water supplied by Anglian Water
Analysis of test concentrations:	Homogeneity tested day 0 of week 1; stability tested days 0, 9 and 18. Additional samples collected weeks 1, 12 and 22 to measure/verify test concentrations. Samples taken from control and treated diets on each occasion.
Test organisms	
Species:	Bobwhite quail (*Colinus virginianus*) approximately 9 months old
Source:	BC Potter, Rosedean, Woodhurst, Cambridgeshire, England
Acclimatisation period:	Not reported. There was a pre-treatment phase of 14 days
Treatment for disease:	A routine course of antibiotics was given on first arrival at the test facility. No other treatment for disease was given.
Weight:	164 to 224 g at test initiation
Test design	
Replication:	20
No. of birds/pen :	Two (1 male and 1 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 pen 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 : 21 and 23 ºC Egg storage prior to incubation: 16 ºC Egg storage during incubation: 37.7 ºC Egg storage during hatching: 39.0 ºC Chicks, mean daily minimum and maximum temperatures: 24 and 25 ºC
Humidity:	Adult birds, mean daily relative humidity: 49 % Egg storage during incubation: 55 % (RH) Chicks, mean daily relative humidity: 51 %
Photoperiod:	Adult birds: 7 hours light per day to Week 6, increasing to 10 hours light per day until Week 10 and followed by two weekly half hour increment increases so that 17 hours light was provided from Week 11 until the end of egg production phase. Mean light intensity was 94 lux with a range of 60 – 120 lux. Chicks: 14 hour light photoperiod

Study Design and Methods

Experimental dates: 6th October 1992 to 16th April 1993

Adult bobwhite quail (80 males and 80 females), approximately 9 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 125, 300 and 700 ppm CGA173506. The mix for the control groups consisted of basal ration only. The birds were housed in batteries of cages measuring 0.31 x 0.39 x 0.24 m, with sloping floors with 0.1 m egg-catchers. During the test all birds were given food and water *ad libitum*.

Adult birds were observed daily for signs of toxicity or abnormal behaviour. Offspring were observed daily from hatching until 14 days old. Adult body weights were measured on weeks -2, 0 (immediately before introduction of test diets), 2, 4, 6, 8 and 22 (study completion). Feed consumption per pen was recorded weekly throughout the pre-treatment and treatment 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 and stored on plastic egg trays in a refrigerator at 16 °C. Following each seven 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 °C and 55 % relative humidity and turned once hourly until incubation day 21, when they were placed in hatching trays.

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

Fecundity is summarised in the table below.

Table 8.1.1.3-32: Summary of effects of CGA173506 on fecundity of northern bobwhite (*Colinus viginianus*) following oral exposure

Parameter	Control	125 ppm CGA173506	300 ppm CGA173506	700 ppm CGA173506
Eggs laid per female	62.6	59.9	55.4	66.2
Eggs damaged	44	26	49	43
Eggs damaged of eggs laid (%)	3.8	2.3	5.0	3.3
Mean egg shell thickness (mm)	0.21	0.20	0.21	0.21
Eggs set	1020	1048	858	1180
Viable embryos	927	946	742	1008
Viable embryos of eggs set (%)	91	90	86	85

Parameter	Control	125 ppm CGA173506	300 ppm CGA173506	700 ppm CGA173506
Live 3-week embryos	915	924	723	991
Live 3-week embryos of viable embryos (%)	99	98	97	98
Normal hatchlings	842	802	626	909
Normal hatchlings of viable embryos (%)	91	85	84	90
Normal hatchlings of live 3-week embryos (%)	92	87	87	92
14-day old survivors	747	705	555	810
14-day old survivors of eggs laid (%)	65	61	57	62
14-day old survivors of normal hatchlings (%)	89	88	89	89
14-day old survivors per female	41.0	36.6	31.5	40.8
Chick bodyweights at hatching (g)	6.9	6.7	6.7	6.7
Chick bodyweights at 14 days (g)	25	25	25	25

There were no treatment-related signs of toxicity during the experimental period and in the *post mortem* examination. The distribution of mortality was variable over the treatments. Three birds of each sex died in the control; one bird of each sex died in the 125 ppm group and three males and four females died in the 300 ppm group. No birds died in the 700 ppm group.

Table 8.1.1.3-43: Total number of eggs laid in the bobwhite quail study (Johnson *et al.* 1993; Syngenta file number CGA173506/0355)

Treatment (ppm)	Initial number of pairs	Total number of eggs laid (weeks 11 to 22)	Female mortality before week 22		Poor laying females alive at end of study (number of eggs produced)		Surviving females at week 22 (excluding poor layers)		Number of eggs per living female (excluding poor layers alive at week 22)
			Number of female mortalities	Eggs laid	Number of poor laying females [c]	Eggs laid	Parents	Eggs laid	
0	20	1146	3 [a]	7	None	-	17	1139	67
125	20	1154	1	20	1	19	18	1115	62
300	20	981	4 [b]	73	1	8	15	900	60
700	20	1316	0	0	None	-	20	1316	66

[a] One died due to spontaneous mortality with no prior symptoms early in study (day 45); two died/ were humanely sacrificed due to injuries from bullying
[b] Two died from bullying, third humanely sacrificed due to injuries from bullying, fourth was a spontaneous mortality with no prior symptoms

Significant ($P < 0.05$) bodyweight reduction was observed in male adults dosed with 700 ppm CGA173506. No statistically significant effects from treatment with CGA173506 were observed for food consumption and egg production.

Table 8.1.1.3-54: Mean adult body weight (g) ~ bobwhite quail (Johnson *et al.* 1993; Syngenta file number CGA173506/0355)

Experimental group (ppm a.s.)	Sex	Week -2	Week 0	Week 2	Week 4	Week 6	Week 8	Term Week 22
0	Male	190	193	195	197	199	202	205
	Female	190	188	189	192	192	204	235

125	Male	192	193	196	197	197	197	203
	Female	189	190	192	192	193	207	223
300	Male	190	192	195	198	199	198	201
	Female	187	189	189	192	191	204	231
700	Male	188	191	193	195	194	196	195*
	Female	187	191	193	192	191	205	225

* Significantly different from the control ($p<0.05$)

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

The no-observed effect concentration was 300 ppm CGA173506.

Conclusions

A statistically significant reduction in male bodyweight was observed in the 700 ppm CGA173506 treatment group at test termination. Based on the statistics performed, the no effect level was 300 ppm CGA173506.

(Johnson A, 1993a)

The conversion of the endpoint from ppm to mg/kg bw proposed in the initial submission (DAR 2005) has been revised at the request of the RMS. The originally calculated average consumption of 188 g/bird/day included a two week pre-exposure period. The new conversion based on study results on the individual body weight for male and female for weeks 0, 2, 4, 6, 8 and 23 and the food consumption reported for each week of exposure (week 1 to 23). The conversion is shown in the table below.

Table 8.1.1.3-6: Recalculation of conversion from ppm to mg/kg bw/d doses for fludioxonil effects on fecundity of bobwhite quail (*Colinus viginianus*) following oral exposure

Dose	Average body weight 1 to 23 weeks (g)	Average food consumption (g/bird/day)	Nominal concentration of dose (ppm)	Mean actual dose (mg/kg bw/d)
125 ppm	198	19	125	12
300 ppm	198	19	300	28
700 ppm	197	18	700	65

The recalcated NOEC for the reproductive toxicity tes of fludioxonil on the bobwhite quail duck is 65 mg/kg bw/d.

In accordance with **Commission Regulation (EU) No 283/2013**, attempts have been made to estimate EC_{10} and EC_{20} values for the avian reproduction study on bobwhite quail conducted by Johnson in 1993 (CGA173506/0355). A summary of the re-evaluation is provided below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.1.1.3/04 Taylor, S. & Howells A. (2015). Fludioxonil (CGA173506) – Bobwhite Quail Dietary Reproduction and Tolerance Study, statistical re-analysis. Report Number CEA.1588, Reference Number CCE2068_001. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11843)

Summary

Huntingdon Life Sciences report number CBG 541/930706 (Johnson *et al*., 1993; Syngenta file number CGA173596/0355), for the reproductive toxicity test of CGA173506 on the bobwhite quail, 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 were intended to be re-analysed in an attempt to provide these values.

The test design consisted of three concentrations of the test substance (nominally 125, 300 and 700 ppm) and a control. There were 20 replicates of each test concentration and the control, each containing an initial population of one male and one female per cage.

Statistical analysis in the original report determined that there were no statistically significant ($P > 0.05$) treatment related effects for any of the parameters evaluated other than for male bodyweight at 700 ppm at test termination. Mean male bodyweights at test termination were 205, 203, 201 and 195 g in the control and 123, 300 and 700 ppm groups, respectively. As only a 4.9% decrease in mean weight in relation to the control value was observed at 700 ppm, it can be reasonably concluded that no EC_{10} or EC_{20} values could be derived from these data.

As a result of these findings, it can be concluded that no reliable EC_x values could be determined and no additional statistical analysis was performed on any of the parameters from the original study.

Results

Statistical analysis in the original report determined that there were no statistically significant ($P > 0.05$) treatment related effects on all the parameters evaluated other than for male bodyweight at 700 ppm at test termination. Mean male bodyweights at test termination were 205, 203, 201 and 195 g in the control and 123, 300 and 700 ppm groups, respectively. As only a 4.9% decrease in mean weight in relation to the control value was observed at 700 ppm, it can be reasonably concluded that no EC_{10} or EC_{20} values could be derived from these data.

Conclusion

Statistical analysis in the original report determined that there were no statistically significant ($P > 0.05$) treatment related effects on all the parameters evaluated other than for male bodyweight at 700 ppm at test termination. As a result of these findings, it can be concluded that no reliable EC_x values could be determined and no additional statistical analysis was performed on any of the parameters from the original study.

(Taylor & Howells, 2015)

Two new studies on bobwhite quail have been conducted since the original EU evaluation. The range finding study is summarised below.

Report:	Conducted to establish a test range for the new definitive reproduction study. K-CA 8.1.1.3/05 Frey LT, Bryden M, Martin KH & Beavers .B (2014). Fludioxonil - A Range-Finding Reproduction Study with the Northern Bobwhite Report Number; 528-427. Wildlife International, A Division of EAG Inc., 8598 Commerce Drive, Easton, MD 21601 USA (Syngenta File No. CGA173506_11722)

Guidelines

OPPTS Number 850.2300

FIFRA Subdivision E Section 71- 4

OECD Guideline 206

GLP: Yes

Executive Summary

A 10-week range-finding reproduction test was conducted in order to assess the effects of CGA173506 on the bobwhite quail (*Colinus virginianus*) were determined in a 10-week reproduction toxicity test. Mortality, body weight, food consumption, reproductive parameters, and any other overt signs of toxicity, were assessed at nominal dietary concentrations of 78.8, 106, 225 and 446 mg a.s./kg body weight/day (800, 1200, 2400 and 4800 ppm a.s.), alongside an untreated control group.

There were no treatment-related differences in adult body weights or food consumption. No avoidance was indicated in any of the treatment groups. When evaluated on a weekly basis, treatment group feed consumption values were similar to the controls.

There were no treatment-related declines in the reproduction parameters. ED_{50} values could not be calculated by a regression analysis. Therefore, for the parameters the ED_{50} values were greater than 446 mg a.s./kg bw/day. There were no treatment-related differences in egg shell quality and offspring body weights.

There were no treatment-related differences in egg shell quality or offspring body weights.

During the withdrawal period, adult body weight, feed consumption and reproduction parameter results from the treated groups were comparable with the control group.

Materials

Test Material	CGA173506 Fludioxonil
Lot/Batch #:	SMO3J0464
Purity:	97.7%
Description:	Brownish powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	End of October 2017

Treatments

Test rates:	Concentrations of 78.8, 106, 225 and 446 mg a.s./kg body weight/day (800, 1200, 2400 and 4800 ppm a.s.) untreated control.
Food:	Basal ration contained at least 27% protein, a minimum of 2% crude fat, and no

	more than 5% fibre.
Water:	Water was supplied by the town of Easton public water supply. All offspring received a water-soluble vitamin and electrolyte mix in their water.
Analysis of test concentrations:	Yes – via HPLC analysis
Test organisms	
Species:	Bobwhite quail (*Colinus virginianus*) 38 weeks old
Source:	M & M Quail Farm, 4090 Campbell Road, Gillsville, GA 30543, U.S.A.
Acclimatisation period:	18 weeks
Treatment for disease:	None reported
Weight :	174 to 219 g
Test design	
Test cage description:	All birds were housed in pens 27 X 51 cm. The pens had sloping floors that resulted in ceiling height ranging from 20 to 25 cm
Replication:	5
No. of birds/pen :	Two (one male, one female)
Duration of test:	Study phases: 1. Acclimation – approximately 18 weeks. 2. Pre-exposure feed consumption – 1 week. 3. Pre-photostimulation with exposure- 1 week. 4. Pre-egg laying (with photostimulation) - 3 weeks. 5. Egg laying - 8 weeks (6 weeks during exposure and 2 weeks during withdrawal). 6. Withdrawal period – 2 weeks. 7. Post-adult termination (final incubation, hatching, and 14-day offspring rearing period) – 6 weeks.
Environmental test conditions	
Temperature:	20.4 ± 1.8°C (SD)
Humidity:	39 ± 13% (SD).
Photoperiod:	During acclimation, pre-exposure feed consumption and the first week of the exposure period was 8 hrs light. The photoperiod was increased to 17 hrs light per day at the beginning of the second week of exposure to induce egg laying and was maintained until test end (266 lux).

Study Design and Methods

Experimental dates: 12th February to 23rd June 2014

Adult northern bobwhite quail, approximately 38 weeks old and in good health, were randomly assigned to the control and the test groups, and each group was further divided into 5 pens containing one female and one male per pen. The test substance doses were prepared using appropriate volumes of CGA173506 and basal ration such that, once incorporated into the final diet, treatment levels of 800, 1200, 2400 and 4800 ppm a.s. were achieved.

The mix for the control groups consisted of basal ration only. The birds were housed in batteries of pens measuring 27 x 51 cm with sloping floors that resulted in ceiling height ranging from 20 to 25 cm. During the test all birds were given food and water *ad libitum*.

Adult birds were observed daily for signs of toxicity or abnormal behaviour. Offspring were observed daily from hatching until 14 days old. Adult body weights were measured at test initiation and then biweekly thereafter except during the egg production period. Feed consumption per pen was measured bi-

weekly. At the start of week 5, birds were induced to lay. At the conclusion of the exposure period, birds that died during the investigation and all surviving adult birds in each group, were euthanized and subjected to a gross necropsy.

At the end of the weekly interval, all eggs were removed from the cold room, counted and eggs selected by indiscriminate draw for eggshell strength and thickness measurements, and content collection. The remaining eggs were candled with an egg-candling lamp (Speed King Model No. 32) to detect egg shell cracks or abnormal eggs. Cracked or abnormal eggs were recorded and discarded. All eggs to be incubated were fumigated with formaldehyde gas in an airtight cabinet with a circulating fan for approximately two hours, to reduce the possibility of pathogen contamination prior to incubation. All eggs not discarded or used for egg shell thickness measurements were placed in an incubator. In the incubator the temperature was maintained at an average 37.4 ± 0.1°C (SD) with an average relative humidity of 55 ± 0%.

On Day 21 of incubation, the eggs were placed in an incubator configured for hatching and allowed to hatch. All hatchlings, unhatched eggs and eggshells were removed from the hatcher on Day 25 or 26 of incubation. The individual body weight of each surviving hatchling was measured. Hatchlings were leg banded for identification by pen of origin and then routinely housed in brooding pens until 14 days of age. The hatchlings were fed untreated diet without the addition of 5% supplemental limestone. At 14 days of age the individual body weights of surviving offspring were measured.

Upon completion of the test, data were evaluated for differences among the groups. Results, except for the daily feed consumption data, were reviewed for declines across the treatment groups approaching or greater than 50%. This was done to determine which, if any, parameters needed to be analysed using non-linear regression analysis to determine the Effect Dose that would cause a 50% reduction in the parameter (ED_{50}) and the 95% confidence levels. However, none of the parameters had declines among the groups that were suitable for the regression analysis. An analysis of variance (ANOVA) was performed to determine statistically significant differences among groups. Dunnett's multiple comparison procedure was then used to compare the five treatment means with the control group mean and assess the statistical significance of the observed differences.

Results and Discussion

Samples were collected during the test to verify test substance concentrations for the 800, 1200, 2400 and 4800 ppm a.s. diets, which were found to have maintained 97%, 108%, 108 and 116% of nominal concentrations, respectively. Analysis of diet samples collected from feeders after being held at ambient temperature for seven days averaged 93% and 102% of the Day 0 values for the 800, and 4800 ppm a.s. test concentrations, respectively.

No adult treatment-related mortalities occurred during the course of the test. No overt signs of toxicity were observed at any of the concentrations tested. When compared to the control group, there were no apparent treatment-related effects upon adult body weight. There appeared to be no treatment-related effects upon egg shell strength or thickness at the 800, 1200, 2400 or 4800 ppm a.s. treatment groups. When compared to the control group, there appeared to be no treatment-related effects upon weekly feed consumption at the any of the test concentrations.

Reproductive effects are summarised in the table below.

Table 8.1.1.3-75: Summary of reproductive performance from a northern bobwhite (*Colinus virginianus*) dietary reproduction range-finding study with fludioxonil

Reproductive parameter	Test concentration (ppm a.s.)									
	Exposure period					Withdrawal period				
	0	800	1200	2400	4800	0	800	1200	2400	4800

Reproductive parameter	Test concentration (ppm a.s.)									
	Exposure period					Withdrawal period				
	0	800	1200	2400	4800	0	800	1200	2400	4800
Dietary Dose (mg a.s./kg bw/day)	0	78.8	106	225	446	--	--	--	--	--
No. of replicates	5	5	5	4	5	5	5	5	5	5
Total Eggs laid	74	101	61	100	78	50	59	46	49	49
Eggs cracked	0	2	1	3	2	1	0	0	2	3
Eggs set	64	87	53	85	66	44	54	41	43	41
Fertile eggs	61	80	48	84	65	44	54	40	43	41
Viable embryos	61	79	46	81	64	44	53	39	42	40
Live 3-week embryos	61	79	46	81	64	44	53	39	42	40
Hatchlings	58	72	44	79	61	43	48	35	41	35
14-day old survivors	55	68	43	78	60	38	45	35	38	35
Eggs laid/hen	15	20	12	25	16	10	12	9	12	10
Eggs Laid / Hen / Day	0.23	0.32	0.19	0.40	0.25	0.71	0.84	0.66	0.88	0.70
14 day survivors per hen	11	14	9	20	12	8	9	7	10	7
Eggs laid as a % of maximum laid	41	56	34	69	43	67	79	61	82	65
Eggs cracked as a % of eggs laid	0	1	1	3	3	3	0	0	4	5
Fertile eggs as a % of eggs set	96	91	95	99	98	100	100	98	100	100
Viable embryos as a % of eggs set	96	89	94	94	97	100	98	97	97	98
Live 3-week embryos as a % of viable embryos	100	100	99	100	97	100	100	98	100	96
Hatchlings as a % of live 3-week embryos	95	86	99	97	98	96	88	93	98	93
14-day survivors as a % of hatchlings	96	96	99	99	99	88	92	100	92	100
Hatchlings as a % of eggs set	92	77	92	92	93	96	87	85	95	87
Hatchlings as a % of fertile eggs	95	84	95	93	95	96	87	87	95	87
14-day survivors as a % of eggs set	88	73	91	91	92	85	82	85	87	87
Hatchlings as a % of maximum set	36	45	28	62	38	61	69	50	73	50
14-day survivors as a % of maximum set	34	43	27	61	38	54	64	50	68	50
Mean egg shell thickness (mm)	0.221 ± 0.022	0.239 ± 0.016	0.227 ± 0.018	0.221 ± 0.016	0.232 ± 0.026	0.228 ± 0.024	0.238 ± 0.011	0.231 ± 0.019	0.227 ± 0.026	0.233 ± 0.025
Mean hatchling body weight (g)	6.0 ± 0.7	6.1 ± 0.4	5.4 ± 0.8	5.9 ± 0.3	6.0 ± 0.7	6.1 ± 0.3	6.2 ± 0.6	5.7 ± 0.6	6.1 ± 0.2	6.0 ± 0.8
Mean 14-day old survivor body weight (g)	27 ± 2	26 ± 5	25 ± 2	26 ± 2	26 ± 4	27 ± 2	28 ± 3	25 ± 1	27 ± 4	23 ± 7

There were no treatment-related reductions in any reproduction parameters at any of the treatment levels. While there were some differences among the treatment groups, they were variable and not concentration responsive. None of the reductions appeared sufficient for regression analysis and possible determination of an ED_{50}. Therefore, regression parameters and an ED_{50}'s could not be calculated (ED_{50} > 446 mg/kg bw/day). Reproductive parameters during the withdrawal period were comparable to the exposure period. Parameters were also comparable among the treatment groups and the control group for the withdrawal period.

Conclusions

Northern bobwhite were exposed to fludioxonil at dietary concentrations of 800, 1200, 2400 or 4800 ppm a.s. for 10 weeks. No treatment-related mortalities or overt signs of toxicity were observed. The estimated daily dietary doses for the 10-week exposure period were 78.8, 106, 225 and 446 mg a.s./kg/day, respectively.

There were no treatment-related differences in adult body weights or food consumption. Food avoidance was evaluated for each day during the first week of exposure. No avoidance was indicated in any of the treatment groups. When evaluated on a weekly basis, treatment group feed consumption values were similar to the controls.

There were no treatment-related declines in the reproduction parameters. ED_{50} values could not be calculated by a regression analysis. Therefore, for the parameters the ED_{50} values were greater than 446 mg a.s./kg bw/day.

There were no treatment-related differences in egg shell quality and offspring body weights. Values among the groups were comparable.

During the withdrawal period, adult body weight, feed consumption and reproduction parameter results from the treated groups were comparable with the control group.

(Frey *et al*. 2014)

The new definitive bobwhite quail reproduction study is summarised below.

Report:	New definitive reproduction study to investigate the NOEC and reduce necessity for risk refinement for seed treatment. K-CA 8.1.1.3/06 Bryden M, Frey LT, Elliott S. & Martin KH (2015). Fludioxonil - A Reproduction Study with the Northern Bobwhite, Report Number 528B-457. Wildlife International A Division of EAG Inc. 8598 Commerce Drive Easton, MD 21601 USA (Syngenta File No. CGA173506_11828)

Guidelines

U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OCSPP Number 850.2300

OECD Guideline 206

GLP: Yes

Executive Summary

The effects of fludioxonil on the northern bobwhite quail (*Colinus virginianus*) were determined in a 20-week reproduction toxicity test. Mortality, body weight, food consumption, reproductive parameters, and any other overt signs of toxicity, were assessed at nominal dietary concentrations of 1200, 2400 and 4800 ppm a.s., alongside an untreated control group.

There were no adult treatment-related mortalities, overt signs of toxicity or treatment-related effects upon adult body weight, feed consumption or reproductive parameters at any test concentration. The no-observed-effect concentration for northern bobwhite exposed to fludioxonil in the diet during the study was 4800 ppm a.s. (389.02 mg a.s./kg bw/day), the highest concentration tested.

Materials

Test Material	fludioxonil tech. CGA173506
Lot/Batch #:	SMO3J0464
Purity:	97.7%
Description:	Brownish powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	31 October 2017
Density:	Not applicable
Treatments	
Test rates:	1200, 2400 and 4800 ppm a.s., alongside an untreated control
Food:	Basal diet (at least 27% protein and 2% crude fat, and no more than 5% crude fibre) *ad libitum* in treated groups diet contained fludioxonil.
Water:	Easton public water supply. All offspring received a water-soluble vitamin and electrolyte mix in their water
Analysis of test concentrations:	Fludioxonil determined in the diet using HPLC analysis
Test organisms	
Species:	Northern bobwhite quail (*Colinus virginianus*) 22 weeks at test initiation
Source:	Birds obtained from M & M Quail Farm, 4090 Campbell Road, Gillsville, GA 30543 U.S.A
Acclimatisation period:	3 weeks
Treatment for disease:	None
Weight:	171 to 219 g at test initiation
Test design	
Replication:	18
No. of birds/pen :	Two (1 male and 1 female)
Duration of test:	Acclimation - 3 weeks. Pre-photostimulation - 8 weeks. Pre-egg laying (with photostimulation) - 2 weeks. Egg laying - 10 weeks. Post-adult termination (final incubation, hatching, and 14-day offspring rearing period) –6 weeks.

Parameters monitored:	Adult Body Weight; Adult Feed Consumption; Eggs Laid/Hen/Day; Eggs Cracked of Eggs Laid; Fertile Eggs of Eggs Set; Viable Embryos of Eggs Incubated; Live 3-Week Embryos of Viable Embryos; Hatchlings of 3-Week Embryos; 14-Day Old Survivors of Hatchlings; Hatchlings of Eggs Incubated; Hatchlings of Fertile Eggs; 14-Day Old Survivors of Eggs Incubated; Hatchlings/Pen/Day; 14-Day Old Survivors/Pen/Day; Egg Shell Thickness; Offspring Body Weights.

Environmental test conditions

Temperature:	Average temperature: 21.3 (range 18.8-23.6) °C Egg storage prior to incubation: 13.4 ºC Egg storage during incubation: 37.4 ºC Egg storage during hatching: 37.3 ºC Chicks average temperature 26.8 ºC
Humidity:	Adult birds, mean daily relative humidity: 33 % Egg storage during incubation: 55% (RH) Chicks, mean daily relative humidity: 50%
Photoperiod:	Adult birds: 8 hrs light (422 lux) during first 8 weeks,. At the beginning of week 9 photoperiod changed to 17 hrs light (232 lux) Chicks: 16 hour light photoperiod

Study Design and Methods

Experimental dates: 29 December 2014 to 29 June 2015

Adult bobwhite quail (72 males and 72 females), approximately 22 weeks 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. The mix for the control groups consisted of basal ration only. The birds were housed in batteries of cages measuring 25 x 51 cm with a sloping roof height of 20 to 26 cm. During the test all birds were given food and water *ad libitum*.

Adult birds were observed daily for signs of toxicity or abnormal behaviour. Offspring were observed daily from hatching until 14 days old. Adult body weights were measured at test initiation, at the end of Weeks 2, 4, 6, 8, and at adult termination. Feed consumption was measured weekly throughout the test. At the conclusion of the exposure period, all surviving adult birds were euthanized with carbon dioxide gas, necropsied and disposed of by incineration.

Eggs were collected daily from all pens, when available. The eggs were stored in a cold room until incubation at a mean temperature of 13.4°C. All eggs laid in a weekly interval were considered as one lot.

At the end of the weekly interval, all eggs were removed from the cold room, counted and eggs selected by indiscriminate draw for egg shell thickness measurement. The remaining eggs were candled with an egg-candling lamp to detect egg shell cracks or abnormal eggs. Cracked or abnormal eggs were recorded and discarded. All eggs not discarded or used for egg shell thickness measurements were placed in an incubator. In the incubator the temperature was maintained at an average 37.4 ± 0.0°C (SD) with an average relative humidity of 55 ± 0 % (SD).

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during odd numbered weeks and from each of the even numbered pens during the even numbered weeks, and were examined for shell thickness. Embryo viability and survival, unhatched eggs, hatchlings, and weight and viability of chicks, were recorded for each hatch.

Upon completion of the test, Dunnett's multiple comparison procedure was used to compare the three treatment means with the control group mean and assess the statistical significance of the observed differences. Sample units were the individual pens within each experimental group, except adult body weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation.

Results and Discussion

Fecundity is summarised in the tables below.

Table 8.1.1.3-86: Summary of effects of fludioxonil on fecundity of northern bobwhite (*Colinus virginianus*) following oral exposure

Parameter	Control	1200 ppm	2400 ppm	4800 ppm [a]
Eggs laid per henfemale	38	46	44	51
Mean egg shell thickness (mm)	0.231	0.234	0.231	0.233
Total Eggs laid	645	825	800	870
Eggs cracked	4	4	12	12
Eggs cracked % of eggs laid	1	1	1	1
Fertile eggs	541	695	674	669
Eggs set	553	736	702	774
Viable embryos	532	687	661	647
Viable embryos of eggs set (%)	96	93	93	85
Live 3-week embryos	529	685	658	645
Live 3-week embryos of viable embryos (%)	99	100	100	100
Normal hatchlings	485	611	597	609
Normal hatchlings of eggs set (%)	88	82	84	80
Normal hatchlings of live 3-week embryos (%)	92	89	91	95
14-day old survivors	450	552522	531	562
14-day old survivors of eggs set (%)	81	75	76	74
14-day old survivors of normal hatchlings (%)	92	90	90	92
14-day old survivors per female	26	31	30	33
Chick bodyweights at hatching (g)	6.1	6.1	5.9	5.8
Chick bodyweights at 14 days (g)	23	23	23	23

Table 8.1.1.3-86: Summary of reproductive performance from northern bobwhite (*Colinus viginianus*) with fludioxonil

Parameter	Control	1200 ppm	2400 ppm	4800 ppm [a]
Number of surviving replicates	17	18	18	17
Eggs cracked	4	4	12	11
Eggs set	553	736	702	719
Fertile eggs	541	695	674	662
Viable embryos	532	687	661	647
Live 3-week embryos	529	685	658	645
Normal hatchlings	485	611	597	609
14-day old survivors	450	552	531	562
Eggs laid per hen	38	46	44	51 [b]
Hatchlings per pen	29	34	33	36
14-day old survivors per pen	26	31	30	33
Mean egg shell thickness (mm)	0.231	0.234	0.231	0.233

[a] As requested by the RMS a clarification was made to explain why this data had been excluded from the study. In Pen 566 both birds were found to be female at necropsy. Therefore the data for these birds has been excluded from the study and tables in the original report marked with '-'.
[b] Clarified that Table 7 in the original report was wrong, Appendix 12 data was correct. Error due to amendment of numbers following discovery of to females in one pen (566); data from this pen was then excluded from the report.

Table 8.1.1.3-9: Summary of reproductive performance, normalised as percentages from northern bobwhite (*Colinus viginianus*) with fludioxonil

Parameter	Control	1200 ppm	2400 ppm	4800 ppm [a]
Number of surviving replicates	17	18	18	17
Total Eggs laid	645	825	800	809
Eggs laid / hen / day	0.45	0.55	0.53	0.60
Eggs cracked / eggs laid (%)	1	1	1	1
Fertile eggs / eggs set (%)	98	94	95	93
Viable embryos / eggs set (%)	96	93	93	90
Live 3-week embryos / viable embryos (%)	99	100	100	100
Hatchlings / live 3-week embryos (%)	92	89	91	95
14-day old survivors / normal hatchlings (%)	92	90	90	92
Hatchlings / eggs set (%)	88	82	84	85
Hatchlings / fertile eggs (%)	90	88	88	87
14-day old survivors / eggs set (%)	81	75	76	78

Parameter	Control	1200 ppm	2400 ppm	4800 ppm [a]
Hatchlings / pen / day	0.34	0.40	0.39	0.45
14-day old survovors / pen / day	0.32	0.37	0.35	0.42
Chick bodyweights at hatching (g)	6.1	6.1	5.9	5.8
Chick bodyweights at 14 days (g)	23	23	23	23

[a] In Pen 566 both birds were found to be female at necropsy. Therefore the data for these birds has been excluded from the study and tables in the original report marked with '-'.

There were no treatment-related effects upon reproductive performance at the 1200, 2400 or 4800 ppm a.s. test concentrations, for adult feed consumption, egg shell thickness, reproduction or offspring body weight.

At the 1200 ppm a.s. treatment group, there was a slight increase in the mean body weight of hens at adult termination that was statistically significant at $p \leq 0.05$. However, the increase was slight, not consistent over time or concentration responsive, and was therefore considered to be unrelated to treatment (see table below).

Table 8.1.1.3-97: Mean ± standard deviation of adult body weight (g) from a northern bobwhite reproduction study with fludioxonil

Experimental group (ppm a.s.)	Sex	Week 0	Δ	Week 2	Δ	Week 4	Δ	Week 6	Δ	Week 8	Δ	Term	Total change
0	Male	196	3	199	1	200	1	202	3	204	9	215	18
		13	3	14	2	14	2	15	2	16	11	19	11
	Female	189	3	192	1	193	0	194	2	196	34	230	41
		11	3	11	4	11	3	12	2	13	14	20	16
1200	Male	196	6	202	-1	202	0	202	2	204	12	216	20
		13	2	13	2	12	2	13	2	13	11	18	11
	Female	193	5	198	-1	198	0	198	2	200	46	246*	52
		9	3	9	3	9	2	9	3	9	17	18	17
2400	Male	195	6	201	0	200	1	201	1	202	9	211	15
		15	4	16	2	16	2	16	3	16	9	19	9
	Female	190	6	196	0	196	0	196	1	197	38	235	45
		10	2	9	3	10	2	10	2	10	13	11	13
4800	Male	194	0	195	3	197	0	198	2	199	8	207	14
		11	2	12	3	13	1	13	3	12	12	17	11
	Female	194	0	193	2	196	-1	195	1	196	44	241	48
		10	5	9	2	9	4	10	3	11	12	15	14

The means for body weights and body weight changes were calculated and rounded separately.
Δ - Change between intervals.
* Significantly different from control at $p \leq 0.05$ (Dunnett's t-test).

Validity criteria

Criteria number:	Parameter	Validity criteria	This study
1	Assignment of birds to control groups	Random	Random
2	Test substance concentrations maintained in diets		
	1200 ppm	≥80%	97-109%

	2400 ppm		101-109%
	4800 ppm		103-110%
Control group criteria:			
3	Mortality	≥10%	3%
4	Average number of eggs laid per hen	≥29	38
5	Viable embryos of eggs set at ~Day 11	≥80%	96%
6	Live 3-week embryos of viable embryos at ~ Day 21	≥97%	99%
7	Hatchlings of live 3-week embryos	≥85%	92%
8	Hatchlings of eggs set for incubation	≥71%	88%
9	14-day old survivors per hen	≥77%	92%
10	Average egg shell thickness	≥0.200 mm	0.231 mm
11	Cracked eggs of eggs laid	≥13%	1%
12	Average number of 14-day old survivors per hen	≥12	26

Conclusions

There were no adult treatment-related mortalities, overt signs of toxicity or treatment-related effects upon adult body weight, feed consumption or reproductive parameters at the 1200, 2400 or 4800 ppm a.s. test concentrations. The no-observed-effect concentration for northern bobwhite exposed to fludioxonil the diet during the study was 4800 ppm a.s. (389 mg a.s./kg bw/day), the highest concentration tested.

(Bryden M., Frey L.T, Elliott S. & Martin K.H, 2015)

Relevant literature on birds

No relevant scientifically peer-reviewed open literature could be found on fludioxonil 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

Organism	Test item	Test type	EU endpoint	Endpoints used in the risk assessment	Reference (author, date, Syngenta Ref)
Rat	Fludioxonil	Acute oral	LD_{50} = >5000 mg/kg bw	LD_{50} = >5000 mg/kg bw	***Hartmann, 1993 (CGA173506/0369)***
Rat	Fludioxonil	Chronic oral	NOAEL 200 mg/kg bw/day	NOAEL 1004~~200~~ mg/kg bw/day	***Yau et al., 1992 (CGA173506/0180)***

CA 8.1.2.1 Acute oral toxicity to mammals

Since fludioxonil 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 original EU review as these data were evaluated previously. Studies have been carried out with fludioxonil for the toxicology assessment; please refer to point **M-CA Section 5 (Toxicology)** for studies with the active substance and major metabolites. No new data are provided.

The original endpoints relevant for the ecotoxicological risk assessment are summarised in Table 8.1.2-1 above and these endpoints remain valid.

For acute toxicity studies 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, or by the wild mammal acute risk assessments according to the current CA 8.1.3 EFSA Guidance Document (2009)[4].

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

Since fludioxonil 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**. No new studies have been carried out for the toxicology assessment as these data were provided previously; please refer to **M-CA Section 5 (Toxicology)** for studies with the active substance and major metabolites.

The original endpoints relevant for the ecotoxicological risk assessment are summarised in Table 8.1.2-1 above and these endpoints have been re-evaluated in the table below~~remain valid~~.

Table 8.1.2.2-1 Data from the mammalian toxicology section, relevant to identify the ecotoxicologically relevant reproductive endpoint for mammals

Endpoint	Studies evaluated	Author/ Syngetna file number / title	Summary
Body weight change, behavioural effects and systemic toxicity	28-day oral toxicity study (OECD 407) Dose levels 0, 10, 100 and 1000 mg/kg/day in distilled water with 0.5% methylcellulose and 0.1% Tween 80	Fankhauser 1990 – CGA173506/0054 Gavage in the rat	No adverse clinical signs. Treatment up to 1000 mg/kg/day (highest dose) slightly affected bodyweight gain (↓8% M/↓35%F). No effect of treatment on gain at lower doses. ≥100 mg/kg/day resulted in effects on clinical pathology and/or organ weight effects of the liver and kidneys but the effects at 100 mg/kg/day are not considered adverse. Pathology findings in liver and kidney at 1000 mg/kg/day only The NOEL is 10 mg/kg/day. The NOAEL = 100 mg/kg/day based on liver pathology and decreased bodyweight gain at 1000 mg/kg/day. The increases in organ weights at 100 mg/kg/day are considered to be non-adverse (↑ ≤20% of control and no correlating pathology at this dose).
	Sub-chronic oral toxicity study (OECD 408) Dose levels 0, 0.8, 6.6, 64, 428 and 1283 mg/kg/day (males); 0, 1, 7.1, 70, 462 and 1288 mg/kg/day (females)	Chang 1990 – CGA172506/0048 Dietary 90 day in the rat	≥428/462 mg/kg/day resulted in bodyweight decreases in both sexes, and a decrease in food consumption in females. No clinical signs ≥428/462 mg/kg/day also resulted in effects on clinical pathology and/or organ weight effects of the liver and kidneys. Plus, statistically significant increases in chronic nephropathy and centrilobular hepatocyte hypertrophy were observed. Centrilobular hypertrophy was also noted at lower doses (but not in controls) but did not attain statistical significance.

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

Endpoint	Studies evaluated	Author/ Syngetna file number / title	Summary
			The NOEL is 0.8/1.0 mg/kg/day. The NOAEL is set at 64/70 mg/kg/day based on kidney effects, and decreases in body weight gain and food intake. The finding centrilobular hypertrophy observed at 6.6/7.0 and 64/70 mg/kg/day were not associated with organ weight changes and did not achieve statistical significance.
	Multi-generational study (OECD 416) Dose levels: 0, 1.88, 18.9 and 187.74 mg/kg/day (F_0-males); 0, 2.11, 21.07 and 211.5 mg/kg/day (F_0-females); 0, 2.08, 21.13 and 212.9 mg/kg/day (F_1-males); 0, 2.33, 22.84 and 236.86 mg/kg/day (F_1-females)	Singh 1992-CGA173506/0180 2-generation study in the rat (dietary)	No adverse clinical signs All animals at the highest dose (in all generations) had reduced body weight gain and food consumption, but difference was no more than 12% of control. No effects on pup survival The parental NOAEL was 18.9/21.1 mg/kg bw/day based on reduced body weight and food consumption. The relevant NOEL for reproduction was 211.5/212.9 mg/kg bw/day owing to no effects.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 1000 mg/kg/day, gavage in 0.5% MC in water	Savary 1989a - CGA173506/0051 Rat developmental toxicity study	1000 mg/kg/day resulted in lower body weight gain (-16%). The maternal NOAEL was set at 100 mg/kg/day.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 300 mg/kg/day, gavage in 0.5% methylcellulose in water	Savary 1989b - CGA173506/0052 Rabbit developmental toxicity study	1 animal died in the 100 mg/kg/day on day 18, considered unrelated to treatment. No adverse clinical signs. Reduced body weight and food consumption were observed at 300 mg/kg/day. The relevant maternal NOAEL is 100 mg/kg bw/day, and the foetal/developmental NOAEL, is 300 mg/kg bw/day.
Indices of gestation, litter size, pup and litter weight	Multi-generational study (OECD 416) Dose levels: 0, 1.88, 18.9 and 187.74 mg/kg/day (F_0-males); 0, 2.11, 21.07 and 211.5 mg/kg/day (F_0-females); 0, 2.08, 21.13 and 212.9 mg/kg/day (F_1-males); 0, 2.33, 22.84 and 236.86 mg/kg/day (F_1-females)	Singh 1992-CGA173506/0180 2-generation study in the rat (dietary)	Reduced pup weight and survival in F_1 and F_2 generations were observed, but were related to severe reductions in bodyweight parameters in the parental females. Gestation and litter size were unaffected. The NOAEL was 18.9/21.1 mg/kg bw/day based on reduced body weight.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 1000 mg/kg/day, gavage in 0.5% MC in water	Savary 1989a - CGA173506/0051 Rat developmental toxicity study	No effects. The developmental NOAEL was set at 1000 mg/kg/day.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and	Savary 1989b - CGA173506/0052 Rabbit	No effects. The developmental NOAEL was set at 300 mg/kg/day.

Endpoint	Studies evaluated	Author/ Syngetna file number / title	Summary
	300 mg/kg/day, gavage in 0.5% methylcellulose in water	developmental toxicity study	
Indices of viability, pre- and post-implantation loss	Multi-generational study (OECD 416) Dose levels: 0, 1.88, 18.9 and 187.74 mg/kg/day (F_0-males); 0, 2.11, 21.07 and 211.5 mg/kg/day (F_0-females); 0, 2.08, 21.13 and 212.9 mg/kg/day (F_1-males); 0, 2.33, 22.84 and 236.86 mg/kg/day (F_1-females)	Singh 1992-CGA173506/0180 2-generation study in the rat (dietary)	No effects.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 1000 mg/kg/day, gavage in 0.5% MC in water	Savary 1989a - CGA173506/0051 Rat developmental toxicity study	No effects.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 300 mg/kg/day, gavage in 0.5% methylcellulose in water	Savary 1989b - CGA173506/0052 Rabbit developmental toxicity study	No effects.
Embryo/foetal toxicity including teratological effects	Multi-generational study (OECD 416) Dose levels: 0, 1.88, 18.9 and 187.74 mg/kg/day (F_0-males); 0, 2.11, 21.07 and 211.5 mg/kg/day (F_0-females); 0, 2.08, 21.13 and 212.9 mg/kg/day (F_1-males); 0, 2.33, 22.84 and 236.86 mg/kg/day (F_1-females)	Singh 1992-CGA173506/0180 2-generation study in the rat (dietary)	No effects.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 1000 mg/kg/day, gavage in 0.5% MC in water	Savary 1989a - CGA173506/0051 Rat developmental toxicity study	No effects.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 300 mg/kg/day, gavage in 0.5% methylcellulose in water	Savary 1989b - CGA173506/0052 Rabbit developmental toxicity study	No effects.
Number aborting and number delivering early	Multi-generational study (OECD 416)	Singh 1992-CGA173506/0180	

Endpoint	Studies evaluated	Author/ Syngetna file number / title	Summary
	Dose levels: 0, 1.88, 18.9 and 187.74 mg/kg/day (F_0-males); 0, 2.11, 21.07 and 211.5 mg/kg/day (F_0-females); 0, 2.08, 21.13 and 212.9 mg/kg/day (F_1-males); 0, 2.33, 22.84 and 236.86 mg/kg/day (F_1-females)	2-generation study in the rat (dietary)	
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 1000 mg/kg/day, gavage in 0.5% MC in water	Savary 1989a - CGA173506/0051 Rat developmental toxicity study	No effects.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 300 mg/kg/day, gavage in 0.5% methylcellulose in water	Savary 1989b - CGA173506/0052 Rabbit developmental toxicity study	No effects.
Systemic toxicity and effects on adult body weight	Multi-generational study (OECD 416) Dose levels: 0, 1.88, 18.9 and 187.74 mg/kg/day (F_0-males); 0, 2.11, 21.07 and 211.5 mg/kg/day (F_0-females); 0, 2.08, 21.13 and 212.9 mg/kg/day (F_1-males); 0, 2.33, 22.84 and 236.86 mg/kg/day (F_1-females)	Singh 1992- CGA173506/0180 2-generation study in the rat (dietary)	No adverse clinical signs All animals at the highest dose (in all generations) had reduced body weight gain, but difference was less than 10% of control. The parental NOAEL was ≥18.9/21.1 mg/kg bw/day based on reduced body weight and food consumption. Considered to be greater due to the large dose spacing and reduced gain is not considered biologically significant.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 1000 mg/kg/day, gavage in 0.5% MC in water	Savary 1989a - CGA173506/0051 Rat developmental toxicity study	1000 mg/kg/day resulted in lower body weight gain (-16%). The maternal NOAEL was set at 100 mg/kg/day.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 300 mg/kg/day, gavage in 0.5% methylcellulose in water	Savary 1989b - CGA173506/0052 Rabbit developmental toxicity study	1 animal died in the 100 mg/kg/day on day 18, considered unrelated to treatment. No adverse clinical signs. Reduced body weight and food consumption were observed at 300 mg/kg/day. The relevant maternal NOAEL is 100 mg/kg bw/day.
Indices of post-natal growth, indices of lactation and data on physical landmarks	Multi-generational study (OECD 416) Dose levels: 0, 1.88, 18.9 and 187.74 mg/kg/day (F_0-males); 0, 2.11, 21.07 and 211.5 mg/kg/day (F_0-females);	Singh 1992- CGA173506/0180 2-generation study in the rat (dietary)	No biologically significant effect on body weight gain, slightly decreased gain for males at 212.9 mg/kg/day but overall difference less than 6% No effect on pup survival, lactation or physical landmarks. The relevant NOAEL for reproduction was

Endpoint	Studies evaluated	Author/ Syngetna file number / title	Summary
	0, 2.08, 21.13 and 212.9 mg/kg/day (F_1-males); 0, 2.33, 22.84 and 236.86 mg/kg/day (F_1-females)		211.5/212.9 mg/kg bw/day owing to no effects.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 1000 mg/kg/day, gavage in 0.5% MC in water	Savary 1989a - CGA173506/0051 Rat developmental toxicity study	No effects.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 300 mg/kg/day, gavage in 0.5% methylcellulose in water	Savary 1989b - CGA173506/0052 Rabbit developmental toxicity study	No effects.
Survival and general toxicity up to sexual maturity	Multi-generational study (OECD 416) Dose levels: 0, 1.88, 18.9 and 187.74 mg/kg/day (F_0-males); 0, 2.11, 21.07 and 211.5 mg/kg/day (F_0-females); 0, 2.08, 21.13 and 212.9 mg/kg/day (F_1-males); 0, 2.33, 22.84 and 236.86 mg/kg/day (F_1-females)	Singh 1992- CGA173506/0180 2-generation study in the rat (dietary)	No effects.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 1000 mg/kg/day, gavage in 0.5% MC in water	Savary 1989a - CGA173506/0051 Rat developmental toxicity study	No effects.
	Developmental studies (OECD 414) Dose levels 0, 10, 100 and 300 mg/kg/day, gavage in 0.5% methylcellulose in water	Savary 1989b - CGA173506/0052 Rabbit developmental toxicity study	No effects.

Conclusion

Test type	Current Annex I endpoint	Proposed ecotoxicologically relevant reproductive endpoint for mammals
Reproductive toxicity (long-term)	Rat NOAEL = 200 mg a.s./kg bw/d Singh (1992) (CGA173506/0180)	Rabbit NOAEL = 100 mg a.s./kg bw/d Savary (1989) (CGA173506/0052)

Justification of Endpoint
A NOAEL from the rabbit developmental study (Savary 1989 – CGA173506/0052) of 100 mg/kg/day was body weight effects at 300 mg/kg/day. Although there was a 38% decrease in body weight gain for females receiving 100 mg//kg/day between GD6-12 compared with controls it is Syngenta's opinion that this difference was not adverse due to interindividual variation in the body weight performance of rabbits in the study (MCA Section 5). There were no other effects in this study.

	LOAEL (mg/kg/day)	NOAEL (mg/kg/day
28 day rat toxicity	1000	100
90 day rat toxicity	428	100
Rat devevelopmental toxicity	1000	100
Rabbit devevelopmental toxicity	300	100 (maternal)/300 (fetal)
Multigeneration study	211.5	19.1

The proposed ecotoxicologically relevant reproductive endpoint for mammal's endpoint is from the parental NOAEL of 100mg/kg bw/day based on reduced body weight and food consumption (Savary 1989 - CGA173506/0052).

Relevant Literature on Terrestrial Vertebrates other than Birds

No relevant scientifically peer-reviewed open literature could be found on fludioxonil or its major metabolites from an ecotoxicological perspective on wild mammals. Please refer to **M-CA Section 5 (Toxicology)** 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** (A8207M) and **Document M-CP Section 10** (A8240D) . As fludioxonil has a log P_{ow} of 4.12 a study was previously submitted for the EU review and summarised in point B.9.2.3 of the DAR ~~therefore an additional study summary is not repeated here~~. At the request of the RMS, the original summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoints are summarised in Table 8.1.3-1.

Table 8.1.3-1: Summary of endpoints for bioaccumulation in fish

Organism	Test item	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
Fathead minnow (*Pimephales promelas*)	Bio-accumulation	EU	BCF (whole fish) = 366	BCF (whole fish) = 366	***Gonzalez-Valero (1994) CGA173506/0517***

Report:	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS: *KIIA 8.2.3 Gonzalez-Valero J.F. (1994): Accumulation and elimination of 14C-CGA 173506 by bluegill sunfish (Lepomis macrochirus) in a dynamic flow-through system, Ciba-Geigy Ltd., Basel, Switzerland, unpublished report No. 93GJ02, September 30, 1994. (CGA173506/0517).*

Guidelines

Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental Fate: EPA 540/09-82-021, Section 165-4: "Laboratory Studies of Pesticide Accumulation in Fish". US Environmental Protection Agency, October 18, 1982.

EPA 540/09-88-051. Addendum 8 on Data Reporting; by Brinson Conerly and Samuel Creeger (Sept. 1988).

Additionally the following guidelines were taken into account:

OECD Guideline for Testing of Chemicals. Guideline 305 E: "Bioaccumulation: Flow-thro

OECD Guideline for Testing of Chemicals. Draft Guideline 305 E: "Bioaccumulation: Flow-through Fish Test, Paris/France, December 1992.

GLP: Yes

Executive Summary

^{14}C-CGA 173506 residues were rapidly concentrated in fish tissues, reaching 95 % of the steadystate concentration within two weeks. At steady state the estimated bioconcentration factors were of 56-58, 741-749 and 365-366 for edible portions, non-edible portions and whole fish, respectively. Fludioxonil residues were rapidly eliminated following the termination of exposure, with DT_{90} values for the whole fish of < 2 days.

Test System: 14C-fludioxonil, Batch No. Le-84.2A, specific activity 2.02 MBq/mg (= 54.54 µCi/mg), Purity ≥98%. Juvenile bluegill sunfish, *Lepomis macrochirus* (length 3.9-6.5 cm, average weight 2.0 g) were exposed to 14C-pyrrole-ring labelled fludioxonil at a concentration of 0.01 mg/l for 28 days under flow-through conditions. At the beginning of the study, the fish biomass to water ratio was 0.5 g per litre passing through the test system during 24 hours. The exposure was followed by a 14-day depuration phase in untreated water.

Stock solution A: Prior to the preparations of the treatment solutions (day 0-18), 122.70 mg unlabelled fludioxonil and about 72 mg of 14C-fludioxonil were dissolved in 20 mL acetone. Thereafter, the amount of radioactive material present was determined by LSC (Liquid Scintillation Counting) to be 193.87 mg, i.e. 143.76 MBq (3.882 mCi) with a specific radioactivity of 0.74 MBq/mg (19.98 µCi/mg). For preparation of the treatment solutions (three times a week), 0.428 ml (= 4.15 mg) of stock solution A were taken, 1 mL of Tween® 80 added and adjusted with tap water to the a final volume of 1 litre. The concentration of active ingredient in this solution was controlled by LSC.

Stock solution B: Prior to the preparations of the treatment solutions (day 19-28), 72.75 mg unlabelled fludioxonil and about 31 mg of 14C-fludioxonil were dissolved in 10 mL acetone. Thereafter, the amount of radioactive material present was determined by LSC (Liquid Scintillation Counting) to be 103.68 mg, i.e. 62.48 MBq (1.687 mCi) with a specific radioactivity of 0.60 MBq/mg (16.2 µCi/mg). For preparation of the treatment solutions (three times a week), 0.4 mL (= 4.15 mg) of stock solution B were taken, 1 mL of Tween® 80 added and adjusted with tap water to the final volume of 1 litre. The concentration of active ingredient in this solution was controlled by LSC. A continuous flow chemical delivery system was

used. The flow of the system was adjusted to 345.6 L per day and aquarium during exposure (corresponding to about 3 aquarium volumes per day). One aquarium served as control, the other two aquaria continuously received the 14Clabelled test compound from the treatment solution.

For each test aquarium, a pump equipped with a ceramic piston in a ceramic cylinder was used to deliver the appropriate amount of chemical stock solution into a mixing flask where it was diluted with water to produce the test solution. Volumes of 0.58 mL/min of the treatment solutions were pumped into the diluter and mixed with 240 mL/min of water, giving a final concentration in exposure water of 0.01 mg a.s./L.

During the depuration phase, no chemical stock solution was added. The flow of the system was adjusted to about 9001 per day and aquarium (corresponding to about 9 aquarium volumes per day). For analysis of the test item and metabolites, samples up to 1000 mL were taken at days 0, 3, 7, 21 and 28 of the exposure phase.

For analysis of radioactivity in edible and non-edible portions of fish samples, 2 to 3 fish were taken from each tank (total of 5 fish) at days 0, 1, 3, 7, 14, 21 and 28 of the exposure phase and at days 1, 2, 5, 7, 9 and 14 of the depuration phase and placed into a tricaine solution for euthanasia. Thereafter, they were dissected into edible (body muscle, skin and skeleton) and non-edible parts (fins, head and organs). The corresponding portions were pooled, placed into glass vials and tissue solubiliser *(Soluene350)* added at a quantity of 1 mL/g fish tissue. After the complete digestion of the tissues, aliquots of the solutions were submitted to LSC. For analysis of the test item and metabolites, 10 fish were removed from each tank (total 20 fish) at days 8 and 28 of the exposure phase, dissected as described above.

In addition to the incubation described above, a total biomass of about 500 g fish was exposed for 14 days to a dose of 50 µg fludioxonil per litre of water in a semi-static system. The water of the system was completely exchanged every 2 days and the concentration of the test compound new adjusted. After 14 days, all fish were removed from the aquaria and prepared as described above, but without dissecting into edible and non-edible portions.

Findings:
The measured temperature was between 19.5 and 20.9°C during the test, pH ranged from 7.38 to 7.66, and the oxygen saturation was between 81% and 118%. Illumination: twilight (14 hours daily). Concentrations of labelled fludioxonil in test water and fish tissue during the 42-day bioconcentration study (28-day uptake phase, followed by 14-day depuration phase) for bluegill sunfish are presented in the following table:

Table 8.1.3-2: Summary of fludioxonil concentrations in watr and fish tissues during uptake and depuration phases for bioaccumulation in fish

		Measured concentration of ^{14}C- fludioxonil			
	Day	Water [mg equiv./l]	Edible [mg equiv./kg]	Non-edible [mg equiv./kg]	Average concentration in fish [mg equiv./kg]
Accumulation	0	0.0092	0.073	0.130	0.098
	1	0.0095	0.315	2.566	1.384
	3	0.0094	0.290	3.690	1.815
	7	0.0097	0.280	4.187	2.132
	14	0.0087	0.441	5.645	2.878
	21	0.0092	0.701	8.449	4.183
	28	0.0087	0.451	6.232	2.987
Average		0.0095*	0.531**	6.775**	3.349**
Depuration	29	0.0002	0.470	1.228	0.828
	30	0.0007	0.235	0.316	0.272
	33	0.0000	0.042	0.421	0.218
	35	0.0001	0.041	0.227	0.128
	37	0.0000	0.022	0.293	0.152
	42	0.0000	0.037	0.213	0.117

* average Day 0-28
** average at steady state (Day 14-28)

Prior and during the experiment, all fish showed normal behaviour and no treatment related symptoms. No mortality was observed in the two treated tanks and in the control tank.

Distribution of radioactivity in water
The concentration of fludioxonil equivalents in water ranged from 0.0081 to 0.0125 mg equiv./L. On average, the level of radioactivity during the accumulation phase was 0.0095 ± 0.00087 mg equiv./L. During the first three days of the depuration phase the residual radioactivity in water was ≤ 0.0007 mg equiv./L. Thereafter, no residual radioactivity (≤ 0.0001 mg equiv./L) could be measured in water.

Nature of radioactivity in water
The nature of radioactivity in water of the treated tanks was proven by TLC analysis at days 0, 3, 7, 21 and 28 of the exposure phase. Besides the parent molecule only low amounts of the unknown metabolite fraction MT2 were detected, ranging around the limit of quantification for 14C-fludioxonil in water of the test aquaria of 0.0004 mg equiv./L. However, the formation of this fraction indicated a slight degradation of the test compound in the test aquaria. As a result of this degradation, the overall analysed exposure concentration of 14C-fludioxonil in the test system during the accumulation period was on average 0.0091 mg/L.

Distribution of radioactivity in fish
14C-fludioxonil residues were rapidly concentrated in the fish tissues, reaching 80 % of the steady-state concentration after 6.4, 7.8 and 7.1 days of exposure in edible portions, non-edibles portions and whole fish, respectively. For the same tissues, 95 % of the steady-state concentration were reached after 12.1, 14.7 and 13.2 days, respectively.

At steady state, the measured concentrations of 14C-fludioxonil equivalents were 0.531, 6.775 and 3.349 mg equiv./kg tissue fresh weight in edible portions, non-edible portions and whole fish, respectively. The calculated concentrations of 14C-fludioxonil equivalents for the same tissues were 0.505, 6.229 and 2.983 mg equiv./kg tissue fresh weight.

In fludioxonil free water, the concentration of 14C-fludioxonil equivalents in fish tissues rapidly dropped to very low values. After two days of depuration more than 90 % of the bioconcentrated radioactivity was depurated from the whole fish tissues. The calculated depuration times for 50 % of fludioxonil residues from the different fish tissues were 2.1, 0.4 and 0.6 days for edible portions, non-edible portions and whole fish, respectively. For the same tissues, the calculated depuration times for 90 % of the total accumulated residues were 6.9, 1.4 and 1.8 days, respectively.

The results obtained from the course of exposure and depuration indicate, that fludioxonil under constant exposure will be bioconcentrated in non-edible fish tissues, but will be almost completely and very fast depurated when exposed to untreated water.

Nature of radioactivity in fish
The identity of radioactivity in the edible and non-edible fish portions was analysed after 8 and 28 days of exposure. In edible fish portions, no other extractable radioactivity than fludioxonil could be detected. The amounts of fludioxonil in tissues decreased during incubation from 0.496 (93.4%) to 0.465 (87.6%) mg/kg edible fish tissues after 8 and 28 days of incubation, respectively. Besides the parent molecule, low amounts of non-extractable radioactivity were measured in edible fish tissues reaching 3.7 and 10.6 % of the total radioactivity in these tissues after 8 and 28 days of incubation, respectively.

In extractable radioactivity of non-edible fish portions after 8 days of incubation, the amount of fludioxonil was only 2.044 (30.2%) mg/kg. Beside the parent molecule one significant (M2) and two

minor metabolite fractions (MT1 and MT2) were observed, reaching 0.724 (10.7%), 0.335 (4.9%) and 0.117 (1.7%) mg equiv./kg, respectively. After 28 days of incubation, the same metabolite fractions had decreased to ≤ 0.579 (≤ 8.6%), 0.317 (4.7%) and ≤ 0.111 (≤ 1.6%) mg equiv./kg. The amount of fludioxonil in this sample was 1.899 mg/kg (28.0%).

Besides the three distinct metabolite fractions, a variety of other very small metabolite fractions ranging around the detection limit were formed. In addition, remainder radioactivity was observed, reaching 23.5 and 20.1 % of the radioactivity in the non-edible tissue extracts after 8 and 28 days of incubation, respectively. For the same samples, the non-extractable radioactivity increased from 22.0 to 33.4 %.

Metabolite fraction M2 could be measured in the unpolar phase of the tissue extracts and did not correspond to any of the reference compounds. Due to the low quantities formed, metabolite fraction M2 could not be further characterised by other spectroscopic methods, e.g. MS or NMR. Metabolite fractions MT1 and MT2 could only be measured in the polar phase of the tissue extracts. MT1 was observed in the starting region of the TLC chromatograms. Both fractions did not correspond to any of the reference compounds and the quantities extracted were too low for further characterisation.

In order to verify the metabolite fractions found in non-edible tissues and to produce higher quantities of material for further characterisation by spectroscopic methods, a biomass of about 500 g fish was exposed for 14 days to a dose of 50 µg fludioxonil per litre of water in a semistatic system. The water of the system was completely exchanged every 2 days and the concentration of the test compound new adjusted. The amounts of radioactivity concentrated in the fish tissues were 24.0 mg equiv./kg.

In the non-polar phase of whole fish extracts, no other radioactivity than fludioxonil could be detected. The amounts of fludioxonil in this sample were 21.9 (91.1 %) mg/kg fish tissues after 14 days of incubation. The polar phase of the fish extracts accounted to 4.7 % of the total radioactivity in the tissue and was not further analysed. Beside the extractable radioactivity, low amounts of non-extractable radioactivity were measured reaching 2.6 % of the total radioactivity in the tissues.

Estimation of Bioconcentration Factors

Bioconcentration factors (BCF's) were determined by calculating the ratio of the steady-state concentration of 14C-fludioxonil equivalents in fish tissues and whole fish to the average concentration of fludioxonil in water during exposure (measured BCF). In addition, bioconcentration factors for the same tissues were estimated calculating the ratio of the uptake rate constant to the depuration rate constant (calculated BCF).

Table 8.1.3-3: Summary of endpoints for bioaccumulation in fish

Bioconcentration factors			
	Edibles	**Non-edibles**	**Total**
Measured (C_{tissue} / C_{water})	58	741	366
Calculated (k_u / k_d)	56	749	365

These values indicate that bioconcentration of fludioxonil in the bluegill *Lepomis macrochirus* is limited and greatly influenced by active elimination mechanisms in fish.

Conclusion

^{14}C-fludioxonil residues were rapidly concentrated in fish tissues, reaching 95 % of the steady-state concentration within two weeks.

In edible fish portions, no other radioactivity than 14C-fludioxonil (0.496-0.465 mg/kg or 93.4- 87.6%) and non-extractables (0.020- 0.056 mg/kg or 3.7-10.6 %) could be detected. In non-edible fish portions, 14C-fludioxonil accounted only for 30.2-28.0% of the residual radioactivity in the tissue (2.044-1.899 mg/kg). Beside the parent molecule, one significant (M2) and two minor metabolite fractions (MT1 and MT2) were observed, reaching maximum concentrations with 0.724 (10.7%), 0.335 (4.9%) and 0.117 (1.7%) mg equiv./kg, respectively. Non-extractable radioactivity in non-edible fish portions increased up to 33.4 % (2.264 mg equiv./kg).

At steady state the estimated bioconcentration factors were of 56-58, 741-749 and 365-366 foredible portions, non-edible portions and whole fish, respectively. Fludioxonil residues were rapidly eliminated following the termination of exposure, with DT_{90} values for the whole fish of < 2 days.

Fludioxonil showed only limited bioconcentration in bluegill, *Lepomis macrochirus,* and bioconcentrated residues were rapidly eliminated in fludioxonil free water. The measured BCF value was 366 (total fish).

(Gonzalez-Valero, 1994)

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

An acute toxicity study has been conducted with *Rana limnocharis*, a species of frog. A summary of this is presented below.

Report:	Provided for relevance assessment against existing endpoints: K-CA 8.1.4/01 Mingqi Z. (2010). Fludioxonil (CGA173506) - Acute toxicity test in frog (*Rana limnocharis*). Report Number CAU2010A194. China Agricultural University, No.2, Yuan Ming Yuan West Road, Haidian District, Beijing, 100193, P.R. China (CGA173506_11704).

Guidelines

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

Report:	The English translation and original test guideline for the Amphibian acute study for China were provided at the request of the RMS: K-CA 8.1.4/02 GB/T 31270 Standardization Administration of the People's Republic of China Test Guidelines on Environmental Safety Assessment for Chemical Pesticides Part 11: Silkworm Acute Toxicity Test GB/T 31270.11-2014 Part 17: Trichogramma Acute Toxicity Test GB/T 31270.17-2014 Part 18: Amphibian Acute Toxicity Test GB/T 31270.18-2014 (Syngenta File No. NA_14530).

GLP: No

Executive Summary

The acute toxicity of fludioxonil (CGA173506) to tadpoles of a species of frog (*Rana limnocharis*) was determined. Tadpoles were exposed to concentrations of 5.00, 3.13, 1.95, 1.22, 0.76 mg/L, and a control

(tap-water and DMF+Tween 80). The 48 hour LC_{50} was 2.32 mg /L (95% confidence intervals 2.15 – 2.50 mg/L).

Materials

Test Material	Fludioxonil (CGA173506) technical
Test concentrations:	Nominal 5.00, 3.13, 1.95, 1.22, 0.76 mg/L and a control (tap-water and DMF+Tween 80)
Vehicle and/or positive control:	DMF and Tween 80; none
Analysis of test concentrations:	No
Test animals	
Species:	Frog (*Rana limnocharis*) tadpole
Test design	
Exposure regime:	Semi-static
Aeration:	24h
Replication:	3
No of tadpoles per replicate:	10
Environmental conditions	
Test temperature:	23 ± 2°C
pH:	7.86
Dissolved oxygen:	8.29 mg/L
Hardness of water:	46 mg/L (by $CaCO_3$)
Lighting:	14 hours light and 10 hours dark
Length of test:	48 hours

Study Design and Methods

Tadpoles of the frog species (*Rana limnocharis*) were exposed for 48 h to fludioxonil (95%). Following a range-finding test, the definitive study was conducted at test concentrations of 5.00, 3.13, 1.95, 1.22, 0.76 mg/L.

Test water was tap-water. Three replicates were prepared for each treatment and control group. Tadpoles were distributed into 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 3.38 mg/L (95% confidence intervals were 2.98 to 3.83 mg/L).

The 48 hour LC_{50} was 2.32 mg/L (95% confidence intervals were 2.15 to 2.50 mg/L).

Conclusions

The acute toxicity of fludioxonil (95%) to tadpoles of a species of frog (*Rana limnocharis*) was determined. The 48 hour LC_{50} was 2.32 mg/L (95% confidence intervals 2.15 – 2.50 mg /L).

(Mingqi Z., 2010)

Relevant literature on other terrestrial vertebrate wildlife

No relevant scientifically peer-reviewed open literature could be found on fludioxonil 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 endocrine-disrupting effects were made in the EU review (**EFSA Scientific Report (2007) 110, 1-85**).

A review of the all relevant data for potential endocrine disruption in mammalian species has been undertaken (**M-CA Section 5 Supplement, CA 5.8.3**). This report reviews and summarises all of the relevant available data, including open scientific literature, on fludioxonil for potential for endocrine disruption using a weight of the evidence approach proposed by the European Chemical Industry Council (CEFIC) Endocrine Modulators Steering Group (EMSG), structured according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disruptors.

Fludioxonil 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 relevant studies individually and a subsequent weight of evidence evaluation, it can be concluded that fludioxonil is not an endocrine disruptor as defined by WHO/IPCS (2002):

"An endocrine disruptor 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."

Report:	K-CA 8.1.5/01 Vaughan M (2015). Fludioxonil – Review for Potential for Endocrine Disruption, Non-mammalian Toxicology. Syngenta Ltd. Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY United Kingdom. (Syngenta File No. CGA173506_11857)

Fludioxonil 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 fludioxonil. Following evaluation of each of the relevant studies individually and a subsequent weight of evidence evaluation, no concerns for potential endocrine disruption were identified.

The following reports are discussed in this endocrine review and are therefore provided below.

Report:	K-CA 8.1.5/02 Graves WC, Marnk MA, Swigert JP. 1994. CGA-173506. An early life-stage toxicity test with the fathead minnow (*Pimephales promelas*). Project No. 108A-153. Wildlife International Ltd., Maryland, USA. (Syngenta File No. CGA173506/0416)

Report:	K-CA 8.1.5/03 Minderhout T, Kendall TZ, Krueger HO. 2011. Fludioxonil – An early life-stage toxicity test with Sheepshead Minnow (*Cyprinodon variegatus*). Report No. 528A-217. Wildlife International Ltd., Easton, USA. (Syngenta File No. CGA173506_11516)

Report:	K-CA 8.1.5/04 Marini, J (2014) Fludioxonil − Early Life-Stage Toxicity Test with Sheepshead Minnow, *Cyprinodon variegatus*, Report Number 1781.6970, Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571 USA. (Syngenta File No. CGA173506_51331)

Report:	K-CA 8.1.5/05 Maynard SJ. 2005. Fludioxonil (CGA 173506) – Determination of the effect on the growth rate of juvenile rainbow trout (*Oncorhynchus mykiss*) using a continuous flow test system. Study report no. BL8185/B. Brixham Environmental Laboratory, AstraZeneca UK Limited, Brixham, UK. (Syngenta File No. CGA173506/6704)

Report:	K-CA 8.1.5/06 Rufli H. 1989a. Report on the *Daphnia*, reproduction test with CGA 173506 technical, Syngenta Crop Protection, Ciba-Geigy Ltd., Basel, Switzerland. Unpublished report No. 88 17 36. (Syngenta File No. CGA173506/0006)

Report:	K-CA 8.1.5/07 Putt AE. 1991. (CGA-173506 technical) – Chronic toxicity to Daphnids (*Daphnia magna*) under flow-through conditions. Study report no. 91-2-3672. Springborn Laboratories Inc., Massachusetts 02571, USA. (Syngenta File No. CGA173506/0179)

Report:	K-CA 8.1.5/08 Fournier AE. 2014. Fludioxonil – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna*, Under Static Renewal Conditions. Study report no. 1781.6936. Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037, USA. (Syngenta File No. CGA173506_51057)

Report:	K-CA 8.1.5/09 Claude MB, Kendall TZ, Krueger HO. 2011. Fludioxonil – a flow-through life-cycle toxicity test with the saltwater mysid (*Americamysis bahia*). Study report no. 528A-213A. Wildlife International Ltd, Easton, MD, USA. (Syngenta File No. CGA173506_11519)

Report:	K-CA 8.1.5/10 Marini, J (2014) Fludioxonil − Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*), Report Number 1781.6969, Smithers Viscient 790 Main Street, Wareham, Massachusetts 02571 USA (Syngenta File No. CGA173506_51333)

Report:	K-CA 8.1.5/11 Grade R. 1998. Toxicity test of CGA 173506 tech. on sediment-dwelling *Chironomus riparius* (syn. *Chironomus thummi*) under static conditions. Study report no. 983752. Novartis Crop Protection AG, CH-4002 Basel, Switzerland. (Syngenta File No. CGA173506/1274)

Report:	K-CA 8.1.5/12 Friedrich S. 2003. Fludioxonil (CGA 173506): Sublethal toxicity of the technical material to the earthworm *Eisenia fetida*. Study report no. 03 10 48 023. BioChem agrar labor für biologische und chemische Analytick GmbH, D-04827 Gerichshain, Germany. (Syngenta File No. CGA173506/5665)

Report:	K-CA 8.1.5/13 Johnson A. 1993a. CGA 173506 Bobwhite Quail dietary reproduction and tolerance studies. Study report no. CBG 540/541/930706. Huntingdon Research Centre Ltd, Cambridgeshire, England. (Syngenta File No. CGA173506/0355)

Report:	K-CA 8.1.5/14 Johnson A. 1993b. CGA 173506 Mallard Duck dietary reproduction and tolerance studies. Study report no. CBG 542/543/930707. Huntingdon Research Centre Ltd, Cambridgeshire, England. (Syngenta File No. CGA173506/0354)

Report:	K-CA 8.1.5/15 Bryden M., Frey L.T, Elliott S. & Martin K.H, (2015), Fludioxonil - A Reproduction Study with the Northern Bobwhite, Report Number 528B-457. Wildlife International A Division of EAG Inc. 8598 Commerce Drive Easton, MD 21601 USA (Syngenta File No. CGA173506_11828)

Report:	K-CA 8.1.5/16 Marini J. (2015) Fludioxonil – Full Life-Cycle Toxicity Test with the Fathead Minnow (*Pimephales promelas*) Following FIFRA Guideline 72-5 and OCSPP Draft Guideline 850.1500. Smithers Viscient, 790 Main Street, Wareham, MA 02571-1037 USA. Laboratory Report Number: 1781.6971. (Syngenta file no. CGA173506_51493)

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.

Potential for endocrine disruption

The RMS rquested clarification for the following points:

It is noted that the ecotoxicity studies cited in Vaughan (2015) and proposed to determine if fludioxonil has a potential for endocrine disruption effects are part of the OECD conceptual framework for testing and assessment of endocrine disruptors. However, despite a fish full life cycle test with fish is available (Marini, 2015, KCA 8.2.2.2/01), no analysis of effects on sexual characteristics (sex ratio, gonadal conditions, ...) is provided in the position paper. The notifier should complete his position by an analysis of the relevant information available in the fish full life cycle test (Marini, 2015, KCA 8.2.2.2/01).

Syngenta reply:

The fish full life cycle study was identified as a data gap during the US DCI specifically in relation to concerns identified in the US fish long-term risk assessment – the Estimated Environmental Concentration (EEC) for many uses was observed to be >0.1 times the NOAEC of the fish ELS and hence investigations on fish reproduction need to be evaluated (page 8 of EPA response). Therefore the fish full life cycle study was not requested due to potential endocrine disruption concerns and no specific endocrine endpoints where therefore measured in the study. Table 8.2.2.2-9 shows the details of the sex ratio of fish from the FFLC Marini 2015 study.

The Toxicology and Ecotoxicology endocrine disruptor reviews do not indicate any major concerns in this area. Gross histological measurements were taken during the study to ascertain phenotypic sex in the fish full life cycle study; the data has been retrospectively analysed to examine the sex ratio of the surviving pooled fish in the study (see Table 8.2.2.9-9). Statistical analysis of the data using a G-test indicates no significant difference between the pooled replicate sex ratio of the control and exposed fish.

CA 8.2 Effects on Aquatic Organisms

RMS comment: Minor non-transient metabolites observed but not yet identified in laboratory studies in soil should also be considered in the assessment for aquatic organisms. If minor non-transient metabolites are identified, ecotoxicological studies would also have to be provided for their assessment

Syngenta response: Syngenta believes that there are no minor non-transient metabolites observed within the aerobic metabolism or soil photolysis studies and therefore there is requirement to include in the risk assessment.

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 fludioxonil

Organism	Test item	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment		Reference (author, date, Syngenta Ref)
Rainbow trout (*Oncorhynchus mykiss*)	Fludioxonil	EU	96 h LC_{50} = $0.47_{(mm)}$ (flow-through)	Geometric mean = 0.38 mg/L		***Holmes & Swigert (1993) CGA173506/0258***
	Fludioxonil	EU	96 h LC_{50} = $0.23_{(mm)}$ (flow-through)			***Biever (1997) CGA173506/0926***
	Fludioxonil	New study	96 h LC_{50} = $0.50_{(mm)}$ (static) [ab]			***Rufli (1989a) CGA173506/0004***
Bluegill sunfish (*Lepomis macrochirus*)	Fludioxonil	EU	96 h LC_{50} = $0.74_{(mm)}$ (flow-through)	Geometric mean = 0.48 mg/L		***Biever (1997) CGA173506/0927***
	Fludioxonil	New study	96 h LC_{50} = $0.31_{(mm)}$ (static) [ab]			***Rufli (1989b) CGA173506/0002***
Carp (*Cyprinus carpio*)	Fludioxonil	New study	96 h LC_{50} = $1.5_{(mm)}$ (static) [ab]	Conducted for Japanese compliance and submitted to enable risk refinement		***Rufli (1989c) CGA173506/0001***
Catfish (*Ictalurus punctatus*)	Fludioxonil	New study	96 h LC_{50} = $0.63_{(mm)}$ (static) [ab]	Provided for comparison with existing data and endpoints.		***Rufli (1989d) CGA173506/0003***
Sheepshead minnow (*Cyprinodon variegatus*)	Fludioxonil	EU	96 h LC_{50} = $1.2_{(mm)}$ (flow-through)	Conducted for US compliance and provided for comparison with existing data and endpoints.	Geometric mean = 0.81 mg/L	***Holmes & Swigert (1993) CGA173506/0257***
		New study	96 h LC_{50} = $0.54_{(mm)}$ (flow-through)	Provided for comparison with existing data and endpoints.		***Suprenant (1990) CGA173506/0056*** [d]
Fathead minnow (*Pimephales promelas*)	Fludioxonil	New study	96 h LC_{50} = $0.70_{(mm)}$ (static renewal) [c]	Conducted for US compliance and provided for comparison with existing data and endpoints.		***Fournier (2014) CGA173506_51203***

New study refers to studies performed since EU review of fludioxonil

[a] The Rufli 1989 studies were not submitted during the first EU review because more recently conducted studies were available. The combination of the old and new data shows similar results and their use adds to the variety of fish species tested and is of use for refinement of risk.

[b] The earliest studies used a static test design which resulted in some loss of some test substance over the 96h test duration. The results were therefore based on mean measured concentrations.

[c] The use of a semi-static test design was found to be sufficient to maintain test concentrations close to initial measured

throughout the short term study with mean measured concentration ranging from 73 to 87% of nominal values.
[d] Additional studies omitted in error from original supplementary dossier. Added for completeness.
mm = Endpoint derived using mean measured concentration

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

Organism	Test item	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
Rainbow trout	CGA339833	EU	96 h LC_{50} >$100_{(nom)}$ (static)	96 h >$100_{(nom)}$ (static)	***Rufli (2000) CGA339833/0005***
	CGA192155 [a]	EU	96 h LC_{50} >$100_{(nom)}$ (static)	96 h >$100_{(nom)}$ (static)	***Volz (2005) CGA192155/0019***

[a] Included in the Final Addendum to the DAR Article 8(2) of Council Directive 91/414/EEC (June 2007)
nom - nominal concentration

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

Organism	Test type	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
Rainbow trout (*Oncorhynchus mykiss*)	Fish juvenile growth test (flow-through	New study	NOEC = $0.040_{(mm)}$	Superseded by FFLC endpoint	***Maynard (2005) CGA173506/6704***
		EC_{10}/EC_{20} [a]	Could not be estimated	-	***Taylor (2015) CGA173506_11868***
Rainbow trout (*Oncorhynchus mykiss*)	Prolonged toxicity (flow-through)	Not considered reliable	NOEC = $0.014_{(nom)}$ [d]	These studies were not considered reliable by the RMS. During the previous evaluation they were superseded by Maynard (2005) CGA173506/6704	***Grade (1993) CGA173506/0377***
			NOEC = $0.01_{(nom)}$ [d]		***Grade (1993) CGA173506/0378***
			NOEC = $0.0066_{(nom)}$ [d]		***Grade (1993) CGA173506/0356***
			NOEC = $0.0000046_{(nom)}$ [d]		***Vial (1991) CGA173506/0097***
Fathead minnow (*Pimephales promelas*)	Early life stage (ELS) (flow-through)	EU	NOEC = $0.039_{(mm)}$	Current EU endpoint. Superseded by FFLC endpoint	***Graves et al. (1994) CGA173506/0416***
		EC_{10}/EC_{20} [a]	EC_{10} = 0.0142 [b] EC_{20} = 0.0455 [b]	-	***Taylor et al. (2016) CGA173506_11911***
Pimephales promelas (ELS)	Early life stage (ELS) (flow-through)	New study	NOEC = $0.019_{(mm)}$	Some caveats on validity	***Suprenant (1992) CGA173506/0391*** [e]
Sheepshead minnow (*Cyprinodon variegatus*)	ELS (flow-through)	New study	NOEC = $0.1_{(nom)}$	Conducted for US compliance and provided for comparison with existing data and endpoints.	***Minderhout et al. (2011) CGA173506_11516***
		EC_{10}/EC_{20} [a]	EC_{10} = 0.0688 [c] EC_{20} = 0.174 [c]	-	***Taylor & Radford 2015 CGA173506_11875***

Organism	Test type	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
	ELS (flow-through)	New study	NOEC = $0.038_{(mm)}$ EC_{10}= $0.036_{(mm)}$	Conducted for US compliance and provided for comparison with existing data and endpoints.	***Marini (2014) CGA173506_51331***
Fathead minnow (*Pimephales promelas*)	Full life-cycle	New study	**NOEC = $0.018_{(mm)}$** EC_{10}= $0.018_{(mm)}$	Conducted for US compliance and provided for comparison with existing data and endpoints. **Proposed new chronic endpoint.**	***Marini (2015) CGA173506_51493***

New study refers to studies performed since EU review of fludioxonil
mm – mean measured concentration
nom - nominal concentration
[a] Estimation attempted in accordance with **Commission Regulation (EU) No. 283/2013**
[b] Represent the lowest EC_{10} and EC_{20} and are based on post-hatch survival
[c] Represent the lowest EC_{10} and EC_{20} and are based on fresh weight
[d] The reliability of these studies was questioned[5] due to uncertainty of the analytical results where the stock solutions were analysed and not the actual test solutions.
[e] Additional studies omitted in error from original supplementary dossier. Added for completeness.

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

Organism	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
Daphnia magna	EU	48 h EC_{50} = $0.40_{(mm)}$ (flow-through)	Geometric mean = 0.73 mg a.s./L	***Suprenant (1990) CGA173506/0059***
		48 h EC_{50} = $0.90_{(mm)}$ (flow-through)		***Holmes & Swigert (1993) CGA173506/0259***
	New study	48 h EC_{50} = ~~1.1 (mm)~~ 1.06 (gm) [c] (static) [a]		***Rufli (1989e) CGA173506/0005***
Mysidopsis bahia [b]	EU	96 h LC_{50} = $0.27_{(mm)}$ (flow-through)	Geometric mean with *Daphnia magna* geomean endpoint = 0.44 mg a.s./L	***Holmes & Swigert (1993) CGA173506/0256***
Eastern oyster (*Crassostrea virginica*)	EU	EC_{50} = $0.37_{(nom)}$ (flow-through)	-	***Suprenant (1990) CGA173506/0390***
Hyalella azteca	New study	10 d LC_{50} >84 mg/kg $dw_{(mm)}$ (static renewal)	Conducted for US data requirements and provided for comparison with existing data and endpoints	***Bradley (2014) CGA173506_51325***
Leptochirus plumulosa		10 d LC_{50} >90 mg/kg $dw_{(mm)}$ (static)		***Bradley (2014) CGA173506_51327***
Chironomus dilutus		10 d LC_{50} = 100 mg/kg $dw_{(mm)}$ (static renewal)		***Bradley (2014b) CGA173506_51329***

New study refers to studies performed since EU review of fludioxonil
mm – mean measured concentration
nom - nominal concentration

[5] Page 25 second paragraph EFSA Scientific Report (2007) 110: 1-85 Conclusion on the peer review of fludioxonil.

[a] The Rufli 1989 study was not submitted during the first EU review because more recently conducted studies were available. The combination of the old and new data shows similar results and their use adds to the variety of studies available and is of use for refinement of risk.
[b] Also known as *Americamysis bahia*
[c] Recalculated based on geometric mean measured concentrations

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

Organism	Test item	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
Daphnia magna	CGA192155 [a]	EU	48 h EC_{50} >$100_{(nom)}$ (static)	48 h EC_{50} >$100_{(nom)}$	***Volz (2005) CGA192155/0018***
	CGA265378 [b]	New data	EC_{50} >1 mg a.s./L	Non-GLP statement	***Volz (2005) CGA265378/0013***
	CGA344623	EU	48 h EC_{50} >$100_{(nom)}$ (static)	48 h EC_{50} >$100_{(nom)}$	***Volz (2004) CGA344623/0001***
	CGA339833	EU	EC_{50} >$100_{(nom)}$ (static)	EC_{50} >$100_{(nom)}$	***Grade (2000) CGA339833/0006***
	SYN545245	New data	EC_{50} >$100_{(nom)}$ (static)	EC_{50} >$100_{(nom)}$	***Sacker (2016) SYN545245_10005***

New study refers to studies performed since EU review of fludioxonil
nom - nominal concentration
[a] Included in the Final Addendum to the DAR Article 8(2) of Council Directive 91/414/EEC (June 2007)
[b] A statement was supplied to the RMS during the discussion of the need for this study. EFSA[6] concluded that the metabolite was too unstable to test and therefore toxicity testing could not be performed; and due to instability CGA265378 represented a low risk to aquatic organisms due to low exposure; OECD summary included for completeness

A *Daphnia* acute toxicity study with the new soil metabolite SYN545245 is currently being conducted. The study will be submitted and the dossier updated as agreed with the RMS.

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

Organism	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
Daphnia magna	EU	21 d NOEC = $0.005_{(mm)}$ (static renewal)	No longer valid (see below and section CA 8.2.5.1)	***Rufli (1989) CGA173506/0006***
	EC_{10}/EC_{20} [a]	EC_{10} repro = 0.028 EC_{20} repro = 0.034		***Taylor & Allen (2015) CGA173506_11856***
	EU	21 d NOEC = $0.019_{(mm)}$ (flow-through)	No longer valid (see below and section CA 8.2.5.1)	***Putt (1991) CGA173506/0179***
	EC_{10}/EC_{20} [a]	EC_{10} repro = 0.019 EC_{20} repro = 0.022		***Taylor & Radford (2015) CGA173506_11866***
	New study	21 d NOEC = $0.035_{(mm)}$ (static renewal) $EC_{10/20}$ repro >0.035	Conducted for US data requirements and provided for comparison with existing data and endpoints	***Fournier (2014) CGA173506_51057***
Americamysis bahia [b]	New study	28 d NOEC = $0.018_{(mm)}$ (flow-through)	Marini 2014 study will be used in preference to this study (see below and section CA 8.2.5.2)	***Claude et al. (2011) CGA173506_11519***
	EC_{10}/EC_{20} [a]	EC_{10} pre-pairing survival = 0.0048 EC_{20} pre-pairing survival = 0.0073	-	***Taylor & Radford (2015) CGA173506_11873***
	New study	**28 d NOEC = $0.024_{(twm)}$**	Conducted for US data	***Marini (2014)***

[6] Page 26 first paragraph EFSA Scientific Report (2007) 110: 1-85 Conclusion on the peer review of fludioxonil.

		(flow-through)	requirements and provided for comparison with existing data and endpoints **Proposed new chronic endpoint.**	***CGA173506_51333***
Chironomus riparius	EU	28 d NOEC = 40 mg a.s./kg $_{(nom)}$ (static)	Proposed endpoint for chronic sediment risk assessment	***Grade (1998) CGA173506/1274***
	EC_{10}/EC_{20} [a]	Could not be estimated	-	***Taylor & Howells (2015)CGA173506_11840***

New study refers to studies performed since EU review of fludioxonil
mm – mean measured concentration
nom - nominal concentration
twm – time weighted mean concentration
[a] Estimation attempted in accordance with **Commission Regulation (EU) No. 283/2013**
[b] Also known as *Mysidopsis bahia*

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

Organism	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
Desmodesmus subspicatus	EU	72h E_bC_{50} = 0.93$_{(mm)}$	~~Does not pass the ≤35%.validity criteria for the section-by-section criteria = 50%~~ Average specific growth rate require ≤7%: Actual: 2.6% (control) Section-by-section criteria required ≤35%: Actual: 25.4% [d]	***Rufli (1989) CGA173506/0007***
	EC_{10}/EC_{20} [a]	72 h E_rC_{10} = 0.4830~~.740~~ $_{(mm)}$ [c] 72 h E_yC_{10} = 0.108$_{(mm)}$ 72 h E_yC_{20} = 0.2100~~.211~~$_{(mm)}$	-	***Pickering & Allen (2017)~~Taylor & Howells (2016)~~ CGA173506_11910*** [e]
Pseudokirchneriella subcapitata	EU	72h E_rC_{50} = 0.41$_{(mm)}$ 96h E_rC_{50} = 0.31$_{(mm)}$ 120h E_rC_{50} = 0.33$_{(mm)}$ 72h E_bC_{50} = 0.029$_{(mm)}$ 96/120h E_bC_{50} = 0.024$_{(mm)}$	Passes section-by-section validity criteria; the value for this study = 21 to 33% for the 72h and 96 h endpoints only. 120h fails at 41%.	***Hoberg (1992) CGA173506/0243*** ***Recalculated based on mean measured in Hoberg (2005)~~1992~~) CGA173506/6950***
	EC_{10}/EC_{20} [a]	120 h E_rC_{10} = 0.0456$_{(mm)}$ [b] 120 h E_rC_{20} = 0.0809$_{(mm)}$ [b] 120 h E_yC_{10} = 0.0085$_{(mm)}$ [b] 120 h E_yC_{20} = 0.0129$_{(mm)}$ [b]	-	***Priestly & Allen (2015) CGA173506_11886***
	New study	72h E_rC_{50} = >0.442$_{(mm)}$ 96h E_rC_{50} = >0.442$_{(mm)}$ 72h E_yC_{50} = 0.244$_{(mm)}$ 96h E_yC_{50} = 0.301$_{(mm)}$	The average specific growth rate is ≤7%.; the value for this study = 2.64% The section-by-section criteria is ≤35%.; the value for this study = 35% This study is valid & to current guidance; it will be used for risk assessment. **Proposed new algal endpoint geomean of Hoberg and Porch *et al* 2011 studies = 72hr E_rC_{50} = 0.43 mg a.s/L.**	***Porch et al. (2011) CGA173506_11518***

Organism	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
	EC_{10}/EC_{20} [a]	72 h E_rC_{10} = 0.255 $_{(mm)}$ [b,c] 72 h E_yC_{10} = 0.0124 $_{(mm)}$ [b] 72 h E_yC_{20} = 0.0349 $_{(mm)}$ [b]	-	***Taylor & Howells (2016) CGA173506_11897***
Anabaena flos-aquae	New study	72h $E_{bry}C_{50}$ >1.3$_{(mm)}$ 96h $E_{br}C_{50}$ >1.3$_{(mm)}$ 96h E_yC_{50} = 1.1$_{(mm)}$ 72/96 h $E_rC_{10/20}$ >1.3$_{(mm)}$ 72 h E_yC_{10} = 0.39$_{(mm)}$ 72 h E_yC_{20} = 0.56$_{(mm)}$	Conducted for US data requirements and provided for comparison with existing data and endpoints	***Soucy (2013) CGA173506_51018***
Skeletonema costatum	New study	72h E_bC_{50} = 0.25$_{(mm)}$ 72h E_yC_{50} = 0.26$_{(mm)}$ 72h E_rC_{50} = 0.37$_{(mm)}$ 96h E_bC_{50} = 0.26$_{(mm)}$ 96h E_yC_{50} = 0.28$_{(mm)}$ 96h E_rC_{50} = 0.43$_{(mm)}$ 72 h E_rC_{10} = 0.17$_{(mm)}$ 72 h E_rC_{20} = 0.23$_{(mm)}$ 72 h E_yC_{10} = 0.13$_{(mm)}$ 72 h E_yC_{20} = 0.16$_{(mm)}$	Conducted for US data requirements and provided for comparison with existing data and endpoints	***Soucy (2013) CGA173506_51017***
Navicula pelliculosa	New study	72h E_bC_{50} = 0.88$_{(mm)}$ 72h E_yC_{50} = 0.86$_{(mm)}$ 72h E_rC_{50} >1.2$_{(mm)}$ 96h E_bC_{50} = 0.86$_{(mm)}$ 96h E_yC_{50} = 0.85$_{(mm)}$ 96h E_rC_{50} >1.2$_{(mm)}$ 72 h E_rC_{10} = 0.68$_{(mm)}$ 72 h E_rC_{20} = 0.80$_{(mm)}$ 72 h E_yC_{10} = 0.44$_{(mm)}$ 72 h E_yC_{20} = 0.63$_{(mm)}$	Conducted for US data requirements and provided for comparison with existing data and endpoints	***Soucy (2013) CGA173506_51014***

New study refers to studies performed since EU review of fludioxonil
mm – mean measured concentration
[a] Estimation attempted in accordance with **Commission Regulation (EU) No. 283/2013**
[b] Represents the lowest endpoint
[c] It was not possible to derive the E_rC_{20} as a significant concentration response could not be plotted
[d] Study satisfied validity criteria following correction from cells /sample to cells/ml in the statistical recalculation
[d] Statistics were recalculated following correction of the underlying data used from cells /sample to cells/ml in the statistical calculations

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

Organism	Test item	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
Pseudokirchneriella subcapitata [d]	CGA192155 [a]	EU	96 h $E_{rb}C_{50}$ >100$_{(nom)}$	96 h $E_{rb}C_{50}$ >100$_{(nom)}$	***Volz (2005) CGA192155/0017***
		EC_{10}/EC_{20} [b]	Could not be estimated	-	***Priestly & Howells (2015) CGA192155_11294***
Pseudokirchneriella subcapitata	CGA339833	EU	72 h E_rC_{50} = 104.7$_{(nom)}$ 72 h E_bC_{50} = 95.8$_{(nom)}$	72 h E_bC_{50} = 95.8$_{(nom)}$	***Grade (2000) CGA339833/0004***
		EC_{10}/EC_{20} [b,c]	72 h E_rC_{10} = 36.6 72 h E_rC_{20} = 79.0	-	***Taylor & Radford (2015) CGA339833_10011***

Pseudokirchneriella subcapitata	SYN545245	New data	72 h E_rC_{50} = $44_{(mm)}$ 72 h E_yC_{50} = $41_{(mm)}$ 72 h E_rC_{10} = $36_{(mm)}$ 72 h E_rC_{20} = $39_{(mm)}$	72 h E_rC_{50} = $44_{(mm)}$	***Vryenhoef (2016) SYN545245_10001***

nom - nominal concentration
[a] Included in the Final Addendum to the DAR Article 8(2) of Council Directive 91/414/EEC (June 2007)
[b] Estimation attempted in accordance with **Commission Regulation (EU) No. 283/2013**
[c] It was not possible to derive $E_yC_{10}/_{20}$ values as a significant concentration response could not be plotted
[d] Noted incorrectly in the EU endpoint list as the species *Scenedesmus subspicatus* (also known as *Desmodesmus subspicatus*)

An algae acute toxicity study with the new soil metabolite SYN545245 is currently being conducted. The study will be submitted and the dossier updated as agreed with the RMS.

Table 8.2-9: Summary of endpoints for macrophytes

Organism	Test item	Endpoints (mg a.s./L)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
Lemna gibba	Fludioxonil	New study	14 d E_bC_{50} >$1.0_{(Im)}$ 14 d E_bC_{50} >$0.11_{(mm)}$	14 d E_bC_{50} >$1.0_{(im)}$ 14 d E_bC_{50} >$0.11_{(mm)}$ Conducted for US compliance and provided for comparison with existing data and endpoints	***Hoberg (1997) 66723***
		EC_{10}/EC_{20} [a]	Could not be estimated	-	***Taylor & Howells (2015) CGA173506_11839***

New study refers to studies performed since EU review of fludioxonil
Im – initial measured concentration
mm – mean measured concentration
[a] Estimation attempted in accordance with **Commission Regulation (EU) No. 283/2013**

Table 8.2-10: Summary of endpoints from mesocosm studies

Organism	Endpoints (mg a.s./L)		Comments	Reference (author, date, Syngenta Ref)
Mesocosm	EU	NOAEC = $0.0164_{(mm)}$	-	***Giddings (1993) CGA173506/0346***

As requested by the RMS a table has been presented below providing clarity on the following information for aquatic studies:

For all the studies using a vehicle or solvent, the table indicates if the concentration of vehicle/solvent in the control and at all tested concentrations are identical. This information is required to judge the acceptability and reliability of all the tests performed with solvent.

The information provided in the table:
- the detailed reference of the studies,
- the amount of solvent in the vehicle/solvent control,
- the amount of solvent in each tested concentrations,
- the page of the report where the information is reported,
- and a statement on the notifier point of view regarding the reliability of the results.

For all the studies using a vehicle or solvent, detail is provided concerning whether the statistics and endpoints are calculated by comparison with blank control, solvent control or pooled control.

For all the aquatic studies for which endpoints should be expressed based on mean measured concentrations, it is recommended to use the geometric mean measured for static and semi-static tests and to use the arithmetic mean for flow-through studies (according to EFSA Supporting publication 201S:EN-924 on the "Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology").

Table 8.2-11: Summary of endpoints for acute fish toxicity of fludioxonil ~ Clarification of solvent use, statistics when solvent used and mean measured Vs geometric or nominal concentrations in ecotoxicology studies ~ at the request of the RMS

Organism	Solvent used in control	Solvent used in highest test concentration	Statistics conducted on control / solvent control / pooled control	Validity & solvent use	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
Rainbow trout *(Oncorhynchus mykiss)*	Acetone		No control or solvent control mortality (pg 22 report) ~ not relevant which control is used to calculate the statistics	Solvent level was the same in solvent control and all test concentrations. Although the level of solvent is higher than the OECD 203, no deleterious effects were apparent, and no mortality occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.	96 h LC_{50} = $0.47_{(mm)}$ (flow-through)	nom	0.19, 0.32, 0.54, 0.9, 1.5	Validity criteria: Required ≤10% mortality in controls: Actual: 0% control Actual: 0% solvent control The study is considered valid for use.	***Holmes & Swigert (1993) CGA173506/0258***
	Solvent control:	1 mL/L (pg 11-12 report)				mm	0.17, 0.26, 0.38, 0.71, 0.69		
	Highest test concentration:	1 mL/L (pg 11-12 report)				gm	0.17, 0.26, 0.38, 0.71, 0.68		
	All test concentrations:	1 mL/L (pg 11-12 report)				Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required.			
Rainbow trout *(Oncorhynchus mykiss)*	Acetone		No control or solvent control mortality (pg 29 report) ~ not relevant which control is used to calculate the statistics.	Solvent level was the same in the solvent control and highest test concentration, no mortality in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.	96 h LC_{50} = $0.23_{(mm)}$ (flow-through)	nom	0.089, 0.14, 0.21, 0.32, 0.50	Validity criteria: Required ≤10% mortality in controls: Actual: 0% control Actual: 0% solvent control The study is considered valid for use.	***Biever (1997) CGA173506/0926***
	Solvent control:	0.072mL/L (pg 16 report)				mm	0.066, 0.11, 0.18, 0.33, 0.54		
	Highest test concentration:	0.072mL/L (pg 16 report)				gm	0.066, 0.11, 0.18, 0.32, 0.54		
	All test concentrations:	0.013 to 0.072 mL/L (pg 16 report)				Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required			
Rainbow trout *(Oncorhynchus mykiss)*	Acetone + [Arkopal surfactant]		No control or solvent control / surfactant	Solvent use is within guideline levels. Surfactant use was	Calculated [e] 96 h LC_{50} = $0.50_{(mm)}$	nom	0.32, 0.58, 1.0, 1.8, 3.2, 5.8	Validity criteria: Required ≤10%	***Rufli (1989a) CGA173506/0004***

Organism	Solvent used in control	Solvent used in highest test concentration	Statistics conducted on control / solvent control / pooled control	Validity & solvent use	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
	Solvent control +[Arkopal]:	91.8 mg/L (= 0.116 mL/L) (pg 6 report) + [Arkopal 0.7 mg/L]	mortality (pg 8 report) ~ not relevant which control is used to calculate the statistics	allowable at the time but is not current practice. Solvent level was the same in the solvent control and highest test concentration and no mortality occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.	(static) [ab]	mm	0.26, 0.42, 0.83, 1.4, 2.7, ND	mortality in controls: Actual: 0% control Actual: 0% solvent control The study is considered valid for use.	
	Highest test concentration+[Arkopal]:	91.8 mg/L (= 0.116 mL/L) (pg 6 report) + [Arkopal 0.7 mg/l]				gm	0.25, 0.42, 0.83, 1.6, 2.7, ND		
	All test concentrations+[Arkopal]:	0.0067 to 0.116 mL/L (top dose excluded from statistical calculation) + [Arkopal 0.04 to 0.7 mg/]				Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required.			
Bluegill sunfish *(Lepomis macrochirus)*	Acetone + [Arkopal surfactant]		No control or solvent control mortality (pg 29 report) ~ not relevant which control is used to calculate the statistics	Solvent level was the same in the solvent control and highest test concentration and no mortality occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.	96 h LC_{50} = $0.74_{(mm)}$ (flow-through)	nom	0.18, 0.27, 0.42, 0.65, 1.0	Validity criteria: Required ≤10% mortality in controls: Actual: 0% control Actual: 0% solvent control The study is considered valid for use.	***Biever (1997) CGA173506/0927***
	Solvent control:	0.072mL/L (pg 16 report)				mm	0.14, 0.26, 0.45, 0.62, 0.96		
	Highest test concentration:	0.072mL/L (pg 16 report)				gm	0.14, 0.25, 0.45, 0.59, 0.96		
	All test concentrations:	0.013 to 0.072 mL/L (pg 16 report)				Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required			
Bluegill sunfish *(Lepomis macrochirus)*	Acetone + [Arkopal surfactant]		No solvent control / surfactant mortality (pg 8 report) ~	Solvent use is within guideline levels. Surfactant use was allowable at the time but is not current	96 h LC_{50} = $0.31_{(mm)}$ (static) [ab]	nom	0.58, 1.0, 1.8, 3.2, 5.8, 10	Validity criteria: Required ≤10% mortality in controls:	***Rufli (1989b) CGA173506/0002***

Organism	Solvent used in control	Solvent used in highest test concentration	Statistics conducted on control / solvent control / pooled control	Validity & solvent use	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
	Solvent control +[Arkopal]:	79.1 mg/L (= 0.1 mL/L) (pg 6 report) + [Arkopal 0.4 mg/L]	neither control was used to calculate the statsitics. Use of calculated statistical test (pg 6-7 report)	practice. Solvent level was the same in the solvent control and highest test concentration and no mortality occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.		mm	0.14, 0.18. 0.53, 1.3, 2.3, 3.4	Actual: 10% control Actual: 0% solvent control The study is considered valid for use.	
	Highest test concentration +[Arkopal]:	79.1 mg/L (= 0.1 mL/L) (pg 6 report) + [Arkopal 0.4 mg/L]				gm	0.13, 0.18. 0.52, 1.3, 2.3, 3.4		
	All test concentrations +[Arkopal]:	0.006 to 0.1 mL/L Details of solvent use in other test concentrations (raw data) + [Arkopal 0.024 to 0.4 mg/L]				Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required			
Carp *(Cyrprinus carpio)*	Acetone + [Arkopal surfactant]		No control or solvent control /surfactant mortality (pg 8 report) ~ not relevant which control is used to calculate the statistics	Solvent use is within guideline levels. Surfactant use was allowable at the time but is not current practice. Solvent level was the same in the solvent control and highest test concentration and no mortality occurred	96 h LC_{50} = $1.5_{(mm)}$ (static) [ab]	nom	1.0, 1.8, 3.2, 5.8, 10	Validity criteria: Required ≤10% mortality in controls: Actual: 0% control Actual: 0% solvent control The study is	***Rufli (1989c) CGA173506/0001***
	Solvent control +[Arkopal]:	79.1 mg/L (= 0.1 mL/L) (pg 6 report) + [Arkopal 0.4 mg/L]				mm	0.53, 1.0, 2.3, 4.2, 6.3		
	Highest test concentration +[Arkopal]:	79.1 mg/L (= 0.1 mL/L) (pg 6 report) = [Arkopal 0.4 mg/L]				gm	0.52, 0.99, 2.3, 4.0, 6.2		

Organism	Solvent used in control	Solvent used in highest test concentration	Statistics conducted on control / solvent control / pooled control	Validity & solvent use	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
	All test concentrations +[Arkopal]:	0.01 to 0.1 ml/L + [Arkopal 0.04 to 0.4 mg/L]		in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.		Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required		considered valid for use.	
Catfish *(Ictalurus punctatus)*	Acetone + [Arkopal surfactant]		No control or solvent control / surfactant mortality (pg 6-7 report) ~ not relevant which control is used to calculate the statistics	Solvent use is within guideline levels. Surfactant use was allowable at the time but is not current practice. Solvent level was the same in the solvent control and highest test concentration and no mortality occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.	Calculated [d] 96 h LC_{50} = $0.63_{(mm)}$ (static) [ab]	nom	0.58, 1.0, 1.8, 3.2, 5.8	Validity criteria: Required ≤10% mortality in controls: Actual: 0% control Actual: 0% solvent control The study is considered valid for use.	***Rufli (1989d) CGA173506/0 003***
	Solvent control +[Arkopal]:	45.9 mg/L (= 0.058 mL/L) (pg 6 report) + [Arkopal 0.2 mg/L]				mm	0.32, 0.68. 0.89, 2.07, 3.67		
	Highest test concentration +[Arkopal]:	45.9 mg/L (= 0.058 mL/L) (pg 6 report) + [Arkopal 0.2 mg/L]				gm	0.31, 0.67, 0.89, 2.07, 3.66		
	All test concentrations +[Arkopal]:	0.006 to 0.058 mL/L Details of solvent use in other test concentrations (raw data) + [Arkopal 0.024 to 0.2 mg/L]				Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required			
Sheepshead minnow *(Cyprinodon variegatus)*	Acetone		No control or solvent control mortality (pg 22 report) ~ not relevant which control	Solvent level was the same in solvent control and all test concentrations. Although higher than the OECD203, no	96 h LC_{50} = $1.2_{(mm)}$ (flow-through)	nom	0.45, 0.76, 1.3, 2.1, 3.5	Validity criteria: Required ≤10% mortality in controls:	***Holmes & Swigert (1993) CGA173506/0 257***
	Solvent control:	1 mL/L (pg 11-12 report)				mm	0.38, 0.67, 0.93, 1.5, 2.0		
	Highest test concentration:	1 mL/L (pg 11-12 report)				gm	0.39, 0.66, 0.93, 1.5, 1.9		

Organism	Solvent used in control	Solvent used in highest test concentration	Statistics conducted on control / solvent control / pooled control	Validity & solvent use	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
	All test concentrations:	1 mL/L (pg 11-12 report)	is used to calculate the statistics	deleterious effects were apparent or mortality occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.		Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required	Actual: 0% control Actual: 0% solvent control The study is considered valid for use.	
Sheepshead minnow *(Cyprinodon variegatus)*	Acetone		No control or solvent control mortality (pg 29 report) ~ not relevant which control is used to calculate the statistics	Solvent level was the same in the solvent control and highest test concentration and no mortality occurred in the solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.	96 h LC_{50} = 0.54 $_{(mm)}$ (flow-through)	nom: 0.89, 1.4, 2.1, 3.3, 5.0 mm: 0.39, 0.57, 0.76, 1.1, 1.4 gm: 0.39, 0.57, 0.75, 1.1, 1.4 Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required	Validity criteria: Required ≤10% mortality in controls: Actual: 0% control Actual: 0% solvent control The study is considered valid for use.	***Surprenant 1990 CGA173506/0056***
	Solvent control:	0.074 mL/L (pg 15 report)						
	Highest test concentration:	0.074 mL/L (pg 15 report)						
	All test concentrations:	0.013 to 0.074 mL/L (pg 16 report)						
Fathead minnow *(Pimephales promelas)*	Acetone		No control or solvent control mortality (pg 21 report) ~ not relevant which control is used to	Solvent level was the same in solvent control and all test concentrations and no mortality occurred in solvent control. Therefore the	96 h LC_{50} = 0.70$_{(mm)}$ (static renewal) [c]	nom: 0.081, 0.16, 0.33, 0.65, 1.3 mm: 0.068, 0.12, 0.24, 0.54, 1.1 gm: 0.068, 0.12, 0.24, 0.54, 1.1	Validity criteria: Required ≤10% mortality in controls: Actual: 0%	***Fournier (2014) CGA173506_51203***
	Solvent control:	0.1 mL/L (pg 12-13 report						
	Highest test concentration:	0.1 mL/L (pg 12-13 report						

Organism	Solvent used in control	Solvent used in highest test concentration	Statistics conducted on control / solvent control / pooled control	Validity & solvent use	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
	All test concentrations:	0.1 mL/L (pg 12-13 report	calculate the statistics	concentration of solvent in the range used did not affect survival of the test species.		Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required	control Actual: 0% solvent control The study is considered valid for use.	

[d] Calculated according to Spearman-Kaeber in D. J. Finney, 524-530, London (1964), pg 6 report.
[e] Calculated according to Berkson JASA 48, 569-599 (1953), pg 6 report)
mm = Endpoint derived using mean measured concentration
gm = geometric mean measured concentration
nom = nominal concentration
ND = Not determined analytically
Arkopal = alkylphenol-polyglykol-ether

The end points and confidence intervals for the acute fish studies are shown in the table below. Syngenta recongnises that some of the studies were conducted a while ago and in some cases the solvent loading was higher than guideline levels. However, no deleterious effects were noted from solvent use and all studies statisfy the OECD 203 validity criteria. And the LC_{50} values from all the acute fish studies range from 0.23 to 1.5 mg/L and so are within a factor of 10 of the lowest value. These indicate that the data from the acute fish studies provide an accurate estimate of fish acute toxicity of fludioxonil. Therefore all acute fish and amphidina studies may be used for the refinement of the risk assessment as there are no contraindications based on the study validty criteria.

Table 8.2-12: Range of acute aquatic vertebrate fludioxonil toxicity endpoints ~ Clarification of solvent use, statistics when solvent used and mean measured Vs geometric or nominal concentrations in ecotoxicology studies

Species	Endpoint (mg a.s./L)	Confidence interval (mg a.s./L)	NOEC mortality (mg a.s./L)	LOEC mortality (mg a.s./L)	Reference (author, date, Syngenta Ref)
Rainbow trout (*Oncorhynchus mykiss*)	0.47	0.38 – 0.69	0.26	0.38	**Holmes & Swigert (1993) CGA173506/0258**
	0.23	0.18 – 0.33	0.11	0.33	**Biever (1997) CGA173506/0926**
	0.50	0.38 – 0.81	0.42	0.83	**Rufli (1989a) CGA173506/0004**
Bluegill sunfish (*Lepomis macrochirus*)	0.74	0.62 – 0.96	0.45	0.96	**Biever (1997) CGA173506/0927**
	0.31	0.18 – 0.53[a]	0.18	0.53	**Rufli (1989b) CGA173506/0002**

Carp (*Cyprinus carpio*)	1.5	1.8 – 3.2[a]	1.0	2.3	**Rufli (1989c) CGA173506/0001**
Catfish (*Ictalurus punctatus*)	0.63	0.54 – 0.75	0.32	0.68	**Rufli (1989d) CGA173506/0003**
Sheepshead minnow (*Cyprinodon variegatus*)	1.2	0.93 – 1.5[a]	0.93	1.5	**Holmes & Swigert (1993) CGA173506/0257**
	0.54	0.39 – 0.76	0.39	0.57	**Surprenant 1990 CGA173506/0056**
Fathead minnow (*Pimephales promelas*)	0.70	0.57 – 0.85	0.54	1.1	**Fournier (2014) CGA173506_51203**
Rana limnocharis	2.32	2.15 – 2.5			**Mingqi (2010) CGA173506_11704**
Total range:	0.23 – 2.3 (all) 0.23 – 1.5 (fish)				

[a] Concentration either side of the LC_{50} used for confidence intervals as 0% and 100% mortality occurred at these rates with no concentration inbetween.

Table 8.2-13: Summary of endpoints for chronic toxicity to fish for fludioxonil ~ Clarification of solvent use, statistics when solvent used and mean measured Vs geometric or nominal concentrations in ecotoxicology studies

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity criteria	Reference (author, date, Syngenta Ref)
Rainbow trout *(Oncorhynchus mykiss)* Fish juvenile growth test (flow-through)	Triethylene glycol		No significant difference between control and solvent control. Pooled control data used for statistical comparison (pg 16 report).	Solvent level was the same in solvent control and all test concentrations, and no mortality occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.	NOEC = $0.040_{(mm)}$	nom	0.005, 0.010, 0.020, 0.040, 0.080	Validity criteria: Control mortality required: Actual: Control: 0% Solvent control: 0% Inrease in body weight required ≥ 50% by 28d: Control: >50% (189%) Solvent control: >50% (190%) The study is considered valid for use.	***Maynard (2005) CGA173506/6704***
	Solvent control:	0.1 mL/L (pg 11-12 report				mm	0.0045, 0.0092, 0.020, 0.040, 0.076		
	Highest test concentration:	0.1 mL/L (pg 11-12 report)				gm	Not applicable as flow through.		
	All test concentrations:	0.1 mL/L (pg 11-12 report)				Based on mean measured results. No recalculation of results required.			
Fathead minnow *(Pimephales promelas)* Early life stage (ELS) (flow-through)	Acetone		No significant difference between control and solvent control. Pooled control data used for statistical comparison (pg 16 report).	Solvent level was the same in the solvent control and all test concentrations, also no statistical difference between	NOEC = $0.039_{(mm)}$	nom	0.0094, 0.019, 0.038, 0.075, 0.15	Validity criteria: Control / SC hatching: 86-88% Control/SC survival: 87-88% The study is considered valid for use.	***Graves et al. (1994) CGA173506/0416***
	Solvent control:	0.28 mL/L (pg 11 report)				mm	0.010, 0.020, 0.039, 0.077, 0.144		
	Highest test concentration:	0.28 mL/L (pg 11 report)				gm	Not applicable as flow through.		

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity criteria	Reference (author, date, Syngenta Ref)
	All test concentrations:	0.28 mL/L (pg 11 report)		controls. Therefore the concentration of solvent in the range used did not affect survival/ hatching of the test species.		All results between 80-120% of nominal so use of mean measured concentrations is acceptable ~ no recalculation of results required		
Sheepshead minnow *(Cyprinodon variegatus)* Early life stage (ELS) (flow-through)	Dimthylformamide (DMF)		Control/SC pooled for hatching success and survival endpoints. The data for growth parameters were based on the solvent control as there was a significant difference between control and solvent control (Pg16 report).	Solvent level was the same in solvent control and all test concentrations. The slight decrease for growth parameters is not considered to be a true effect of the solvent as the reduction was not consistent in concentrations ≤NOEC. Therefore the concentration of solvent in the range used did not affect the test species.	NOEC = $0.1_{(nom)}$	nom: 0.0064, 0.016, 0.040, 0.10, 0.25, 0.625 mm: 0.0062, 0.016, 0.040, 0.101, 0.246, 0.575 gm: Not applicable as flow through. All results between 80-120% of nominal so use of nominal concentrations is acceptable ~ no recalculation of results required (pg 17-18, 23 report)	Validity criteria: Hatching success: >75% Control: 96% Solvent control: 95%% Pooled control: 96% Laval survival: >80% Control: 100% Solvent control: 99% Pooled control: 99% The study is considered valid for use.	***Minderhout et al. (2011) CGA173506_11516***
	Solvent control:	0.1 mL/L (pg 14 report)						
	Highest test concentration:	0.1 mL/L (pg 14 report)						
	All test concentrations:	0.1 mL/L (pg 14 report)						
Sheepshead minnow *(Cyprinodon*	None	None	Control compared to test concentrations	No solvent used.	NOEC = $0.038_{(mm)}$	nom: 0.019, 0.038, 0.075, 0.15, 0.30	Validity criteria: Control hatching: >75%	***Marini (2014) CGA173506_51331***

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity criteria	Reference (author, date, Syngenta Ref)
variegatus) Early life stage (ELS) (flow-through)			for statistical analysis.			mm: 0.021, 0.038, 0.092, 0.16, 0.33 gm: Not applicable as flow through. Based on mean measured results. No recalculation of results required.	(Actual: 90%) Control survival: >80% (Actual: 100%). The study is considered valid for use.	
Fathead minnow *(Pimephales promelas)* Fish full life cycle (flow through)	None	None	Control compared to test concentrations for statistical analysis.	No solvent used.	NOEC = $0.018_{(mm)}$	nom: 0.0070, 0.012, 0.019, 0.032, 0.054, 0.090 twm: 0.0065, 0.011, 0.018, 0.030, 0.051, 0.086 mm: 0.0066, 0.011, 0.019, 0.030, 0.052, 0.086 Based on time weighted mean measured results. Arithmetic mean measured concentrations are similar so no recalculation of results is required.	Validity criteria: F_0 Control hatching required: 70% (Actual: 86%) F_0 60d post hatch larval survival required: 80% (Actual: 90%) F_1 hatch success required: 70% (Actual: 87%) F_1 30d post hatch larval survival required: 70% (Actual: 79%) The study is considered valid for use.	***Marini (2015) CGA173506_51493***

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity criteria	Reference (author, date, Syngenta Ref)
RMS query: For chronic toxicity studies on fish, in addition to the EC1O values proposed, it is recommended to provide a justification of the robustness and the reliability of the NOEC values.	The valid fish chronic studies, relevant to the risk assessment, were all conducted according to the current guideline at the time. Both ELS studies with sheepshead minnow (*Minderhout, 2011* and *Marini 2014*) meet the current guideline (*OECD 210 (2013)*, *OCSPP 850:1400 (1996)*) requirement with regard to number of organisms and number of replicates. The fathead minnow ELS study (*Graves, 1994*), employed only two reps, rather than four (standard at the time), however the number of eggs used was up to current standards. The proposed new chronic endpoint is taken from a Fish Full Life Cycle study (*Marini, 2015*), conducted to the current guideline (*OCSPP 850.1500 (1996)*). Each study is considered valid (meeting current criteria) and robust statistical tests were used to identify clear NOECs. EFSA have confirmed that assessing the statistical power of a NOEC from a lower tier laboratory test is not necessary (*EFSA; Outcome of the pesticides peer review meeting (133) on general recurring issues in ecotoxicology, 2015*).							

mm – mean measured concentration
nom - nominal concentration
twm – time weighted mean
gm – geometric mean measured concentration

Table 8.2-14: Summary of acute endpoints for aquatic invertebrates for fludioxonil ~ Clarification of solvent use, statistics when solvent used and mean measured Vs geometric or nominal concentrations in ecotoxicology studies

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
Daphnia magna	Acetone		Immobility: 5% controls 0% solvent control (pg 29 report) ~ not sure about	Solvent control same as highest test concentration and no immobility in solvent control. Therefore the	48 h EC_{50} = $0.40_{(mm)}$ (flow-through)	nom	0.13, .22, 0.36, 0.60, 1.0	Validity criteria: Immobility required ≤ 10 % 5% controls 0% solvent	***Suprenant (1990) CGA173506/0059***
	Solvent control:	0.029 mL/L				mm	0.12, .21, 0.31, 0.54, 0.76		

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
	Highest test concentration:	0.029 mL/L	which control used to calculate the statistics	concentration of solvent in the range used did not affect survival of the test species.		gm; mm correct as flow through test	control. The study is considered valid for use.	
	All test concentrations:	0.0038 to 0.029 mL/L				Based on mean measured results ~ so no recalculation of results required		
Daphnia magna	Acetone		Mortality (%) 5% controls 0% solvent control (pg 22) ~ not sure about which control used to calculate the statistics	Solvent level was the same in solvent control and all test concentrations. Although higher than the OECD202, no deleterious effects were apparent, and no mortality occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.	48 h EC_{50} = $0.90_{(mm)}$ (flow-through)	nom; 0.65, 1.1, 1.8, 3.0, 5.0 mm; 0.50, 0.83, 1.1, 1.6, 1.9 gm; 0.50, 0.83, 1.1, 1.6, 1.9	Validity criteria: Immobility required ≤ 10 % 5% controls 0% solvent control. The study is considered valid for use.	***Holmes & Swigert (1993) CGA173506/0259***
	Solvent control:	1 mL/L (pg 11-12 report)						
	Highest test concentration:	1 mL/L (pg 11-12 report)						
	All test concentrations:	1 mL/L (pg 11-12 report)				Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required		

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
Daphnia magna	Acetone + [Arkopal surfactant]		Minor solvent control /surfactant immobility (5%) within guideline allowable limits ~ from examination of the raw data neither control of solvent control appear to have been used in the statistical calculation. In recalculated statistics the controls showed no significant difference. The solvent control was used for comparison with the treatments.	Solvent control same as highest test concentration and 5% immobility in solvent control (within guideline allowable guideline). Therefore the concentration of solvent in the range used did not affect survival of the test species.	48 h EC_{50} = $1.1_{(mm)}$ (static) [a] Recalculated on geomean concentrations: 48 h EC_{50} = $1.06_{(gm)}$ (static) [a]	nom	0.32, 0.58, 1.0, 1.8, 3.2, 5.8, 10	Validity criteria: Immobility required ≤ 10 % 0% controls 5% solvent control. The study is considered valid for use.	***Rufli (1989e) CGA173506/0005***
	Solvent control + [Arkopal]:	73.7 mg/L (= 0.1 mL/L) (pg 6 report) [0.4 mg/L]				mm	0.28, 0.51, 1.6, 1.2, 2.1, 3.4, 5.8		
	Highest test concentration [Arkopal]:	73.7 mg/L (= 0.1 mL/L) (pg 6 report) [0.4 mg/L]				gm	0.28, 0.51, 0.79, 1.2, 2.0, 3.0, 4.4		
	All test concentrations + [Arkopal]:	0.00322 to 0.10 mL/L (pg 6 report and raw data examination) [0.040 to 0.4 mg/L]				Results were based on nominals ~ recalculated based on geometric mean measured concentrations.			
Americamysis bahia [b]	Acetone		Mortality (%) 0% controls 10% solvent control ~ not sure which control is used to calculate the statistics (pg 15 report)	Solvent level was the same in solvent control and all test concentrations. Although higher than the test guidelines, no deleterious effects	96 h LC_{50} = $0.27_{(mm)}$ (flow-through)	nom	0.10, .17, 0.29, .48, 0.80	Validity criteria: Immobility required ≤ 10 % 0% controls 10% solvent control. The study is	***Holmes & Swigert (1993) CGA173506/0256***
	Solvent control:	1 mL/L (pg 11-12 report)				mm	0.075, .15, 0.24, 0.37, 0.63		
	Highest test concentration:	1 mL/L (pg 11-12 report)				gm	0.073. .15, 0.24, 0.37, 0.63		

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
	All test concentrations:	1 mL/L (pg 11-12 report)		were apparent with 10% mortality occurring in solvent control (allowable level in guidance). Therefore the concentration of solvent in the range used did not affect survival of the test species.		Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results is required	considered valid for use.	
Eastern oyster (*Crassostrea virginica*)	Acetone		Survival (%) 100% controls 100% solvent control. Growth rates for the controls were not significantly different and statistics was conducted using the pooled control data (pg .18 report)	Solvent control levels are the same as highest test concentration and no mortality in occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.	EC_{50} = 0.37$_{(mm)}$ (flow-through)	nom: 0.084, .14, 0.23, 0.39, 0.65 mm: 0.093, 0.15, .22, 0.38, .54 gm: 0.093, 0.15, .21, 0.38, .54 Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results is required	Validity criteria: Mortality ≤10% 0% controls 0% solvent control. Minimum shell deposition ≥ 2mm: 2.5 mm controls 2.4 mm solvent control 2.4 mm pooled control The study is considered valid for use.	***Suprenant (1990) CGA173506/0390***
	Solvent control:	0.075 mL/L (pg 16 report)						
	Highest test concentration:	0.075 mL/L (pg 16 report)						
	All test concentrations:	0.0097 to 0.075 mL/L (pg 16 report)						

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
Hyalella azteca	Acetone Solvent control: Highest test concentration: All test concentrations:	10 mLs added to 0.050 kg silica sand and allowed to evaporate. The sand mixed with 2 kg of wet sediment. (pg 13-14)	Survival (%) 99% controls 96% solvent control. Controls were not significantly different and statistics was conducted using the negative control. (pg 18-19	Solvent control same as all test concentrations and 4% mortality occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of the test species.	10 d LC_{50} >84 mg/kg $dw_{(mm)}$ (static renewal)	nom mm gm Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required	6.3, 13, 25, 50, 100 mg/kg sediment 5.2, 11, 21, 41, 84 5.2, 11, 21, 41, 83	Validity criteria: 80% control survival: 99% controls 96% solvent control Measurable growth in the controls: 0.21 mg controls 0.20 mg solvent control The study is considered valid for use.	***Bradley (2014) CGA173506_51325***
Leptochirus plumulosa	Acetone Solvent control: Highest test concentration:	10 mLs added to 0.050 kg silica sand and allowed to evaporate. The sand mixed with 2 kg of wet sediment.	Survival (%) 99% controls 99% solvent control. Controls were not significantly different and statistics was conducted using the negative control. (pg 18)	Solvent control same as all test concentrations and 1% mortality occurred in solvent control. Therefore the concentration of solvent in the range used did not affect survival of	10 d LC_{50} >90 mg/kg $dw_{(mm)}$ (static)	nom mm gm	6.3, 13, 25, 50, 100 mg/kg sediment 5.7, 11, 23, 45, 90 5.6, 11, 23, 45, 90	Validity criteria: ≥ 90% mean survival: 99% controls 99% solvent control The study is considered valid for use.	***Bradley (2014) CGA173506_51327***

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
	All test concentrations:	10 mLs added to 0.050 kg silica sand and allowed to evaporate. The sand mixed with 2.75 kg of wet sediment. (pg 13-14)		the test species.		Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required			
Chironomus dilutus	Acetone	10 mLs added to 0.050 kg silica sand and allowed to evaporate. The sand mixed with 2 kg of wet	Survival (%) 98% controls 98% solvent control. Controls were significantly different using the Equal variance two sample t-test (but not	Solvent control same as all test concentrations. No difference in control and solvent control survival. The significant difference	10 d LC_{50} = 100 mg/kg $dw_{(mm)}$ (static renewal)	nom	0.80, 2.0, 5.0, 13, 31, 100	Validity criteria: ≥70% mean survival: 98% controls 98% solvent control. 0.60 mg ash-free	***Bradley (2014b) CGA173506_51329***
	Solvent control:					mm	0.85, 2.0, 5.3, 13, 31, 100		
	Highest test concentration:					gm	0.84, 1.9, 5.3, 13, 31, 99		

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
	All test concentrations:	sediment. 10 mLs added to 0.050 kg silica sand and allowed to evaporate. The sand mixed with 2.75 kg of wet sediment (pg 14-15)	Wilcoxon), so comparisons were made to the negative control. Pg 18-20).	between control and solvent control for growth is not perceived as being a function of solvent interference and is instead related to natural variability commonly observed for *Chironomus dilutus* larval growth. Validity criteria for survival were amply satisfied, therefore the concentration of solvent in the range used did not affect survival of the test species.		Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required	dry weight per midge larvae) 1.46 mg control 1.89 mg solvent control. The study is considered valid for use.	

mm – mean measured concentration
nom - nominal concentration
gm – geometric mean measured concentration
Arkopal = alkylphenol-polyglykol-ether

Table 8.2-15: Summary of chronic endpoints for aquatic invertebrates for fludioxonil ~ Clarification of solvent use, statistics when solvent used and mean measured Vs geometric or nominal concentrations in ecotoxicology studies

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
Daphnia magna	Acetone + [Arkopal surfactant]		Unclear which control data has been used to calculate the statistics. In addition, the data from dead parents has not been removed. And the number of both live and dead young have been included in calculations. This is not current practice.	Solvent use is within guideline levels. Surfactant use was allowable at the time but is not current practice. The performance of the solvent control does not show it has any deleterious effect for survival, although reproductive performance is below guideline validity criteria. Therefore the concentration of solvent in the range used may have affected the reproduction of the test species.	21 d NOEC = 0.005$_{(mm\ expired\ only)}$ (static renewal) ~ based on cumulative number of dead young from live and dead parents. Recalculation from raw data excluding data from dead parents, and excluding dead young from live parents and using the geometric mean of both new and old test solutions provides a 21d NOEC = 0.042 mg/L.	nom	0.00074, 0.0022, 0.0067, 0.020, 0.060, 0.18	Validity criteria: Parental mortality ≤20% Control: 10% Solvent control : 10% Other test concentrations 0 - 50% (not dose responsive) Cumulative number of live neonates criteria ≥ 60 Control = 55 (recalculated = 53*) Solvent control = 47 (recalculated = 46*) * from raw data excluding data from dead parents, and excluding dead young from live parents Invalid study as too few live neonates per female in both control and solvent control.	***Rufli (1989) CGA173506/0006***
	Solvent control + {Arkopal]:	0.09 mL/L + [Arkopal 0.0072 mg/L]				mm	0.00038, 0.0009, 0.0052, 0.0109, 0.033, 0.11		
	Highest test concentration + [Arkopal]:	0.09 mL/L + [Arkopal 0.0072 mg/L]				gm	0.0005, 0.0013, 0.0055, 0.014, 0.042, 0.13		
	All test concentrations + [Arkopal]:	Proportionally diluted so 0.00037 to 0.09 mL/L + [Arkopal 0.000030 to 0.0072 mg/L]				Results calculated on expired concetrations nly. Geometric means should be used to take account of fresh concentrations too ~ results recalculated with geomeans			
Daphnia magna	Acetone		Significantly reduced survival of solvent control (not considered in report to be of biological importance).	The large difference (35% decrease) in cumulative number of neonates in the solvent control compared to the	21 d NOEC = 0.019$_{(mm)}$ (flow-through)	nom	0.02, 0.04, 0.08, 0.16, 0.32 ug/L	Validity criteria: Parental mortality ≤20% Control: 7% Solvent control : 0%	***Putt (1991) CGA173506/0179***
	Solvent control:	0.017mL/L				mm	0.019, 0.034, 0.062, 0.086, 0.19		
	Highest test concentration:	0.017mL/L				gm	mm correct as flow through test		

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
	All test concentrations :	0.0011 to 0.017 mL/L	Statistics was performed in comparison to the solvent control (pg 19-21) Notably reduced number of neonates (35%) in solvent control but not mentioned in the report if this difference is statistically significant. Additional statistics are in Appendix V (pg 75) but the comparisons between the control and solvent control are not very clear.	control is a source of cernern about the validty of the results of this study (although validty criteria met on this point). The solvent levels used are 5x lower than the maximum guideline level. No reason can be found to explain such a large difference. Therefore this study is of questionable validity.		Based on mean measured results ~ so no recalculation of results required		Cumulative number of live neonates criteria ≥ 60 Control = 170 Solvent control = 111 Validity of study questionable due to the large difference (35% decrease) in cumulative number of neonates in the solvent control Vs the control. Several offspring were noted as immobilized in the solvent control on d21. US EPA deemed this study invalid on this basis.	
Daphnia magna	None used (pg 14-15)		Statistics was conducted on the negative control (pg 17-18 report)	No solvent was used.	21 d NOEC = 0.035$_{(mm)}$ (static renewal) EC$_{10/20}$ repro >0.035	nom	0.0038, 0.0075, 0.015, 0.030, 0.060	Validity criteria: Parental mortality ≤20% Control: 0% Cumulative number of live neonates criteria ≥ 60 Control = 82 The study is considered valid	***Fournier (2014) CGA173506_51057***
	Solvent control:	-				mm	0.0022, 0.0044, 0.0089, 0.018, 0.035		
	Higest test concentration:	-				gm	0.0022, 0.0044, 0.0088, 0.018, 0.035		

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
	All test concentrations :	-				Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required	for use.	
Americamysis bahia [b]	Dimethylformamide (DMF) Solvent control: 0.1 ml/L (pg 14) Highest test concentration: 0.1 ml/L (pg 14) All test concentrations : 0.1 ml/L (pg 14)		Control and solvent control were not significantly different for survival, reproduction, male or female dry weight or female length data the control data were pooled for comparison among the treatment groups for these parameters. Controls were significantly different for male weight only (very minor difference), so comparisons were made to the solvent control. (pg 16-17)	The solvent level was the same in the solvent control and all test concentrations. The survival and reproductive perfromance of the control and solvent control were similar therefore solvent use was acceptable and no deleterious effects were noted. Therefore the concentration of solvent in the range used did not affect the survival and reproduction of the test species.	28 d NOEC = $0.018_{(mm)}$ (flow-through)	nom: 5, 10, 20, 40, 80 mm: 4.6, 9.1, 18, 36, 70 gm: mm correct as flow through Based on mean measured results ~ so no recalculation of results required	Validity criteria: F0 post-pairing survival >70% Control: 98% Solvent control: 96% Pooled control: 97% Reproductively active females ≥75% Control: 90% Solvent control: 89% Pooled control: 90% Reproductive performance ≥ 3 offspring per female: Control: 7.6 Solvent control: 4.6 Pooled control: 6.1 The study is considered valid for use.	***Claude et al. (2011) CGA173506_11519***
Americamysis bahia [b]	None used (pg 13-14) Solvent control: -		Statistics was conducted on the negative control (pg 19 report)	No solvent was used.	28 d NOEC = $0.024_{(twm)}$ (flow-through)	nom: 0.0025, 0.005, 0.010, 0.020, 0.040 mm: 0.0032, 0.0055, 0.012, 0.023, 0.044, 0.093	Validity criteria: F0 post-pairing survival >70% Control: 80% Reproductively active females	***Marini (2014) CGA173506_51333***

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
	Highest test concentration:	-				twm 0.0032, 0.0055, 0.012, 0.024, 0.044, 0.096	≥75% Control: 100% Reproductive performance ≥ 3 offspring per female: Control: 18.3 The study is considered valid for use.	
	All test concentrations :	-				Based on time weighted mean measured results. Time weighted mean measured and arithmetic mean measured results are similar ~ so no recalculation of results required.		
Chironomus riparius	Acetone	2.0 mLs added to 15.5 g sand and allowed to evaporate. The dry sand then mixed with 1100 g of wet sediment. (pg 14)	Statistics was conducted on the negative control (pg 23 report)	The solvent level was the same in the solvent control and all test concentrations. The emergence and development of the control and solvent control were similar therefore solvent use was acceptable and no deleterious effects were noted. Therefore the concentration of solvent in the range used did not affect survival or reproductive performance of the test species.	28 d NOEC = 40 mg a.s./kg $_{(nom)}$ (static)	nom 5.0, 10, 20, 40, 80, 160 mg a.s./kg sediment	Validity criteria: Emergence in control required >80%: Actual control: 90% Actual solvent control: 90% Mean development time in controls required ≤ 23d: Actual control: by day 20 Actual solvent control: by day 19 The study is considered valid for use.	***Grade (1998) CGA173506/1274***
	Solvent control:					mm 79, 158 mg a.s./kg (rates below 80 mg/kg not measured)		
	Highest test concentration:					Water 0d 0.03, 0.04, 0.07, 0.12, 0.25, 0.22 mg a.s./L		
	All test concentrations :					Water 28d 0.02, 0.04, 0.14, 0.27, 0.36, 0.43 mg a.s./L		

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
						Based on nominal concentrations in sediment. Measured concentrations in sediment confirm correct dosing for top 2 test concentrations (two concentrations ncluding top dose is the OECD 218 requirement) and stability in sediment during study. Water, pore water and sediment were analysed at 0, 7, 28d as required by GD. Geometric mean measured results are not required. No recalculation of results is required.		

Organism	Solvent used in study	Solvent levels in solvent control and test concentrations	Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
RMS query: For chronic toxicity studies on aquatic invertebrates, in addition to the EC10 values proposed, it is recommended to provide a justification of the robustness and the reliability of the NOEC values.	The *Daphnia* and Mysid chronic studies providing the proposed chronic invertebrate endpoints for fludioxonil, were all conducted according to the most recent versions of the internationally recognised test guidelines (OECD 211 (2012), US EPA OCSPP 850.1300 (1996), US EPA OCSPP 850.1350 (1996)). The replication and number of organisms proposed in these guidelines is in place to derive a reliable and robust NOEC. While the chironomid study was conducted prior to the finalisation of the OECD guideline, there is nothing to call the study endpoint into question (EC50 and NOEC ≥highest test concentration). EFSA have confirmed that assessing the statistical power of a NOEC from a lower tier laboratory test is not necessary (EFSA; Outcome of the pesticides peer review meeting (133) on general recurring issues in ecotoxicology, 2015).							

mm – mean measured concentration
nom - nominal concentration
gm – geometric mean measured concentration
twm – time weighted mean

Table 8.2-16: Summary of endpoints for algae for fludioxonil ~ Clarification of solvent use, statistics when solvent used and mean measured Vs geometric or nominal concentrations in ecotoxicology studies

Organism	Solvent use in study		Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
Desmodesmus subspicatus	None used		No solvent used (pg 6 report)	No solvent used	72h E_bC_{50} = 0.93 (expired measured)	nom	0.063, 0.13, 0.25, 0.50, 1.0	Validity criteria: Recalculated based on cell number/ml: Control increase required 16x: Actual: 143 (control) Average specific growth rate require ≤7%: Actual: 2.6% (control) Section-by-section criteria required ≤35%: Actual: 25.4% The study is considered valid for use.	***Rufli (1989) CGA173506/0007***
	Solvent control:	-				Expired measured	0.039, 0.081, 0.050, 0.46, 0.71		
						mm	0.061, 0.12, 0.10, 0.52, 0.96		
	Highest test concentration:	-				gm	0.057, 0.11, 0.88, 0.51, 0.93		
	All test concentrations:	-				Expired measured concentrations used to calculate endpoints ~ more conservative than geomeans so no recalculation is required			
Pseudokirchneriella subcapitata	Acetone		Page 6 ~A t-Test compared the control data and if similar, they were pooled for comparison with the treatments. If data not similar, the solvent control was used for	The solvent level was the same in the solvent control and all test concentrations and complies with guideline limits.	72h E_rC_{50} = 0.41 (mm) 96h E_rC_{50} = 0.31 (mm) 120h E_rC_{50} = 0.33 (mm)	nom	0.026, 0.050, 0.10, 0.20, 0.40, 0.80	Validity criteria: Control increase required 16x: Actual: 87.2 (solvent control) Average specific	***Hoberg (1992) CGA173506/0243 Recalculated based on mean measured in Hoberg (1992) CGA173506/6950***
	Solvent control:	0.1 ml/L				im	0.028, 0.062, 0.11, 0.20, 0.36, 0.43		

Organism	Solvent use in study		Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
	Highest test concentration:	0.1 ml/L	comparison. This was done at each time point; e.g. 48, 72, 96 and 120 hours. In Tables 3 and 5 of this report, the footnotes describe which control was used for comparison with the treatments. It is clear that at 48, 72 and 96 hours, the pooled control was used (as there was no difference between controls) and then at 120 hours endpoints for the inhibition data and the statistically significant differences were derived by comparing to the solvent control (as there was a difference between controls).	Therefore the concentration of solvent in the range used did not affect growth of the test species.	72h E_bC_{50} = 0.029$_{(mm)}$ 96/120h E_bC_{50} = 0.024$_{(mm)}$	gm	0.014, 0.020, 0.027, 0.085, 0.22, 0.51	growth rate require ≤7%: Actual: 2.0% (solvent control) Section-by-section criteria required ≤35%: Actual: 21.6% (solvent control) The study is considered valid for use.	
	All test concentrations:	0.1 ml/L				The four lowest test concentrations were <LOQ. So geomean calculated using 0.5 x LOQ for expired concentrations. Inhibitory effects occur at concentrations above gm: 0.014 mg/L. The calculations are still conservative for the lower 4 test concentrations therefore this makes no difference to the validity of the results. Results based on geometric mean measured concentrations as required during original regaNo recalculation is required.			

Organism	Solvent use in study		Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
Pseudokirchneriella subcapitata	Dimethylformamide (DMF)		Significant differences between the control groups were observed in cell density and yield on Day 4. The significant difference observed was slight. The results for cell density, biomass and growth rates were evaluated on the basis of the solvent control. (Pg 16 report)	The solvent level was the same in the solvent control and all test concentrations. The levels of solvent used complied with guideline limits and and the study passed the validty criteria. Therefore the concentration of solvent in the range used did not affect growth of the test species.	72h E_rC_{50} = >0.442$_{(mm)}$ 96h E_rC_{50} = >0.442$_{(mm)}$ 72h E_vC_{50} = 0.244$_{(mm)}$ 96h E_vC_{50} = 0.301$_{(mm)}$	nom	0.0051, 0.013, 0.032, 0.080, 0.20, 0.50	Validity criteria: Control increase required 16x: Actual: 100 (solvent control) Average specific growth rate require ≤7%: Actual: 2.64% (solvent control) Section-by-section criteria required ≤35%: Actual: 35% (solvent control) The study is considered valid for use.	***Porch et al. (2011) CGA173506_11518***
	Solvent control:	0.1 ml/L				mm	Not calculated		
	Highest test concentration:	0.1 ml/L				gm	0.0026, 0.0043, 0.014, 0.037, 0.132, 0.442		
	All test concentrations:	0.1 ml/L				The two lowest test concentrations (gm: 0.0026, 0.0043) were <LOQ. So geomean calculated using 0.5 x LOQ for expired concentration. Inhibitory effects occur at concentrations above gm: 0.132 mg/L, therefore this makes no difference to the validity of the results. No recalculation is required.			
Anabaena flos-aquae	Dimethylformamide (DMF)		No significant difference between the control and solvent control. The results	Solvent control levels are the same as highest test concentration,	72h $E_{brv}C_{50}$ >1.3$_{(mm)}$ 96h $E_{br}C_{50}$ >1.3$_{(mm)}$	nom	0.13, 0.25, 0.50, 1.0, 2.0	Validity criteria: Control increase required 16x:	***Soucy (2013) CGA173506_51018***

Organism	Solvent use in study		Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
	Solvent control:	0.1 ml/L	were compared to the pooled control data (pg 21 report).	and no significant differences were detected between the controls. Therefore the concentration of	96h E_vC_{50} = $1.1_{(mm)}$ 72/96 h $E_rC_{10/20}$ $>1.3_{(mm)}$ 72 h E_yC_{10} =	mm	0.095, 0.15, 0.30, 0.62, 1.3	Actual: 96h 240 (pooled control) Average specific growth rate require ≤10%:	
	Highest test concentration:	0.1 ml/L				gm	0.092, 0.15, 0.29, 0.62, 1.3		

Organism	Solvent use in study		Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
	All test concentrations:	0.065 to 0.1 mL/L		solvent in the range used did not affect growth of the test species.	$0.39_{(mm)}$ 72 h E_rC_{20} = $0.56_{(mm)}$	Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required. No concentrations <LOQ.	Actual: 96h 6.4% (pooled control) Section-by-section criteria required ≤35%: Actual: 96h 72% (pooled control) The study is considered valid for use. Although criteria for section by section growth rate) exceed the above noted criteria, cell counts for *A. flos-aquae* are typically more variable than single cell green algae due to its growth in chains and groups of cells. Toxicity observed with *A. flos-aquae* is consistent with the other algal species tested (*N. pelliculosa* and *S. costatum*) therefore, these results are considered acceptable and clearly define the toxicity of fludioxonil to *A. flos-aquae*.	

Organism	**Solvent use in study**		**Statistics conducted on control / solvent control / pooled control**	**Comment on solvent use in study**	**Endpoints (mg a.s./L)**	**Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)**		**Validity**	**Reference (author, date, Syngenta Ref)**
Skeletonema costatum	Dimethylformamide (DMF)		No significant difference between the control and solvent control. The results were compared to the pooled control data (pg 21 report)	Solvent control levels are the same as highest test concentration and no significant differences were detected between the controls. Therefore the	72h E_bC_{50} = $0.25_{(mm)}$ 72h E_vC_{50} = $0.26_{(mm)}$ 72h E_rC_{50} = $0.37_{(mm)}$ 96h E_bC_{50} = $0.26_{(mm)}$ 96h E_yC_{50} =	nom	0.13, 0.25, 0.50, 1.0, 2.0	Validity criteria: Control increase required 16x: Actual: 96h 47 (pooled control) Average specific growth rate require ≤10%:	***Soucy (2013) CGA173506_51017***
	Solvent control:	0.1 ml/L				mm	0.074, 0.14, 0.31, 0.62, 1.3		
	Highest test concentration:	0.1 ml/L				gm	0.073, 0.14, 0.30, 0.62, 1.2		

Organism	Solvent use in study		Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)	Validity	Reference (author, date, Syngenta Ref)
	All test concentrations:	0.065 to 0.1 mL/L		concentration of solvent in the range used did not affect growth of the test species.	$0.28_{(mm)}$ 96h E_rC_{50} = $0.43_{(mm)}$ 72 h E_rC_{10} = $0.17_{(mm)}$ 72 h E_rC_{20} = $0.23_{(mm)}$ 72 h E_vC_{10} = $0.13_{(mm)}$ 72 h E_vC_{20} = $0.16_{(mm)}$	Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required. No concentrations <LOQ.	Actual: 96h 6.4% (pooled control) Section-by-section criteria required ≤35%: Actual: 96h 83% (pooled control) The study is considered valid for use. Cell counts for *S. costatum* are typically more variable than single-cell green algae due to its growth in chains and groups of cells. OECD Guideline #201 does not specifically require testing with marine algae or diatoms, and as such, the acceptance criteria stated in the protocol were based on freshwater algal species mentioned in the guideline. *Skeletonema costatum*, and diatoms in general, reproduce more slowly than most other algal species. Therefore, the criteria proposed in the test guideline and protocol may not be appropriate for this test organism. Based on this laboratory's	

Organism	Solvent use in study		Statistics conducted on control / solvent control / pooled control	Comment on solvent use in study	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
Navicula pelliculosa	Dimethylformamide (DMF)		Minor significant difference detected between the negative control and solvent control data for yield and growth rate at 96 hours. But the mean yield and growth rate values for the two lowest treatments (where no statistical effects are observed) fall between the control and solvent control mean values, it is unlikely there is a true solvent effect. Therefore the pooled control data was used for comparison.	Solvent control levels are the same as highest test concentration, no significant effects of the solvent were considered to be evident, and the study passed the validty criteria. Therefore the concentration of solvent in the range used did not affect growth of the test species.	72h E_bC_{50} = $0.88_{(mm)}$ 72h E_vC_{50} = $0.86_{(mm)}$ 72h E_rC_{50} >$1.2_{(mm)}$ 96h E_bC_{50} = $0.86_{(mm)}$ 96h E_vC_{50} = $0.85_{(mm)}$ 96h E_rC_{50} >$1.2_{(mm)}$ 72 h E_rC_{10} = $0.68_{(mm)}$ 72 h E_rC_{20} = $0.80_{(mm)}$ 72 h E_vC_{10} = $0.44_{(mm)}$ 72 h E_vC_{20} = $0.63_{(mm)}$	nom	0.13, 0.25, 0.50, 1.0, 2.0	Validity criteria: Control increase required 16x: Actual: 96h 163 (pooled control) Average specific growth rate require ≤10%: Actual: 96h 2.8% (pooled control) Section-by-section criteria required ≤35%: Actual: 96h 22% (pooled control) The study is considered valid for use.	***Soucy (2013) CGA173506_51014***
	Solvent control:	0.1 ml/L				mm	0.074, 0.14, 0.30, 0.63, 1.2		
	Highest test concentration:	0.1 ml/L				gm	0.073, 0.14, 0.30, 0.62, 1.2		
	All test concentrations:	0.065 to 0.1 mL/L				Based on mean measured results. Mean measured and geomean measured results are similar ~ so no recalculation of results required. No concentrations <LOQ.			
pH variation	The pH variation during an algal study frequently shows variation during the study. The duration of an algal study affects the final pH. The longer the study, the higher the pH tends to go. If flasks are shaken enough, or aerated, this will reduce the pH rise. However some algal studies were conducted over 5 days which is significantly longer than the 72-h OECD test. Even the US OCSPP test is 96 hour; in that test there is no corresponding no statement regarding pH change during the test. In studies which use a high initial inoculum (e.g. 10,000 cells/mL) this can also result in high final pHs. The high pH reached at the end such studies is expected and is indicative of strong, healthy algal growth. pH change during such tests result from the algae absorbing the dissolved CO_2 (carbonic acid), and releasing oxygen into the test medium. As the CO_2 is absorbed and oxygen released by the photosynthesising algae the pH of the medium rises. The stronger the algal growth the greater the pH rise. A study by Dubinsky & Rotem 1974* examines the effect of pH on algae and found that algae grew well between pH 5 and 12, while sterile controls grew under pH 4 to 10. Therefore the rise in pH during the studies presented here is not considered to be of any consequence for the validity of these studies, and the absence of this criteria from the OCSPP guidelines validates this statement. * Dubinsky, Z. and Rotem, J. (1974) Relations between algal populations and the pH of their media. Oecologia (Berl.)16: 53-60.								

nom - nominal concentration

mm – mean measured concentration
gm – geometric mean measured concentrations

Table 8.2-17: Summary of endpoints for macrophytes ~ Clarification of solvent use, statistics when solvent used and mean measured Vs geometric or nominal concentrations in ecotoxicology studies

Organism	Solvent used in control and all test concentration		Statistics conducted on control / solvent control / pooled control	Validity & solvent use	Endpoints (mg a.s./L)	Endpoint calculated using Mean measured or geometric mean measured concentrations (mg/L)		Validity	Reference (author, date, Syngenta Ref)
Lemna gibba	Acetone		No significant difference between the control and solvent control. The results were compared to the pooled control data (pg 12 report)	Solvent use had no significant effects on this study.	14 d E_bC_{50} >0.11$_{(mm)}$	nom	0.094, 0.16, 0.34, 0.61, 1.0	Does not pass the ≤2.5d doubling time validity criteria. Control 6d = 30 Control 14d = 15 S. Control 6d = 20 S. control 14d = 70 Pooled control 6d = 19 Pooled control 14d = 22 Although the study is not valid it provides supplementary evidence that *Lemna* is not sensitive to fludioxonil.	***Hoberg (1997) 66723***
	Solvent control:	0.1 ml/L (pg. 16-18 report)				im	0.095, 0.16, 0.34, 0.61, 1.0		
	Highest test concentration:	0.1 ml/L (pg. 16-18 report)				gm	0.034, 0.045, 0.065, 0.087, 0.11		
	All test concentrations:	0.1 ml/L (pg. 16-18 report)				Geometric means should be used to take account aged test concentrations too ~ results recalculated with geomeans. All aged solutions were below LOQ (0.025 mg/L); geomeans calculated using 0.5 x LOQ.			

Im – initial measured concentration
mm – mean measured concentration

Long-term fish endpoint

A new fish full life cycle (FFLC) study has been conducted for fludioxonil with fathead minnow (*Pimephales promelas*). Effects were found on reproductive parameters, namely number of eggs per female, hatching success of the F_0 and survival of the F_1 fry. The fish early life stage studies gave NOECs of 0.039 mg/L. Therefore, the endpoint from the fish full life cycle is proposed as the new long term endpoint for risk assessment as it provides a lower NOEC of 0.018 mg a.s./L.

Long-term invertebrate endpoint

Daphnia magna

Three studies were conducted for chronic toxicity to *Daphnia magna*, Rufli (1989); Putt (1991) and Fournier (2014). The Putt (1991) study was recently rejected by the US EPA on grounds of a significant difference between the solvent control and control (EPA reference MRID 4852 26201, 2008 provided as requested by RMS).

Report:	Submitted as requested by RMS: K-CA 8.2/01 Pease A., Felthousen R (2008). EFED: Fludioxonil: Reclassification of Freshwater Aquatic Invertebrate Life Cycle Test From Acceptable to Invalid; MRID 43080015; Data Evaluation Record Included, EPA reference MRID 4852 26201 (Syngenta File No. CGA173506_50848).

The Rufli (1989) study was not submitted to the EPA due to concerns with quality. As a consequence, all three studies have therefore been reassessed for quality and reliability in the following two independent reviews.

Independent reviews of fludioxonil *Daphnia* reproduction studies

Two reviews of the three *Daphnia* reproduction studies have been conducted; Bealing 2015 (Syngenta file number CGA173506_11921) and Hargreaves 2016(Syngenta file number CGA173506_11935). They highlight the potential effects that the changes in test guideline practice with regard to test media, feeding and solvent/surfactant use since 1989/1991 may have had on the quality of the data from the older studies.

Report:	Submitted to aid evaluation of *Daphnia* reproduction position: K-CA 8.2/~~0~~102 Bealing D (2015). A review of disparate NOEC endpoints for the long-term toxicity of fludioxonil to *Daphnia magna* in various tests, TSGE Consulting, St James Business Park, Knaresborough, North Yorkshire, HG5 8QB, UK. Owner: Lanxess, LoA provided (Syngenta File No. CGA173506_11921).

Executive Summary

This paper reviews long-term NOEC endpoints for the active substance fludioxonil that are based on effects on reproduction of the freshwater crustacean *Daphnia magna*. Differing NOEC values have been obtained by three GLP-compliant studies performed at different times, using different versions of the relevant test procedure. The endpoint obtained in the earliest (Rufli 1989, Syngenta file number CGA173506/0006)) study according to a now obsolete version of the test guideline is lower – by ×7 –

than the corresponding value obtained in the most recent study (Fournier 2014, Syngenta file number CGA173506_51057) that followed current methodology updated and adapted to technical progress.

The endpoint obtained in the earliest study is considered to have been influenced by sub-optimal test conditions that subjected the test organisms to a combination of stresses, manifested in the feeble reproductive performance of the controls. These background influences are also likely to have exacerbated their susceptibility to the technical grade fludioxonil active substance. In addition, solvent systems used to enhance the solubilisation of fludioxonil in the test media, with the carrier present at different concentrations in the various test substance treatments, appear likely to have compromised the outcomes of the first two studies.

The NOEC values of 0.005 mg fludioxonil/L (Rufli, 1989) and 0.019 mg fludioxonil/L (Putt, 1991, Syngenta file number CGA173506/0179) are therefore considered to be unreliable.

The definitive 21-day NOEC for effects on the reproduction of *D. magna* is considered to be 0.035 mg a.s./L, as determined by Fournier (2014).

In depth examination of key technical points regarding study conduct

The following tables summarise the main points to illustrate the influence of media, feeding regime, use of solubilizing agents which have changed as the test guidance developed between OECD TG 202 (II) (1984) and the current OECD 211 (2012).

Table 8.2.5.1-185: Study details

Test substance and reference	Fludioxonil active substance: Rufli (1989)	Fludioxonil active substance: Putt (1991)	Fludioxonil active substance: Fournier (2014)
Guideline followed	OECD 202 Part II (1984) (with modifications from EEC/OECD ring-test, 1985/6. Full GLP study with confirmatory analysis.	US EPA FIFRA 72-4 (1985); Full GLP study, with confirmatory analysis.	OECD 211 (2012), US EPA OCSPP Draft 850 1300 (1996a), OCSPP 850, 1000 (1996b); Full GLP study, with confirmatory analysis.
Exposure regime	Semi-static. Nominal concentrations: 0 (control and vehicle control), 0.00074, 0.0022, 0.0067. 0.020, 0.060 and 0.18 mg a.s./L.	Flow –through Nominal concentrations: 0 (control), 0.020, 0.040, 0.080, 0.160 and 0.320 mg a.s./L.	Semi-static. Nominal concentrations: 0 (control), 0.0038, 0.0075, 0.015. 0.030 and 0.060 mg a.s./L.

Table 8.2.5.1-196: Use of solubilising agents

Test substance and reference	Fludioxonil active substance: Rufli (1989)	Fludioxonil active substance: Putt (1991)	Fludioxonil active substance: Fournier (2014)
Test substance vehicle	**Prescribed by guideline (OECD 202):** Preferably none. Solubilising agents permitted, e.g. organic solvents, emulsifiers or dispersants of low toxicity to *Daphnia*. Concentration limited to ≥ 100 mg/L. If used, the study design should include a vehicle control loaded with the vehicle at the same concentration as used at the highest test substance concentration.	**Prescribed by guideline:** Solvents other than water to be used sparingly when necessary, concentration not to exceed 0.5 ml/L in static systems. DMF, triethylene glycol, methanol, acetone and ethanol considered to be acceptable solvents.	**Prescribed by guideline (OECD 211):** Preferably none, to be used only if unavoidable. Only solvents or dispersants known to have minimal impact on the response variable should be used, e.g. acetone, ethanol, methanol, DMF and triethylene glycol (solvents) and Cremophor TR40, methylcellulose 0.01% and hydrogentated castor oil HC0-40 (dispersants). Final solvent or dispersant concentration not to exceed 0.1 mL/L and the concentration should be identical in the vessels of all treatments except the dilution water control.
	Used in study: Acetone (71.2 mg/L) combined with alkylphenol-polyglycol-ether surfactant (0.04 mg/L) at the top dose and in the vehicle control. The report implies the vehicle was present at proportionately lower concentrations at lower doses prepared by dilution of a primary stock dispersion.	**Used in study:** Acetone. The solvent concentration was 6.6 uL/382 mL (=17.3 uL/L) at the highest fludioxonil concentration and was proportionally diluted at each of the lower treatments.	**Used in study:** None
Comments:	**Comments:** The vehicle used complied with the guideline requirements current at the time the study was performed. The identity of the surfactant is not reported and the possibility that it was an octyl-or nonyl-phenol derivative cannot be excluded. Mention of the presence of the surfactant is omitted from the summary of this study which appears at Point B.9.2.5. of the 2005 DAR for fludioxonil. Current recommended practice is to apply identical vehicle concentrations in the vehicle control and all the test substance treatments where the vehicle is present.	**Comments:** The vehicle used complied with the guideline requirements current at the time the study was performed and the highest acetone concentration was below 0.1 mL/L. Current recommended practice is to apply identical vehicle concentrations in the vehicle control and all the test substance treatments where the vehicle is present	**Comments** None

Table 8.2.5.1-2017: Test medium

Test substance and reference	Fludioxonil active substance: Rufli (1989)	Fludioxonil active substance: Putt (1991)	Fludioxonil active substance: Fournier (2014)
Test medium	**Used in study:** Reconstituted water prepared by amending 2 x distilled water with 65 mg $NaHCO_3$, 294 mg CaCl2.2H20, 123 mg $MgSO_4.7H_20$ and 6 mg KCl per litre. Total hardness: 240 mg $CaCO_3$/L. No mineral fortification or organic growth supplements were provided	**Used in study:** Well water fortified to ASTM standard hard water specification. Total hardness: 160-180 mg $CaCO_3$/L; Total alkalinity: 110-130 mg $CaCO_3$/L	**Used in study:** Well water fortified to ASTM standard hard water specification. Total hardness: 160-170 mg $CaCO_3$/L; Total alkalinity: 88-100 mg $CaCO_3$/L
Comments:	**Comments:** The study complied with the guidelines requirement current at the time the study was performed, which permitted any water, provided it supported "good" reproduction of *Daphnia*, as defined by a low and – by current standards – non-discriminatory validity criterion (mean cumulative juvenile production ≥20/adult after three broods). The composition of the test medium was identified as one of the underlying factors causing variability in results obtained by OECD 202 (II) and this was one of the several aspects specifically addressed in the development of the new OECD 211 guideline. Compared to the requirements prescribed in OECD 211, the test medium used in the Rufli (1989) study is likely to have been deficient in macro-and micro-elements and vitamin supplements. Conditions for growth and reproduction are therefore likely to have been sub-optimal for the test species. Between 0 and 50% adult mortality occurred in all groups and without a clear dose response during the last week of the study.	**Comments:** The study complies with the guideline requirements current at the time the study was performed. Adult mortality was uniformly 0% in all treatments throughout the study.	**Comments:** The study complies with the optimised guideline requirements current at the time the study was performed. Adult mortality was uniformly 0% in all treatments throughout the study.

Table 8.2.5.1-2118: Feeding regime

Test substance and reference	Fludioxonil active substance: Rufli (1989)	Fludioxonil active substance: Putt (1991)	Fludioxonil active substance: Fournier (2014)
Feeding regime	**Prescribed by guideline:** Food of any kind and in any quantity (without over-loading) that permits the achievement of the reproduction criterion for gauging test validity. **Used in study:** Addition on 6 days/week of a *Desmodesmus subspicatus* suspension (added at 17-33 mg TOC equivalent per L medium), supplemented with a Tetramin© extract (1.8 mg TOC-equiv/L). No other medium supplements as previously noted – see above.	**Prescribed by guideline:** Algal suspension (10^8 cells/L) and trout food (5 mg/L) extract, at initiation and 3x/week. **Used in study:** Additions of *Ankistrodesmus falcatus*, 1.2 x 10^8 cells, supplemented with 10 mg trout food and 0.5 mL Selco® (a proprietary mixture of proteins and fatty acids). Additions 3 x daily on weekdays; 2 x per day on weekends and holidays. The algal ration was not defined in terms of C-equivalent.	**Prescribed by guideline:** Algal suspension , daily addition preferred, but at least at every renewal of media in semi-static tests. The feeding rate should be in the range 0.1-0.2 mg organic carbon per adult daphnid per day. **Used in study:** Daily additions of *Ankistrodesmus falcatus*, 8 x 10^6 cells per day, supplemented with a 50 uL suspension of YCT (yeast, ground cereal leaves and flaked fish food). The daily algal ration was equivalent to ca. 0.48 mg C per daphnid per day.
Comments:	**Comments:** The study complied with the guideline requirement current at the time the study was performed. However, the feeding regime was identified as one of the underlying factors causing variability in results obtained by OECD 202 (II) and this was one of several aspects that were refined in the development of the new OECD 211guideline.	**Comments:** Compatible with the optimised guideline requirement. The food ration was not quantified in terms of organic carbon, though the performance of the control groups provides assurance that the feeding regime was adequate.	**Comments:** Complies with the optimised guideline requirement, except that the daily algal ration of 0.48 mg C/daphnid/d exceeded the guideline recommendation of 0.1 to 0.2 mg C/daphnid/d. There is no evidence that this deviation caused any adverse impact.

Table 8.2.5.1-2219: Reproductive performance

Test substance and reference	Fludioxonil active substance: Rufli (1989)	Fludioxonil active substance: Putt (1991)	Fludioxonil active substance: Fournier (2014)
Reproduction in the control group	**Prescribed by guideline:** First juveniles to have been produced by day 9 in the control group, ≥ 3 broods, ≥ 20 juveniles per adult. **Achieved in study:** 9 of 10 unamended control adults produced juveniles by day 9. In the vehicle control only 7 of 10 adults produce juveniles by day 9. Cumulative 37 to 101 (mean = 62 total, of which 55 live) juveniles per adult in unamended control; fewer than 60 in the majority (6/10) of replicates. Cumulative 32 to 74 (mean = 54 total, of which 47 live) juveniles per adult in vehicle control; fewer than 60 in majority (7/10) of replicates.	**Prescribed by guideline:** ≥ 40 mean cumulative juveniles per adult after 21 days. **Achieved in study:** Cumulative mean 170 juveniles/female (Std Dev: 48) in the unamended control treatment. Cumulative mean 111 juveniles/female (Std Dev: 13) in the solvent control treatment.	**Prescribed by guideline:** ≥ 60 mean cumulative juveniles per adult after 21 days. **Achieved in study:** 63 to 115 (mean = 82) per adult in the unamended control.
Comments:	**Comments:** The study complied with the guideline requirement current at the time the study was performed, except that a number of the control adults did not produce their first juveniles by day 9; only 7 adults met this criterion in the vehicle control group. However the requirement prescribed in the current OECD 211 is for at least 60 juveniles per control group adult during the 21-day test. The under-realisation of reproductive potential in this study with fludioxonil therefore implies that juvenile production was limited by parameters of the study design. In other words the test conditions were sub-optimal with respect to reproduction of *D.magna*.	**Comments:** The study complied with the validity requirement of the US guideline followed when the study was performed and additionally complies with the requirement of the current OECD 211 guideline. Note that the US EPA method was similarly revised and the reproductive validity limit increased to ≥ 60 offspring/adult in OPPTS 850.1300 (1996).	**Comments:** Complies with the optimised OECD guideline requirement.

Table 8.2.5.1-2320: Other aspects of the studies

Test substance and reference	Fludioxonil active substance: Rufli (1989)	Fludioxonil active substance: Putt (1991)	Fludioxonil active substance: Fournier (2014)
Other aspects	• Unusually high numbers of dead neonates in the control and fludioxonil treatments. • The coefficient of variation of numbers of juveniles produced in the control (36%) exceeds the maximum limit (25%) for a complaint study according to the current test guideline. • No adjustment was made in the juvenile production data to compensate for adult mortalities. Poor data reporting prevents retrospective compensation. • Mean measured concentrations were calculated using "aged" samples from days 7, 14 and 21. Freshly prepared ("new") solutions were only analysed on day 0. The current OECD guideline requires that new and aged samples be analysed and these analyses should be made on a sample of the same solution. Additionally, there were no measurements on media aged for 72 h, which was the longest span between renewal intervals. Insufficient chemical analyses were performed to appropriately characterise the exposure conditions.	• Test concentrations were applied above the functional solubility limit of fludioxonil in the test medium. • Statistically significant difference in terms of adult survival between the untreated and solvent control groups. • 35% difference in juveniles per female between the untreated and solvent control groups. Report does not state whether this difference was tested for statistical significance.	• Test concentrations were adequately maintained without the use of an auxiliary carrier solvent.

Summary

Although it complied with the guideline requirement current at the time it was performed, the Rufli (1989) study failed to meet the reproductive performance criteria for studies of long-term toxicity to *Daphnia magna* according to the current state-of-the-art as embodied in OECD Guideline 211 (1998 *et seq*.). Hence the untreated control organisms each produced an average of only 55 live juveniles, whereas the current validity requirement is for at least 60, and even this level is normally easily exceeded. It is also notable that adult mortalities were recorded in the latter days of the study, with the numbers of immobilised parent animals unrelated to dose. Taken together, these findings are indicative of sub-optimal test conditions that limited reproductive output as well as survival. The presence of the solvent

system comprising acetone and an unidentified alkylphenol-based surfactant may have potentially reduced juvenile production further. The NOEC of 0.005 mg fludioxonil/L obtained in this study is considered to have been influenced by a combination of stress factors in addition to the presence of the test substance and for that reason it is considered to be invalid.

Higher juvenile counts were recorded in the Putt (1991) study: the current OECD validity criterion of ≥ 60 per adult was fulfilled and there were additionally no adult mortalities in any treatment. It may therefore be concluded that the nutritional requirements of *D. magna* were adequately met in this study. However, there was a substantial difference in reproduction between the two control groups, with mean juvenile production reduced by 35% (not reported) in the acetone solvent control relative to the untreated control group. This difference receives no attention in the study report and it is not stated whether it was tested for statistical significance. The potential influence of acetone in the responses observed in the various fludioxonil treatments is confounded by the fact that the proportional diluter system used to generate the various concentrations of fludioxonil will have delivered media containing unequal concentrations of the acetone carrier solvent to the vessels of the various treatment groups. According to Hutchinson *et al*. (2006)[7], co-solvents should be applied only when necessary, as a last resort. Hutchinson *et al*. (2006) note that differing and unexplained effects of acetone have been observed in reproduction toxicity studies with *D. magna*, possibly linked to feeding regime and nutritional status. Whatever the mechanism, circumspection seems justified when acetone is used in studies of reproductive toxicity in *D. magna*. An endpoint from a study where co-solvent is absent (provided that it has been properly conducted and fulfils reliability criteria) should therefore take precedence over one in which a potential confounding influence of the presence of a co-solvent cannot be excluded.

The most recent study of long-term toxicity of fludioxonil to *D. magna* (Fournier, 2014) was performed without an auxiliary solvent and exposure concentrations were satisfactorily maintained throughout the test under semi-static conditions. All the validity criteria were met, including the number of juveniles per adult in the control treatment as well as the limit set on the coefficient of variation between the cumulative juvenile counts of the individual control replicates. It may therefore be concluded that the test medium and feeding regime provided all the nutrients essential for healthy growth and development. The complicating factor of the co-solvent that compromises the earlier two studies was absent in this test.

For these reasons, the Putt (1991) study is considered to be unreliable and the endpoint provided by the technically superior and reliable Fournier (2014) study shall be considered to provide the definitive long-term endpoint for the long-term toxicity of fludioxonil to *D. magna*. Accordingly, the definitive 21-day NOEC for effects on the reproduction of D. magna is considered to be 0.035 mg a.s./L.

(Bealing, D. 2015)

Report:	Submitted to aid evaluation of *Daphnia* reproduction position: K-CA 8.2/~~02~~03 Hargreaves T (2016). Evaluation of Available Data for the Determination of the Chronic Toxicity of Fludioxonil to *Daphnia magna,* Blue Frog Scientific Limited, Scott House, South St. Andrew Street, Edinburgh, EH2 2AZ, UK. (Syngenta File No. CGA173506_11935).

Guidelines

Not applicable.

[7] Hutchinson, T.H., Shillabeer, N., Winter, M.J. and Pickford, D.B. (2006). Acute and chronic effects of carrier solvents in aquatic organisms: A critical review. *Aquatic Toxicology* **76** (2006) pp. 69-92.

GLP: No

Executive Summary

Three studies were available for chronic toxicity to *Daphnia magna*, Rufli (1989); Putt (1991) and Fournier (2014). Each study was assessed for reliability, based on adherence to the current test guideline (OECD 211).

Both the Rufli (1989) and Putt (1991) had deficiencies that rendered the studies unreliable for derivation of a robust endpoint. The study conducted by Fournier (2014) adhered to the conditions stipulated in the current test guideline.

Based on the deficiencies in the two older studies, it was concluded that the overall NOEC for daphnid reproduction and development should be based on the reliable NOEC from the Fournier (2014) test, i.e. 35 µg/L.

Study Design and Methods

The evaluation was conducted by reviewing the report for each study and where necessary the raw data. Each study was evaluated for adherence to current guidance and quality of data recording. Where necessary data were reanalysed according to current guidance.

Results and Discussion

The study by Rufli (1989) had the following issues:

- Insufficient chemical analysis to express the exposure concentrations;
- non-dose related parental mortality;
- a large and unusual amount of dead neonates in both controls and test concentrations with no discernible dose-response relationship;
- solvent control did not meet the minimum validation criteria for neonate production;
- the coefficient of variation for the control was 36%, outside the maximum suggested to be applicable to a well run study[8];
- neonates from dead parental animals were not discounted;
- poor data recording hampers accurate reconstruction of study.

The results suggested a similar NOEC for reproduction as for the Fournier (2014) test, however all endpoints in the older studies could not be considered to be robust. The secondary endpoints, fraction of dead neonates (non-standard) were considered to be equally unreliable. The number of dead neonates seen in all the test groups (including the control) would not normally be expected with healthy cultures and if the health status of the parent animals is in question then the time to first brood is also in question. The fact that satellite vessels were deemed necessary for the appearance of males suggested that there may have been an issue in the culture conditions in the laboratory.

The second study by Putt (1991) appeared to have been generally well conducted but had some fundamental issues in its execution:

[8] OECD 211 (2012): paragraph 38: "Generally in a well-run test, the coefficient of variation around the mean number of living offspring produced per parent animal in the control(s) should be ≤ 25%, and this should be reported for test designs using individually held animals."

- The test concentrations were prepared with unequal concentrations of carrier solvent[9]. The impact of this on the validity of the study is exacerbated by the observation that there is a significant reduction (35%) in reproductive output in the solvent control compared with the dilution water control. There was no appropriate control for comparison for the lowest five concentrations.

- Furthermore the exposure concentrations appear to have been made by diluting suspensions of the test material prepared above the limit of solubility capable in this study. This causes variability in test results as the suspended solids would not result in a homogenous test substance mixture.

The most recent study by Fournier (2014) builds on the experiences of the previous tests, aiming to avoid the issues encountered. Hence in the Fournier (2014) test, the method followed the semi-static renewal method without the use of solvents, was conducted at suitable concentrations below the limit of functional solubility and with suitable replication. In this study the adult mortality was zero across all treatments and the coefficient of variation for mean number of neonates per parent animal was <25% for both controls, indicating that the study was well conducted.

For the key endpoint of reproduction, the Fournier (2014) test clearly provides the most stable and robust results for the low end range.

In the Rufli (1989) and Putt (1991) study there was an indication of delayed production of the first brood. However when the results are expressed as the total number of neonates produced per test group as a proportion of the total produced of the entire test, (thereby normalizing the data with respect to time of the observation, which may not include neonates produced later the same day) total proportions are similar over the same time period for each test. This would indicate no overall delay in reproduction. This is not an issue for the Fournier (2014) study as the number of neonates produced was recorded daily, whereas values were recorded at renewal in the Rufli (1989) study, and recorded at specific days for Putt (1991). On this basis the data of Fournier (2014) is more comprehensive. Both the Rufli (1989) and Putt (1991) studies are inherently compromised by the study methodology for this endpoint. Additionally in the Putt (1991) study there is an obvious difference between the control and solvent control, further compromising any obtained endpoints.

Based on aspects of study design and quality, the Rufli (1989) and Putt (1991) studies have major deficiencies and methodological flaws, particularly when compared to modern guideline criteria, which invalidate their results. The Fournier test was well conducted, meets all the modern validation criteria and provides non-ambiguous endpoints for reproduction and development.

Conclusion

The NOEC endpoint for reproduction and development is considered to be 35 μg/L.

(Hargreaves, 2016)

[9] OECD 211 (2012), paragraph 37: "When used, the solvent or dispersant concentration should be the same as that used in the vessels containing the test substance."

Key details regarding the materials and methods from each of the three studies are presented in the table below.

Table 8.2-2411: Summary of endpoints from *Daphnia* reproduction studies

	Rufli, 1989	***Putt, 1991***	***Fournier, 2014***
Test guideline	OECD Guideline No. 202, Phase 2, Paris 1984, modified according to the EEC/OECD-ring test 1985/86	EPA Pesticide assessment guidelines, E, Wildlife and aquatic organisms, 1989. § 72-4	OECD Guideline No. 211: *Daphnia magna* Reproduction test (2012)
Test duration (days)	21	21	21
Age of organisms at initiation (hours)	< 24	< 24	< 24
Replication (# organisms)	10/treatment 1/vessel[a]	40/treatment 10/vessel	10/treatment 1/vessel
Exposure method	Semi-static Solution renewal on Monday, Wednesday and Friday Solvent (acetone) used Surfactant (alkylphenol-polyglycol-ether) used	Flow-through 6 volume replacements per 24 hour Solvent (acetone) used	Semi-static Solution renewal every 48 to 72 hours No solvent used
Nominal concentrations	0.73, 2.2, 6.7, 20, 60 and 180 µg a.s./L	20, 40, 80, 160 and 320 µg a.s./L	3.8, 7.5, 15, 30 and 60 µg a.s./L
Analysis of test solutions	Mean measured concentrations calculated based on day 7 (aged), day 14 (aged) and day 21 (aged) analytical results [b]	Mean measured concentrations calculated based on day 0, day 7, day 14 and day 21 analytical results [c]	Mean measured concentrations calculated based on day 0 (new), day 2 (aged and new), day 5 (aged), day 9 (new), day 12 (aged), day 16 (new), day 19 (aged and new) and day 21 (aged) analytical results
Mean measured concentrations	0.38, 0.9, 5.2, 10.9, 33 and 110 µg a.s./L [b]	19, 34, 62, 86 and 190 µg a.s./L	2.2, 4.4, 8.9, 18 and 35 µg a.s./L
Endpoints evaluated	Immobilisation Appearance of 1st brood Fraction of dead neonates Total number of neonates	Immobilisation Total number of live neonates Total adult length Adult dry weight	Immobilisation Total number of live neonates Total adult length Adult dry weight

[a] Five satellite organisms were maintained in case of the appearance of males.
[b] Test solutions and controls were also analysed on day 0 (new samples) however these results were not included in the mean measured concentrations reported in this study. Geometric mean measured concentrations with the Day 0 samples were 0.54, 1.3, 5.5, 14, 41 and 130 µg a.s./L.

The endpoints determined from each of the studies are presented in the table below.

Table 8.2-2512: Summary of endpoints from *Daphnia* reproduction studies

Endpoints		*Rufli, 1989*	*Putt, 1991*	*Fournier, 2014*	Overall conclusion
Immobilisation	EC_{50} (mg/L)	>0.11	>0.19	>0.035	>0.035
	LOEC (mg/L)	>0.11	0.19	>0.035	>0.035
	NOEC (mg/L)	>0.11	0.086	0.035	>0.035
Reproduction:					
Appearance of 1st brood	Not OECD 211 endpoint Used as an endpoint in Rufli 1989	0.005 mg/L reported* 0.0004 to 0.033 mg/L Days 9-12 0.11 mg/L Days 12-19 Not counted daily so unreliable	0.019-0.034 mg/L Day 10 (in line with controls) 0.062 to 0.086 mg/L Day 13 -14 (considered an effect)	0.0022 to 0.035 mg/L Day 8-12	Not a current endpoint. A delay potentially occurs at ≥0.062 mg/L (but data confounded by use of solvents/ surfactants and inadequate media/feeding regimes)
Fraction of dead neonates	Not OECD 211 endpoint Used as endpoint in Rufli 1989	0.0052 mg/L But not dose related so unreliable	--	--	Not a current endpoint & not a dose responsive effect
Total number of neonates	NOEC (mg/L)	0.033	0.019	0.035	0.035
Control validity criteria:	≥60 neonates per female	47/55	111	82	-
Growth:					
Total adult length	NOEC (mg/L)	--	0.019 (6% decrease)	0.035	0.035
Adult dry weight	NOEC (mg/L)	--	0.034	0.035	0.035

* In Rufli 1989 the report states effects were apparent in time to first brood at 0.005 mg/L however neonates were not counted daily so this endpoint is unreliable. Data suggests a delay at 0.10 mg/L but is not unduly delayed at the concentrations <0.10 mg/L. This is also in line with the results from the Putt 1991 and Fournier 2014.

Rufli (1989):

Several study deficiencies were identified in the Rufli (1989) study:

- Media was considered deficient in macro-and micro-nutrients as unfortified with minerals or growth supplement. Feeding was inadequate in type of feed and daily ration (only fed 6 days/week). Both were major causes of variability during the ring test of OECD 211.
- Solvent control and control had low numbers of live offspring (47 and 55, respectively); validity criteria ≥60. Coefficient of variation for control reproduction was 36%; validity criteria <25%10. Also many dead offspring in the control groups (11 to 13%).

[10] OECD 211 (2012): paragraph 38: "Generally in a well-run test, the coefficient of variation around the mean number of living offspring produced per parent animal in the control(s) should be ≤ 25%, and this should be reported for test designs using individually held animals."

- Broodstock questionable as satellite vessels were included in case males appeared; potentially indicative of an unhealthy culture 11. Also sieving used to isolate neonates for testing is not current practice, as traps air under carapace of neonates.
- Parental immobility was unusually high is the study but not concentration responsive (0 to 50% across the treatment range); potentially indicative of a poor culture.
- The number of immobile parents was not removed in calculation number of offspring per female (significant number see above) and live and dead offspring were included; endpoint unreliable.
- Lowest NOEC based on time to first brood, but data not collected daily (missing days 8, 10, 11 and 13). Endpoint unreliable as data missing and not a current endpoint.
- Lowest NOEC also based on cumulative number of dead offspring which was not dose-responsive. Not an endpoint in either 1984 or 2012 guideline; endpoint unreliable.
- Insufficient number of analytical samples did not cover 72h renewal interval[12]. Measured concentrations did not include freshly prepared solutions; therefore the mean concentrations used underestimate actual exposure.
- Solvent used in unequal concentrations in test concentrations. Unusual surfactant used (alkylphenol-polyglycol-ether); surfactants are not permitted in current guidance.
- Poor documentation of raw data hampers accurate reconstruction of study.

[11] Feeding rate and frequency are important in maintaining the organisms in optimal condition so that they achieve maximum reproduction. Stock cultures which are stressed because they are not adequately fed may produce low numbers of young, large numbers of males, and ephippial females. When the young taken from these inadequately fed *Daphnia* cultures are used in toxicity tests, they may show higher than acceptable mortality in controls and greater than normal sensitivity to toxicants. EPA 2002 **Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. 5th edition.**

[12] In Point 48 of OECD 211(2012): "For tests where the concentration of the test substance is not expected to remain within ± 20 per cent of the nominal, it is necessary to analyse all test concentrations, when freshly prepared and at renewal. However, for those tests where the measured initial concentration of the test substance is not within ± 20 per cent of nominal but where sufficient evidence can be provided to show that the initial concentrations are repeatable and stable (i.e. within the range 80 - 120 per cent of initial concentrations), chemical determinations could be reduced in weeks 2 and 3 of the test to the highest and lowest test concentrations. In all cases, determination of test substance concentrations prior to renewal need only be performed on one replicate vessel at each test concentration."

Statistical revaluation of the Rufli 1989 data, excluding data from dead parents, dead nonates from live parents, and recalculating the data based on geometric mean measured concentrations (i.e. including the fresh solution measured on Day 0) provides a NOEC of 0.042 mg a.s./

Table 8.2-26: Re-calculated (from raw data) and re-interpreted according to OECD 211 ~ Mortality and reproduction ~ data from dead parent removed, dead neonates removed and geometric mean measured concentrations (Rufli 1989 Syngenta file no CGA173506/0006)

Nominal concentrations (mg a.s./L)	Geometric mean measured concentrations (mg a.s./L) *Based on geomean of new and old solutions*	Immobilised parental daphnids at Day 21 (n = 10)	Mean cumulative juveniles per live female at Day 21		
			Dead and alive	Live	Dead
Control	-	1	62	53	9
Solvent control	-	1	52	46	6
0.00074	0.00054	5	73	52	21
0.0022	0.0014	1	70	57	13
0.0067	0.0056	2	76	58	18
0.020	0.014	1[a]	62	43	19
0.060	0.042	5	71	64	7
0.18	0.13	4	12	8	5
NOEC	-	No dose response so not calculable	0.042	0.042[b]	No dose response so not calculable

[a] 15 replicates were initially prepared. Replicate 8 died on Day 6 and was excluded and replicate 11 was used instead. However, there was no justification for exclusion in the data (i.e. injury) so the Replicate 8 data has been included instead of replicate 11 in the data set. Using replicate 8 data as part of the original 10 replicates changes the parental mortality from 0 to 1.
[b] Recalculated using excluding data from dead parents and excluding dead neonates from live parents and using the geometric mean measured concentrations.

Putt (1991):

The second study by Putt (1991) has some deficiencies:

- Number of neonates in solvent control 35% lower than in the control, and some dead neonates also present in the solvent control. Scale of difference suggests data is may be unreliable.
- Solvent used in unequal concentrations in test concentrations13.
- Test concentrations produced by diluting suspensions of fludioxonil prepared above limit of functional solubility. Insufficient number of analytical samples did not cover 72h renewal interval. Undissolved test material visible on day 21. As fludioxonil is not easy to get into solution, the analytical results are unreliable.

Fournier (2014):

- Modern study to current OECD guideline. Well-conducted, avoided use of solvents, met all current validation criteria, provides clear endpoints for reproduction and development.

[13] OECD 211 (2012), paragraph 37: "When used, the solvent or dispersant concentration should be the same as that used in the vessels containing the test substance."

Conclusion: Based on the results of this evaluation, the reproductive endpoint (NOEC) for *Daphnia magna* exposed to fludioxonil is considered to be 35 µg a.s./L.

Americamysis bahia (also known as *Mysidopsis bahia*)

Two mysid chronic studies have been conducted for US Data Call In (DCI) compliance; Claude *et al*. (2011) and Marini (2014). The NOEC endpoints of 0.018 and 0.024 mg a.s./L, respectively are very similar.

The Claude *et al*. (2011) had some deviations from the OPPTS guideline, namely:

- Offspring counted and discarded. According to OPPTS 850.1530 the offspring (G2) should be transferred to retention chambers where mortality should be observed, number of males and females counted and body length measured.
- Body lengths of male and female mysids measured at test end. According to guideline, body lengths should also be determined when possible to distinguish the sexes e.g. 10-12 days after test start.
- The time to first brood release was not recorded.

There was other evidence that the conduct of the study was less than optimal. For instance, anomalous endpoints were recorded for the lowest mean measured concentration of 0.046 mg a.s./L in that a) the mortality of the adults was significantly lower than for the pooled control and this was not the case for the 4 other higher test concentrations, b) the mean number of G2 per female were considerably lower than for the other test concentrations, in fact >50% lower than for 0.018 mg a.s./L test concentration.

The number of young produced per female in the Claude *et al*. (2011) study varied between 3.1 to 7.6 young per female in the controls and concentrations ≤ 0.018 mg a.s/L. This is now considered low. In the Marini (2014) study, the number of young per female ranged from 16.5 to 20.5 in the control and concentrations ≤ 0.024 mg a.s/L. The improved reproductive performance results from better handling and rearing techniques during culturing, and non-inclusion of solvent for test concentration preparation.

Both studies will be compared using the most sensitive endpoint, growth; as the LOEC for reproduction was 0.070 mg a.s./L. The individual growth data is shown in Table 8.2.5.2-3 and Table 8.2.5.2-8 for Claude *et al*. (2011) and Marini (2014) below in the OECD summaries, respectively. The data has been combined in an approach used in the EFSA Bird and Mammal Guidance document which helps to compare the results of both studies in the table immediately below. Due to the position of the NOEC and LOEC values from the two studies, the value of 24 µg a.s./L is appropriate for use in the risk assessment.

Table 8.2-2714: Combined data on growth effects of fludioxonil on *Americamysis bahia* (Claude *et al.* 2011 & Marini 2014)

Study	Time-Weighted Mean Concentration (µg/L)	F_0 Total Body Length		F_0 Dry Weight	
		Male (mm)	Female (mm)	Male (mg)	Female (mg)
Marini 2014	Control (2014)	7.52	7.64	0.92	1.26
Claude *et al*. 2011	Pooled control (2011)	8.25	8.30	1.06	1.32
Marini 2014	3.2	7.40	7.70	0.89	1.22
Claude *et al*. 2011	4.6	8.01	8.22	0.99	1.21
Marini 2014	5.5	7.32	7.53	0.87	1.17
Claude *et al*. 2011	9.1	8.04	8.43	1.00	1.33
Marini 2014	12	7.15[b]	7.32	0.89	1.16
Claude *et al*. 2011	18	8.13	8.44	0.98	1.38
Marini 2014	24	7.35	7.64	0.89	1.22
Claude *et al*. 2011	36	8.04	8.27	0.93*	1.13* [a]
Marini 2014	44	7.03*	7.36	0.81	1.07
Claude *et al*. 2011	70	7.85*	8.10	0.91*	1.21
Marini 2014	96	7.05*	7.37	0.79*	1.10
Combined endpoint Summary (µg/L)	NOEC	24			
	LOEC	36			

* Significantly different compared to the corresponding control ($p \leq 0.05$).
[a] While the decrease in dry weight was statistically significant in comparison to the pooled control, it was not considered to be treatment-related since the difference was slight and was not concentration-responsive.
[b]Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test. However, due to the lack of a concentration response in the 24 µg/L level, the effect observed at this treatment level was not considered to be treatment related.

The overall invertebrate chronic endpoint used for risk assessment is the NOEC of 0.024 mg a.s./L for *Americamysis bahia.*

Classification according to EC 1272/2008

The relevant endpoints for classification of fludioxonil, together with the classification criteria, are given in table 8.2-15. Table 8.2-16 gives the criteria for deriving the associated M-factor.

Table 8.2-2815: Fludioxonil aquatic endpoints and criteria for classification

Acute toxicity			Chronic toxicity		
Trophic group	**Criteria for category 1**	**Endpoint (mg/L)**	**Trophic group**	**Criteria for category 1**	**Endpoint (mg/L)**
Fish: Rainbow trout	≤ 1 mg/L	96 h LC_{50} = 0.23	Fish: Fathead minnow	≤ 0.1 mg/L	NOEC = 0.018
Crustacea: *Americamysis bahia*	≤ 1 mg/L	96 h LC_{50} = 0.27	Crustacea: *Americamysis bahia*	≤ 0.1 mg/L	NOEC = 0.024
Algae/ aquatic plants: *Pseudokirchneriella subcapitata*	≤ 1 mg/L	72h E_rC_{50} = 0.41	Algae/ aquatic plants	≤ 0.1 mg/L	-

Table 8.2-2916: Multiplying (M) factors for highly toxic components of mixtures

Acute toxicity	M factor	Chronic toxicity	M factor	
L/EC_{50} value		**NOEC value**	**NRD**	**RD**
$0.1 < L(EC)_{50} \leq 1$	1	$0.01 < NOEC \leq 0.1$	1	-
$0.01 < L(EC)_{50} \leq 0.1$	10	$0.001 < NOEC \leq 0.01$	10	1
$0.001 < L(EC)_{50} \leq 0.01$	100	$0.0001 < NOEC \leq 0.001$	100	10
$0.0001 < L(EC)_{50} \leq 0.001$	1000	$0.00001 < NOEC \leq 0.0001$	1000	100
$0.00001 < L(EC)_{50} \leq 0.0001$	10000	$0.000001 < NOEC \leq 0.0001$	10000	1000
(continue in factor 10 intervals)		(continue in factor 10 intervals)		

NRD: Not rapidly degradable
RD: Rapidly degradable

The lowest reliable short term L/EC_{50} endpoints for the three main trophic groups of aquatic organisms (fish, invertebrates and algae) are all < 1 mg/L and > 0.1 mg/L. **Classification as aquatic environmental hazard acute category 1 (H400) therefore applies to fludioxonil with an M-factor of 1.**

Fludioxonil is not rapidly degradable and adequate chronic toxicity endpoints are available. The lowest reliable chronic NOEC endpoints for fish (0.018 mg/L) and invertebrates (0.024 mg/L) are both < 0.1 and > 0.01 mg fludioxonil/L. C**lassification as aquatic environmental hazard chronic category 1 (H410) therefore applies to fludioxonil with an M-factor of 1.**

Sediment dwelling organism testing on the fludioxonil metabolite CGA192155

The metabolite CGA192155 is present in sediment from the water-sediment study (Adam 2004; Syngenta file number CGA173506/5918). CGA192155 appears gradually in the sediment starting at 0.6% AR after 1d in the pond sediment to a maximum of 5.5% AR at 100d (study end); a similar pattern is evident in the river sediment. It is therefore relevant in sediment.

In order to determine if CGA192155 is likely to adsorb to sediment, the partitioning co-efficients have been calculated as recommended in the TGD part II 2.3.5.3 (EC, 2003)[14] for both fludioxonil and CGA192155 (see table below). The low value of 1.01 for CGA192155 in the sediment matrix indicates that it is not likely to adsorb to the sediment to any great extent. The partitioning coefficient for water shows that the metabolite is most likely to occur in the water matrix but at low levels.

Table 8.2-3017: Partitioning coefficients of fludioxonil and CGA192155 in water and sediment

Partitioning coefficients;				
Partitioning between solids and water in soil, sediment and suspended matter			**Fludioxonil**	**CGA192155**
Partition coefficient organic carbon-water	Koc		80341	20.2
Partition coefficient solid-water in suspended matter	Kpsusp	L . Kg-1	8034	2.02
Partition coefficient solid-water in sediment	Kpsed	L . Kg-1	4017	1.01
Partition coefficient solid-water in soil	Kpsoil	L . Kg-1	1607	0.404

[14] EC (European Commission), 2003. Technical Guidance Document on risk assessment in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.

As CGA192155 resides mainly in the water matrix then it is relevant to look at the toxicity to aquatic organisms (see table below). These indicate that the metabolite is of very low toxicity to fish, *Daphnia magna* and algae with endpoints L/EC_{50} >100 mg a.s/L); this is in contrast to the effect of fludioxonil where the same endpoints range from 0.024 to 0.40 mg a.s./L showing greater toxicity. As CGA192155 is likely to reside in the pore water of the sediment, the main route of exposure is likely to be through water. Therefore, the standard aquatic organisms tested (see table below) are considered to cover the risk posed by CGA192155 to sediment dwelling organisms.

Table 8.2-318: Summary of acute endpoints for aquatic invertebrates for metabolites

Organism	Test item	Endpoints (mg a.s./L)		Reference (author, date, Syngenta Ref)
Rainbow trout	CGA192155 [a]	EU	96 h LC_{50} >$100_{(nom)}$ (static)	***Volz (2005) CGA192155/0019***
Daphnia magna	CGA192155 [a]	EU	48 h EC_{50} >$100_{(nom)}$ (static)	***Volz (2005) CGA192155/0018***
Pseudokirchneriella subcapitata	CGA192155 [a]	EU	96 h E_rbC_{50} >$100_{(nom)}$	***Volz (2005) CGA192155/0017***

[a] Included in the Final Addendum to the DAR Article 8(2) of Council Directive 91/414/EEC (June 2007)

In addition, the risk from CGA192155 in sediment exposure has also been conducted using the assumption that the metabolite has the same toxicity as parent in **M-CP Section 10** (A8240D (see Section 10.2, table 10.2-63) and **M-CP Section 10** (A8207M**,** see section 10.2, table 10.2-11). These indicate that exposure of sediment dwellers to CGA192155 shows an acceptable risk as the PEC/RAC ratios were ≤0.023, therefore much below the trigger of one.

CA 8.2.1 Acute toxicity to fish

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 (2013)[15].

Fish acute toxicity studies with fludioxonil

The studies previously submitted in the EU were summarised in point B.9.2.1 of the DAR ~~therefore additional study summaries are not repeated here~~. The endpoints are summarised in Table 8.2-1. At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

RMS comment: For the acute toxicity studies conducted with fludioxonil. The 96h-LC50 values have been determined graphically. A more suitable calculation should be provided

Syngenta response: All of the study reports for the fish studies referenced, contain both a calculated LC50 and a graphically determined LC50. The OECD summaries contained the LC50s calculated using a model, however the wording in the summary mistakenly referred to both the calculated and graphically determined endpoints. This has been rectified in the MCA.

[15] 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.

Summaries of studies not previously reviewed are included below.

Report:	Submitted to enable risk refinement: K-CA 8.2.1/01 Rufli H (1989a). Test for acute toxicity of CGA173506 technical to Rainbow trout *(Salmo gairdneri),* Report Number 881739, CIBA-GEIGY Ltd. CH-4002 Basle, Switzerland. (Syngenta File No. CGA173506/0004).

Guidelines

OECD Guidelines 203: Fish, Acute Toxicity Test (1984)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to rainbow trout (*Oncorhynchus mykiss*) was determined under static conditions. Fish were exposed to a range of nominal concentrations of 0.32, 0.58, 1.0, 1.8, 3.2, and 5.8 mg/L (corresponding to 0.26, 0.42, 0.83, 1.6, and 2.7 mg/L mean measured for the lowest concentrations (the highest concentration was not determined analytically). These concentrations were tested alongside a dilution water control and solvent control. Based on mean measured concentrations, the 96 hour LC_{50} was 0.50 mg/L.

Materials

Test material	CGA173506 technical
Lot/Batch #:	805002
Purity:	97.5%
Description:	Beige solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	June 1992
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 0.32, 0.58, 1.0, 1.8, 3.2, and 5.8 mg/L (corresponding to 0.26, 0.42, 0.83, 1.6, and 2.7 mg/L mean measured)
Solvent:	Acetone
Analysis of test concentrations:	Yes at 0 and 96 hours using HPLC analysis
Test organisms	
Species:	Rainbow trout (*Salmo gairdneri*)
Source:	P. Hohler / CH-4341 Zeiningen, Switzerland
Acclimatisation period:	27 days
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	Mean length: 63 mm (range: 58 - 70 mm) Mean weight: 2.45 g (range: 2.00 – 3.26 g)

Feeding:	None during test
Test design	
Test vessels:	20 L glass vessels containing 15 L water
Test medium:	Dechlorinated water
Replication:	2
No of fish per tank:	5
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	14° C
pH:	7.8 to 8.5
Dissolved oxygen:	91 to 103 % gentle aeration provided
Hardness of dilution water:	170 mg/L $CaCO_3$
Lighting:	16 hours fluorescent light and 8 hours dark

Study Design and Methods

Experimental dates: 12th to 20th January 1989

0.5 g CGA173506 and 60 mg alkylphenol-polyglykol-ether were mixed and made up to 10 mL with acetone. Appropriate volumes of the stock were then made up to 15 L of dilution water in each test vessel to give the test concentrations. The control consisted of dilution water only.

At the start of the test ten fish were randomly allocated to each of the test concentrations and the dilution water control. Observations for mortalities and symptoms of toxicity were made at 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 CGA173506 at 0 and 96 hours using HPLC analysis.

Results and Discussion

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

Table 8.2.1-1: Analytical results

Nominal concentration (mg/L)	% of nominal 0 hours	% of nominal 96 hours	Mean measured concentration (mg a.s./L)
0.32	88	72	0.256
0.58	81	65	0.424
1.0	84	83	0.833
1.8	91	84	1.57

3.2	92	75	2.68
5.8	n.d.	n.d.	n.d.

n.d. – not determined; actual concentrations were not analysed

The LC_{50} values were calculated according to Berkson. ~~LC-values were graphically determined on gausso-logarithmic probability paper.~~

Mortalities were observed at nominal concentrations of 0.32 mg a.s./L and above. Symptoms of toxicity were observed at concentrations of 0.32 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 8.2.1-2: Effects of CGA173506 on the survival of *Oncorhynchus mykiss*

Nominal concentration (mg/L)	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
0.32	0.256	0	1	1	1
0.58	0.424	0	0	0	0
1.0	0.833	8	8	10	10
1.8	1.57	10	10	10	10
3.2	2.68	10	10	10	10
5.8	n.d.	10	10	10	10
LC_{50} mg/L	-	0.71	0.60	0.50	0.50
95% confidence interval	-	0.52-0.89	0.44-0.83	0.38-0.81	0.38-0.81

n.d. – not determined; actual concentrations were not determined analytically

Conclusions

Based on mean measured concentrations, the 96-hour LC_{50} for CGA173506 to rainbow trout (*Oncorhynchus mykiss*) was 0.50 mg a.s./L and the 96-hour NOEC was 0.42 mg/L.

(Rufli H. 1989a)

Whilst there are minor exceedances of the 1992 validity criteria for acute fish toxicity studies, this study (which pre-dates the criteria) is considered valid for use in the risk assessment. The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure (which ranged between 73% and 87% of nominal values).

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Rufli, 1989 CGA173506/0004	OECD No. 203 (1984)	OECD No. 203 (1992)	Measured concentration deviated >20% during the test. No measured concentrations are available for the highest nominal test concentration of 5.8 mg/L. Mean fish length was 6.3 cm; range 5.8 to 7.0 cm; (Guideline recommendation: 5.0 ± 1.0 cm).	Most of the validity criteria were met: 1. Mortality in the negative control and the solvent control was ≤10% (0%). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 3. Evidence that the concentration being tested has been satisfactorily maintained (see explanation below) 4. DO ≥ 60% ASV (91 to 103%) CGA173506 has a solubility of 1.53 mg/L in water at 20ºC, consequently the mean measured concentrations were in some cases below 80% (73 to 87% of nominal) and a slight deposit was noted at nominal concentrations of 3.2 to 5.8 mL/L at 72 hours. The 96 hr LC_{50} value was based on the mean measured concentrations (with the highest concentration of 5.8 mg/L apparently excluded from the calculation), thus the lower mean measured concentrations and the lack of analytical data for the highest concentration did not adversely affect the results of the study. The solvent (acetone and alkylphenol-polyglykol-ether) concentrations in the treatment and solvent control groups were 91.8 mg and 0.7 mg/L, respectively; this concentration is considered to be within the tolerance of rainbow trout for acute toxicity testing (as confirmed by the absence of effects observed in the solvent control). Whilst there are minor exceedances of the 1992 validity criteria for acute fish studies, this study (which pre-dates the criteria) is considered valid for use in the risk assessment.

Report:	Submitted to enable risk refinement: K-CA 8.2.1/02 Rufli H (1989b). Test for acute toxicity of CGA173506 technical to bluegill (*Lepomis macrochirus*), Report Number 881741, CIBA-GEIGY Ltd. CH-4002 Basle, Switzerland. (Syngenta File No. CGA173506/0002).

Guidelines

OECD Guidelines 203: Fish, Acute Toxicity Test (1984)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to bluegill (*Lepomis macrochirus*) was determined under static conditions. Fish were exposed to a range of nominal concentrations of 0.58, 1.0, 1.8, 3.2, 5.8, and 10 mg/L (corresponding to 0.14, 0.18, 0.53, 1.3, 2.3 and 3.4 mg/L mean measured), alongside a dilution water control and solvent control. Based on mean measured concentrations, the 96 hour LC_{50} was 0.31 mg/L.

Materials

Test material	CGA173506 technical
Lot/Batch #:	805002
Purity:	97.5%
Description:	Beige solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	June 1992
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 0.58, 1.0, 1.8, 3.2, 5.8, and 10 mg/L (corresponding to 0.14, 0.18, 0.53, 1.3, 2.3 and 3.4 mg/L mean measured).
Solvent:	Acetone
Analysis of test concentrations:	Yes at 0 and 96 hours using HPLC analysis
Test organisms	
Species:	Bluegill (*Lepomis macrochirus*)
Source:	Osage Catfish./Box 222/Missouri 65065/USA
Acclimatisation period:	45 days
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	Mean length: 34 mm (range: 30 - 41 mm) Mean weight: 0.45 g (range: 0.29 – 0.69 g)
Feeding:	None during test
Test design	
Test vessels:	20 L glass vessels containing 15 L water
Test medium:	Dechlorinated water
Replication:	1
No of fish per tank:	10
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	23° C

pH:	7.8 to 8.5
Dissolved oxygen:	82 to 103 % gentle aeration provided
Hardness of dilution water:	167 mg/L $CaCO_3$
Lighting:	16 hours fluorescent light and 8 hours dark

Study Design and Methods

Experimental dates: 16th to 24th February 1989

1.0 g CGA173506 and 40 mg alkylphenol-polyglykol-ether were mixed and made up to 10 mL with acetone. Appropriate volumes of the stock were then made up to 15 L of dilution water in each test vessel to give the test concentrations. The control consisted of dilution water only.

At the start of the test ten fish were randomly allocated to each of the test concentrations and the dilution water control. Observations for mortalities and symptoms of toxicity were made at 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 CGA173506 at 0 and 96 hours using HPLC analysis.

Results and Discussion

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

Table 8.2.1.1-3: Analytical results

Nominal concentration (mg/L)	% of nominal 0 hours	% of nominal 96 hours	Mean measured concentration (mg a.s./L)
0.58	28	19	0.14
1.0	19	17	0.18
1.8	34	24	0.53
3.2	41	39	1.28
5.8	44	36	2.31
10	39	30	3.43

The LC_{50} values were calculated according to Spearman-Kaerber. ~~LC-values were graphically determined on gausso-logarithmic probability paper.~~

Mortalities were observed at nominal concentrations of 1.8 mg a.s./L and above. Symptoms of toxicity were observed at concentrations of 0.58 mg/L and above. No symptoms of toxicity were observed in the control; one mortality was observed in the control at the end of the test.

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

Table 8.2.1.1-4: Effects of CGA173506 on the survival of *Lepomis macrochirus*

Nominal concentration (mg/L)	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	1
Solvent control	-	0	0	0	0
0.58	0.14	0	0	0	0
1.0	0.18	0	0	0	0
1.8	0.53	1	2	10	10
3.2	1.28	10	10	10	10
5.8	2.31	10	10	10	10
10.0	3.43	10	10	10	10
LC_{50} mg/L	-	0.75	0.68	0.31	0.31
95% confidence interval	-	0.62 – 0.92	0.52 – 0.89	n.d.	n.d.

n.d. – not determined

Conclusions

Based on mean measured concentrations, the 96-hour LC_{50} for CGA173506 to bluegill (*Lepomis macrochirus*) was 0.31 mg a.s./L and the 96-hour NOEC was 0.18 mg/L

(Rufli H. 1989b)

Whilst there are minor exceedances of the 1992 validity criteria for acute fish toxicity studies, this study (which pre-dates the criteria) is considered valid for use in the risk assessment. The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure (which ranged between 18% and 41% of nominal values).

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Rufli, 1989 CGA173506/0002	OECD No. 203 (1984)	OECD No. 203 (1992)	Measured concentration deviated >20% during the test. Mean fish length was 3.4 cm; range 3.0 to 4.1 cm; (Guideline recommendation: 2.0 ± 1.0 cm).	Most of the validity criteria were met: 1. Mortality in the negative control and the solvent control was ≤10% (1% and 0% respectively). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 3. Evidence that the concentration being tested has been satisfactorily maintained (see explanation below) 4. DO ≥ 60% ASV (82 to 103%) CGA173506 has a solubility of 1.53 mg/L in water at 20ºC, consequently the mean measured concentrations were below 80% (18 to 41% of nominal) and a slight deposit was noted in the 5.8 and 10 mg/L treatments at 24 hrs. The 96 hr LC_{50} value was based on the mean measured concentrations, thus the lower mean measured concentrations did not adversely affect the results of the study. The solvent (acetone and alkylphenol-polyglykol-ether) concentrations in the treatment and solvent control groups were 79.1 mg and 0.4 mg/L, respectively; this concentration is considered to be within the tolerance of Bluegill for acute toxicity testing (as confirmed by the absence of effects observed in the solvent control). Whilst there are minor exceedances of the 1992 validity criteria for acute fish studies, this study (which pre-dates the criteria) is considered valid for use in the risk assessment.

Report:	Submitted to enable risk refinement: K-CA 8.2.1/03 Rufli H (1989c). Test for acute toxicity of CGA173506 technical to common carp (*Cyprinus carpio*), Report Number 881740 (Syngenta File No. CGA173506/0001).

Guidelines

OECD Guidelines 203: Fish, Acute Toxicity Test (1984)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to common carp (*Cyprinus carpio*) was determined under static conditions. Fish were exposed to a range of nominal concentrations of 1.0, 1.8, 3.2, 5.8, and 10 mg/L (measured concentration 0.53, 1.0, 2.3, 4.2, and 6.3 mg/L), alongside a dilution water control. Based on mean measured concentrations, the 96-hour LC_{50} for CGA173506 to common carp (*Cyprinus carpio*) was 1.5 mg ai/L and the 96-hour NOEC was 1.8 mg/L

Materials

Test material	CGA173506 technical
Lot/Batch #:	805002
Purity:	97.5%
Description:	Beige solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	June 1992
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 1.0, 1.8, 3.2, 5.8, and 10 mg/L (measured concentration 0.53, 1.0, 2.3, 4.2, and 6.3 mg/L)
Solvent:	None
Analysis of test concentrations:	Yes at 0 and 96 hours using HPLC analysis
Test organisms	
Species:	Common carp (*Cyprinus carpio*)
Source:	Supplier: P. Hohler/ CH-4314 Zeiningen, Switzerland
Acclimatisation period:	66 days
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	Mean length: 53 mm (range: 49 - 59 mm) Mean weight: 1.85 g (range: 1.29 – 2.41 g)
Feeding:	None during test
Test design	
Test vessels:	Glass vessels containing 15 L water
Test medium:	Dechlorinated water
Replication:	2
No of fish per tank:	5
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	22° C
pH:	7.8 to 8.5
Dissolved oxygen:	54 to 100% gentle aeration provided

Hardness of dilution water: 184 mg/L $CaCO_3$

Lighting: 16 hours fluorescent light and 8 hours dark

Study Design and Methods

Experimental dates: 5th to 13th January 1989

1.0 g CGA173506 and 40 mg alkylphenol-polyglykol-ether were dissolved in and made up to 10 mL with acetone. Appropriate volumes of the stock were then made up to 15 L of dilution water in each test vessel to give the test concentrations. The control consisted of dilution water only.

At the start of the test ten fish were randomly allocated to each of the test concentrations and the dilution water control. 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 CGA173506 at 0 and 96 hours using HPLC analysis.

Results and Discussion

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

Table 8.2.1-5: Analytical results

Nominal concentration (mg/L)	% of nominal 0 hours	% of nominal 96 hours	Mean measured concentration (mg a.s./L)
1.0	59	46	0.53
1.8	61	49	1.0
3.2	81	63	2.3
5.8	91	52	4.2
10	73	52	6.3

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 according to Spearman-Kaerber. ~~LC-values were graphically determined on gausso-logarithmic probability paper.~~ Mortalities were observed at nominal concentrations of 3.2 mg a.s./L and above. Symptoms of toxicity observed were observed at concentrations of 1.0 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 8.2.1-6: Effects of CGA173506 on the survival of *Cyprinus carpio*

Nominal concentration (mg/L)	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
1.0	0.53	0	0	0	0
1.8	1.0	0	0	0	0
3.2	2.3	10	10	10	10
5.8	4.2	10	10	10	10
10	6.3	10	10	10	10
LC_{50} mg/L	-	1.5			
95% confidence interval	-	n.d.			
NOEC	-	1.8			

n.d. – not determined

Conclusions

Based on mean measured concentrations, the 96-hour LC_{50} for CGA173506 to common carp (*Cyprinus carpio*) was 1.5 mg ai/L and the 96-hour NOEC was 1.8 mg/L

(Rufli H. 1989c)

Whilst there are minor exceedances of the 1992 validity criteria for acute fish toxicity studies, this study (which pre-dates the criteria) is considered valid for use in the risk assessment. The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure (which ranged between 53% and 72% of nominal values).

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Rufli, 1989 CGA173506/0001	OECD No. 203 (1984)	OECD No. 203 (1992)	Measured concentration deviated >20% during the test. DO was measured as 54% ASV in the solvent control at 96 hours only; all other value met guideline recommendation (DO ≥ 60% ASV) Mean fish length was 5.3 cm; range 4.9 to 5.9 cm; (Guideline recommendation: 3.0 ± 1.0 cm).	Most of the validity criteria were met: 1. Mortality in the negative control and the solvent control was ≤10% (0%). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 3. Evidence that the concentration being tested has been satisfactorily maintained (see explanation below) 4. DO ≥ 60% ASV (86 to 100% in all CGA173506 treatments and the negative control; the single exception in the solvent control (54%) is not considered to have negatively impacted the study) CGA173506 has a solubility of 1.53 mg/L in water at 20ºC, consequently the mean measured concentrations were below 80% (53 to 72% of nominal) and a slight deposit was noted in all treatments at Time 0. The 96 hr LC_{50} value was based on the mean measured concentrations, thus the lower mean measured concentrations did not adversely affect the results of the study. The solvent (acetone and alkylphenol-polyglykol-ether) concentrations in the treatment and solvent control groups were 79.1 mg and 0.4 mg/L, respectively; this concentration is considered to be within the tolerance of common carp for acute toxicity testing (as confirmed by the absence of effects observed in the solvent control). Whilst there are minor exceedances of the 1992 validity criteria for acute fish studies, this study (which pre-dates the criteria) is considered valid for use in the risk assessment.

Report:	Submitted to enable risk refinement: K-CA 8.2.1/04 Rufli H (1989d). Test for acute toxicity of CGA173506 technical to catfish (*Ictalurus punctatus),* Report Number 881742, CIBA-GEIGY Ltd. CH-4002 Basle, Switzerland. (Syngenta File No. CGA173506/0003).

Guidelines

OECD Guidelines 203: Fish, Acute Toxicity Test (1984)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to channel catfish (*Ictalurus punctatus*) was determined under static conditions. Fish were exposed to a range of nominal concentrations of 0.58, 1.0, 1.8, 3.2, and 5.8 mg/L (corresponding to 0.32, 0.68, 0.89, 2.1 and 3.7 mg/L mean measured), alongside a dilution water control and solvent control. Based on mean measured concentrations, the 96 hour LC_{50} was 0.63 mg/L.

Materials

Test material	CGA173506 technical
Lot/Batch #:	805002
Purity:	97.5%
Description:	Beige solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	June 1992
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 0.58, 1.0, 1.8, 3.2, and 5.8 mg/L (corresponding to 0.32, 0.68, 0.89, 2.1 and 3.7 mg/L mean measured)
Solvent:	Acetone
Analysis of test concentrations:	Yes at 0 and 96 hours using HPLC analysis
Test organisms	
Species:	Catfish (*Ictalurus punctatus*)
Source:	Osage Catfish./Box 222/Missouri 65065/USA
Acclimatisation period:	61 days
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	Mean length: 62 mm (range: 56 - 67 mm) Mean weight: 2.01 g (range: 1.50 – 2.67 g)
Feeding:	None during test
Test design	
Test vessels:	20 L glass vessels containing 15 L water
Test medium:	Dechlorinated water
Replication:	2
No of fish per tank:	5
Exposure regime:	Static
Duration:	96 hours
Environmental conditions	
Test temperature:	22 to 23° C
pH:	7.7 to 8.4

Dissolved oxygen:	78 to 101 % gentle aeration provided
Hardness of dilution water:	184 mg/L $CaCO_3$
Lighting:	16 hours fluorescent light and 8 hours dark

Study Design and Methods

Experimental dates: 5th to 13th January 1989

1.0 g CGA173506 and 40 mg alkylphenol-polyglykol-ether were mixed and made up to 10 mL with acetone. Appropriate volumes of the stock were then made up to 15 L of dilution water in each test vessel to give the test concentrations. The control consisted of dilution water only.

At the start of the test ten fish were randomly allocated to each of the test concentrations and the dilution water control. Observations for mortalities and symptoms of toxicity were made at 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 CGA173506 at 0 and 96 hours using HPLC analysis.

Results and Discussion

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

Table 8.2.1-7: Analytical results

Nominal concentration (mg/L)	% of nominal 0 hours	% of nominal 96 hours	Mean measured concentration (mg a.s./L)
0.58	55	53	0.32
1.0	69	66	0.68
1.8	53	46	0.89
3.2	68	61	2.07
5.8	62	64	3.67

The LC_{50} values were calculated according to Spearman-Kaerber. ~~LC-values were graphically determined on gausso-logarithmic probability paper.~~

Mortalities were observed at nominal concentrations of 1.0 mg a.s./L and above. Symptoms of toxicity were observed at concentrations of 0.58 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 8.2.1-8: Effects of CGA173506 on the survival of *Ictalurus punctatus*

Nominal concentration (mg/L)	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
0.58	0.32	0	0	0	0
1.0	0.68	0	0	3	4
1.8	0.89	2	8	10	10
3.2	2.07	10	10	10	10
5.8	3.67	10	10	10	10
LC_{50} mg/L	-	1.2	0.87	0.67	0.63
95% confidence interval	-	1.1-1.4	0.75-1.0	0.57-0.78	0.54-0.75

Conclusions

Based on mean measured concentrations, the 96-hour LC_{50} for CGA173506 to catfish (*Ictalurus punctatus*) was 0.63 mg a.s./L and the 96-hour NOEC was 0.32 mg/L.

(Rufi H. 1989d)

Whilst there are minor exceedances of the 1992 validity criteria for acute fish toxicity studies, this study (which pre-dates the criteria) is considered valid for use in the risk assessment. The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure (which ranged between 49% and 68% of nominal values).

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Rufli, 1989 CGA173506/0003	OECD No. 203 (1984)	OECD No. 203 (1992)	Measured concentration deviated >20% during the test.	Most of the validity criteria were met: 1. Mortality in the negative control and the solvent control was ≤10% (0%). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 3. Evidence that the concentration being tested has been satisfactorily maintained (see explanation below) 4. DO ≥ 60% ASV (78 to 101%) CGA173506 has a solubility of 1.53 mg/L in water at 20ºC, consequently the mean measured concentrations were below 80% (49 to 68% of nominal) and a slight deposit was noted in all test concentrations from Time 0. The 96 hr LC_{50} value was based on the mean measured concentrations, thus the lower mean measured concentrations did not adversely affect the results of the study. The solvent (acetone and alkylphenol-polyglykol-ether) concentrations in the treatment and solvent control groups were 45.9 mg and 0.2 mg/L, respectively; this concentration is considered to be within the tolerance of catfish for acute toxicity testing (as confirmed by the absence of effects observed in the solvent control). Whilst there are minor exceedances of the 1992 validity criteria for acute fish studies, this study (which pre-dates the criteria) is considered valid for use in the risk assessment.

A fish acute toxicity study has been conducted as part of a US EPA Data Call-In (DCI), using the fathead minnow, a freshwater species, is summarised below:

Report:	New fish acute study conducted for relevance comparison with existing endpoints: K-CA 8.2.1/05 Fournier A (2014a). Fludioxonil - Acute Toxicity Test with Fathead Minnow (*Pimephales promelas*) Under Static-Renewal Conditions, Report Number 1781.6967, Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571-1037, USA, (Syngenta File No. CGA173506_51203)

Guidelines

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

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

GLP: Yes

Executive Summary

The acute toxicity of fludioxonil to fathead minnow, *Pimephales promelas,* was determined under static-renewal conditions. Fish were exposed to a range of nominal concentrations of 0.081, 0.16, 0.33, 0.65 and 1.3 mg/L (0.068, 0.12, 0.24, 0.54 and 1.1 mg/L, mean measured), alongside a dilution water control and a solvent (acetone) control. Based on mean measured concentrations, the 96 hour LC_{50} was 0.70 mg/L. The NOEC was determined to be 0.12 mg/L.

Materials

Test material	Fludioxonil
Lot/Batch #:	SMO3J0464
Purity:	97.7% (w/w); Tested as 100%
Description:	Brownish powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	End of October 2017
Treatments	
Test concentrations:	Dilution water control, solvent (acetone) control and nominal concentrations of 0.081, 0.16, 0.33, 0.65 and 1.3 mg/L (mean measured 0.068, 0.12, 0.24, 0.54 and 1.1 mg/L)
Solvent:	Acetone (CAS No. 67-64-1)
Analysis of test concentrations:	Yes, analysis of fludioxonil in freshly prepared solutions at 0 and 48 hours and in aged solutions at 48 and 96 hours
Test organisms	
Species:	Fathead minnow, *Pimephales promelas*
Source:	Continuous laboratory cultures
Acclimatisation period:	14 days
Treatment for disease:	None
Mean weight and length of representative sample of the fish population:	Mean length: 23 mm (ranged from 16 to 32 mm), N = 30 Mean weight: 0.28 g (ranged from 0.15 to 0.53 g), N = 30
Feeding:	The fish were fed a dry commercial flake fish food, *ad libitum*, daily during culture. The fish were not fed during the 48-hour period prior to exposure initiation or during the 96-hour definitive exposure.
Test design	
Test vessels:	Glass aquaria containing 15 L water

Test medium:	Well water
Replication:	None
No of fish per tank:	7
Exposure regime:	Static-renewal; test solutions renewed at 48 hours
Duration:	96 hours
Environmental conditions	
Test temperature:	22 to 23° C
pH:	6.9 to 7.2
Dissolved oxygen:	7.0 to 9.7 mg/L (no aeration)
Hardness of dilution water:	60 mg/L $CaCO_3$
Lighting:	500 to 550 lux. 16 hours fluorescent light and 8 hours dark; sudden transitions of light to dark and dark to light were avoided

Study Design and Methods

Experimental dates: 31st March to 4th April 2014

Prior to exposure initiation, a 13 mg/mL primary stock solution was prepared by placing 0.3154 g of the test substance into a 25-mL volumetric flask and diluting to volume with acetone. This primary stock solution was used to prepare solvent stock solutions at concentrations of 6.5, 3.3, 1.6 and 0.81 mg/L. Aliquots (1.5 mL) of each of the solvent stock solutions were diluted to 15 L with well water. The control consisted of well water only. The solvent control was prepared by bringing 1.5 mL of acetone to a final volume of 15 L with well water.

At the start of the test seven fish were randomly allocated to each of the test concentrations and controls. The test was conducted in a temperature controlled water-bath. Observations for mortalities and symptoms of toxicity were made at 6, 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 fludioxonil at 0 (new solutions), 48 (new and aged solutions) and 96 hours (aged solutions) using liquid chromatography/mass spectrometry (LC/MS/MS).

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 Spearman-Kärber Estimates. 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.

Results and Discussion

Measured concentrations in the new solutions ranged from 67 to 92% of the nominal concentrations. Measured concentrations in the aged solutions ranged from 63 to 86% of the nominal concentrations. Mean measured fludioxonil concentrations ranged from 73 to 87% of the nominal concentrations and defined the treatment levels tested as 0.068, 0.12, 0.24, 0.54 and 1.1 mg/L.

The limit of quantification (LOQ) for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. In this study, the LOQ at 0, 48 and 96 hours was 0.0029, 0.0031 and 0.0037 mg/L, respectively. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.1-9: Analytical results

Nominal concentration (mg/L)	Measured Concentration (mg/L)					
	0 Hour (New)	48 Hour (Aged)	48 Hour (New)	96 Hour (Aged)	Mean	Mean Percent Nominal
Control	< 0.0029[a]	< 0.0031	< 0.0031	< 0.0037	NA[b]	NA
Solvent control	< 0.0029	< 0.0031	< 0.0031	< 0.0037	NA	NA
0.081	0.075	0.063	0.069	0.067	0.068	84
0.16	0.13	0.12	0.12	0.11	0.12	75
0.33	0.27	0.26	0.22	0.21	0.24	73
0.65	0.54	0.52	0.57	0.52	0.54	83
1.3	1.1	1.1	NM[c]	NM	1.1	87

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.
[b] NA = Not Applicable.
[c] NM= Not Measured. Test solution was not sampled due to 100% mortality in the treatment level.

Mortalities were observed at mean measured concentrations of 0.12 and 1.1 mg/L. Due to the lack of a concentration response, the mortality observed at 0.12 mg/L was not considered to be toxicant-related, but was considered to be due to biological variability. Symptoms of toxicity observed included lethargy, partial and complete loss of equilibrium and fish on the bottom of the test vessel and were observed at 0.24 mg/L and above. No mortality or symptoms of toxicity were observed in the controls.

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

Table 8.2.1-10: Effects of fludioxonil on the survival of *Pimephales promelas*

Mean measured concentration (mg/L)	% mortality observed (Cumulative number of dead fish)				
	6 hour	24 hours	48 hours	72 hours	96 hours
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.068	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.12[a]	14 (1)	14 (1)	14 (1)	14 (1)	14 (1)
0.24	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.54	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.1	57 (4)	71 (5)	100 (7)	100 (7)	100 (7)
LC_{50} (mg/L)	--	0.88[b]	0.70[c]	0.70[c]	0.70[c]
95% confidence interval	--	0.68 – 1.1	0.57 – 0.85	0.57 – 0.85	0.57 – 0.85
NOEC (mg/L)	--	--	--	--	0.12

[a] Due to the lack of a concentration response, the mortality observed at this treatment level is not considered to be toxicant-related, but is considered to be due to biological variability.
[b] LC value and corresponding 95% confidence intervals were determined using Trimmed Spearman-Kärber Estimates.
[c] LC value and corresponding 95% confidence intervals were determined using Spearman-Kärber Estimates.

Conclusions

Based on mean measured concentrations, the 96 hour LC_{50} for *Pimephales promelas* exposed under static-renewal conditions to fludioxonil was 0.70 mg/L. The NOEC was determined to be 0.12 mg/L.

(Fournier A, 2014a)

The following study was previously submitted in the EU and was summarised in point B.9.2.1 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-1 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.1 Holmes C.M. & Swigert J.P., (1993). CGA-173506: A 96-Hour Flow Through Acute Toxicity Test with the Rainbow Trout (Oncorhynchus mykiss), Report Number 108A-136, Wildlife International Ltd., 8598 Commerce Drive, Easton, Maryland 21601, USA. (Syngenta File No. CGA173506/0258)*

Guidelines

U.S. EPA Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, EPA 540/9-82-024 (1982)

ASTM Standard E 729-88. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians, American Society for Testing and Materials (1988)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to rainbow trout, *Oncorhynchus mykiss*, was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.19, 0.32, 0.54, 0.90 and 1.5 mg a.s./L (mean measured concentrations 0.17, 0.26, 0.38, 0.71 and 0.69 mg a.s./L), alongside a solvent and a dilution water control. Based on mean measured concentrations, the 96-hour LC_{50} for CGA173506 to rainbow trout was 0.47 mg a.s./L, with 95 % confidence limits of 0.38 to 0.69 mg a.s./L.

Materials

Test material	CGA173506 Technical
Lot/Batch #:	P905004/5
Purity:	95.4 %
Description:	Fluffy white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	16 April 1994

Treatments	
Test concentrations:	Dilution water control, vehicle control and nominal concentrations of 0.19, 0.32 0.54, 0.90 and 1.5 mg a.s./L (mean measured 0.17, 0.26, 0.38, 0.71 and 0.69 mg a.s./L
Solvent:	Acetone
Analysis of test	Yes, based on analysis of CGA173506 at 0 and 96 hours using HPLC analysis

concentrations:

Test organisms

Species:	Juvenile rainbow trout, *Oncorhynchus mykiss*
Source:	Mt. Lassen Trout Farm, Rt. 5, Box 36, Red Bluff, California 98080
Acclimatisation period:	70 hours
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	Mean length: 44 mm (range: 38 to 48 mm) Mean weight: 1.45 g (range: 0.81 to 1.79 g)
Loading rate:	0.16 g fish/L
Feeding:	None during the acclimatisation or test

Test design

Test vessels:	Teflon®-lined polyethylene aquaria containing 15 L water
Test medium:	Filtered well water
Replication:	2 replicates
No of fish per tank:	10
Exposure regime:	Flow-through
Duration:	96 hours

Environmental conditions

Test temperature:	11.5 – 12.5 °C measured continuously in a single control replicate
pH:	8.0 – 8.5
Dissolved oxygen:	7.8 – 9.0 mg/L
Hardness of dilution water:	140 – 152 mg/L as $CaCO_3$
Lighting:	430 Lux 16 hours fluorescent light and 8 hours dark with 30 minute dawn and dusk transition periods

Study Design and Methods

Experimental dates: 29th May to 2nd June 1992

A stock solution with a nominal concentration of 1.6 mg/mL was prepared by dissolving CGA173506 in acetone. Aliquots of the stock were diluted with acetone to prepare four additional stocks at concentrations of 0.96, 0.58, 0.35 and 0.21 mg/mL. A continuous-flow diluter was used to deliver each test concentration, the solvent control and dilution water control. The stock solutions were injected into the diluter mixing chambers and mixed with dilution water to produce the test concentrations. The diluter was adjusted so that each chamber received approximately 6 volume additions of water every 24 hours. The solvent concentration in the treatment and solvent control groups was 1.0 mL/L. The negative control consisted of dilution water only.

At the start of the test ten fish were randomly allocated to each of the test concentrations, the solvent control 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 2, 24, 48, 72 and 96 hours. The LC_{50} was calculated by the binomial method. The NOEC was determined by visual inspection of the data.

Daily measurements of the test solutions were undertaken throughout the 96 hour period for pH and dissolved oxygen concentration. The temperature was measured at test initiation and termination. Temperature was also measured continuously in one negative control replicate.

The test concentrations were verified by chemical analysis of CGA173506 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 limit of quantification in this study was 0.02 mg a.s./L. The measured concentrations at study initiation (day 0) were 93, 79, 68, 73 and 41 % of nominal values, at study termination measured values were 91, 84, 71, 84 and 51 % of nominal. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.1-11: Analytical results

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)[1] 0 hours	Measured concentration (mg a.s./L) 96 hours	Mean measured concentration (mg a.s./L)
Negative control	< LOQ	< LOQ	< LOQ
Solvent control	< LOQ	< LOQ	< LOQ
0.19	0.1771	0.1721	0.17
0.32	0.2533	0.2675	0.26
0.54	0.3682	0.3842	0.38
0.90	0.6612	0.7527	0.71
1.5	0.6111	0.7597	0.69

[1]The limit of quantification (LOQ) was based upon the lowest matrix fortification level (0.02 mg a.s./L) established during method validation and analysed concurrently with the test sample

Mortalities were observed at mean measured concentrations of 0.38 mg a.s./L and above. Symptoms of toxicity including loss of equilibrium, lethargy and discolouration were observed at concentrations of 0.19 mg a.s./L and above. No mortality or symptoms of toxicity were observed in the negative and solvent control.

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

Table 8.2.1-12 Effects of CGA173506 on the survival of *Oncorhynchus mykiss*

Measured concentration (mg a.s./L)	Mortality observed (Cumulative number of dead fish) (n = 20)				
	2 hours	24 hours	48 hours	72 hours	96 hours
Dilution water control	0	0	0	0	0
Solvent control	0	0	0	0	0
0.17	0	0[b]	0[b]	0[bcd]	0[bcd]
0.26	0[a]	0[bc]	0[bcd]	0[bcd]	0[bcd]
0.38	0[b]	0[bc]	0[bcd]	1[bcd]	3[bcd]
0.71	0[b]	20	20	20	20
0.69	0[b]	13*	20	20	20
LC_{50}	-	0.62	0.51	0.49	0.47
95% confidence interval	-	0.38 – 0.71	0.38 – 0.69	0.38 – 0.69	0.38 – 0.69

[a] Some surviving fish experienced loss of equilibrium
[b] All surviving fish experienced loss of equilibrium
[c] All surviving fish showed signs of lethargy
[d] All surviving fish showed signs of discolouration
* Observations of these fish inadvertently not noted at 24 hours

Validity Criteria

No validity criteria are reported.

Conclusions

Based on mean measured concentrations, the 96-hour LC_{50} for CGA173506 to rainbow trout (*Oncorhynchus mykiss*) was 0.47 mg a.s./L. The 96 NOEC was 0.26 mg a.s./L.

(Holmes C.M., & Swigert J.P., 1993)

This study complies with the current validity criteria for acute toxicity testing with fish, with the exception of the measured concentration deviating from nominal >20%. The validity criteria state that if results do deviate >20%, results should be based on the measured concentration. The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure, taking into account the low solubility of the test item at the highest test concentration. Therefore this study is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Holmes, Swigert, 1993, CGA173506/0258	ASTM Standard E 729-88 FIFRA No. 72-1	OECD No. 203 (1992)	Measured concentration deviated >20% during the test. Test temperature range: 11.3 to 11.9ºC (Guideline recommended test temperature range: 13 to 17ºC)	Most of the validity criteria were met: 5. Mortality in the negative control and the acetone solvent control was ≤10% (0%). 6. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 7. Evidence that the concentration being tested has been satisfactorily maintained (see explanation below) 8. DO ≥ 60% ASV (60% saturation at 12ºC = 6.5 mg/L; measured range during the study was 8.0 to 9.0 mg/L) CGA173506 has a solubility of 1.53 mg/L in water. The mean measured concentration in the highest treatment group (1.5 mg/L nominal concentration) was 46% of nominal. Mean measured concentrations in the other treatment groups, whose target concentrations were less than the limit of solubility, ranged from 70 to 89% of nominal. Since 100% mortality occurred at the two highest test concentrations, an LC_{50} could be calculated without using the data associated with the highest treatment group. Consequently the low mean measured concentration in the highest treatment group did not adversely affect the results of the study. The solvent (acetone) concentration in the treatment and solvent control groups was 1.0 mL/L; this concentration is considered to be within the tolerance of rainbow trout for acute toxicity testing (as confirmed by the absence of effects observed in the solvent control). The study is reliable and the endpoint can be used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.1 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-1 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.1 Biever R.C. (1997) CGA-173506 Technical – Acute Toxicity to Rainbow Trout, Oncorhynchus mykiss, Under Flow-through Conditions. Report Number 90-03-3245. Springborn Laboratories, Inc. Environmental Sciences Division, 790 Main Street, Wareham, Massachusetts 02571, USA. (Syngenta File No. CGA173506/0926)*

Guidelines

US EPA FIFRA Guideline No. 72-1(c)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to rainbow trout *Oncorhynchus mykiss* was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.50, 0.32, 0.21, 0.14 and 0.089 mg CGA173506/L, alongside dilution water and solvent controls. Based on mean measured concentrations, the 96 hour LC_{50} was 0.23 mg CGA173506/L with 95 % confidence limits of 0.18 to 0.33 mg a.s./L.

Materials

Test material	CGA173506 Technical
Lot/Batch #:	P805002 (FL 881677)
Purity:	97.5 %
Description:	Yellow powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	Not reported

Treatments	
Test concentrations:	Dilution water and solvent controls, and nominal concentrations of 0.50, 0.32, 0.21, 0.14 and 0.089 mg CGA173506/L
Solvent:	Acetone
Analysis of test concentrations:	Yes, based on analysis of CGA173506 at 0 and 96 hours using GC-MS

Test organisms	
Species:	Rainbow trout *Oncorhynchus mykiss*
Source:	Mount Lassen Trout Farms, California (SLI lot # 88A83c)
Acclimatisation period:	At least 14 days
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	Mean length: 47 mm (range: 42 - 58 mm) Mean weight: 0.91 g (range: 0.52 - 1.81 g)
Feeding:	None during test

Test design	
Test vessels:	Glass test aquaria (39 x 20 x 25 cm) with a 14.5 standpipe which maintained the 11 L test medium volume.

Test medium:	Well water
Replication:	2
No. of fish per replicate:	10
Exposure regime:	Flow-through
Duration:	96 hours

Environmental conditions

Test temperature:	12 ± 1 °C
pH:	6.7 – 7.1
Dissolved oxygen:	7.3 - 9.4 mg/L (79 to 87%)
Hardness of dilution water:	28 mg/L $CaCO_3$
Total alkalinity:	22 mg/L $CaCO_3$
Specific conductance range:	90 - 110 µmhos/cm
Lighting:	16 hours fluorescent light and 8 hours darkness with a light intensity of 60 - 120 footcandles

Study Design and Methods

Experimental dates: 14th to 18th February 1989.

A stock solution with a nominal concentration of 6.9 mg CGA173506/mL was prepared by dissolving 3.548 g of the test item (3.459 g a.s.) in 500 mL of acetone. A 50 mL gas-tight syringe pump was constructed to deliver the CGA173506 stock solution to the exposure system mixing cell (0.031mL/min). Dilution water was continuously delivered to the mixing cell at a rate of 0.429 L/min and was mixed with appropriate amounts of the stock solution to produce the highest treatment level. This solution was diluted to provide the lower exposure solutions.

The diluter delivered 4.5 L/hour of solution to each replicate test aquarium, providing approximately 9.8 volume replacements per aquarium every 24 hours. The control consisted of dilution water only, and the solvent control contained dilution water and acetone (0.0723 mL/L). The maximum concentration of acetone present in any test variant was 0.0723 mL/L.

At the start of the test ten fish were randomly allocated to each of the test concentrations and control treatments. 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. The LC_{50} was calculated by the Probit method. The NOEC was determined by visual inspection of the data.

Daily measurements of pH, temperature and dissolved oxygen concentration were recorded throughout the 96-hour exposure period.

The test concentrations were verified by chemical analysis of CGA173506 at 0 and 96 hours using a wide-bore capillary gas chromatograph equipped with a nitrogen phosphorus detector.

Results and Discussion

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

Table 8.2.1-13: Analytical results

Nominal concentration (mg a.s./L)	Measured concentrations 0-hours[a] (mg a.s./L)		Measured concentrations 96-hours[a] (mg a.s./L)		Mean measured concentration in mg a.s./L[b]
	Replicate A	Replicate B	Replicate A	Replicate B	
Control	< 0.010	< 0.010	< 0.008	< 0.008	N/A
Solvent control	< 0.010	< 0.010	< 0.008	< 0.008	N/A
0.089	0.066	0.072	0.062	0.065	0.066
0.14	0.10	0.11	0.11	0.11	0.11
0.21	0.14	0.17	0.37[c]	0.42[c]	0.18
0.32	0.25	0.28	0.22[c]	0.20[c]	0.33
0.50	0.50	0.49	0.60	0.56	0.54

N/A = Not Applicable
[a] Measured concentrations presented in this table have been rounded to two significant figures
[b] Mean measured concentrations calculated using the analytical results rather than the rounded values presented in this table
[c] Inversion of these values apparently resulted from a labelling error occurring during the analytical process. Values were reversed in order to calculate the mean measured concentrations

Following 96-hour exposure, 100 % mortality was observed in the two highest treatment levels (0.54 and 0.33 mg a.s./L). Mortalities were also observed at a measured concentration of 0.18 mg a.s./L. Further symptoms of toxicity observed included loss of equilibrium were observed at concentrations of 0.11 mg a.s./L and above. No mortality or symptoms of toxicity were observed in the controls or the 0.066 mg a.s./L treatment group..

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

Table 8.2.1-14: Effects of CGA173506 on the survival of *Oncorhynchus mykiss*

Mean measured concentration (mg a.s./L)	% Mortality observed (Cumulative number of dead fish) (n = 10)											
	24-Hour			48-Hour			72-Hour			96-Hour		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	0	0	0	0	0	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0	0	0	0	0	0
0.066	0	0	0	0	0	0	0	0	0	0	0	0
0.11	0	0	0[cd]	0	0	0[b]	0	0	0[b]	0	0	0[a]
0.18	0	0	0[b]	0	0	0[ae]	10	10	10[ce]	10	10	10[a]
0.33	20	20	20[a]	60	40	50[a]	100	100	100	100	100	100
0.54	100	100	100	100	100	100	100	100	100	100	100	100
LC_{50} (mg a.s./L)	0.38			0.33			0.23			0.23		
95% Confidence interval (mg a.s./L)	0.33 - 0.54			0.18 - 0.54			0.18 - 0.33			0.18 - 0.33		
NOEC	-			-			-			0.066		

[a] All surviving fish exhibited a complete loss of equilibrium
[b] All surviving fish exhibited a partial loss of equilibrium
[c] Several surviving fish exhibited a partial loss of equilibrium
[d] Several surviving fish exhibited lethargic behaviour
[e] Several fish exhibited a complete loss of equilibrium
[g] LC_{50} value estimated by nonlinear interpolation; 95% confidence interval calculated by binomial probability

Validity Criteria

No validity criteria are reported.

Conclusions

Based on mean measured concentrations, the 96-hour LC_{50} for CGA173506 to rainbow trout (*Oncorhynchus mykiss*) was 0.23 mg CGA173506/L and the 96-hour NOEC was 0.066 mg CGA173506/L.

(Biever R.C., 1997)

This study complies with the current validity criteria for acute toxicity testing with fish, with the exception of the measured concentration deviating from nominal >20%. The validity criteria state that if results do deviate >20%, results should be based on the measured concentration. The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure (which ranged between 74% and 108% of nominal values), therefore this study is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Biever, 1997, CGA173506/0926	US EPA FIFRA No. 72-1 (c)	OECD No. 203 (1992)	Measured concentration deviated >20% during the test. Test temperature: 12 ± 2ºC (Guideline recommended test temperature range: 13 to 17ºC)	Most of the validity criteria were met: 1. Mortality in the negative control and the acetone solvent control was ≤10% (0%). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 3. Evidence that the concentration being tested has been satisfactorily maintained (see explanation below) 4. DO ≥ 60% ASV (79 to 87%) The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure (which ranged between 74% and 108% of nominal values). Consequently the lower mean measured concentrations in the two lowest treatment groups did not adversely affect the results of the study. The solvent (acetone) concentration in the treatment and solvent control groups was 0.0723 mL/L; this concentration is considered to be within the tolerance of rainbow trout for acute toxicity testing (as confirmed by the absence of effects observed in the solvent control). The study is reliable and the endpoint can be used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.1 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-1 above.

RMS comment:

In the acute toxicity test on rainbow trout with fludioxonil (Biever, 1997, CGA173506/0926, KIlA 8.2.1/02), in view of the preparation of the stock solution and test concentrations, it seems that the amounts of solvent in the control and in the different test concentrations are different. A justification of the reliability of the study should be provided.

Syngenta response

Clarification of solvent control:

The test solution was prepared by creating a primary stock (see page 16 of 47). 3.548 g of material (equivalent to 3.459g AI) was brought to a total volume of 500 using acetone (this is equivalent to 6.918 mg AI/mL of stock). 0.031 ml of stock was delivered to the mixing cell, where it was diluted with 0.429 L of dilution water. Page 15 of 48 states "The solvent control solution contained the maximum amount of acetone (CAS# 67-64-1) present in any test concentration (0.0723 mL/L). The 0.029 mL/L calculation can be performed by dividing 0.031ml of stock by 0.429 L of stock.

The concentration achieved in the mixing well is equivalent to the highest test concentration.

0.0031 mL of stock/diluter cycle x 6.918 mg AI/mL of stock = 0.0214458 mg AI/diluter cycle

0.0214458 mg AI/diluter cycle x 1 diluter cycle/0.429 L of dilution water = 0.04999 mg AI/L of dilution water

The amount of solvent per treatment decreased proportionally to the dilution factor (0.65). Therefore, the 0.54, 0.33, 0.18, 0.11, and 0.066 mg AI/L treatment contained 0.0723 mL stock/L, 0.046995 ml stock/L, 0.03054 mL stock/L, 0.019855 mL stock/L, and 0.012906 mL stock/L.

At the time of the study, it was very common for laboratories to only dilute the primary stock and therefore often the amount of solvent in each treatment decreases as treatment level decrease.

Statistical Comparisons:

Table 5 on page 29 of 47 shows that the cumulative mortality in the negative and solvent control were 0% and 0%, respectively. The solvent and negative controls were therefore not significantly different. Whether pooled controls, or either individual controls were used, there will be negligible, if any, impact on subsequent statistical analysis.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.1 Biever R.C. (1997), CGA-173506 Technical - Acute Toxicity to Bluegill Sunfish, Lepomis macrochirus, Under Flow-Through Conditions. Report Number 90-03-3248. Springborn Laboratories, Inc., Health and Environmental Sciences, 790 Main Street, Wareham, Massachusetts, 02571-1075, USA. (Syngenta File No. CGA173506/0927)*

Guidelines

ASTM, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. Standard E729-80 (1980)

US EPA, Standard Evaluation Procedures for Acute Toxicity Test for Freshwater Fish. (1985)

US EPA, Toxic Substances Control Act Test Guidelines, 'Fish Acute Toxicity Test' (1985)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to bluegill sunfish (*Lepomis macrochirus*) was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.18, 0.27, 0.42, 0.65 and 1.0 mg a.s./L (mean measured: 0.14, 0.26, 0.45, 0.62 and 0.96 mg a.s./L) alongside a dilution water control and solvent control. Based on mean measured concentrations of CGA173506 the 96 hour LC_{50} was 0.74 mg a.s./L, with 95 % confidence limits of 0.62 to 0.96 mg/L.

Materials

Test material	CGA173506 Technical
Lot/Batch #:	P805002
Purity:	97.5 %
Description:	Yellow powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	Not reported

Treatments	
Test concentrations:	Dilution water control, solvent control and nominal formulation concentrations of 0.18, 0.27, 0.42, 0.65 and 1.0 mg a.s./L (mean measured: 0.14, 0.26, 0.45, 0.62 and 0.96 mg a.s./L)
Solvent:	Acetone
Analysis of test concentrations:	Yes, based on measurement of CGA173506 at 0 and 96 hours using GC-NPD analysis

Test organisms	
Species:	Bluegill sunfish, *Lepomis macrochirus*
Source:	Bybrook Bass Hatchery, Connecticut
Acclimatisation period:	At least 14 days
Treatment for disease:	None reported
Weight and length of a representative sample from test population (n = 30):	Mean length: 38 mm (range 29 – 47 mm) Mean weight: 0.77 g (range 0.13 – 1.7 g)
Feeding:	None during test

Test design	
Test vessels:	Glass aquaria (39 x 20 x 25 cm) containing 11 L water, with a 14.5 cm-high standpipe
Test medium:	Well water
Replication:	2 replicates of the test concentrations, control and solvent control
No of fish per tank:	10 per replicate

Exposure regime:	Flow-through
Duration:	96 hours

Environmental conditions

Test temperature:	21 °C
pH:	6.8 – 7.3
Dissolved oxygen:	8.1 – 9.9 mg/L, no aeration
Hardness of dilution water:	26 – 30 mg/L $CaCO_3$
Lighting:	16 hours light and 8 hours dark, 60 – 120 footcandles

Study Design and Methods

Experimental dates: 20th to 24th January 1989

A flow-through test system was employed. A stock solutions of 13.8 mg a.s./mL was prepared by dissolving 7.100 g of the test item with acetone to a total volume of 500 mL. A 50 mL gas-tight syringe and a syringe pump were calibrated to deliver the stock solution directly to the exposure system's chemical mixing cell at a rate of 0.031 mL/min. Dilution water was continuously delivered to the mixing cell at a rate of 0.429 L/min. The delivery rate provided a solution at a test concentration equal to the highest treatment level, and was then diluted (65 % dilution factor) to provide the remaining nominal test concentrations. The diluter solutions were delivered at a rate of 75 mL per minute into each test aquarium providing approximately 9.8 volume replacements per aquarium every 24 hours. The diluter system was allowed to equilibrate for a minimum of 24 hours prior to the addition of the test organisms.

The control consisted of dilution water only, and the solvent control contained dilution water and acetone. The solvent control contained greater than the amount of acetone present in any test concentration (0.14 mL/L).

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

Daily measurements of pH, temperature and dissolved oxygen concentration within the test solutions were undertaken throughout the 96-hour exposure period. Total hardness and alkalinity as $CaCO_3$ and specific conductivity was measured on Day 0 in one replicate of each treatment level and control. Additionally, test solution temperature was continuously monitored in one replicate of the control solution.

The test concentrations were verified by chemical analysis of CGA173506 at 0 and 96 hours using an GC – nitrogen phosphorus detection (NPD) method. Samples for analysis were taken from the centre of the test solutions.

The LC_{50} was calculated by nonlinear interpolation. The NOEC was also determined.

Results and Discussion

Analyses of the treatment solutions throughout the concentration range resulted in mean measured concentrations which averaged 94 % of the nominal levels. The coefficients of variation for all mean measured concentrations averaged 21 %. The minimal detectable concentration in this study was 0.00100 mg a.s./L for a 1000 mL sample. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.1-15: Analytical results

Nominal concentrations (mg a.s./L)	Measured concentrations* (mg a.s./L) 0 hours		Measured concentrations* (mg a.s./L) 96 hours		Mean measured concentrations* (mg a.s./L)
	A	B	A	B	
Control	< 0.0077	< 0.0077	< 0.013	< 0.013	n.a.
Solvent Control	< 0.0077	< 0.0077	< 0.013	< 0.013	n.a.
0.18	0.17	0.15	0.12	0.13	0.14
0.27	0.27	0.32	0.21	0.23	0.26
0.42	0.50	0.51	0.37	0.42	0.45
0.65	0.79	0.80	0.45	0.42	0.62
1.0	1.2	0.93	0.96	0.76	0.96

* Measured concentrations have been rounded to two significant figures. Mean measured concentrations calculated using the analytical results not the rounded values.
n.a. = not applicable

Mortalities were observed at mean measured concentrations of 0.62 mg a.s./L and above. Symptoms of toxicity observed included partial or complete loss of equilibrium and were observed at concentrations of 0.26 mg a.s./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 8.2.1-16: Effects of CGA173506 on the survival of *Lepomis macrochirus*

Mean measured concentration (mg a.s./L)	Mortality observed (cumulative % of dead fish) (n = 20)			
	24 hours	48 hours	72 hours	96 hours
Dilution water control	0	0	0	0
Solvent control	0	0	0	0
0.14	0	0	0	0
0.26	0[a]	0[a]	0[a]	0[a]
0.45	0[a,b]	0[c]	0[c]	0[c]
0.62	0[c]	0[c]	0[c]	10[c]
0.96	100	100	100	100
LC_{50} (mg a.s./L)	0.77	0.77	0.77	0.74
95 % confidence interval (mg a.s./L)	0.62 – 0.96	0.62 – 0.96	0.62 – 0.96	0.62 – 0.96
NOEC (mg a.s./L)	0.14			

[a] Several surviving fish exhibited a partial loss of equilibrium
[b] Several surviving fish exhibited a complete loss of equilibrium
[c] All surviving fish exhibited a complete loss of equilibrium

Validity Criteria

No validity criteria are reported.

Conclusions

Based on the mean measured concentrations of CGA-173506, the 96-hour LC_{50} for CGA-173506 Technical to bluegill sunfish (*Lepomis macrochirus*) was 0.74 mg CGA-173506/L and the 96-hour NOEC was 0.14 mg CGA-173506/L.

(Biever R.C., 1997)

This study complies with the current validity criteria for acute toxicity testing with fish, with the exception of the measured concentration deviating from nominal >20%. The validity criteria state that if results do deviate >20%, results should be based on the measured concentration. The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure (which ranged between 77.8% and 107% of nominal values), therefore this study is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Biever, 1997, CGA173506/0927	US EPA FIFRA No. 72-1 (a);	OECD No. 203 (1992)	Measured concentration deviated >20% during the test. Mean length of test fish: 3.8 cm; (Guideline recommended total length of test fish: 2.0 ± 1.0 cm)	Most of the validity criteria were met: 1. Mortality in the negative control and the acetone solvent control was ≤10% (0%). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 3. Evidence that the concentration being tested has been satisfactorily maintained (see explanation below) 4. DO ≥ 60% ASV (8.1 – 9.9 mg/L). The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure (which ranged between 77.8% and 107% of nominal values). Consequently the lower mean measured concentration in the lowest treatment group did not adversely affect the results of the study. The solvent (acetone) concentration in the solvent control groups was 0.14 mL/L; the solvent control contained a concentration of solvent approximately 2-fold that of the highest test concentration. This concentration is considered to be within the tolerance of Bluegill Sunfish for acute toxicity testing (as confirmed by the absence of effects observed in the solvent control). The study is reliable and the endpoint can be used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.1 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-1 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.1 Holmes C.M. & Swigert J.P., (1993). CGA 173506 – A 96-Hour Flow Through Acute Toxicity Test with the Sheepshead Minnow (Cyprinodon variegatus). Report Number 108A-135. Wildlife International Ltd., 8598 Commerce Drive, Easton, MD 21601, USA. (Syngenta File No. CGA173506/0257)*

Guidelines

U.S. EPA, Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, EPA 540/9-82-024, Series 72-3 (1982)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to sheepshead minnow *Cyprinodon variegatus* was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 0.45, 0.76, 1.3, 2.1 and 3.5 mg a.s./L, alongside dilution water and solvent controls. Based on mean measured concentrations, the 96 hour LC_{50} was 1.2 mg a.s./L. The no mortality concentration, determined by visual examination, was 0.93 mg a.s./L.

Materials

Test material	CGA173506 Technical
Lot/Batch #:	P905004/5 (FL 891552)
Purity:	95.4 %
Description:	Fluffy white powder
Stability of test compound:	Stable under test conditions
Solubility:	1.53 mg/L at 20 °C
Reanalysis/expiry date:	16 April 1994

Treatments

Test concentrations:	Nominal concentrations: 0.45, 0.76, 1.3, 2.1 and 3.5 mg a.s./L Mean measured concentrations: 0.38, 0.67, 0.93, 1.5 and 2.0 mg a.s./L
Solvent:	Acetone
Analysis of test concentrations:	Yes, based on analysis at 0 and 96 hours using HPLC analysis

Test organisms

Species:	Sheepshead minnow *Cyprinodon variegatus*
Source:	Wildlife International Ltd. Cultures, 8651 Brooks Drive, Easton, Maryland 21601
Acclimatisation period:	76 hours
Age:	Juvenile
Treatment for disease:	None
Weight and length of dilution water control fish at end of exposure period:	Mean length: 21 mm (range: 18 - 24 mm) Mean weight: 0.32 g (range: 0.19 - 0.45 g)
Loading rate:	0.02 g/L/day
Feeding:	None during test

Test design

Test vessels:	Test chambers were Teflon®-lined, 25 L polyethylene aquaria filled with 15 L of test medium
Test medium:	Saltwater blended with dechlorinated water
Replication:	2
No of fish per replicate:	10
Exposure regime:	Flow-through
Duration:	96 hours

Environmental conditions

Test temperature:	22 ± 1 °C
pH:	8.2 to 8.3
Dissolved oxygen:	5.4 to 7.4 mg/L (4.5 mg/L represents 60% saturation at 22 °C and a salinity of 22 ‰)
Salinity:	25 - 26 ‰
Lighting:	16 hours fluorescent light and 8 hours dark daily, with 30 minute dawn and dusk transition periods. Light intensity ≈ 463 lux at the water surface

Study Design and Methods

Experimental dates: 5th to 10th June 1992

A stock solution with a nominal concentration of 3.7 mg a.s./mL was prepared. The stock was inverted to aid solubilisation of the test substance. Aliquots of the stock were diluted with acetone to prepare four additional stocks at nominal concentrations of 2.2, 1.3, 0.80 and 0.48 mg a.s./mL. The stock solutions where injected into the diluter mixing chambers and mixed with dilution water to achieve the desired concentrations.

A peristaltic pump and continuous flow-through diluter delivered each concentration of test substance, a solvent control and a negative (saltwater) control, providing approximately 12 volume replacements per aquarium every 24 hours. The solvent concentration in the treatment and solvent control groups was 1.0 mL acetone/L. The dilution water control consisted of dilution water only.

At the start of the test ten fish were randomly allocated to each of the test concentrations and the controls. The test was conducted in a temperature controlled water-bath enclosed in a plexiglass ventilation hood to minimise cross-contamination. Observations for mortalities and symptoms of toxicity were made at 6, 24, 48, 72 and 96 hours.

The LC_{50} was calculated by the Probit or binomial method. The NOEC was determined by visual inspection of the data.

Daily measurements of the pH, temperature and dissolved oxygen concentration in the test solutions were recorded throughout the 96-hour exposure period.

The test concentrations were verified by chemical analysis of CGA173506 at 0 and 96 hours using HPLC mass spectrometry.

Results and Discussion

The measured concentrations are shown in the table below. The test concentrations were maintained throughout the study. The limit of quantification in this study was 0.04 mg a.s./L. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.1-17: Analytical results

Nominal concentration (mg CGA 173506/L)	Measured concentration 0 hours (mg a.s./L)	Measured concentration 96 hours (mg a.s./L)	Mean measured concentration (mg a.s./L)
Negative control	< 0.04	< 0.04	< 0.04
Solvent control	< 0.04	< 0.04	< 0.04
0.45	0.36 - 0.37	0.41	0.38
0.76	0.62	0.71	0.67
1.3	0.88	0.98	0.93
2.1	1.40	1.56	1.5
3.5	1.51	2.43	2.0

* All samples were centrifuged prior to analysis
** The limit of quantification (LOQ) was based upon the lowest matrix fortification level (0.04 mg a.s./L) analysed concurrently with the test samples

Mortalities were observed at mean measured concentrations of 1.5 mg a.s./L and above. No mortality or symptoms of toxicity were observed in the controls.

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

Table 8.2.1-18: Effects of CGA173506 on the survival of *Cyprinodon variegatus*

Mean measured concentration (mg a.s./L)	Replicate	Percentage Mortality observed (Cumulative number of dead fish, %) (n = 10)				
		6 hours	24 hours	48 hours	72 hours	96 hours
Negative control	A	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)
	B	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)
Solvent control	A	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)
	B	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)
0.38	A	0 (10AN)	0 (7N, 3AN)	0 (10AN)	0 (8N, 2AN)	0 (10AN)
	B	0 (10AN)	0 (8N, 2AN)	0 (10AN)	0 (8N, 2AN)	0 (10AN)
0.67	A	0 (10N)	0 (10N)	0 (10C, N)	0 (10C, N)	0 (10C, N)
	B	0 (10N)	0 (10N)	0 (10C, N)	0 (10C, N)	0 (10C, N)
0.93	A	0 (10C, N)	0 (10C, N)	0 (10C, N)	0 (10C, N)	0 (10C, N)
	B	0 (10C, N)	0 (10C, N)	0 (10C, N)	0 (10C, N)	0 (10C, N)
1.5	A	0 (10C, N)	10 (9C, N)	30 (7C, N)	100	100
	B	0 (10C, N)	0 (10C, N)	30 (7C, N)	80 (2AN)	100
2.0	A	0 (10C, N)	0 (10C, N)	90 (1N, C)	100	100
	B	0 (10C, N)	0 (10C, N)	50 (5N, C)	100	100
LC_{50} (mg a.s./L)		-	> 2.0	1.7	1.2	1.2
95% confidence interval		-	N/A	1.6 - 2.0	0.93 - 1.5	0.93 - 1.5
Statistical method		-	Visual	Probit	Binomial	Binomial

Observed effects: C = Lethargy; N = Loss of equilibrium; AN = Appears normal
N/A = Not applicable

Validity Criteria

No validity criteria are reported.

Conclusions

Based on mean measured concentrations, the 96 hour LC_{50} to sheepshead minnow (*Cyprinodon variegatus*) was 1.2 mg a.s./L, and the no mortality concentration, determined by visual examination, was 0.93 mg a.s./L.

(Holmes C.M., & Swigert J.P., 1993)

This study complies with the current validity criteria for acute toxicity testing with fish, with the exception of the measured concentration deviating from nominal >20%. The validity criteria state that if results do deviate >20%, results should be based on the measured concentration. The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure (which ranged between 57% and 88% of nominal values), therefore this study is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Holmes, Swigert, 1993, CGA173506/0257	US EPA FIFRA No. 72-3	OECD No. 203 (1992)	Measured concentration deviated >20% during the test.	Most of the validity criteria were met: 5. Mortality in the negative control and the acetone solvent control was ≤10% (0%). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 3. Evidence that the concentration being tested has been satisfactorily maintained (see explanation below) 4. DO ≥ 60% ASV; equivalent to 4.5 mg/L in saltwater with a salinity of 25‰ at 22ºC (measured values: 5.4 to 7.8 mg/L). CGA173506 has a solubility of 1.53 mg/L in water at 20ºC. The mean measured concentrations in the 1.3, 2.1 and 3.5 mg/L treatment groups were consequently below 80% (71.5%, 71.4% and 57% of nominal respectively). Mean measured concentrations in the other 2 treatment groups, whose target concentrations were less than the limit of solubility, were 84% and 88% of nominal. The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure, consequently the lower mean measured concentrations in the three highest treatment groups did not adversely affect the results of the study. The solvent (acetone) concentration in the treatment and solvent control groups was 1.0 mL/L. This concentration is considered to be within the tolerance of Sheepshead minnow for acute toxicity testing (as confirmed by the absence of effects observed in the solvent control), and was necessary to enhance the solubility of the test item in water. The study is reliable and the endpoint can be used in the risk assessment.

~~The following study was previously submitted in the EU and was summarised in point B.9.2.1 of the Monograph.~~ At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-1 above. It was clarified that the following study is new and had not been included in the original EU evaluation so the report has also been provided in this supplementary dossier.

Report:	New fish acute study provided for completeness and for relevance comparison with existing endpoints: ~~Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:~~ ~~KHA 8.2.1~~ KCA 8.2.1/06 Surprenant D. (1990), (CGA-173506 Technical) - Acute toxicity to sheepshead minnow (Cyprinodon variegatus) under flow through conditions. Report Number SLI-90-03-3257, CIBA-GEIGY Agricultural Division, 410 Swing road, P.O. Box 18300, Greensboro, North Carolina 27419 (Syngenta File No. CGA173506/0056)

Guidelines

US EPA FIFRA Guideline 72-3

GLP: Yes

Executive Summary

The toxicity of CGA173506 to sheepshead minnow (*Cyprinodon variegatus*) was determined under flow-through conditions. Fish were exposed to a range of nominal concentrations of 5, 3.3, 2.1, 1.4 and 0.89 mg a.s./L, alongside a solvent control and a dilution water control. Based on mean measured concentrations the 96 hr LC_{50} was 0.54 mg a.s/L.

Materials

Test material	CGA173506 Technical
Lot/Batch #:	P805002
Purity:	97.5%
Description:	Yellow powder
Stability of test compound:	Stable under test conditions

Treatments

Test concentrations:	Dilution water control, solvent control and nominal concentrations of 5, 3.3, 2.1, 1.4 and 0.89 mg a.s./L
Solvent:	Acetone
Analysis of test concentrations:	Yes, based on analysis of CGA173506 at 0 and 96 hours using a gas chromatograph equipped with a nitrogen phosphorus detector

Test organisms

Species:	Sheepshead minnow (*Cyprinodon variegatus*)
Source:	Aquatic bioassay laboratory, Baton Rouge, Louisiana
Acclimatisation period:	10 days
Treatment for disease:	None stated
Weight and length of dilution water control fish at end of exposure period:	Mean length: 13 mm (10 - 19 mm) Mean weight: 0.06 g (0.03 – 0.13 g)
Feeding:	None during test; (dry commercial flake food *ad libitum* until 24 hrs prior to testing)

Test design

Test vessels:	Glass vessels containing 11 L water
Test medium:	Filtered natural seawater (salinity 32 ‰)
Replication:	2

No of fish per tank: 10 (maximum organism loading = 0.008 g biomass/L of flowing test solution/day.)
Exposure regime: Flow-through (6.5 volume replacements per aquarium every 24 hours)
Duration: 96 hours

Environmental conditions
Test temperature: 22° C
pH: 7.8
Dissolved oxygen: 5.9 – 7.9 mg/l
Lighting: 16 hour light 8 hour dark

Study Design and Methods

Experimental dates: 13th to 17th March 1989

A stock solution with a nominal concentration of 75.3 mg/L was prepared by dissolving 7.72 g of CGA-173506 technical with acetone to a total volume of 100. Appropriate volumes of the stock were then made up in dilution water in each test vessel to give the test concentrations. The highest concentration contained 0.074 mL/L of acetone. The solvent control contained acetone at 0.074 mL/L. The negative control consisted of dilution water only. Dosing was conducted on a continuous flow serial diluter delivering at 286mL/minute. This delivery rate provided a solution containing a CGA-173506 concentration equal to the highest treatment level, which was then diluted (65%) to provide the desired nominal concentrations. The diluter system was allowed to equilibrate for at least 24 hours prior to the addition of the fish.

At the start of the test, 10 fish (20 per treatment level) were randomly allocated to each replicate aquaria. . Observations for symptoms of toxicity were made at 0, 24, 48, 72 and 96 hours.

Water samples were taken from each aquarium for analysis on Days 0 and 4. Measurements of the test solutions were undertaken throughout the test period for pH, temperature and dissolved oxygen concentration.

To calculate the LC_{50}, three statistical methods were used: moving average angle analysis, probit analysis and nonlinear interpolation with 95% confidence intervals calculated by binominal probability.

Results and Discussion

At the start of the test, the concentrations of CGA173506 were in the range 23 to 42% of the nominal values and at the end of the test were in the range 33 to 46% (see table below). Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.1-19: Analytical results

Nominal concentrations (mg a.s./L)	Measured concentrations at 0 hours (mg a.s./L)	% of nominal measured at 0 hours	Measured concentrations at 96 hours (mg a.s./L)	% of nominal measured at 96 hours	Mean measured concentrations (mg a.s./L)
Control	-	-	-	-	-
Solvent control	-	-	-	-	-
0.89	0.38	42	0.41	46	0.39
1.4	0.52	37	0.63	45	0.57
2.1	0.69	33	0.83	40	0.76
3.3	0.86	26	1.4	41	1.1

5	1.2	23	1.7	33	1.4

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 moving average angle analysis at 24 hrs, the Probit method at 48 and 72 hours, and nonlinear interpolation with 95% confidence intervals calculated by binominal probability at 96 hours . 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 mean measured concentrations of 0.57 mg/L and above. Symptoms of toxicity included erratic behaviour and loss of equilibrium and were observed in all surviving fish at concentrations ranging from 0.39 to 1.1 mg/L. No mortality or symptoms of toxicity were observed in the negative control or solvent controll. The mortality data and estimated LC_{50} values are shown in the table below:

8.2.1-20: Effects of CGA173506 on the survival of *Cyprinodon variegatus*

Mean measured concentration (mg a.s./L)	**Percent mortality observed (cumulative number of dead fish) (n = 20)**			
	24 hours	**48 hours**	**72 hours**	**96 hours**
Dilution water control	0	0	0	0
Solvent control	0	0	0	0
0.39	0	0	0	0[a,b]
0.57	5	15	45	60[c,d]
0.76	35	70	85	100
1.1	90	100	100	100
1.4	100	100	100	100
LC_{50} mg a.s./L	0.8	0.69	0.6	0.54
95% confidence interval	0.74 – 0.88	0.63 – 0.75	0.55 – 0.66	0.39 – 0.76
NOEC	<0.39	<0.39	<0.39	<0.39

[a] All surviving fish exhibited erratic behaviour
[b] Two surviving fish exhibited a partial loss of equilibrium
[c] All surviving fish exhibited complete loss of equilibrium
[d] Two surviving fish were observed at the surface of the test solution

Conclusions

The toxicity of CGA173506 to sheepshead minnow (*Cyprinodon variegatus*) was determined under flow-through conditions. Based on mean measured concentrations the 96 hr LC_{50} was 0.54 mg a.s/L, and the NOEC was determined to be < 0.39 mg a.s./L (the lowest concentration tested).

(Surprenant D. 1990)

This study complies with the current validity criteria for acute toxicity testing with fish, with the exception of the measured concentration deviating from nominal >20%. The validity criteria state that if results do deviate >20%, results should be based on the measured concentration. The 96 hr LC_{50} value was based on the mean measured concentrations of CGA173506 during the exposure (which ranged between 28% and 44% of nominal values), therefore this study is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Surprenant, 1990, CGA173506/0056	US EPA FIFRA No. 72-3	OECD No. 203 (1992)	Measured concentration deviated >20% during the test.	Most of the validity criteria were met: 6. Mortality in the negative control and the acetone solvent control was ≤10% (0%). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 3. Evidence that the concentration being tested has been satisfactorily maintained (see explanation below) 4. DO ≥ 60% ASV; equivalent to 4.5 mg/L in saltwater with a salinity of 25‰ at 22ºC (measured values: 5.9 to 7.9 mg/L). CGA173506 has a solubility of 1.53 mg/L in water at 20ºC, consequently the mean measured concentrations were below 80% (28 to 44% of nominal) and during the exposure period, undissolved test material was observed at the highest test concentration (5.0 mg/L nominal). The 96 hr LC_{50} value was based on the mean measured concentrations, thus the lower mean measured concentrations did not adversely affect the results of the study. The solvent (acetone) concentration in the treatment and solvent control groups was 0.074 mL/L. This concentration is considered to be within the tolerance of Sheepshead minnow for acute toxicity testing (as confirmed by the absence of effects observed in the solvent control), and was necessary to enhance the solubility of the test item in water. The study is reliable and the endpoint can be used in the risk assessment.

Fish acute toxicity studies with metabolites of fludioxonil

The endpoint for study on CGA339833 by Rufli 2000 (Syngenta file number; CGA339833/0005) previously submitted in the EU was provided in point B.9.2.1 of the DAR**,** ~~therefore an additional study summary is not repeated here~~. The endpoint is summarised in Table 8.2-2.

The endpoint for study on CGA192155 by Volz 2005 (Syngenta file number; CGA192155/0019) previously included in the Final Addendum to the DAR (June 2007)**,** ~~therefore an additional study summary is not repeated here~~. The endpoint is summarised in Table 8.2-2.

At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.1 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk

assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-2 above.

Report:	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS: *KIIA 8.2.1 Rufli H, (2000). Acute Toxicity Test of CGA339833 (Metabolite of CGA173506) to Rainbow Trout (Oncorhynchus mykiss) Under Static Conditions. Report Number 2003513, Novartis Crop Protection AG, Product Portfolio Management, Environmental Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. CGA339833/0005)*

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, Methods for the Determination of Ecotoxicity, C.1: Acute Toxicity For Fish (1992)

US EPA Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, EPA 570/9-82-024, Guideline No. 72-1 (1982)

GLP: Yes

Executive Summary

The acute toxicity of CGA339833 (metabolite of CGA173506) to rainbow trout *Oncorhynchus mykiss* was determined in a limit test under static conditions. Fish were exposed to a nominal concentration of 100 mg CGA339833/L, alongside a dilution water control. Based on nominal concentrations, the 96 hour LC_{50} was > 100 mg CGA339833/L.

Materials

Test Material	CGA339833
Parent:	CGA173506
Lot/Batch #:	KI 6119/6
Purity:	90 ± 2 %
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	May 2002

Treatments	
Test concentrations:	Dilution water control and nominal concentration of 100 mg CGA339833/L
Solvent:	None
Analysis of test concentrations:	Yes, based on analysis of CGA339833 0 and 96 hours using HPLC analysis with UV detection

Test organisms	
Species:	Rainbow trout *Oncorhynchus mykiss*
Source:	P. Hohler, CH-4314 Zeiningen, Switzerland
Acclimatisation period:	39 days
Treatment for disease:	None

Weight and length of dilution water control fish:	Mean length: 57 mm (range 56 to 59 mm) Mean weight: 1.54 g (range 1.40 to 1.70 g)
Loading rate:	0.72 g/L
Feeding:	None 24 hours prior to the start of the test and during test

Test design

Test vessels:	20 L glass aquaria (35 x 22 x 26 cm) containing 15 L water (20 cm water depth)
Test medium:	Dechlorinated tap water
Replication:	None
No of fish per tank:	7
Exposure regime:	Static
Duration:	96 hours

Environmental conditions

Test temperature:	13.3 to 13.4 °C
pH:	7.9 to 8.1
Dissolved oxygen:	89 to 100 % gentle aeration provided
Hardness of dilution water:	198 mg/L $CaCO_3$
Lighting:	16 hours fluorescent light daily with 30 minute transition period

Study Design and Methods

Experimental dates: 15th to 20th May 2000

A stock solution was prepared by mixing 2.0 g of the test item and making up to 1000 mL with dilution water with additional ultrasonication for approximately 10 minutes. Appropriate volumes of the stock were then added to the dilution water in the test vessel to give the test concentration. The test solutions were equilibrated for 4.25 hours before the start of the test. The control consisted of dilution water only.

At the start of the test seven fish were allocated to the test concentration 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 2, 24, 48, 72 and 96 hours.

Daily measurements of the pH, temperature and dissolved oxygen concentration in the test solutions were recorded throughout the 96 hour exposure period. The total hardness was measured in the blank control immediately before the start of the test.

The test concentrations were verified by chemical analysis of CGA339833 at 0 and 96 hours using high performance liquid chromatography with ultra-violet detection.

Results and Discussion

At the start of the test, the measured concentrations were in the range 97.5 to 98.0 % of the nominal values and at the end of the test were in the range 98.4 to 99.0 % (see table below). The limit of quantification (LOQ) in this study was 0.03 mg CGA339833/L. Nominal concentrations were used for the calculation and reporting of results.

Table 8.2.1-21: Analytical results

Nominal concentration (mg CGA339833/L)	Measured concentrations at 0 hours (mg CGA339833/L)	% of nominal measured at 0 hours	Measured concentrations at 96 hours (mg CGA339833/L)	% of nominal measured at 96 hours
Blank	< LOD	-	< LOD	-
100	97.8	98	98.7	99

LOD = 0.01 mg CGA339833/L

No mortalities were observed in this study, therefore no statistical interpretation was possible.

No sublethal symptoms, such as a change in pigmentation, loss of equilibrium, exophthalmus, change in swimming behaviour or in respiratory function, were observed in the control or treatment. The mortality data and estimated LC_{50} values are shown in the table below:

Table 8.2.1-22 Effects of CGA339833 on the survival of *Oncorhynchus mykiss*

Nominal concentration (mg CGA339833/L)	Mortality observed (Cumulative number of dead fish) (n = 7)				
	2 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
100	0	0	0	0	0
LC_{50} mg CGA339833/L	> 100	> 100	> 100	> 100	> 100

Validity criteria

No validity criteria were reported.

Conclusions

Based on nominal concentrations, the 96-hour LC_{50} for CGA339833 to rainbow trout (*Oncorhynchus mykiss*) was > 100 mg CGA339833/L. The highest concentration with no sublethal and lethal effects was 100 mg CGA339833/L.

(Rufli H, 2000)

This study complies with the current validity criteria for acute toxicity testing with fish and thus is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Rufli, 2000, CGA339833/0005	OECD No. 203 (1992), US EPA FIFRA No. 72-1	OECD No. 203 (1992)	Compliant with both OECD and US EPA guidelines	The validity criteria were met: 1. Mortality in the negative control was ≤10% (0%). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 3. Evidence that the concentration being tested has been satisfactorily maintained (measured concentrations were in the range 98 to 99.0% throughout the test) 4. DO ≥ 60% ASV (89 to 100%) No solvent was used in this study. The study is reliable and the endpoint can be used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.1 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-2 above.

Report:	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS: *KIIA 8.2.1 Volz E,(2005). CGA192155 (Metabolite of Fludioxonil): Acute toxicity to rainbow trout (Oncorhynchus mykiss) in a 96-hour static test, Report Number 857596, RCC Ltd, Itingen, Switzerland. (Syngenta File No. CGA192155/0019)*

Guidelines

OECD (1992). OECD Guidelines for Testing of Chemical 203. Fish, Acute Toxicity Test. Adopted 17 July 1992.

Official Journal of the European Communities, L 383 A, Part C.1, Acute Toxicity for Fish. 29 December 1992.

GLP: Yes

Executive Summary

Toxicity of CGA192155 (metabolite of fludioxonil) to rainbow trout (*Oncorhynchus mykiss*) was determined. This study was run with a culture medium control and with a single nominal test item concentration of 100 mg CGA192155/L. Thus, a limit test was performed in accordance with the test guidelines to demonstrate that the test item has no toxic effect on the test organisms at the nominal concentration of 100 mg/L. The 96-hour LC_{50} was determined to be >100 mg/L based on nominal test item concentration.

Materials

Test Material: CGA192155

Description:	White solid
Lot/Batch #:	MLA-336/4
Purity:	96 %

Treatments:

Test concentrations:	Dilution water control and a single nominal test item concentration of 100 mg CGA192155/L
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes (0 and 96 hours)

Test organism

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Source:	P. Hohler, trout breeding station Zeiningen, Switzerland
Acclimatisation period:	7 days
Treatment for disease:	None
Length of fish	5.5 ± 0.3 cm (mean ± SD)
Weight of fish	1.7 ± 0.3 g (mean ± SD)
Loading rate:	0.84 g fish /L
Feeding:	None during test

Test design

Test vessel:	Glass aquarium containing 14 liters test solution
Dilution medium:	Non chlorinated well water
Exposure regime:	Static
Aeration:	Gentle aeration during the test.
Replication:	Seven fish were exposed in each test vessel, one replicate per treatment.

Environmental conditions

Test temperature:	13 to 14 °C
pH:	8.1 to 8.5
Dissolved oxygen:	9.4 to 9.9 mg/L
Total hardness of dilution water:	198 mg/L ($CaCO_3$)
Lighting:	A 16-hour light to 8-hour dark photoperiod (with a 30 minute transition period). Light intensity during the light period was approximately within the range of 50 to 500 Lux.
Length of test:	96 hours

Study Design and Methods

Experimental dates: 25th November to 10th December 2004.

The single test medium of nominal 100 mg/L was prepared by completely dissolving 700.1 and 700.2 mg of the test item in 7 liters of test water each using ultrasonic treatment for 35 minutes and stirring for 80 minutes at room temperature. Both the prepared volumes of test medium were mixed in the test aquarium under intensive stirring.

One glass aquarium with 14 liters test medium was used for each treatment (the single test concentration and the control). Seven fish were randomly allocated to each prepared test vessel. The test was conducted under static conditions with gentle aeration of the test media. Observations for mortality and visible abnormalities were made at 3, 24, 48, 72 and 96 hours of test duration.

The concentrations of the CGA192155 in the test solutions were measured at 0 and 96 hours using a high performance liquid chromatography method.

The water temperature, pH-values and dissolved oxygen concentrations were measured at the start of the test and once every day during the test in the single test concentration and in the control.

At the same dates the appearance of the test medium was recorded. The total hardness of the water used in the test was measured at the start of the test in the control.

Results and Discussion

The analytically determined test item concentration in the test medium of 100 mg/L was 103 and 104% of the nominal value at the start and end of the test, respectively. The nominal test item concentrations were used for the calculation and reporting of the results.

At the beginning and the end of the test period, the pH ranged from 8.1 to 8.5 and dissolved oxygen concentrations were in the range of 9.4 to 9.9 mg/L. The water temperature was 13 to 14 °C. The mean light intensity was approximately 50 to 500 Lux. The water hardness was 198 mg/L as $CaCO_3$.

The LC_{50} value could not be determined due to the absence of a toxic effect of the test item in this test (Table 8.2.1-23).

Table 8.2.1-23 Effects of CGA192155 on the survival of rainbow trout (*Oncorhynchus mykiss*) following exposure for 96 hours in a static test

Nominal concentration of formulation (mg CGA192155/L)	Cumulative percentage mortality observed[a]				
	3 h	24 h	48 h	72 h	96 h
Dilution water control	0	0	0	0	0
100	0	0	0	0	0

[a]: Seven fish were exposed in each test vessel, one replicate per treatment

No mortality was observed at the CGA192155 concentration of 100 mg/L. The 96-hour LC_{50} was therefore concluded to be >100 mg CGA192155/L (95% confidence limits: not determined).

Validity criteria

None reported.

Conclusions

Based on nominal test item concentration, the 96-hour LC_{50} for CGA192155 was >100 mg CGA192155/L to the rainbow trout (*Oncorhynchus mykiss*).

(Volz E, 2005)

Reference	*Guidance followed*	*Current guidance in force*	*Differences from guidance in force*	*Critical assessment*
Volz 2005, Syngenta file number	*OECD 203 (1992)* *EU L383A Part C1*	*OECD No. 201 (2006)*	*Compliant with both OECD and US EPA*	*Following critical validity criteria were*

CGA192155/0019	*(1992)*	*or* *US EPA OPPTS 850.5400 (1996)*	*guidelines*	*met:* *1. Control mortality :≤10% or 1/7 fish (actual: 0% control).* *This study is considered reliable.*

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 early life stage (FELS) test is now the recommended test method. However, the existing data are retained here for completeness. The studies previously submitted in the EU were summarised in point B.9.2.2.1 of the DAR therefore additional study summaries are not repeated here. The endpoints are summarised in Table 8.2-3.

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.

During the previous EU review, four fish prolonged studies (Grade 1993; Syngenta file number CGA173506/0377, Grade 1993; Syngenta file number CGA173506/0378, Grade 1993 Syngenta file number CGA173506/0356, Vial 1991; Syngenta file number CGA173506/0097) were submitted which the RMS considered as not reliable and that further considerations or studies were needed to address the potential risk. After subsequent discussions with the RMS, a new 28-d juvenile fish toxicity study with rainbow trout (Maynard, 2005; Syngenta file number CGA173506/6704) was conducted in order to clarify the uncertainty the RMS had over the appropriate fludioxonil juvenile growth chronic fish study endpoint to use in the risk assessment. The study was included in the Final Addendum to the DAR (June 2007). The new study was considered reliable and used to set the EU endpoint for chronic toxicity to fish, in conjunction with the study by Graves *et al.* 1994; Syngenta file number CGA173506/0416, superseding the four previously submitted studies.

Report:	Conducted to clarify the fish juvenile prolonged toxicity endpoint at the request of the original RMS in 2004 and included in Final Addendum to the DAR Article 8(2) of Council Directive 91/414/EEC (June 2007); included for completeness here: K-CA 8.2.2/01 Maynard SJ (2005). Fludioxonil (CGA173506): Determination of the effects on the growth rate of juvenile rainbow trout (*Oncorhynchus mykiss*) using a continuous flow test system, Report Number BL8185/B, Brixham Environmental Laboratory, Brixham, UK (Syngenta File No. CGA173506/6704)

Guidelines

OECD Guidelines for Testing of Chemicals. Test Guideline 215, Fish, Juvenile Growth Test. Adopted 21 January 2000.

GLP: Yes

Executive Summary

The effects of fludioxonil on the growth of juvenile rainbow trout (*Oncorhynchus mykiss*) were evaluated. This study was run with nominal concentrations of 5.0, 10, 20, 40 and 80 µg fludioxonil/L together with a dilution water control and solvent control (triethyleneglycol).

Analysis of variance was used to test for differences between the treatments and controls. Based on the Pseudo Specific Growth Rate (PSGR) for weight and length and mean measured concentrations the NOEC was determined to be 40 µg fludioxonil/L.

A Lowest observed effect concentration on length and weight (LOEC) could not be determined due to the absence of significant effects on length and weight on surviving fish

Materials

Test Material:	Fludioxonil
Description:	Yellow brown powder
Lot/Batch #:	P 910007
Actual content of a.s.:	96.4%
Stability of test compound:	Not given
Test concentrations:	Dilution water control and nominal concentrations of 5.0, 10, 20, 40 and 80 µg fludioxonil/L
Vehicle and/or positive control:	Triethyleneglycol (100 µL/L)
Analysis of test concentrations:	Yes
Test animals	
Species:	Rainbow trout *Oncorhynchus mykiss*
Source:	Houghton Springs Fish Farm, Dorset, UK
Acclimatisation period:	In excess of 2 weeks
Treatment for disease:	Amoxycillin at 80 mg/kg for 10 days, completed 61 days prior to the test
Weight and length of dilution water control fish at start of exposure period:	Weight: range 1.80 - 2.71 g, mean 2.28 g Length: range 47 - 55mm, mean 52 mm
Feeding:	Commercial fish food (batch ref FF601) at a rate of 4% of the body weight of the total number of fish present per day. From day 0 to 12 the ration was based on the initial total weight of fish for each vessel. The ration was re-calculated when the fish were weighed again on day 14. The ration was divided into two feeds per day on each weekday and one feed per day at the weekends.
Environmental conditions	
Test temperature:	15 ± 1°C (14.6 to 15.1°C)
pH range:	7.3 to 7.8
Dissolved oxygen:	8.6 to 10.2
Total hardness of dilution water:	38.7 - 44.3 mg/L $CaCO_3$
Conductivity of dilution water	230 - 245 µS/cm @ 25°C
Lighting:	16 hours fluorescent light and 8 hours dark with 20 minute dawn and dusk transition periods
Length of test:	28 days

Study Design and Methods

Exposure dates: 11th August to 8th September 2005

Test procedure and apparatus

The apparatus used in this test was a continuous flow-through system. The test vessels, dosing lines, mixing chambers and stock vessels were all constructed of glass. A minimum quantity of silicone rubber tubing was used to connect the components.

Glass vessels (610 × 305 × 310 mm, length × width × height) with a maximum capacity of 54 litres and a working volume of 45 litres were used to hold the test fish. The test vessels had loosely fitting glass covers. The test solutions were renewed at a nominal rate of 250 mL/min, to give water removal rates of >10 litres per g of fish per day on day 0.

The stock solutions were fed to the glass mixing chambers using a peristaltic pump and the dilution water was supplied using a capillary flow control system. Magnetic stirrers in the mixing chambers ensured thorough mixing before the test solutions passed into the exposure vessels. The dilution ratio of the stock solutions to dilution water was nominally 1:10 000 in all concentrations.

At the start of the test, individual fish were randomly selected and anaesthetised using a general anaesthetic (MS222 0.1 g/L, neutralised by the addition of 2 parts sodium bicarbonate to 1 part MS222). Each fish was then placed on a pad of moist tissue before being weighed and measured. The fish were transferred to a labelled bucket containing aerated dilution water from each tank to recover from the anaesthetic. When all fish had recovered, they were transferred to the test vessels. Each test vessel contained 10 fish.

Preparation of test solutions

Five nominal concentrations, 5.0, 10, 20, 40 and 80 µg fludioxonil/L, together with a dilution water and solvent control were used in the study.

A 0.8 g fludioxonil/L stock concentrate was prepared by dissolving 0.52 g of fludioxonil in 650 ml of triethyleneglycol (trigol). The solution was then stirred for a minimum of 30 minutes to ensure thorough mixing and used to prepare a series of stock solutions.

The series of stock solutions were prepared by the addition of the required volume of stock concentrate and trigol to 500 ml stock jars.

Each stock solution was connected to a peristaltic pump and the solutions were delivered at a nominal flow rate of 0.025 ml/min to the mixing chambers. The stock solutions were replenished at approximately weekly intervals during the exposure phase of the study (on exposure days 1, 8, 15 and 22).

The solvent control and each concentration of fludioxonil contained a constant nominal 100 µl/L of trigol. The test solutions were not aerated during the test.

Observations for mortality and symptoms of toxicity

Observations for mortalities were made daily. Detailed observations of feeding response and symptoms of toxicity were made on days 4, 7, 10, 15 and 22. Any dead fish were removed, but not replaced, and the feeding rate adjusted accordingly.

Fish weights and lengths

The weight and length of each fish were recorded on days 0, 14 and on termination of the study at day 28. Fourteen days after the start of the test, after having been starved for 24 hours, the fish were anaesthetised and measured for weight and length. Following this repeat weighing, the fish were returned to their respective tanks for a further 14 days exposure. At the end of the 28 day exposure period, the fish were again anaesthetised and measured, after having been starved for 24 hours.

Growth rates

For each individual fish Pseudo Specific Growth Rates (PSGR), based fish weight and length, were calculated. The PSGR is defined as follows:

$$PSGR = \frac{\log_e W_2 - \overline{\log_e W_1}}{t_2 - t_1} \times 100$$

where:

W_1, W_2 = weights (or lengths) of a particular fish at times t_1, and t_2 respectively

$\log_e W_2$ = logarithm of the weight (or length) of an individual fish at the end of the study period

$\overline{\log_e W_1}$ = average of the logarithms of the values W_1 for the fish in the tank at the start of the study period

t_1, t_2 = time (days) at start and end of study period

Growth rates were calculated over the following time periods:

(i) $t_1 = 0$, $t_2 = 14$ days
(ii) $t_1 = 14$, $t_2 = 28$ days
(iii) $t_1 = 0$, $t_2 = 28$ days

Statistical analysis

The Pseudo Specific Growth Rate (PSGR) for each individual fish were entered into electronic data files and analysed using statistical procedures. Firstly the dilution water and solvent controls were compared to determine whether the controls could be pooled.

In each case the data were tested for normality and Levene's or Bartlett's test for homogeneity of variances was applied. If the data were normal and had homogeneous variances a parametric statistical procedure such as analysis of variance followed by Dunnett's T test was performed to test for significant differences between treatments. If the data had heterogeneous variances or was found to be non-normal then the data were analysed using a non-parametric statistical procedure such as Kruskal Wallis followed by Mann-Whitney to test for significant differences between treatments.

Analytical method

The concentrations of fludioxonil in the test solutions were measured on days 0, 7, 14, 21 and 28 using a high performance liquid chromatography method.

Physical and chemical parameters

Calibration of the dilution water flow rates was undertaken daily for the first two days of the test and thereafter three times per week.

Temperature values in the test vessels were determined daily for the first two days of the test and thereafter three times per week. Hourly temperature measurements were also recorded automatically in the dilution water control using an electronic recording system.

Measurement of pH and dissolved oxygen concentration in the test vessels was undertaken daily for the first two days of the test and thereafter twice per week. During the test, the pH, alkalinity, conductivity and hardness of the dilution water control was measured once per week in two test vessels. The salinity of the dilution water was measured prior to the start of the exposure and thereafter once per week.

In addition, the pH, conductivity, alkalinity, total hardness, free chlorine and residual chlorine, ammonium-N, solids-suspended, total organic carbon and chemical oxygen demand of the freshwater supply were determined periodically.

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. Variability in the measured concentration did not demonstrate time dependency. The mean measured concentrations ranged from 90 to 100% of nominal values. The highest limit of quantification throughout the study was 0.58 µg fludioxonil/L. Mean measured concentrations have been used for the calculation and reporting of the results.

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. All fish in all concentrations were observed to be feeding eagerly by day 4. Fish in the highest nominal concentration of 80 µg fludioxonil/L showed loss of balance on exposure day 5 and were terminated due to the severity limit of the study being exceeded. No mortality was observed in the dilution water control, satisfying the validity criteria of <10% specified in the guideline.

Length and weight data

The mean weight of fish in the dilution water control increased from 2.28 g to 6.60 g between days 0 and 28, satisfying the criteria of achieving at least 50% increase in body weight by the end of the test.

No significant differences (P=0.05) based on length or weight, were observed for any of the time intervals or at any of the concentrations tested compared to the pooled control. Therefore, based on mean measured concentrations, the No Observed Effect Concentration (NOEC) was 40 µg fludioxonil/L. A Lowest Observed Effect Concentration (LOEC) could not be determined on surviving fish.

Mean weights and lengths and Pseudo Specific Growth Rates re given in the tables below.

Table 8.2.2-1: Effects of fludioxonil on the length and weight of juvenile *Oncorhynchus mykiss* following exposure for 28 days in a flow-through test (Maynard, 2005)

Mean measured concentration (µg/L)	Day 0		Day 14		Day 28	
	Weight (g) [±SD]	Length (mm) [±SD]	Weight (g) [±SD]	Length (mm) [±SD]	Weight (g) [±SD]	Length (mm) [±SD]
0 (dilution water control)	2.28 [0.29]	52 [2.37]	3.91 [0.46]	63 [3.02]	6.60 [1.17]	73 [6.75]
0 (solvent control)	2.14 [0.30]	52 [2.27]	3.79 [0.42]	61 [3.73]	6.62 [1.08]	74 [6.38]
4.5	2.19 [0.18]	52 [1.37]	3.85 [0.36]	63 [2.01]	6.53 [0.62]	75 [2.72]
9.2	2.18 [0.28]	51 [1.77]	3.71 [0.31]	61 [1.79]	6.29 [0.63]	73 [3.22]
20	2.13 [0.36]	52 [2.49]	3.65 [0.52]	60 [2.71]	6.19 [0.65]	72 [2.79]
40	2.08 [0.25]	51 [2.00]	3.73 [0.33]	60 [2.13]	6.46 [0.72]	73 [2.92]
80	2.17 [0.31]	52 [2.33]	-	-	-	-

- 100% mortality had occurred by Day 14

Table 8.2.2-2: Effects of fludioxonil on the Pseudo Specific Growth Rate of juvenile *Oncorhynchus mykiss* following exposure for 28 days in a flow-through test (Maynard, 2005)

Mean measured concentration (µg/L)	Mean Pseudo Specific Growth Rate (PSGR) (% increase in weight/day) [±SD]			Mean Pseudo Specific Growth Rate (PSGR) (% increase in length/day) [±SD]		
	0-14 days	14-28 days	0-28 days	0-14 days	14-28 days	0-28 days
0 (dilution water control)	3.85 [0.88]	3.66 [1.51]	3.76 [0.76]	1.35 [0.35]	1.02 [0.72]	1.19 [0.36]
0 (solvent control)	4.09 [0.78]	3.94 [1.18]	4.02 [0.59]	1.19 [0.44]	1.42 [0.64]	1.30 [0.32]
4.5	4.01 [0.66]	3.78 [0.71]	3.89 [0.35]	1.30 [0.23]	1.26 [0.26]	1.28 [0.13]
9.2	3.85 [0.61]	3.76 [0.74]	3.80 [0.37]	1.23 [0.21]	1.27 [0.32]	1.25 [0.16]
20	3.89 [1.06]	3.80 [0.76]	3.85 [0.38]	1.02 [0.32]	1.33 [0.28]	1.18 [0.14]
40	4.18 [0.61]	3.92 [0.81]	4.05 [0.40]	1.11 [0.26]	1.40 [0.28]	1.25 [0.14]

No statistical differences (P=0.05) were found at any of the concentrations tested compared to the pooled control
Growth rates for the 76 (80) µg fludioxonil/L mean measured concentration could not be calculated

Conclusions

For both weight and length Pseudo Specific Growth Rate (PSGR), the NOEC was 40 µg fludioxonil/L based on mean measured concentrations. A Lowest Observed Effect Concentration (LOEC) could not be determined on surviving fish.

(Maynard S J, 2005)

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 8.2.2/02 Taylor, S. (2015). Fludioxonil (CGA173506) – Determination of the Effects on the Growth Rate of Juvenile Rainbow Trout (*Oncorhynchus mykiss*). Statistical Reanalysis. Report Number CEA.1597. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN/UK. (Syngenta File No: CGA173506_11868).

Summary

Brixham Environmental Laboratory report number BL8185/B 108A-153 (Maynard, 2005; Syngenta file number CGA173506/6704), for the juvenile growth test of CGA173506 with rainbow trout (*Oncorhynchus mykiss*), 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 were intended to be re-analysed in an attempt to provide these values.

The test was conducted at nominal concentrations of 5, 10, 20, 40 and 80 µg a.s./L. The measured concentrations of the test item in the individual vessels ranged from 76 to 110% of the nominal values. The mean measured concentrations ranged from 90 to 100% of the measured values. As a result and in keeping with the approach used in the original study, this analysis used the mean measured exposure concentrations of 4.5, 9.2, 20, 40 and 76 µg a.s./L.

The following parameters were intended to be analysed statistically:

- Pseudo specific growth rates based on fish weight and length (day 0-14, 14-28, 0-28)

For both weight and length pseudo specific growth rates, the No Observed Effect Concentration (NOEC) provided in the original report was 40 µg a.s./L due to 100% mortality at 76 µg a.s./L, whilst a Lowest Observed Effect Concentration (LOEC) could not be determined on surviving fish due to the absence of any treatment related effects at the remaining concentrations.

Closer inspection of the pseudo specific growth rate data supports the conclusion that no effects were observed on the surviving fish and it can be reasonably concluded that no EC_{10} or EC_{20} values could be reliably determined from the data. As a result, no additional statistical analysis was performed.

Results

For both weight and length pseudo specific growth rates, the No Observed Effect Concentration (NOEC) provided in the original report was 40 µg a.s./L due to 100% mortality at 76 µg a.s./L, whilst a Lowest Observed Effect Concentration (LOEC) could not be determined on surviving fish due to the absence of any treatment related effects at the remaining concentrations.

Closer inspection of the pseudo specific growth rate data supports the conclusion that no effects were observed on the surviving fish and it can be reasonably concluded that no EC_{10} or EC_{20} values could be reliably determined from the data. As a result, no additional statistical analysis was performed.

The data for pseudo specific growth rates are summarised below:

Table 8.2.2-3: Effects of fludioxonil on the Pseudo Specific Growth Rate of juvenile *Oncorhynchus mykiss* following exposure for 28 days in a flow-through test (Maynard, 2005)

Mean Measured Conc. (µg a.s./L)	Pseudo specific growth rate for increases in weight			Pseudo specific growth rate for increases in length		
	0-14d	14-28d	0-28d	0-14d	14-28d	0-28d
Diluent control	3.85	3.66	3.76	1.35	1.02	1.19
Solvent control	4.09	3.94	4.02	1.19	1.42	1.30
4.5	4.01	3.78	3.89	1.30	1.26	1.28
9.2	3.85	3.76	3.80	1.23	1.27	1.25
20	3.89	3.80	3.85	1.02	1.33	1.18
40	4.18	3.92	4.05	1.11	1.40	1.25

Conclusion

Inspection of the pseudo specific growth rate data supported the originally reported findings that no effects were observed on the surviving fish and it can be reasonably concluded that no EC_{10} or EC_{20} values could be reliably determined from the data. As a result, no additional statistical analysis was performed.

(Taylor S, 2015)

CA 8.2.2.1 Fish early life stage toxicity test

The studies previously submitted in the EU were summarised in point B.9.2.2.2 of the DAR ~~therefore additional study summaries are not repeated here~~. The endpoints are summarised in Table 8.2-3.

The RMS requested that a justification is provided for the robustness and the reliability of the NOEC values. Syngenta's response is below:

The valid fish chronic studies, relevant to the risk assessment, were all conducted according to the current guideline at the time.

Both ELS studies with sheepshead minnow (*Minderhout, 2011* and *Marini 2014*) meet the current guideline (*OECD 210 (2013)*, *OCSPP 850:1400 (1996)*) requirement with regard to number of organisms and number of replicates. The fathead minnow ELS study (*Graves, 1994*), employed only two reps, rather than four (standard at the time), however the number of eggs used was up to current standards. The proposed new chronic endpoint is taken from a Fish Full Life Cycle study (*Marini, 2015*), conducted to the current guideline (*OCSPP 850.1500 (1996)*).

Each study is considered valid (meeting current criteria) and robust statistical tests were used to identify clear NOECs.

EFSA have confirmed that assessing the statistical power of a NOEC from a lower tier laboratory test is not necessary (*EFSA; Outcome of the pesticides peer review meeting (133) on general recurring issues in ecotoxicology, 2015*).

A study has been conducted with *Pimephales promelas* (Graves *et al*., 1994; Syngenta file number CGA173506/0416). This study was evaluated during the EU review and used to set the EU endpoint for chronic fish toxicity, in conjunction with the fish prolonged study on rainbow trout (Maynard 2005; Syngenta file number CGA173506/6704).

At the request of the RMS, the summary of the Graves *et al*., 1994 report (Syngenta file number CGA173506/0416) is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoints are summarised in Table 8.2-3 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.2.2 Graves W. C., Mank M. A. & Swigert J. P. (1994). CGA-173506 Early-Life Stage Toxicity test with the fathead minnow (Pimephales promelas), Report Number 108A-153. Wildlife International Ltd., 8598 Commerce Drive, Maryland 21601, USA. (Syngenta File No. CGA173506/0416)*

Guidelines

FIFRA Subdivision E, Series 72-4

GLP: Yes

Executive Summary

The toxicity of CGA173506 to early-life stages of fathead minnow (*Pimephales promelas*) was determined using a flow-through exposure system. Fish were exposed to the following range of nominal concentrations of 9.4, 19, 38, 75 and 150 µg a.s./L, a solvent control and a dilution water control. The mean measured concentrations were 10, 20, 39, 77 and 144 µg a.s./L.

Post hatch survival was the most sensitive biological parameter, with a significant reduction at 77 and 144 µg a.s./L when compared to the control groups. No significant adverse effects on growth were found at 10, 20 and 39 µg a.s./L, although mean lengths and weights at the 20 and 39 µg a.s./L treatment levels were slightly shorter and lower than those in the control groups.

The NOEC for survival and growth was 39 µg a.s./L. The LOEC was 77 µg a.s./L, based upon a statistically significant reduction in survival at that concentration.

Materials

Test Material	CGA173506
Description:	White Powder
Lot/Batch #:	P905004/5
Actual content of a.i.:	95.4%
Stability of test compound:	Reanalysis date 16 April 1994

Treatments	
Test concentrations:	Nominal: 9.4, 19, 38, 75 and 150 µg a.s./L Mean measured 10, 20, 39, 77 and 144 µg a.s./L
Control:	Dilution water is dechlorinated tap water
Vehicle and/or positive control:	Acetone
Analysis of test concentrations:	Yes, days 0, 7, 14, 21, 28 and 33 (based on measurement of CGA173506)

Test animals	
Species:	Fathead minnow (*Pimephales promelas*)
Source:	Cultures maintained at Wildlife International Ltd. Laboratories
Treatment for disease:	None
Feeding:	Fed shrimp nauplii (Artemia) 3 times a day for first 7 days. From days 8 to 26 they were fed 3 times daily on weekdays and twice daily on weekends

Test design	
Exposure regime:	Flow-through
Replication:	2
Test vessels:	9 L glass tanks with a working volume of 7.5 L
No of eggs per tank:	40
Duration:	33 days

Environmental conditions

Test temperature:	24.6 to 25.3 ºC
pH:	7.9 to 8.4
Dissolved oxygen:	5.2 to 8.2 mg/L
Hardness of dilution water:	140 - 144 mg/L as $CaCO_3$
Conductivity of dilution water:	345 - 380 µS/cm
Lighting:	16 hours fluorescent light and 8 hours dark with 30 minute dawn and dusk transition periods (269 lux)

Study Design and Methods

Experimental dates: 15th September to 20th October 1993

A flow-through test system was employed. At the start of the test 40 eggs were randomly allocated to 2 egg cups suspended in each of the 2 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 were exposed to mean measured concentrations of 10, 20, 39, 77 and 144 µg a.s./L, a solvent control and a dilution water control.

One test substance stock solution was prepared at weekly intervals for each of the five test concentrations. The primary stock solution was prepared by dissolving technical grade CGA173506 in acetone at a concentration of 0.562 mg/mL. The stock solution was inverted several times to facilitate mixing. Aliquots of the primary stock solution then were diluted with acetone to prepare four additional stock solutions at concentrations of 0.281, 0.141, 0.0703 and 0.0351 mg/ml. The stock solutions were delivered by peristaltic pumps to the diluter mixing chambers where they were mixed with well water to achieve the desired test concentrations. Test substance concentrations were adjusted for purity of the active substance and are presented as micrograms of active substance per litre. The solvent concentration in the treatment and the solvent control test chambers was 0.28 mL/L. Although the solvent concentration exceeded the concentration recommended in the testing guidelines, this was done to enhance solubilisation of the test substance. A concentration of 0.28 mL/L was considered to be within the tolerance of the fathead minnow for early life stage toxicity testing based on solvent control data.

The concentrations of CGA173506 in test solutions were measured at 0, 7, 14, 21, 28 and 33 days using an HPLC method. Samples for analysis were taken from the centre of the test solutions.

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) were estimated from the data obtained as follows:

Quantal responses

Egg survival: the number of eggs at the start of the test (day 0) minus the number of dead eggs identified on day 5, expressed as a percentage of the number of eggs at the start of the test.

Hatching success: the number of live larvae on the day they are transferred from the egg cups to the test vessels (day 5), expressed as a percentage of the number of eggs at the start of the test (day 0).

Survival at the end of the test: the number of surviving fry at the end of the test (day 33) expressed as a percentage of the number of live larvae on the day they were transferred from the egg cups to the test vessels (day 5).

Non-quantal responses

Length: mean total length of surviving fry per replicate at test end.

Dry weight: mean dry weight of surviving fry per replicate at test end.

Statistical analysis

Hatching success, post hatch survival, wet weight, dry weight and total length of the juvenile fish were test endpoints that were analysed statistically.

Data collected during this test were either continuous-variable data (e.g. weight and length) or discrete-variable data (e.g. mortality proportions). Discrete-variable data were first transformed using the arc-sine-square root transformation and then analysed in a manner similar to continuous variable data described below.

All continuous-variable data and transformed discrete-variable data were evaluated for normality using either a chi square test or Kolmogorov-Smirnov test and for homogeneity of variance using the Bartlett's test. Negative control and solvent control groups were compared using the students T-test. Since no differences were detected between the two control groups, they were pooled and used to assess treatment level effects. For data with unequal replicate sample sizes which passed both homogeneity of variance and normality tests, Bonferronni's test or Scheffe's multiple comparison test were used to evaluate differences between treatment and control means. If the data set failed to pass the tests for either normality or homogeneity, then the Kruskal-Wallis test was used to evaluate differences between the treatment and control groups.

The result of the statistical analyses were used to aid in the determination of the NOEC and the LOEC. The maximum acceptable toxicant concentration (MATC) was calculated as a geometric mean of the NOEC and LOEC.

Results and Discussion

Analytical data

The concentrations of CGA173506 were determined in the test solutions. The mean measured concentrations ranged from 81% to 125% of their nominal concentrations. The limit of detection was 0.2 µg/L. The mean measured concentrations were used for calculating and reporting the results.

Table 8.2.2.1-1: Analytical results

Nominal Concentration (µg a.s./L)	Measured Concentration (mg/L)							Percent of Nominal (%)
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 33	Mean	
Control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	na	na
Solvent control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	na	na
9.4	8.77	10.94	11.73	9.73	10.59	9.75	10	106
19	16.5	21.7	22.3	19.5	21.3	19.0	20	105
38	34.6	42.5	40.6	37.9	40.9	39.2	39	103
75	67.6	83.1	79.4	75.7	82.7	75.6	77	103
150	122	156	147	140	151	149	144	96

na not applicable

Biological data

Table 8.2.2.1-2: Effects of CGA173506 on the survival and growth of early life stages of fathead minnow

Mean measured concentration	Quantal responses		Non quantal responses	
(µg a.s./L)	Hatching success (%)	Post-hatch survivial (%)	Mean length (mm) ± SD	Mean dry weight (mg) ± SD
Control	86	88	19.2 (2.9)	12.7 (4.6)
Solvent control	88	87	19.6 (2.6)	11.8 (3.5)
10	82	89	20.1 (2.9)	14.1 (5)
20	90	88	19.0 (2.6)	12.5 (4.5)
39	81	87	18.5 (3.1)	12.0 (5.4)
77	86	70*	17.1 (2.9)**	9.8 (3.9)**
144	84	65*	15.8 (2.3)**	8.2 (3.1)**

* Significantly different from the pooled control
** Not included in statistical analysis due to significant reductions in survivial

Validity Criteria

No validity criteria are reported.

Conclusions

Post-hatch survival was the most sensitive biological parameter with a significant reduction at 77 and 144 µg a.s./L when compared to the control groups. No significant adverse effects on growth were found at 10, 20 and 39 µg a.s./L, although mean lengths and weights at the 20 and 39 µg a.s./L treatment levels were slightly shorter and lower than those in the control groups.

The NOEC for the effects of CGA173506 on the early life stages of fish was determined to be 39 µg a.s./L. The LOEC was 77 µg a.s./L, based upon a statistically significant reduction in survival at that concentration. The geometric mean of the NOEC and the LOEC was 55 µg a.s./L.

(Graves et al. 1994)

This study complies with the current validity criteria for early life-stage testing with fish (OECD 210; 2013) and thus is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Graves et.al., 1994, CGA173506/0416	US EPA FIFRA No. 72-4	OECD No. 210 (2013), or US EPA OPPTS 850.1400 (2016)	Compliant with OECD No. 210 (2013). US EPA OPPTS 850.1400 (2016): Solvent concentration was > 0.1 mL/L	The validity criteria were met: 1. Overall survival of fertilised eggs and post-hatch success in the controls and solvent controls was ≥70 and 75% respectively (all values ≥86%). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits 3. Evidence that the concentration being tested had been satisfactorily maintained (mean measured concentrations were in the range 96 to 106%) 4. DO ≥ 60% ASV (4.9 mg/L represents 60% saturation at 25ºC (measured values were 5.2 to 8.2 mg/L) The solvent (acetone) concentration in the treatment and solvent control groups was 0.28 mL/L; this concentration is considered to be within the tolerance of fathead minnow for early life stage toxicity testing (as confirmed by the absence of effects observed in the solvent control). No statistically significant differences were detected between the negative controls and the solvent controls (p>0.05); negative control and solvent control data were pooled prior to statistical comparison with the treatment data. The study is reliable and the endpoint can be used in the risk assessment.

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., Priestly S. & Allen M. (2016). Fludioxonil (CGA173506) – An Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*) Statistical reanalysis, Report Number CEA.1592. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN/UK. (Syngenta File No: CGA173506_11911).

Summary

The report from Wildlife International Ltd. report number 108A-153 (Graves *et al*., 1994; Syngenta file number CGA173506/0416), for the toxicity test of CGA173506 with fathead minnow (*Pimephales promelas*) embryo and larvae, 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 have been re-analysed in an attempt to provide these values.

The following parameters were analysed statistically:

- Survival of organisms at hatch
- Post-hatch survival
- Mean larval fresh and dry weight
- Mean total larval length

Statistical analyses of the available data for posh-hatch survival, fresh weight, dry weight and total length revealed that the following EC_{10} and EC_{20} values were reliably calculated:

Table 8.2.2.1-13: Statistical analysis for EC_{10} and EC_{20} endpoints for the fludioxonil fish early life stage study on fathead minnow *Pimephales promelas* (Graves *et al.*, 1994; Syngenta file number CGA173506/0416)

Parameter	Post-Hatch Survival		Fish Fresh Weight	
	EC_{10}	EC_{20}	EC_{10}	EC_{20}
Value [µg/L]	14.023	45.544	37.761	65.840
lower 95%-cl	4.058	27.626	17.527	32.260
upper 95%-cl	23.996	73.219	81.355	134.622
Parameter	**Fish Dry Weight**		**Fish Total Length**	
	EC_{10}	EC_{20}	EC_{10}	EC_{20}
Value [µg a.s./L]	43.404	66.384	58.047	136.300
lower 95%-cl	20.386	33.096	33.139	75.621
upper 95%-cl	92.412	132.586	101.678	245.256

cl: confidence limits

No EC_{10} or EC_{20} values could be reliably calculated from the available data for hatching.

Statistical Analysis

Where possible, non-linear regression was used to derive the ECx values. However, in cases where this approach did not provide reliable estimates, linear max likelihood regression was used in an attempt to derive these values.

Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

A 3-parameter normal cumulative distribution function (Bruce and Versteeg, 1992) was fitted to the original data and a non-linear regression without weighting was performed using the Levenberg-Marquardt algorithm to calculate the function parameters. Analysis of variance (ANOVA) and a test for the lack of fit was then performed to determine the probability that the variance explained by the regression analysis was due to chance. Where p (F:Regression) <= 0.05, a significant amount of the variance is explained by the regression and where p(F:Lack of Fit) > 0.05, there is no significant lack of fit. If both of these criteria are met, the results of the regression analysis are accepted and the point estimates from the 3-parameter normal cumulative distribution function are meaningful.

Results

Survival of organisms at hatch

The relationship between dose and response was not significant, p(F) = 0.999 and as a result, no reliable EC_{10} and EC_{20} values could be calculated.

Post-hatch larval survival

The relationship between dose and response was significant, p(F) = 0.030 and the slope function was 14.552. The EC_{10} and EC_{20} values and their 95% confidence limits presented were reliably determined.

Fish fresh weight

A significant amount of variance was explained by the regression model (p(F):Regression = <0.001) and there was no significant lack of fit (p(F):Lack of fit = 0.183). As a result, the EC_{10} and EC_{20} values and their 95% confidence limits presented were reliably determined.

Fish dry weight

A significant amount of variance was explained by the regression model (p(F):Regression = <0.001) and there was no significant lack of fit (p(F):Lack of fit = 0.138). As a result, the EC_{10} and EC_{20} values and their 95% confidence limits presented were reliably determined.

Fish total length

A significant amount of variance was explained by the regression model (p(F):Regression = <0.001) and there was no significant lack of fit (p(F):Lack of fit = 0.411). As a result, the EC_{10} and EC_{20} values and their 95% confidence limits presented were reliably determined.

Conclusion

The mean measured concentrations of CGA173506 ranged from 96 to 106% of the nominal concentrations. In keeping with the approach used in the original study, the mean measured values of 10, 20, 39, 77 and 144 µg a.s./L were used in the calculation the results from this analysis.

Statistical analyses of the available data for posh-hatch survival, fresh weight, dry weight and total length revealed that the following EC_{10} and EC_{20} values were reliably calculated:

Table 8.2.2.1-24: Statistical analysis for EC_{10} and EC_{20} endpoints for the fludioxonil fish early life stage study on fathead minnow *Pimephales promelas* (Graves *et al.*, 1994; Syngenta file number CGA173506/0416)

Parameter	Post-Hatch Survival		Fish Fresh Weight	
	EC_{10}	EC_{20}	EC_{10}	EC_{20}
Value [µg/L]	14.023	45.544	37.761	65.840
lower 95%-cl	4.058	27.626	17.527	32.260
upper 95%-cl	23.996	73.219	81.355	134.622
Parameter	**Fish Dry Weight**		**Fish Total Length**	
	EC_{10}	EC_{20}	EC_{10}	EC_{20}
Value [µg a.s./L]	43.404	66.384	58.047	136.300
lower 95%-cl	20.386	33.096	33.139	75.621
upper 95%-cl	92.412	132.586	101.678	245.256

cl: confidence limits

No EC_{10} or EC_{20} values could be reliably calculated from the available data for hatching.

(Taylor S, Priestly S & Allen M, 2016)

Two fish ELS studies have been conducted as part of a US EPA Data Call-In (DCI), using the Sheepshead minnow, an estuarine species are summarised below:

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.2.1/02 Minderhout T., Kendall T.Z., & Krueger H.O. (2011) Fludioxonil - An Early Life-Stage Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*), Report Number 528A-217, Wildlife International, Ltd., 8598 Commerce Drive, Easton, MD 21601 USA. (Syngenta File No. CGA173506_11516

Guidelines

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

OPPTS 850.1400 Fish, Early-life Stage Toxicity test (1996)

GLP: Yes

Executive Summary

The toxicity of fludioxonil to early-life stages of Sheepshead minnow (*Cyprinodon variegatus*) was determined. Fish were exposed to the following range of nominal concentrations of 6.4, 16, 40, 100, 250 and 625 µg a.s./L, a solvent control and a dilution water control under flow-through conditions for 34 days (a 6-day hatching period plus a 28-day post-hatch growth period). The mean measured concentrations were 6.2, 16, 40, 101, 246 and 575 µg a.s./L.

There were statistically significant treatment-related effects on hatching success and survival at the 625 µg a.s./L treatment level and on growth at the 250 µg a.s./L treatment level. Consequently, the NOEC (based on nominal concentrations) was 100 µg a.s./L. The LOEC was 250 µg a.s./L.

Materials

Test Material	Fludioxonil technical
Description:	Solid
Lot/Batch #:	428860
Purity:	95.4%
Density:	n/a
Stability of test compound:	Reanalysis date: February 2012
Treatments	
Test concentrations:	Nominal: 6.4, 16, 40, 100, 250 and 625 µg a.s./L Mean measured 6.2, 16, 40, 101, 246 and 575 µg a.s./L
Control:	Filtered seawater
Vehicle and/or positive control:	Dechlorinated tap water with dimethylformamide (DMF) No positive control
Analysis of test concentrations:	Yes, days 0, 7, 14, 21, 28 and 34
Test animals	
Species:	Sheepshead minnow (*Cyprinodon variegatus*)
Source:	Aquatic Bio Systems, Inc., Fort Collins, Colorado. Embryos were 25 hours post-fertilization at test initiation.

Acclimatisation period:	None
Treatment for disease:	None
Feeding:	Newly-hatched larvae were fed live brine shrimp nauplii (*Artemia* sp.) three times per day during the test.
Test design	
Exposure regime:	Flow-through
Aeration:	Gentle
Replication:	4
Test vessels:	9 L glass tanks with a working volume of 7 L
No of eggs per tank:	20
Duration:	34 days (a 6-day hatching period plus a 28-day post-hatch growth period)
Environmental conditions	
Test temperature:	24.7 to 25.2°C
pH:	8.0
Dissolved oxygen:	6.6 to 7.1 mg/L
Lighting:	16 hours fluorescent light and 8 hours dark with 30 minute dawn and dusk transition periods

Study Design and Methods

Experimental dates: 18th March to 29th April 2011.

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 6.4, 16, 40, 100, 250 and 625 µg a.s./L, a solvent control and a dilution water control. All test solutions and the solvent control contained 0.1 mL DMF per litre, with the control containing dilution water only.

Primary test substance stock solution was prepared in HPLC-grade DMF at a nominal concentration of 6.25 or 2.5 mg a.s./mL. Proportional dilutions of the primary stock were made in DMF to prepare additional stock solutions at nominal concentrations of 0.064, 0.16, 0.40, 1.0 and 2.5 mg a.s./mL. The stock solutions were delivered to the diluter mixing chambers (at a rate of 20 L/minute) where they were mixed with dilution water (at a rate of 200 mL/minute) to achieve the desired test concentrations of 6.4, 16, 40, 100, 250 and 625 µg a.s./L.

The concentrations of fludioxonil in test solutions were measured at 0, 7, 14, 21, 28 and 34 days using an HPLC method. Samples for analysis were taken from the centre of the test solutions.

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) were estimated from the data obtained.

Statistical analysis

Test endpoints analysed statistically for the juvenile fish were hatching success, larval survival and growth (total length, wet weight and dry weight). The results of the statistical analyses were used to determine the NOEC and LOEC. However, scientific judgement was used to determine if statistical

differences were biologically meaningful, and if the data followed a concentration-dependent response. The NOEC was defined as the highest test concentration that produced no significant treatment-related effects on hatching success, survival or growth. The LOEC was defined as the lowest test concentration that produced a significant treatment-related effect on hatching success, survival or growth.

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 ($p \leq 0.05$) from the pooled controls. All continuous-variable data were evaluated for normality using Shapiro-Wilk's test, and for homogeneity of variance using Bartlett's and/or Levene's tests ($p = 0.01$). Since the data passed the assumptions of normality and homogeneity of variances, those treatments that were significantly different from the control means were identified using Dunnett's one-tailed test ($p \leq 0.05$). All statistical tests were performed using SAS software.

Results and Discussion

The mean measured concentrations ranged from 92 to 101% of their nominal concentrations. The limit of quantification was 3.00 µg a.s./L. The nominal concentrations were used for calculating and reporting the results.

Table 8.2.2.1-35: Analytical results

Nominal Concentration (µg/L)	Measured Concentration (µg/L)							Percent of Nominal (%)
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 34	Mean (SD[b])	
Control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	na	na
Solvent control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	na	na
6.4	5.71	7.09	5.90	6.24	6.30	6.40	6.2	97
16	16.6	16.8	15.5	14.8	16.5	15.2	16	100
40	40.8	40.6	40.9	39.6	39.7	39.7	40	100
100	99.7	97.4	95.2	97.9	121	102	101	101
250	236	253	248	221	237	294	246	98
625	545	667	567	591	-	-	575	92

na not applicable
- no data as 100% mortality occurred

Time to hatch

Embryos in the controls and 6.4 to 250 µg a.s./L treatment replicates hatched on Days 5 to 8 of the test, and larvae were released into the test chambers on Days 6 to 8 of the test. Embryos in the 625 µg a.s./L hatched from Day 6 to Day 12 of the test. 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 fludioxonil treatment groups, with the exception of the 625 µg a.s./L group

Hatching success

There were statistically significant decreases in hatching success at 16, 40 and 625 µg a.s./L treatment groups when compared to the pooled controls ($p \leq 0.05$). However, the reductions noted in the 16 and 40 µg a.s./L treatment groups were not dose-responsive. All of the treatment groups except the highest treatment level (625 µg a.s./L) had percent hatching success above the control validity criterion of 75%, and these very minor differences in percent hatching success were not considered to be treatment-related. Therefore, the NOEC for hatching success was 250 µg a.s./L and the LOEC was 625 µg a.s./L.

Larval survival

Statistically significant decreases in larval survival were noted at the 625 µg a.s./L treatment group when compared to the pooled controls data ($p \leq 0.05$). Consequently, the NOEC and LOEC based on percent larval survival at test termination were 250 and 625 µg a.s./L, respectively.

Biological observations

The majority of the fish in the negative control and solvent control and the 6.4, 16 and 40 µg a.s./L treatment groups appeared normal throughout the test, with occasional observations of small or lethargic fish. Fish in the 100 µg a.s./L treatment groups were observed to be small and/or lethargic. Fish in the 250 and 625 µg a.s./L treatment groups were noted with observations of sublethal effects including lethargy, erratic swimming, loss of equilibrium, small, weak, lying on the bottom of the test chamber and/or discoloration throughout the test. All fish in the 625 µg a.s./L treatment group were dead by Day 17 of the test (Day 11 post-hatch). Since the observations of small and/or discoloration of fish in the 6.4 to 100 µg a.s./L treatment groups were generally restricted to a few individuals, did not increase in a dose-responsive manner and were generally comparable to the control groups, none of the sublethal effects noted for the fish in the 6.4 to 100 µg a.s./L treatment groups were considered to be treatment-related. However, the sublethal effects noted in the 250 and 625 µg a.s./L treatment groups were considered to be treatment-related.

Growth

Since there were statistically significant differences in the growth parameters between the negative and solvent control groups ($p \leq 0.05$), the treatment data were compared to the solvent control data. Statistically significant reductions in mean total length, mean wet and dry weight were noted among fish in the 250 µg a.s./L treatment groups in comparison to the solvent control (Dunnett's one-tailed test, $p \leq 0.05$). Consequently, the NOEC was 100 µg a.s./L and the LOEC was 250 µg a.s./L, based on growth.

The results are presented in the table below.

Table 8.2.2.1-46: Effects of fludioxonil on the growth of sheepshead minnow (*Cyprinodon variegatus*)

Nominal concentration (µg a.s./L)	Hatching success (%) [1]	Percent Survival to Day 28 Post-Hatch	Day 28 post hatch		
			Mean Total Length ± SD (mm)	Mean Wet Weight ± SD (mg)	Mean Dry Weight ± SD (mg)
Control	96	100	20.0 ± 0.52	101.6 ± 9.2	24.1 ± 1.6
Solvent control	95	99	19.1 ± 0.34	85.4 ± 4.2	20.1 ± 0.93
Pooled control	96	99	--[2]	--[2]	--[2]
6.4	90	99	19.4 ± 0.24	93.3 ± 3.7	22.4 ± 0.85
16	89*	100	19.2 ± 0.22	88.6 ± 3.6	20.6 ± 0.83
40	89*	100	19.5 ± 0.10	91.2 ± 2.2	22.4 ± 0.54
100	93	96	20.0 ± 0.082	97.7 ± 3.8	23.6 ± 0.59
250	90	97	17.0 ± 0.51 3	55.9 ± 5.2 3	13.7 ± 1.1 3
625	64*	0*	--[4]	--[4]	--[4]

* Statistically significant differences in hatching success and survival at test end from the pooled controls (Fisher's Exact test, $p \leq 0.05$).

[1] There were statistically significant reductions in hatching success noted at 16 and 40 µg a.s./L treatment groups in comparison to the pooled controls (Fisher's Exact test, $p \leq 0.05$). However, the reductions noted were not dose responsive.

[2] The negative control and solvent control data for mean total length, mean wet and dry weights were significantly different ($p \leq 0.05$). Therefore, the treatment data for all growth parameters were compared to the solvent control data.
[3] There were statistically significant reductions in mean total length, mean wet and dry weights in comparison to the solvent control (Dunnett's one-tailed test, $p \leq 0.05$).
[4] There were no growth data for the 625 µg a.s./L treatment replicates due to 100% mortality prior to test termination.

Conclusions

For sheepshead minnows (*Cyprinodon variegatus*) exposed to fludioxonil under flow-through conditions for 34 days (a 6-day hatching period plus a 28-day post-hatch growth period)., statistically significant treatment-related effects on hatching success and survival were noted at the 625 µg a.s./L treatment level. Growth was the most sensitive end point in this study with statistically significant reductions noted at the 250 µg a.s./L treatment level. Consequently, the NOEC was 100 µg a.s./L and the LOEC was 250 µg a.s./L..

(Minderhout T, *et al*., 2011)

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 8.2.2.1/03 Taylor, S. & Radford F. (2015). Fludioxonil (CGA173506) – An Early Life-Stage Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*) Statistical Reanalysis. Report Number CEA.1606. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN/UK. (Syngenta File No: CGA173506_11875).

Summary

Wildlife International Ltd. report number 528A-217 (Minderhout *et al.*, 2011; Syngenta file number CGA173506_11516) for the toxicity of CGA173506 to the sheepshead minnow (*Cyprinodon variegatus*) did not provide estimates of the EC_{10}, EC_{20} or EC_{50} for the response variables evaluated as part of the original study. Consequently the data generated have been re-analysed in an attempt to provide these values

The study was conducted for a period of 34 days (6 days hatch and 28 days post-hatch) at nominal concentrations of 6.4, 16, 40, 100, 250 and 625 µg a.s./L. Measured concentrations of CGA173506 in the test media confirmed that the nominal values were achieved and maintained throughout the test giving mean measured values of 6.2, 16, 40, 101, 246 and 575 µg a.s./L. As a result and in line the approach used in the original study, the results of this analysis are based on the nominal test item concentrations.

The following parameters were analysed statistically using the methods described in the Section 3.0 of this report:

- Egg hatchability
- Post-hatch survival
- Fish weight (fresh and dry)
- Fish length

Statistical analyses of the available data revealed that the following $EC_{10,}$ EC_{20} or EC_{50} values were reliably calculated (no $EC_{10,}$ EC_{20} or EC_{50} values could be determined for hatchability and post-hatch survival and no EC_{20} or EC_{50} values could be determined for fish length due to the lack of suitable response functions):

Table 8.2.2.1-57: Statistical analysis for EC_{10} and EC_{20} endpoints for the fludioxonil fish early life stage study on Sheepshead minnow *Cyprinodon variegatus* (Minderhout *et al.*, 2011; Syngenta file number CGA173506_11875)

Parameter	Fresh Weight			Dry Weight		
	EC_{10}	EC_{20}	EC_{50}	EC_{10}	EC_{20}	EC_{50}
Value [µg a.s./L]	68.760	174.084	1029.288	71.989	196.819	1348.021
lower 95%-cl	58.267	144.401	722.168	61.088	161.508	916.566
upper 95%-cl	80.920	216.990	1625.313	84.873	249.343	2218.104

Parameter	Length EC_{10}
Value [µg a.s./L]	485.615
lower 95%-cl	353.784
upper 95%-cl	725.941

cl: confidence limits

Statistical Analysis

Where possible, non-linear regression was used to derive the ECx values. However, in cases where this approach did not provide reliable estimates, linear max likelihood regression was used in an attempt to derive these values.

Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

A 3-parameter normal cumulative distribution function (Bruce and Versteeg, 1992) was fitted to the original data and a non-linear regression without weighting was performed using the Levenberg-Marquardt algorithm to calculate the function parameters. Analysis of variance (ANOVA) and a test for the lack of fit was then performed to determine the probability that the variance explained by the regression analysis was due to chance. Where p(F:Regression) <= 0.05, a significant amount of the variance is explained by the regression and where p(F:Lack of Fit) > 0.05, there is no significant lack of fit. If both of these criteria are met, the results of the regression analysis are accepted and the point estimates from the 3-parameter normal cumulative distribution function are meaningful.

Results

Survival of organisms at hatch

Although the relationship between dose and response was significant, p(F) = 0.045, no reliable EC_{10}, EC_{20} and EC_{50} values could be calculated as no confidence limits were able to be estimated.

Post-hatch larval survival

The relationship between dose and response was not significant, p(F) = 0.256 and as a result, no reliable EC_{10}, EC_{20} and EC_{50} values could be calculated.

Fish fresh weight

The relationship between dose and response was significant, p(F) = <0.001 and the slope function was 8.261. As a result, the EC_{10}, EC_{20} and EC_{50} values and their 95% confidence limits presented were reliably determined.

Fish dry weight

The relationship between dose and response was significant, p(F) = <0.001 and the slope function was 9.837. As a result, the EC_{10}, EC_{20} and EC_{50} values and their 95% confidence limits presented were reliably determined.

Fish total length

The relationship between dose and response was significant, p(F) = <0.001 and the slope function was 438.51. Although the EC_{10} value and the 95% confidence limits presented were reliably determined, no EC_{20} or EC_{50} values were calculated due to the lack of suitable response functions.

Conclusion

The study was conducted for a period of 34 days (6 days hatch and 28 days post-hatch) at nominal concentrations of 6.4, 16, 40, 100, 250 and 625 µg a.s./L. Measured concentrations of CGA173506 in the test media confirmed that the nominal values were achieved and maintained throughout the test giving mean measured values of 6.2, 16, 40, 101, 246 and 575 µg a.s./L. As a result and in line the approach used in the original study, the results of this analysis are based on the nominal test item concentrations.

Statistical analyses of the available data revealed that the following $EC_{10,}$ EC_{20} or EC_{50} values were reliably calculated (no $EC_{10,}$ EC_{20} or EC_{50} values could be determined for hatchability and post-hatch survival and no EC_{20} or EC_{50} values could be determined for fish length due to the lack of suitable response functions):

Table 8.2.2.1-68: Statistical analysis for EC_{10} and EC_{20} endpoints for the fludioxonil fish early life stage study on Sheepshead minnow *Cyprinodon variegatus* (Minderhout *et al.*, 2011; Syngenta file number CGA173506_11516)

Parameter	Fresh Weight			Dry Weight		
	EC_{10}	EC_{20}	EC_{50}	EC_{10}	EC_{20}	EC_{50}
Value [µg a.s./L]	68.760	174.084	1029.288	71.989	196.819	1348.021
lower 95%-cl	58.267	144.401	722.168	61.088	161.508	916.566
upper 95%-cl	80.920	216.990	1625.313	84.873	249.343	2218.104

Parameter	Length EC_{10}
Value [µg a.s./L]	485.615
lower 95%-cl	353.784
upper 95%-cl	725.941

cl: confidence limits

(Taylor S, Radford F, 2015)

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.2.1/04 Marini J (2014) Fludioxonil − Early Life-Stage Toxicity Test with Sheepshead Minnow, *Cyprinodon variegatus*, Report Number 1781.6970, Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571-1037 USA, (Syngenta File No. CGA173506_51331)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 210 (draft): Fish, Early-Life Stage Toxicity Test (2013)

US EPA Ecological Effects Test Guidelines, OCSPP 850.1400 (public draft): Fish Early Life-Stage Toxicity Test, Freshwater and Marine (1996)

GLP: Yes

Executive Summary

The chronic toxicity of fludioxonil to sheepshead minnow, *Cyprinodon variegatus,* embryos and larvae was determined under flow-through conditions for a period of 34 days (28 days post-hatch). Embryos were exposed to nominal concentrations of 19, 38, 75, 150 and 300 µg/L (21, 38, 92, 160 and 330 µg/L mean measured), alongside a dilution water control. Following 28 days post-hatch exposure, data for embryo hatching success, live, normal larvae at hatch, larval survival, mean total length, mean wet weight and mean dry weight were evaluated. Based on mean measured concentrations and growth endpoints (determined to be the most sensitive), the 28-day NOEC and LOEC values were determined to be 38 and 92 µg/L, respectively. The EC_{10} and EC_{20} values for wet weight were determined to be 36 and 81 µg/L, respectively. The EC_{10} and EC_{20} values for dry weight were determined to be 38 and 85 µg/L, respectively.

Materials

Test material	Fludioxonil
Lot/Batch #:	SMO3J0464
Purity:	97.7% (w/w); tested as 100%
Description:	Brownish powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	End of October 2017
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 19, 38, 75, 150 and 300 µg/L
Solvent:	None
Analysis of test concentrations:	Yes, analysis of fludioxonil on days 0, 6, 13, 20, 27 and 34
Test organisms	
Species:	Sheepshead minnow, *Cyprinodon variegatus*
Source:	Continuous laboratory cultures
Treatment for disease:	None

Feeding:	Beginning on day 7 (day 1 post-hatch), the larvae were fed live brine shrimp nauplii (*Artemia salina*) three times daily. At each feeding, larvae were fed live brine shrimp such that all larvae were afforded equal proportions of food (nauplii supplied *ad libitum*). Fish were not fed during the 24 hours prior to study termination.

Test design

Test vessels:	The exposure aquaria were fabricated of glass and silicone sealant. Each exposure aquarium measured 30 x 14.5 x 20 cm with a 12.5-cm high side drain that maintained a constant exposure solution volume of 5.5 L.
Test medium:	Dilute, filtered, natural seawater
Replication:	4 replicate aquaria
No of embryos per aquaria:	30
Exposure regime:	Flow-through, exposure system consisted of an intermittent-flow proportional diluter, a temperature-controlled water bath and a set of 24 exposure aquaria
Duration:	34 days (28 days post-hatch)

Environmental conditions

Test temperature:	24.6 to 26.5° C
pH:	7.3 to 8.6
Dissolved oxygen:	5.94 to 7.55 mg/L; 80.5 – 103% saturation
Salinity:	19 to 21‰
Lighting:	540 to 760 lux 16 hours fluorescent light and 8 hours dark; 30-minute transition period

Study Design and Methods

Experimental dates: 27th June to 31st July 2014

Based on the solubility and chemical properties of the test substance, a glass wool saturator column was used to deliver fludioxonil to the exposure system. The columns were designed to provide a constant flow of saturated aqueous solutions (1.3 mg/L) of the test substance to the diluter system without the use of a carrier solvent. Columns were constructed entirely of chemically inert materials (glass and Teflon®). The columns were coated by diluting approximately 20 grams of fludioxonil with 300 mL of acetone (CAS No. 67-64-1) and the solution was poured into the glass column. New saturator columns were prepared approximately every two weeks during testing.

Prior to exposure initiation, an FMI Pump was calibrated to deliver 0.45 L/cycle of the 1.3 mg/L stock solution to the diluter's mixing chamber which also received 1.48 L of dilution water per cycle for a total volume of 1.93 L per cycle. The solution contained in the mixing chamber constituted the highest nominal test concentration (300 µg/L) and was subsequently proportionately diluted (50%) to provide the remaining nominal exposure concentrations (150, 75, 38 and 19 µg/L). A set of control vessels was also established which contained the same dilution water and was maintained under the same conditions as the treatment level vessels.

The flow-through exposure system consisted of an intermittent-flow proportional diluter, a temperature-controlled water bath and a set of 24 exposure aquaria. Flow-splitting cells were employed to equally distribute the solutions to the replicate vessels at a rate of 250 mL of control and test solution per vessel per cycle. The diluter delivered the control and test solutions to the exposure aquaria (40.3 L/aquarium/day) at a rate sufficient to provide approximately seven aquarium volumes per 24-hour period, with a 90% replacement time of approximately seven hours.

Embryo incubation cups were round glass jars (5 cm O.D., 8 cm high) with the bottoms removed and 475-µm Nitex® mesh screen bottoms applied. A rocker arm apparatus driven by a 1 rpm electric motor was used to gently oscillate the incubation cups in the test solutions.

At the start of the exposure, 30 embryos (approximately 23 hours old; developmental stage range of 11-14, flat blastula to one-half epiboly) were allocated to each replicate aquaria. The exposure was conducted in a temperature-controlled water bath. At the completion of hatch (day 6), the 28-day post-hatch larval exposure was initiated with 20 impartially selected larvae from each incubation cup. Daily observations were made for larval survival, behaviour and appearance.

At 28 days post-hatch, the exposure was terminated. The surviving larvae in each exposure aquarium were euthanized, counted to determine larval survival and then measured individually to determine total length and wet weight. Larvae were then dried in an oven at a temperature of approximately 100 ºC for approximately 24 hours, cooled and individually weighed to determine dry weight.

At exposure initiation, dissolved oxygen concentration, pH, temperature and salinity were measured in each replicate aquarium. For the remainder of the exposure, one alternating replicate of each concentration and control was measured for these parameters.

The test concentrations were verified by chemical analysis of fludioxonil at days 0 (experimental initiation), 6, 13, 20, 27 and 34 (experimental termination) using liquid chromatography/mass spectrometry (LC/MS/MS).

Percent survival, percent hatch success and percent live, normal larvae at hatch were analysed using Fisher's Exact with Bonferroni-Holm Adjustment Test to establish exposure level effects. Wet weight and dry weight data were evaluated using Williams' Multiple Comparison Test, a parametric procedure, to establish exposure level effects. Length data were analysed using Jonckheere-Terpstra's Step-Down Test, a nonparametric procedure.

The endpoints evaluated for EC_x were wet and dry weight. EC_x values (EC_{10} and EC_{20}) were calculated using a 2P Asymptotic regression model. The test concentrations bracketed the EC_x so that the EC_x came from interpolation rather than an extrapolation. The EC_x values were estimated so that (i) the 95% confidence intervals reported for the EC_x did not contain zero and was not overly wide (i.e., were not greater than approximately 20% of the EC_x), (ii) the 95% confidence interval for the predicted mean at the EC_x did not contain the control mean and (iii) there was no significant lack-of-fit of regression model to the data.

Results and Discussion

Mean measured concentrations ranged from 100 to 120% of nominal and defined the treatment levels as 21, 38, 92, 160 and 330 µg/L. Percent of mean measured concentrations ranged from 76-110% with coefficient of variance (% CV) ranging from 4.2-14%. Analytical results are presented in the table below.

Table 8.2.2.1-79: Analytical results

Nominal Concentration (µg/L)	Measured Concentration (µg/L)							Percent of Nominal (%)
	Day 0	Day 6	Day 13	Day 20	Day 27	Day 34	Mean (SD[b])	
Control	< 0.85[a]	< 0.89	< 0.81	< 0.88	< 0.90	< 0.78	NA[c](NA)	NA
19	19	16	23	22	23	21	21 (2.9)	110
38	34	34	41	38	42	38	38 (3.6)	100
75	87	84	99	93	94	96	92 (5.6)	120
150	150	150	170	160	170	160	160 (6.7)	110
300	290	310	330	350	350	330	330 (25)	110

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.
[c] SD = Standard Deviation.
[b] NA = Not Applicable.

The effects observed and the endpoint summary is shown in the table below:

Table 8.2.2.1-810 Effects of fludioxonil on early life-stages of *Cyprinodon variegatus*

Mean Measured Concentration (µg/L)	Mean Embryo Hatching Success (%)[a]	Mean Live, Normal Larvae at Hatch (%)	Larvae (28 days post-hatch)			
			Mean Larval Survival (%)	Mean Total Length[b] (mm)	Mean Wet Weight[b] (g)	Mean Dry Weight[b] (g)
Control	90	98	100	19.3 (0.23)	0.0936 (0.0057)	0.0230 (0.0016)
21	93	98	98	19.4 (0.15)	0.0935 (0.0035)	0.0229 (0.0010)
38	90	99	100	19.2 (0.59)	0.0908 (0.0091)	0.0222 (0.0027)
92	88	98	96	18.4[c] (0.11)	0.0750[d] (0.0023)	0.0192[d] (0.0004)
160	93	95	96	18.2[c] (0.61)	0.0651[d] (0.0066)	0.0168[d] (0.0018)
330	84	98	84[e]	15.9[c] (1.16)	0.0470[d] (0.0108)	0.0112[d] (0.0028)
Endpoint Summary (µg/L)						
NOEC	330	330	160	38	38	38
LOEC	>330	>330	330	92	92	92
EC_{10}	--	--	--	--	36	38
95% Confidence Interval	--	--	--	--	24 – 49[f]	23-53[f]
EC_{20}	--	--	--	--	81	85
95% Confidence Interval	--	--	--	--	66 - 96	67 - 103

[a] Values presented represent hatching success at the completion of hatch (test day 6).
[b] Standard deviation for each replicate is presented in parentheses.
[c] Significantly reduced compared to the control, based on Jonckheere-Terpstra Step-Down Test.
[d] Significantly reduced compared to the control, based on Williams' Multiple Comparison Test.
[e] Significantly reduced compared to the control, based on Fisher's Exact Test with Bonferroni-Holm's Adjustment.
[f] Confidence intervals do not meet OECD 210 guideline criteria (% difference between EC_{10} and upper/lower limits are greater than the % effect estimated)

The biological performance of the control organisms exceeded the minimum guideline acceptability criteria since the average hatchability of the control was > 75% and the average survival (post-hatch) of larvae in the control was > 80%.

Based on length and wet weight the NOEC was estimated to be 38 µg/L. The EC_{10} and EC_{20} values for wet weight were determined to be 36 and 81 µg/L, respectively. The EC_{10} and EC_{20} values for dry weight were determined to be 38 and 85 µg/L, respectively.

Conclusions

In a test to evaluate the early life stage toxicity of fludioxonil to sheepshead minnow, (*Cyprinodon variegatus)* based on mean measured concentrations and growth endpoints (determined to be the most sensitive), the 28-day NOEC and LOEC values were determined to be 38 and 92 µg/L, respectively. The EC_{10} and EC_{20} values for wet weight were determined to be 36 and 81 µg/L, respectively. The EC_{10} and EC_{20} values for dry weight were determined to be 38 and 85 µg/L, respectively.

(Marini J, 2014)

At the request of the RMS, the summary of the Suprenant 1992 report (Syngenta file number CGA173506/0391) is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoints are summarised in Table 8.2-3 above. It was clarified that the following study is new and had not been included in the original EU evaluation so the report has been provided in this supplementary dossier.

Report:	New fish early life stage study provided for completeness and for relevance comparison with existing endpoints:~~Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS~~: ~~KIIA 8.2.2.2~~ KCA 8.2.2.1/05 Surprenant D. (1992). (CGA173506 Technical) – Toxicity to fathead minnow (Pimephales promelas) embryos and larvae, Report Number 90-5-3319, Springborn laboratories, Inc. 790 Main street Wareham, Massachusetts 02571 (Syngenta File No. CGA173506/0391)

Guidelines

EPA Guidelines No: 72-4

GLP: Yes

Executive Summary

The toxicity of CGA173506 to early-life stages of fathead minnow (*Pimephales promelas*) was determined using a flow-through exposure system. Fish were exposed to the following range of nominal concentrations of 0.019, 0.037, 0.075, 0.15 and 0.30 mg a.s./L, a solvent control and a dilution water control. The mean measured concentrations were 0.019, 0.040, 0.070, 0.14 and 0.27 mg a.s./L.

Based on significant adverse effects on larval wet weight, the maximum acceptable toxicant concentration (MATC) of CGA173506 for fathead minnows was estimated to be <0.040 mg a.s./L and > 0.019 mg a.s./L (geometric mean MATC = 0.028 mg a.s./L). Consequently the NOEC is 0.019 mg a.s./L.

Materials

Test material	CGA173506 Technical
Lot/Batch #:	P805002
Purity:	97.5%
Description:	Yellow powder
Stability of test compound:	Stable under test conditions

Treatments

Test concentrations:	Dilution water control and nominal concentrations of 0.3, 0.15, 0.075, 0.037 and 0.019 mg a.s./L (mean measured: 0.27, 0.14, 0.07, 0.04, 0.019 mg a.s./L)
Solvent:	Acetone
Analysis of test concentrations:	Yes, based on analysis of CGA173506 0, 4, 11, 18, 25, 32 and 35 days using HPLC analysis

Test organisms

Species:	Fathead minnow (*Pimephales promelas*)
Source:	Brood stock maintained at Springborn laboratories
Treatment for disease:	None
Feeding:	Live brine shrimp nauplii (*Artemia salina*)

Test design

Test vessels:	Glass vessels containing 15 L water (39 x 20 x 25 cm)
Test medium:	Well water
Replication:	2
No of eggs per tank:	60 (40 larvae were impartially selected following egg hatch)
Exposure regime:	Flow-through
Duration:	35 days

Environmental conditions

Test temperature:	24 - 26° C
pH:	6.8 – 7.3
Dissolved oxygen:	7.2 – 7.9 mg/L
Hardness of dilution water:	26 - 30 mg/L $CaCO_3$
Lighting:	16 hour light 8 hour dark (30-75 footcandles)

Study Design and Methods

Experimental dates: 30th January to 6th March 1989

The test was conducted using an exposure system consisting of a constant-flow serial diluter, a temperature controlled water bath and a set of 14 exposure aquaria. Treatments included five concentrations of CGA173506, a water control and a solvent control.

Stock solutions (16.7 mg a.s./mL) were prepared on test days -4, 0, 1, 7, 11, 18 and 24 by diluting 0.854 g CGA173506 with acetone to a total volume of 50 mL.

A syringe connected to a pump was used to deliver 4.9 µL/min. of stock solution into the diluter's mixing chamber which received 272 mL/min of dilution water. The solution contained in the mixing chamber constituted the highest test concentration (nominal 0.30 mg/L) and was subsequently diluted (50%) to provide the range of nominal exposure concentrations. The solvent control contained the maximum amount of acetone present in any test concentration (0.018 mL/L).

The exposure of the fathead minnow embryos and larvae to CGA173506 was initiated when the egg incubation cups, each containing 60 embryos were distributed to each of the 14 test aquaria. The diluter system was allowed to equilibrate for at least 72 hours prior to the addition of the test organisms.

Dead embryos were counted daily until hatching was complete. Hatching was deemed complete (exposure day 5) when no more than 10% unhatched viable embryos remained in any control or treatment

level egg incubation cup. Calculations of percentage survival of organisms at hatch were based on the number of live larvae and embryos per incubation cup after hatching was completed compared to the number of embryos per cup on test day 0.

The 30 day post hatch larval exposure was initiated when 40 live larvae were impartially selected from the surviving larvae in each incubation cup on test day 5 and placed into their respective exposure aquaria. Dead larvae were removed when observed and behaviour and appearance of larvae were observed and recorded daily. Larvae survival was estimated at least twice weekly. At 31 days post hatch exposure (test termination), the percentage larval survival was determined. The surviving larvae were anesthetized with MS-222 (tricaine methanesulfonate) and measured for mean total length and mean wet weight. The larvae were measured and weighed individually to calculate the mean and standard deviation of total length and wet weight for each replicate treatment level.

Measurements of the test solutions were undertaken throughout the test period for pH, temperature and dissolved oxygen concentration.

At the termination of the study, data obtained on organism survival at hatch, larval survival and larval growth (wet weight and total length) were statistically analysed to establish significant differences between the treatment level and control organisms. Analyses were performed using the mean organism response in each replicate aquarium rather than individual response values. All statistical conclusions were made at the 95% level of certainty except in the case of the Chi-Square goodness of fit test and the Bartlett's test in which 99% level of certainty was applied.

Results and Discussion

The concentrations of CGA173506 were determined in the test solutions. The mean measured concentrations ranged from 90% to 108% of their nominal concentrations. The limit of detection was 0.001 mg/L. The mean measured concentrations were used for calculating and reporting the results.

Table 8.2.2.1-11: Analytical results

Nominal Concentration (mg a.s./L)	Mean Measured Concentrations (mg a.s./L) [S.D.]	Percent of nominal
0.30	0.27 [0.044]	90
0.15	0.14 [0.0083]	93
0.075	0.07 [0.012]	93
0.037	0.04 [0.015]	108
0.019	0.019 [0.0087]	100

Biological data

Table 8.2.2.1-12: Effects of CGA173506 on the survival and growth of early life stages of fathead minnow

Mean Measured Concentrations (mg a.s./L)	Survival of organisms at hatch (day 5) %	Larval survival (%)	Mean total length (mm)	Mean wet weight (g)
0.27	45 [a]	0 [a]	- [b]	- [b]
0.14	88	33 [a]	28 [b]	0.19 [b]
0.07	92	88	29	0.22 [a]
0.04	90	91	30	0.23 [a]
0.019	93	98	30	0.24
Solvent Control	88	98	30	0.24

Control	93	94	31	0.25
Pooled Control [c]	90	96	- [d]	0.24

[a] Significantly different (P≤0.05) as compared to the pooled control (dilution water & solvent control)
[b] Growth data (total length & wet weight) were not statistically analysed at concentrations which significantly affected larval survival.
[c] Pooled mean dilution water control and solvent control data
[d] Solvent control significantly different from dilution water control; treatment comparisons are made with solvent control only.

Validity Criteria

No validity criteria are reported.

Conclusions

In an early life stage in which fathead minnow embryos and larvae were exposed to CGA173506, based on significant adverse effects on larval wet weight, the maximum acceptable toxicant concentration (MATC) was estimated to be <0.040 mg a.s./L and > 0.019 mg a.s./L (geometric mean MATC = 0.028 mg a.s./L). Consequently the NOEC is 0.019 mg a.s./L.

(Surprenant D. 1992)

This study broadly complies with the current validity criteria for early life-stage testing with fish (OECD 210; 2013), but has some limitations as indicated in the table below.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Surprenant , 1992, CGA173506/0391	US EPA FIFRA No. 72-4	OECD No. 210 (2013), or US EPA OPPTS 850.1400 (2016)	OECD No. 210 (2013): Analysis from Days 25 and 32 were discounted due to inaccurate QC sample results US EPA OPPTS 850.1400 (2016): Solvent concentration was > 0.1 mL/L	The following validity criteria were met: 1. Overall survival of fertilised eggs and post-hatch success in the controls and solvent controls was ≥70 and 75% respectively (all values ≥88%). 2. Constant conditions throughout test (temperature and pH were maintained within acceptable limits) 3. DO ≥ 60% ASV (4.9 mg/L represents 60% saturation at 25ºC (measured values were 7.2 to 7.9 mg/L) Reported mean measured concentrations (90 to 108%) are based on analysis from Days 0, 4, 11, 18 and 35. Analytical results from Days 25 and 32 were discounted due to inaccurate QC sample results, thus there is no clear evidence of maintained concentrations for this period. This validity criteria is not fully met. On Day 27 (Day 23 post-hatch), diluter malfunction resulted in 100% mortality of larvae in one replicate at the mean measured concentration of 0.14 mg a.s./L, therefore results at this dose level are based on one replicate only. The solvent (acetone) concentration in the treatment and solvent control groups was 0.018 mL/L. No statistically significant differences were detected between the negative controls and the solvent controls for survival of organisms at hatch, larval survival post-hatch or wet weight; for these parameters negative control and solvent control data were pooled prior to statistical comparison with the treatment data. Statistically significant differences were detected (1-way ANOVA) between the negative controls and the solvent controls based on total length at test termination; for this parameter, the treatment were compared with the solvent control only. This study provides supporting data for the risk assessment, but has limitations as indicated above.

CA 8.2.2.2 Fish full life cycle test

The RMS requested that a justification is provided for the robustness and the reliability of the NOEC values. Syngenta's response is below:

The valid fish chronic studies, relevant to the risk assessment, were all conducted according to the current guideline at the time.

The proposed new chronic endpoint is taken from a Fish Full Life Cycle study (*Marini, 2015*), conducted to the current guideline (*OCSPP 850.1500 (1996)*). The study is considered valid (meeting current criteria) and robust statistical tests were used to identify clear NOECs.

EFSA have confirmed that assessing the statistical power of a NOEC from a lower tier laboratory test is not necessary (*EFSA; Outcome of the pesticides peer review meeting (133) on general recurring issues in ecotoxicology, 2015*).

No previous data were provided. A fish full life cycle (FFLC) study has been conducted as part of a US EPA Data Call-In (DCI), using the fathead minnow, a freshwater species, and a summary is provided below:

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.2.2/01 Marini, J (2015) Fludioxonil: Full Life-Cycle Toxicity Test with Fathead Minnow (*Pimephales promelas*) Following FIFRA Guideline 72-5 and OCSPP Draft Guideline 850.1500, Report Number 1781.6971, Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571 USA, (Syngenta File No. CGA173506_51493)

Guideline

U.S. EPA, Ecological Effects Test Guidelines, OCSPP 850.1500 (public draft): Fish Life Cycle Toxicity Test (1996)

GLP: Yes

Executive Summary

This study was performed to determine the effects of fludioxonil on the full life-cycle of fathead minnow (*Pimephales promelas*). Newly fertilized embryos (F_0 generation) were exposed to fludioxonil at nominal concentrations of 7.0, 12, 19, 32, 54 and 90 µg/L (6.5, 11, 18, 30, 51 and 86 µg/L time-weighted mean concentrations). Data were compiled on the effects of exposure on hatching success, time-to-hatch, survival, growth (total length and wet weight) and reproductive success (eggs/spawn, spawns/female, and eggs/female) of first generation (F_0) fish and the hatching success, time-to-hatch, survival and growth (total length and wet weight) of their progeny (F_1).

Based on time-weighted mean measured concentrations and the most sensitive endpoints determined (F_0 eggs/female/reproductive day, F_1 hatching success and F_1 30-day survival), the NOEC and LOEC values were determined to be 18 and 30 µg/L, respectively. The EC_{20} and EC_{50} values for F_0 spawns/female were determined to be 42 and 50 µg/L, respectively. The EC_{20} and EC_{50} values for F_0 eggs/spawn were determined to be 28 and 34 µg/L, respectively. The EC_{10}, EC_{20} and EC_{50} values for F_1 hatching success were determined to be 18, 22 and 31 µg/L, respectively.

Materials

Test material	Fludioxonil
Lot/Batch #:	SMO3J0464
Purity:	97.7% (w/w); Tested as 100%
Description:	Brownish powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	End of October 2017
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 7.0, 12, 19, 32, 54 and 90 µg/L (time-weighted mean concentrations: 6.5, 11, 18, 30, 51 and 86 µg/L)
Solvent:	None
Analysis of test concentrations:	Yes, analysis of fludioxonil on days 0, 4, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 82, 88, 95, 102, 109, 116, 123, 129, 137, 144, 151 and 161
Test organisms	
Species:	Fathead minnow (*Pimephales promelas*)
Source:	Continuous laboratory cultures
Acclimatisation period:	14 days
Treatment for disease:	None
In-Life Feeding Regime:	The F_0 and F_1 generation fish were fed a measured amount of live brine shrimp nauplii, three times daily, beginning on day 1 post-hatch. For the F_0 generation, after approximately 30 days of exposure, one of the three daily feedings was replaced with a measured amount of flake fish food. At approximately day 60 post-hatch, the two daily feedings of live brine shrimp nauplii were replaced with a measured amount of frozen brine shrimp. Food type and feeding rates were adjusted based on the demands of the various life stages of the fish throughout the exposure (i.e., larval, juvenile, adult). The amount, frequency and specific type of each feeding were recorded in the study data. Fish were not fed during the 24-hour period prior to their respective termination.
Test design	
Test vessels:	Each exposure aquarium measured 60 × 30 × 30 cm (L x W x H) with a 15-cm high side drain that maintained a constant exposure solution volume of approximately 27 L.
Test medium:	Well water
Replication:	4 replicate aquaria
No. of embryos per aquaria:	50
Exposure regime:	Flow-through, exposure system consisted of an intermittent-flow proportional diluter, a temperature-controlled water bath and a set of 24 exposure aquaria
Duration:	F_0: 151 days F_1: Approximately 30 days post hatch Total: 161 days

Environmental conditions

Test temperature:	23 to 26° C
pH:	6.9 to 8.0
Dissolved oxygen:	5.06 to 8.45 mg/L; 60.7 - 103% of saturation
Lighting:	560 to 780 lux 16 hours fluorescent light and 8 hours dark; with a 30-minute transition period

Study Design and Methods

Experimental dates: 11th July to 14th December 2014

Based on the solubility and chemical properties of the test substance, a glass wool saturator column was used to deliver fludioxonil to the exposure system. The columns were designed to provide a constant flow of saturated aqueous solutions (1.8 mg/L) of the test substance to the diluter system without the use of a carrier solvent. Columns were constructed entirely of chemically inert materials (glass and Teflon®). New saturator columns were prepared approximately every four weeks during testing.

Prior to exposure initiation, an FMI Pump was calibrated to deliver 0.239 L/cycle of the 1.8 mg/L stock solution to the diluter's mixing chamber which also received 4.53 L of dilution water per cycle for a total volume of 4.77 L per cycle. The mixing chamber was positioned over a water-driven magnetic stir plate. The continuous stirring aided the mixing of the test substance with the dilution water. The solution in the mixing chamber was equivalent to that of the highest nominal test concentration (90 µg/L) and was proportionally diluted (60%) to produce the remaining nominal test concentrations (54, 32, 19, 12 and 7.0 µg/L). A set of control vessels was also established which contained the same dilution water and was maintained under the same conditions as the treatment level vessels, but contained no fludioxonil.

The flow-through exposure system consisted of an intermittent-flow proportional diluter, a temperature-controlled water bath and a set of 24 exposure aquaria.

The full life-cycle exposure was initiated when the embryo incubation cups were distributed to each of the exposure aquaria (one cup in each replicate aquarium; each containing 50 embryos).

A summary of the experimental design and biological endpoints is presented in the table below.

Table 8.2.2.2-1: Summary of experimental design and biological endpoints for fish full life cycle study

Phase	Test Days	# Organisms	Endpoint[a]
I. F_0 Hatching Success	0 – 4	200 embryos per concentration 50 per replicate 50 per incubation cup	% Hatching Success Time-To-Hatch
II. F_0 Larval Exposure	4 – 33	120 larvae per concentration 30 per replicate 30 per growth chamber	% Survival Total Length
III. F_0 Juvenile Exposure	34 – 103	120 larvae per concentration 30 per replicate	% Survival Total Length
V. F_0 Reproduction	104 – 151	4 groups of 2 males/ 4 females per concentration; 1 group per replicate	# eggs/female/reproductive day # spawns/female # eggs/spawn

Phase	Test Days	# Organisms	Endpoint[a]
VI. F_0 Non-Spawning Exposure	116	All surplus (non-spawning) F_0 fish	Male Total Length Male Wet Weight Female Total Length Female Wet Weight % Survival
VII. F_0 Termination	151	All surviving spawning F_0 fish; approximately 24 per concentration, 6 per replicate	Male Total Length Male Wet Weight Female Total Length Female Wet Weight
VIII. F_1 Hatching Success	105 – 137	≥250 embryos/replicate	% Hatching Success Time-to-Hatch
IX. F_1 Larval Exposure	116 – 161	4 larval groups (30 individuals/group) per concentration; 1 larval group per replicate	% Survival Total Length Wet Weight

NA = Not Applicable. No endpoints.

[a] Additional observations included evaluation of secondary sexual characteristics at maturation (approximately day 100 post-hatch) and gonadal examinations at non-spawning and spawning fish at termination.

During the in-life phase of the definitive study, samples were removed from a single, alternating replicate solution of each treatment level and the control on days 0, 4, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 82, 88, 95, 102, 109, 116, 123, 129, 137, 144, 151 and 161. The test concentrations were verified by chemical analysis of fludioxonil using liquid chromatography/mass spectrometry (LC/MS/MS). Since the sampling intervals were not equally spaced (e.g., days 0, 4, 11 etc.), a time-weighted mean was calculated and used for reporting of endpoints.

Data were compiled on the effects of exposure on hatching success, time-to-hatch, survival, growth (total length and wet weight) and reproductive success (eggs/spawn, spawns/female, and eggs/female) of first generation (F_0) fish and the hatching success, time-to-hatch, survival and growth (total length and wet weight) of their progeny (F_1). The observed endpoints were used to determine both the highest time-weighted mean measured concentration producing no statistically significant adverse effect on the exposed organisms (NOEC) and the lowest time-weighted mean concentration where statistically significant toxicant-related effects were established (LOEC).

Statistical analyses of continuous data (growth and reproduction) were performed using the mean biological response for each treatment level based on replicate means. All continuous data endpoints passed the assumptions for normality and homogeneity of variance. Since all reproductive data sets (i.e., eggs per female, eggs per spawn and spawns per female) resembled a monotonic dose response, these endpoints were compared to the control data using one-tailed Williams' Multiple Comparison Test (U.S. EPA, 2002). The remaining continuous data endpoints (F_0 and F_1 growth) were compared to the control data using one- tailed Williams' Multiple Comparison Test. Statistical analyses of binomial data (F_0) hatching success and survival and F_1 survival) were performed using Fisher's Exact Test with Bonferroni-Holm's Adjustment. Statistical analysis on F_1 hatch success was performed with one-tailed Williams' Multiple Comparison Test since a monotonic dose response was observed and the data set had an unbalanced design due to pooling (i.e., a different number of total loaded embryos in each treatment level**).** The F_1 Time to Hatch endpoint was compared to control data using the Steel Many-One Rank Sum Test.

ECx values were calculated for several endpoints when the data sets met the regression model criteria.

Results and Discussion

Time-weighted measured concentrations of fludioxonil ranged from 93% to 96% of nominal concentrations with percent CV (coefficient of variation) ranging from 9.9 to 15% and defined the treatment levels tested as 6.5, 11, 18, 30, 51 and 86 µg/L (presented as time-weighted mean measured concentrations). The limit of quantification (LOQ) for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. In this study, the LOQ from each interval ranged from 0.42 to 0.58 µg/L.

The analytical results are presented in the table below.

Table 8.2.2.2-2: Analytical results

Nominal Concentration (µg/L)	Time-Weighted Mean Concentration[ab] (µg/L)	Standard Deviation[b]	Percent of Nominal[b] (%)	% CV[b]
Control	NA[c]	NA	NA	NA
7.0	6.5	0.98	93	15
12	11	1.1	93	10
19	18	1.9	96	11
32	30	3.0	93	10
54	51	5.1	95	9.9
90	86	9.2	96	11

[a] Time-weighted mean of measured fludioxonil concentrations from days 0, 4, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 82, 88, 95, 102, 109, 116, 123, 129, 137, 144, 151 and 161.
[b] Values were calculated using actual analytical results and not the rounded values (2 significant figures) presented in this table.
[c] NA = Not Applicable

The biological effects observed are shown in the tables below:

Table 8.2.2.2-3: Effects of fludioxonil on *Pimephales promelas* – 29 and 62 days post-hatch

Time-Weighted Mean Concentration (µg/L)	Hatching Success (%)	Time-to-Hatch (Days)	29 Days Post-Hatch		62 Days Post-Hatch	
			Larval Survival (%)	Total Length (mm)	Survival (%)	Total Length (mm)
Control	86 (5.0)	4.0 (0.0)	91 (5.0)	19.46 (0.57)	90 (6.1)	46.98 (0.56)
6.5	94 (5.2)	4.0 (0.0)	96 (6.3)	20.42 (0.45)	95 (6.4)	47.60 (0.62)
11	87 (5.8)	4.0 (0.0)	92 (5.8)	20.70 (0.71)	91 (5.0)	46.66 (1.27)
18	92 (3.8)	4.0 (0.0)	95 (4.3)	20.53 (0.87)	91 (7.9)	47.13 (0.76)
30	92 (3.8)	4.0 (0.0)	96 (4.2)	20.37 (0.47)	93 (5.4)	46.47 (1.30)
51	95 (3.0)	4.0 (0.0)	88 (8.8)	20.43 (0.41)	87 (6.1)	46.80 (0.36)
86	92 (2.8)	4.0 (0.0)	88 (5.7)	19.39 (0.69)	83 (6.9)	45.08[a] (0.79)

[a] Significant difference compared to the control, based on Williams' Multiple Comparison Test.
NOTE: Mean values for each treatment are presented with the corresponding standard deviation (SD) in parentheses.

Table 8.2.2.2-4: Effects of fludioxonil on *Pimephales promelas* – non-spawning fish at 116 days post-hatch

Time-Weighted Mean Concentration (µg/L)	Survival (%)	Total Length (mm)		Wet Weight (g)	
		Male	Female	Male	Female
Control	87 (5)	63.73 (1.02)	54.33 (0.78)	3.80 (0.23)	2.25 (0.20)
6.5	95 (6)	61.85 (2.98)	52.53 (1.46)	3.63 (0.34)	2.07 (0.09)
11	91 (5)	62.97 (1.70)	53.00 (1.73)	3.80 (0.18)	2.18 (0.28)
18	91 (8)	63.11 (1.18)	52.54 (2.10)	3.64 (0.16)	1.98 (0.33)
30	93 (4)	64.54 (2.58)	52.49 (1.63)	4.01 (0.55)	2.03 (0.30)
51	86 (5)	64.45 (1.78)	53.36 (0.71)	4.09 (0.40)	2.20 (0.05)
86	83 (7)	63.73 (2.02)	52.68 (2.31)	3.73 (0.35)	2.00 (0.12)

NOTE: Mean values for each treatment are presented with the corresponding standard deviation (SD) in parentheses.

Table 8.2.2.2-5: Effects of fludioxonil on *Pimephales promelas* – F_0 growth at exposure termination

Time-Weighted Mean Concentration (µg/L)	Total Length (mm)		Wet Weight (g)	
	Male	Female	Male	Female
Control	66.71 (3.42)	57.36 (0.93)	5.44 (0.81)	2.78 (0.16)
6.5	67.52 (1.74)	57.61 (1.16)	5.28 (0.34)	2.93 (0.44)
11	67.82 (2.53)	56.75 (1.41)	5.36 (0.71)	2.71 (0.18)
18	65.95 (1.63)	58.10 (1.39)	5.01 (0.42)	2.84 (0.20)
30	66.46 (5.59)	57.65 (0.76)	5.19 (1.14)	2.75 (0.22)
51	65.82 (1.03)	59.51 (2.38)	4.84 (0.29)	3.06 (0.22)
86	68.79 (3.08)	59.41 (1.02)	5.44 (0.89)	3.28 (0.12)

NOTE: Mean values for each treatment are presented with the corresponding standard deviation (SD) in parentheses.

Table 8.2.2.2-6: Effects of fludioxonil on *Pimephales promelas* – F_0 reproductive performance at exposure termination

Time-Weighted Mean Concentration (µg/L)	Eggs/Female/ Reproductive Day	Spawns/Female	Eggs/Spawn
Control	44.5 (8.6)	9.7 (1.4)	215.0 (12.7)
6.5	58.9 (4.7)	11.4 (0.4)	242.0 (14.7)
11	47.6 (10.6)	9.7 (1.4)	229.4 (27.0)
18	52.8 (5.6)	10.4 (0.5)	238.8 (17.1)
30	34.0[a] (10.0)	8.6 (1.3)	186.0 (49.4)
51	17.9[a] (4.5)	7.1[a] (1.8)	118.2[a] (14.9)
86	2.4[a] (4.8)	0.81[a] (1.6)	138.5[a] (NA[b])

[a] Significant difference compared to the control, based on Williams' Multiple Comparison Test.
[b] NA = Not Applicable. Eggs were only produced in one of the replicates for this treatment level.
NOTE: Mean values for each treatment are presented with the corresponding standard deviation (SD) in parentheses.

Table 8.2.2.2-7: Effects of fludioxonil on *Pimephales promelas* – F_1 endpoints at 30 days post-hatch

Time-Weighted Mean Concentration (µg/L)	Hatching Success (%)	Time-to-Hatch (Days)	30 Days Post-Hatch		
			Survival (%)	Total Length (mm)	Wet Weight (g)
Control	87 (3.1)	4.2 (0.2)	79 (15)	22.23 (0.63)	0.0982 (0.0104)
6.5	88 (4.0)	4.4 (0.4)	87 (7.2)	21.29 (0.76)	0.0836 (0.0147)
11	86 (5.9)	4.1 (0.1)	63[d] (25)	22.00 (1.69)	0.0968 (0.0261)
18	78 (5.8)	4.2 (0.2)	79 (9.2)	23.16 (1.44)	0.1077 (0.0201)
30	48[b] (11)	4.4 (0.1)	65[e] (24)	23.72 (1.65)	0.1183 (0.0252)
51[f]	3[bc] (5.2)	4.0 (NA[a])	87 (NA)	21.60 (NA)	0.0922 (NA)
86	0[c] (0)	NA (NA)	NA (NA)	NA (NA)	NA (NA)

NOTE: Mean values for each treatment are presented with the corresponding standard deviation (SD) in parentheses

[a] NA = Not Applicable.

[b] Significant difference compared to the control, based on Williams' Multiple Comparison Test.

[c] Treatment level was excluded from further statistical analysis since an insufficient number of larvae hatched.

[d] Significantly reduced compared to the control, based on Fisher's Exact Test with Bonferroni-Holm's Adjustment. However, due to the lack of statistical significance at the 18 µg/L treatment level, this effect was determined to not be biologically relevant.

[e] Significantly reduced compared to the control, based on Fisher's Exact Test with Bonferroni-Holm's Adjustment.

[f] Since only one replicate produced surviving embryos (87%), this concentration was empirically determined as an effect level and was excluded from statistical analysis of F_1 survival, total length and wet weight.

The endpoint summary is presented in the table below. The NOEC and LOEC for each endpoint are presented in terms of µg/L. LOEC with > symbols indicate that no adverse effects were observed for the endpoint at the highest test concentration. EC_x calculations were determined for population relevant endpoints, only where effects had been reported.

Table 8.2.2.2-8: Effects of fludioxonil on *Pimephales promelas*

	Endpoints presented as µg/L				
Endpoint	**NOEC**	**LOEC**	**EC_{10}**	**EC_{20}**	**EC_{50}**
F_0 hatching success and time to hatch	86	> 86	NA (NA)[b]	NA (NA)[b]	NA (NA)[b]
F_0 survival, weight and length	86	> 86	NA (NA)[b]	NA (NA)[b]	NA (NA)[b]
F_0 eggs/female/reproductive day	18	30	NA (NA)[b]	NA (NA)[b]	NA (NA)[b]
F_0 spawns/female	30	51	NA (NA)[d]	42 (31 - 49)	59 (52 - 66)
F_0 eggs/spawn	30	51	NA (NA)[c]	28 (23 - 33)	34 (29 - 42)
F_1 hatching success	18	30	18 (15 - 20)	22 (20 - 24)	31 (30 -33)
F_1 time to hatch	30	51	--	--	--
F_1 30-day survival	18	30[a]	--	--	--
F_1 30-day total length	30	> 30 [a]	NA (NA)[b]	NA (NA)[b]	NA (NA)[b]
F_1 30-day wet weight	30	> 30 [a]	NA (NA)[b]	NA (NA)[b]	NA (NA)[b]

NOTE: Mean values for each treatment are presented with the corresponding standard deviation (SD) in parentheses

NA = Not Applicable

[a] Treatments in which significant reductions in survival relative to the control were observed were excluded during the analysis of this endpoint.

[b] EC_x values were not calculated due to a significant lack of fit in all applicable regression models.

[c] An EC_{10} was not calculated; since the percent difference between the control estimate and the control estimate confidence interval was ±12%, calculating an EC_{10} was deemed not plausible as stated in OECD 210 Guideline, Annex 6.

[d] An EC_{10} was not calculated; since the percent difference between the control estimate and the control estimate confidence interval was ±18%, calculating an EC_{10} was deemed not plausible as stated in OECD 210 Guideline, Annex 6.

Conclusions

This study was performed to determine the effects of fludioxonil on the full life cycle of the fathead minnow (*Pimephales promelas*). Based on time-weighted mean measured concentrations and the most sensitive endpoints determined (F_0 eggs/female/reproductive day, F_1 hatching success and F_1 30-day survival), the NOEC and LOEC values were determined to be 18 and 30 µg/L, respectively. The EC_{20} and EC_{50} values for F_0 spawns/female were determined to be 42 and 50 µg/L, respectively. The EC_{20} and EC_{50} values for F_0 eggs/spawn were determined to be 28 and 34 µg/L, respectively. The EC_{10}, EC_{20} and EC_{50} values for F_1 hatching success were determined to be 18, 22 and 31 µg/L, respectively.

(Marini J, 2015)

RMS comment:

It is noted that the ecotoxicity studies cited in Vaughan (2015) and proposed to determine if fludioxonil has a potential for endocrine disruption effects are part of the OECD conceptual framework for testing and assessment of endocrine disruptors. However, despite a fish full life cycle test with fish is available (Marini, 2015, KCA 8.2.2.2/01), no analysis of effects on sexual characteristics (sex ratio, gonadal conditions, ...) is provided in the position paper. The notifier should complete his position by an analysis of the relevant information available in the fish full life cycle test (Marini, 2015, KCA 8.2.2.2/01).

Syngenta reply:

The fish full life cycle study was identified as a data gap during the US DCI specifically in relation to concerns identified in the US fish long-term risk assessment – the Estimated Environmental Concentration (EEC) for many uses was observed to be >0.1 times the NOAEC of the fish ELS and hence investigations on fish reproduction need to be evaluated (page 8 of EPA response). Therefore the fish full life cycle study was not requested due to potential endocrine disruption concerns and no specific endocrine endpoints where therefore measured in the study.

The Toxicology and Ecotoxicology endocrine disruptor reviews do not indicate any major concerns in this area. Gross histological measurements were taken during the study to ascertain phenotypic sex in the fish full life cycle study; the data has been retrospectively analysed to examine the sex ratio of the surviving pooled fish in the study (see table below). Statistical analysis of the data using a G-test indicates no significant difference between the pooled replicate sex ratio of the control and exposed fish.

For calculation of sex ration the following examinations were conducted during the study:

Day 100 2 males & 4 females assessed phenotypically and removed from each replicate for spawning assessment
Day 116 remaining fish culled and sex confirmed via gross examination of gonads
Day 151 spawning fish culled and sex confirmed via gross examination of gonads

Table 8.2.2.2-9: Sex ratio in fish full life cycle study for fludioxonil on *Pimephales promelas* at Day 151 (Day 116 fish plus day 151 fish)

Conc (ug/L)	Replicate	male	female	total	sex ratio (% male)	G statistic	df	p value
Control	1	15	10	25	60.0	1.01	1	0.316
	2	12	14	26	46.2	0.15	1	0.695
	3	12	16	28	42.9	0.57	1	0.449
	4	14	11	25	56.0	0.36	1	0.548

	total					2.10	4	0.718
	pooled	53	51	104	51.0	0.04	1	0.845
	heterogeneity					2.06	3	0.561
7	1	12	18	30	40.0	1.21	1	0.272
	2	14	12	26	53.8	0.15	1	0.695
	3	15	13	28	53.6	0.14	1	0.705
	4	15	15	30	50.0	0.00	1	1.000
	total					1.51	4	0.826
	pooled	56	58	114	49.1	0.04	1	0.851
	heterogeneity					1.47	3	0.689
12	1	14	12	26	53.8	0.15	1	0.695
	2	14	14	28	50.0	0.00	1	1.000
	3	15	14	29	51.7	0.03	1	0.853
	4	9	12	21[a]	42.9	0.43	1	0.512
	total					0.62	4	0.961
	pooled	52	52	104	50.0	0.00	1	1.000
	heterogeneity					0.62	3	0.892
19	1	11	18	29	37.9	1.71	1	0.191
	2	13	11	24	54.2	0.17	1	0.683
	3	14	13	27	51.9	0.04	1	0.847
	4	17	7	24[a]	70.8	4.30	1	0.038
	total					6.21	4	0.184
	pooled	55	49	104	52.9	0.35	1	0.556
	heterogeneity					5.86	3	0.119
32	1	17	10	27[b]	63.0	1.84	1	0.175
	2	12	14	26	46.2	0.15	1	0.695
	3	15	13	28[b]	53.6	0.14	1	0.705
	4	17	10	27[b]	63.0	1.84	1	0.175
	total					3.97	4	0.410
	pooled	61	47	108	56.5	1.82	1	0.177
	heterogeneity					2.15	3	0.542
54	1	17	9	26[b]	65.4	2.50	1	0.114
	2	9	18	27	33.3	3.06	1	0.080
	3	13	11	24	54.2	0.17	1	0.683
	4	10	15	25	40.0	1.01	1	0.316
	total					6.73	4	0.151
	pooled	49	53	102	48.0	0.16	1	0.692
	heterogeneity					6.58	3	0.087
90	1	12	14	26[b]	46.2	0.15	1	0.695
	2	14	12	26	53.8	0.15	1	0.695
	3	13	9	22[b]	59.1	0.73	1	0.392
	4	12	11	23	52.2	0.04	1	0.835
	total					1.08	4	0.897
	pooled	51	46	97	52.6	0.26	1	0.612
	heterogeneity					0.82	3	0.843

[a] 5 indiscernible fish exluded from analysis (they are indiscernible as too young to establish sex)
[b] 1 indiscernible fish exluded from analysis (they are indiscernible as too young to establish sex)

CA 8.2.2.3 Bioconcentration in fish

Please refer to original EU review. No new data have been generated since original evaluation of fludioxonil. The study previously submitted in the EU were summarised in point B.9.2.3 of the DAR ~~therefore additional study summaries are not repeated here~~. The endpoints are summarised in Table 8.1.3-1. At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.3 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.1.3 -1 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.3 Gonzalez-Valero J.F. (1994). Accumulation and Elimination of ^{14}C-CGA 173506 by Bluegill Sunfish (Lepomis macrochirus) in a Dynamic Flow-Through System, Report Number PR 27/94, CIBA-GEIGY Limited, Plant Protection Division, CH-4002 Basle, Switzerland. (Syngenta File No. CGA173506/0517)*

Guidelines

US EPA Environmental Fate Subdivision N, EPA-540/9-82-021, Section 165-4: Laboratory Studies of Pesticide Accumulation in Fish, October 1982.

OECD Guideline for Testing Chemicals. Draft 305E: "Bioaccumulation: Flow-through Fish Test"/. Paris/France, December 1992.

GLP: Yes

Executive Summary

The study was undertaken to determine the accumulation and elimination of ^{14}C-CGA173806 in bluegill sunfish (*Lepomis macrochirus*) over a 28-day exposure phase and 14-day depuration phase under dynamic flow-through conditions. The study was conducted with a nominal concentration of 0.01 mg ^{14}C-labelled CGA173506/L and a control. Calculated bioconcentration factors (BCF) were based on analyses of water and fish tissues for total radioactive residues.

Steady-state concentrations of ^{14}C-CGA173506 residues in edible portions, non-edible portions and whole fish reached 80 % after 6 to 8 days of exposure and 95 % after 12 to 15 days. At steady-state, the measured concentrations of ^{14}C-CGA173506 equivalents were 0.531, 6.775 and 3.349 mg CGA173506 equiv./kg tissue fresh weight in edible portions, non-edible portions and whole fish, respectively. The calculated elimination half-lives were 2.1, 0.4 and 0.6 days for edible portions, non-edible portions and whole fish, respectively, and for 90 % depuration were 6.9, 1.4 and 1.8 days, respectively.

Measured bioconcentration factors for ^{14}C-CGA173506 residues on edible portions, non-edible portions and whole fish were 58, 741 and 366, respectively, and the calculated BCFs were 56, 749 and 365, respectively.

Materials

Radiolabelled Test Substance

Test Material:	^{14}C-pyrrole ring labelled CGA173506
Description:	Not reported
Lot/Batch #:	Le-84.2A
Purity:	≥ 98 %
Specific activity:	2.02 MBq/mg (= 54.54 µCi/mg)
Stability of test compound:	Stable under test conditions

Non-Radiolabelled Test Substance

Test Material:	CGA173506
Description:	White crystalline solid
Lot/Batch #:	AMS 273/102
Purity:	99 %
Stability of test compound:	Stable under test conditions
Expiry date:	November 1994

Test design:

Test concentration:	Nominal concentration of 0.01 mg ^{14}C-labelled CGA173506/L
Vehicle and/or positive control:	Basle drinking water/Acetone
Analysis of test concentration:	Yes - HPLC, TLC

Test animals

Species:	Bluegill sunfish (*Lepomis macrochirus*)
Source:	Osage Catfisheries Inc., Lake Road 54-56, Route 4, Box 1500, Osage Beach, MO65065, USA
Acclimatisation period:	At least 14 days
Treatment for disease:	None reported
Weight and length of fish at test start:	Mean weight: 2.0 ± 0.5 g Mean length: 5.2 ± 0.5 cm
Feeding:	Commercial fish food (red midge larva, dito or TETRA Discus food) once daily (except weekends) at a rate between 1 and 2 % of total biomass

Environmental conditions

Test temperature:	19.5 to 20.9 °C
pH range:	7.38 to 7.66
Dissolved oxygen:	81 to 118 % saturation
Organic Carbon	0.7 mg/L
Total hardness of dilution water:	229.85 mg/L as $CaCO_3$
Lighting:	14 hours light
Length of test:	Uptake phase: 28 days Depuration phase: 14 days

Study Design and Methods

Experimental dates: 20th October 1993 to 4th May 1994

Apparatus

A dynamic flow-through system was used to expose fish to a nominal concentration of 0.01 mg ^{14}C-CGA173506/L for a 28-day exposure period, followed by a 14-day depuration period. Three 100-L glass aquaria (one for control and two for treatment), maintained at a water depth of 35 cm, were set up with a dynamic flow-through of nominally 345.6 L per day during the exposure phase and approximately 900 L per day during the depuration phase.

The stock solution for days 0 to 18 of the exposure phase was prepared by dissolving exactly 122.70 mg unlabelled CGA173506 and about 72 mg ^{14}C-CGA173506 in 20 mL of acetone. The specific radioactivity was determined by Liquid Scintillation Counting (LSC) to be 0.74 MBq/mg. For days 19 to 28, the stock solution was prepared by dissolving exactly 72.75 mg unlabelled CGA173506 and about 31 mg ^{14}C-CGA173506 in 10 mL of acetone. The specific radioactivity was determined to be 0.60 MBq/mg. The treatment solutions were prepared three time a week by adding 1 mL of Tween® 80 to 4.15 mg of the respective stock solution and adjusting with tap water to a final volume of 1 L.
Treatment solutions were pumped into the diluter with a FMI® metering pump at exactly 0.58 mL/min and mixed with a diluent stream of water at an exact rate of 240 mL/min.

Test procedure

At the beginning of the study, the fish biomass to water ratio was 0.5 g/L passing through the test system during 24 hours. The population of fish in each tank were fed with red midge larva, dito or TETRA Discus food daily (except weekends) at a rate of 1 to 2 % of the total biomass. During the depuration phase, no chemical stock solution was added to the tank. The depuration phase lasted 14 days.

Analysis of fish tissues

Fish were sampled throughout the uptake and depuration period. Two to three fish were taken from each tank (total of 5 fish) for analysis at 0, 1, 3, 7, 14, 21 and 28 days during the exposure phase, and on days 1, 2, 5, 7, 9 and 14 during the depuration phase. Fish samples were dissected into edible and non-edible parts, and portions of each tissue type were pooled and solubilised with Soluene350. Aliquots of digested tissue solutions were subsequently submitted to LSC.

Test substance and metabolites were measured in ten fish from each tank (total of 20 fish) taken on days 8 and 28 of the exposure phase. The fish were dissected into edible and non-edible portions and homogenised. The homogenised samples were extracted three times with acetone followed by one extraction with chloroform:methanol (2:1, v/v) by shaking (20 min at 300 rpm) at room temperature and a final reflux extraction with chloroform:methanol (2:1, v/v) for 7 hours. All extracts were combined and the radioactivity was determined by LSC. The combined extracts were contrated at 40 °C to approximately 50 mL. The remaining aqueous phase (25 mL aliquots) was partitioned five times with dichloromethane and adjusted to pH <2.0 and partitioned a final time with dichloromethane. The organic phases were dried by filtering over 2 – 4 g sodium sulphate and combined. Thereafter, the organic phases were concentrated to *ca.* 5 mL and adjusted tp 50 mL with hexane. The hexane solution was partitioned twice with 50 mL acetonitrile. The acetonitrile phases were combined, concentrated to 5 mL for HPLC and TLC analysis.

An additional incubation was performed to verify degradates. A total biomass of about 500 g fish was exposed to 50 µg CGA173506/L test solution in a semi-static system for 14 days. The water was completely exchanged every 2 days and the concentration of the test compound new adjusted. After 14 days, all fish were removed and prepared for analysis by HPLC as described above, without dissection into edible and non-edible portions.

Analysis of test water

Water samples were taken from the exposure and control tanks daily (except weekends) to monitor the levels of radioactivity in the tank water. Samples up to 1000 mL were taken on days 0, 3, 7, 21 and 28 of the exposure phase to analyse CGA173506 and its metabolites in the tank water. A 500 mL aliquot of the collected sample was passed through a Bond-Elut C_{18} cartridge, preconditioned with 5 mL dichloromethane (DCM) and 10 mL water. Samples were eluted with 25 mL DCM and submitted for analysis by TLC and HPLC. The aqueous phase of the day 0 sample was centrifuged for 20 min at 2800 rpm and concentrated to *ca.* 2 mL for TLC analysis.

Physical and chemical parameters

Dissolved oxygen, pH and temperature were measured twice a week. Organic carbon and hardness were determined at the beginning of the experiment.

Calculation of Bioconcentration Factors (BCF)

The bioconcentration factors were calculated as ratios of the steady-state concentration of ^{14}C-CGA173506 equivalents in fish tissues and whole fish compared to the average concertation of CGA173506 in water (measured BCF) and as ratios of the uptake rate constant to the depuration rate constant (calculated BCF).

The measured BCF_{ss} was calculated for edible, non-edible and whole fish tissues using mean measured equilibrium tissue concentrations and mean measured concentrations at steady state in the test solutions using the following equation:

Measured bioconcentration factor (BCF_{ss})

$$BCF_{ss} = \frac{C_{tissue}}{C_{water}}$$

The calculated bioconcentration factor, BCF_k, was calculated for edible, non-edible and whole fish tissues as follows:

Calculated bioconcentration factor (BCF_k)

$$BCF_k = \frac{k_u}{k_d}$$

Where k_u is uptake constant and k_d is depuration constant from fish tissue.

The half-life and 90 % depuration time (DT_{50} and DT_{90}, respectively) were determined using the following equation:

Calculated half-life (DT-50) and the time for depuration of 90 % (DT-90)

$$DT50 = \frac{\ln 2}{k_e} \qquad DT90 = \frac{\ln 10}{k_e}$$

Where k_e is elimination constant from fish tissue.

Results and Discussion

The concentration of radioactivity in the test medium corresponded to mean values of 9.5 ± 0.87 µg CGA173506 equiv./L during the exposure phase. The residual radioactivity in water was ≤ 0.7 µg CGA173506 equiv./L during the first three days of the depuration phase, and was ≤ 0.1 µg CGA173506 equiv./L thereafter. The limit of quantification (LOQ) for CGA173506 was 0.4 µg CGA173506 equiv./L. Besides the parent molecule, an unknown metabolite fraction MT2 was detected at approximately the parent LOQ. The formation of this fraction indicated a slight degradation of the test compound in the test

aquaria, therefore the overall mean analysed exposure concentration during the accumulation period was determined to be 9.1 ± 0.3 µg CGA173506 equiv./L. The analytical results of test water are summarised in the table below.

Table 8.2.2.3-1: Analytical results - concentrations of ^{14}C-CGA173506 in tank water during the exposure phase

Day	% of the radioactivity in water		Mean concentration of ^{14}C-CGA173506 (µg CGA173506 equiv./L)
	CGA173506	MT2	
0	92.4	5.7	8.7
3	99.0	<3.0	9.4
7	97.1	<4.5	9.2
21	95.3	4.7	9.0
28	100.0	<3.0	9.5
Mean ± standard deviation	96.8 ± 3.0	-	9.1 ± 0.3

^{14}C-CGA173506 residues reached 80 % of the steady-state concentration after 6.4, 7.8 and 7.1 days of exposure in edible portions, non-edible portions and whole fish, respectively, and 95 % of the steady-state concentrations after 12.1, 14.7 and 13.2 days, respectively. At steady-state, the measured concentrations of ^{14}C-CGA173506 equivalents were 0.531, 6.775 and 3.349 mg CGA173506 equiv./kg tissue fresh weight in edible portions, non-edible portions and whole fish, respectively.

After two days of depuration more than 90 % of the bioconcentrated radioactivity was depurated from the whole fish tissues. The calculated elimination half lives (DT_{50}) were 2.1, 0.4 and 0.6 days for edible portions, non-edible portions and whole fish, respectively, and for 90 % depuration (DT_{90}) were 6.9, 1.4 and 1.8 days, respectively.

The level of radioactivity in fish tissues and BCF results are given in the tables below.

Table 8.2.2.3-2: Level of radioactivity in fish tissues of bluegill sunfish *Lepomis macrochirus* and BCF results

Incubation		Fish portions (mg equiv./kg)		
Phase	Time (days)	Edibles	Non-edibles	Total
Accumulation	0	0.073	0.130	0.098
	1	0.315	2.566	1.384
	3	0.290	3.690	1.815
	7	0.280	4.187	2.132
	14	0.441	5.645	2.878
	21	0.701	8.449	4.183
	28	0.451	6.232	2.987
Depuration	29	0.470	1.228	0.828
	30	0.235	0.316	0.272
	33	0.042	0.421	0.218
	35	0.041	0.227	0.128
	37	0.022	0.293	0.152
	42	0.037	0.213	0.117
Average at steady-state (days 14 to 28)		**0.531**	**6.775**	**3.349**
Measured BCF_{ss} (C_{tissue}/C_{water})		**58**	**741**	**366**

Calculated BCF (k_u/k_d)	**56**	**749**	**365**

In edible fish portions, no other extractable radioactivity other than CGA173506 could be detected. The amounts of CGA173506 in these tissues decreased during incubation from 0.496 to 0.465 mg/kg edible fish tissues after 8 and 28 days of incubation, respectively, corresponding to 93.4 and 87.6 % of the steady state concentration. Besides the parent molecule, low amounts of non-extractable radioactivity were measured in edible fish tissues reaching 3.7 and 10.6 % of the total radioactivity in these tissues after 8 and 28 days of incubation, respectively.

In non-edible fish portions, the content of CGA173506 was 2.044 (30.2 %) and 1.899 (28.0 %) mg/kg after 8 and 28 days of exposure, respectively. One significant (M2) and two minor metabolite fractions (MT1 and MT2) were observed, reaching 0.724 (10.7 %), 0.335 (4.9 %) and 0.117 (1.7 %) mg equiv./kg, respectively, after 8 days of exposure, and were ≤ 0.579 (≤ 8.6 %), 0.317 (4.7 %) and ≤ 0.111 (≤ 1.6 %) mg equiv./kg, respectively after 28 days. Metabolite fraction M2 was quantifiable in the non-polar phase of the tissue extracts, whereas metabolite fractions MT1 and MT2 could only be measured in the polar phase of the tissue extracts. A variety of other small metabolite fractions at approximately the detection limit were observed.

Measured steady state bioconcentration factors for ^{14}C-CGA173506 residues in edible portions, non-edible portions and whole fish were 58, 741 and 366, respectively, and the calculated BCFs were 56, 749 and 365, respectively.

Conclusions

^{14}C-CGA173506 residues in edible portions, non-edible portions and whole fish reached 80 % of the steady state concentrations after 6 to 8 days of exposure and 95 % after 12 to 15 days. At steady-state, the measured concentrations of ^{14}C-CGA 173506 equivalents were 0.531, 6.775 and 3.349 mg CGA173506 equiv./kg tissue fresh weight in edible portions, non-edible portions and whole fish, respectively. The calculated elimination half-lives were 2.1, 0.4 and 0.6 days for edible portions, non-edible portions and whole fish, respectively, and for 90 % depuration were 6.9, 1.4 and 1.8 days, respectively.

Measured steady state bioconcentration factors for ^{14}C-CGA173506 residues on edible portions, non-edible portions and whole fish were 58, 741 and 366, respectively, and the calculated BCFs were 56, 749 and 365, respectively.

(Gonzalez-Valero JF, 1994)

This study complies with the current validity criteria for bioaccumulation testing in fish (OECD 305; 2012), and thus is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Gonzalez-Valero, 1994, CGA173506/0517	US EPA Environmental Fate Subdivision N, EPA-540/9-82-021, Section 165-4 (1982)	OECD No. 305 (2012), or US EPA OPPTS 850.1730 (2016)	OECD No. 305 (2012): Fish weights and lengths are presented, however it is unclear whether these are measurements at test initiation or termination. Fish lipid contents were not measured. US EPA OPPTS 850.1730 (2016): Adjuvant (Tween^{80}) used at a concentration of 2.42 µL/L in the dilution water	The following validity criteria were met: 1. Water temperature variation was less than ± 2°C (19.5 to 20.9°C). 2. DO ≥ 60% ASV (>80%). 3. Concentration of the test substance in the chambers was maintained within ± 20% of the mean of the measured values during the uptake phase (exposure concentration: 0.01 mg/L; overall analysed exposure concentration of ^{14}C-CGA 173506 in the test systems during the accumulation period was 9.1 ± 0.3µg/L). 4. The concentration of the test substance was below its limit of solubility in water (CGA173506 has a solubility of 1.53 mg/L in water). 5. Mortality or other adverse effects/disease in both control and treated fish were < 10% at the end of the test (0%). The solvent (acetone) and adjuvant (Tween^{80}) concentrations in the dilution water were 1.0 µL/L and 2.42 µL/L respectively. The study is reliable and the endpoint can be used in the risk assessment.

CA 8.2.3 Endocrine disrupting properties

An extensive ecotoxicology database exists for fludioxonil. Following evaluation of each of the relevant studies individually and a subsequent weight of evidence evaluation, no concerns for potential endocrine disruption were identified. See M-CA Section 8.1.5 for details of the study reviews conducted and the subsequent reports for mammalian studies and Ecotoxicity studies.

Relevant Literature on Endocrine Disrupting Properties

No scientifically peer-reviewed open literature could be found on fludioxonil or its major metabolites. Details of the literature search undertaken can be found in **Document 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*

Please refer to original EU review. The studies previously submitted in the EU were summarised in point B.9.2.4 of the DAR ~~therefore additional study summaries are not repeated here~~. The endpoints are summarised in Tables 8.2-4 and 8.2-5.

At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

EC_{10} and EC_{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 202: *Daphnia* sp. Acute Immobilisation Test), or by the acute risk assessments according to the current Aquatic Guidance Document (2009)[16]

The following study was previously submitted in the EU and was summarised in point B.9.2.4 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-4 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Holmes C.M. & Swigert J.P., (1993). CGA-173506 – A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (Daphnia magna). Report number 108A-133, Wildlife International Ltd., 8598 Commerce Drive, Easton, Maryland 21601, USA. (Syngenta File No. CGA173506/0259)*

Guidelines

ASTM Standard E 729-88, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians (1988)

US EPA Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, EPA 540/9-82-024, Guideline No. 72-2 (1982)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to *Daphnia magna* was determined under flow-through conditions. Daphnids were exposed to a range of nominal concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg CGA173506/L (mean measured: 0.50, 0.83, 1.1, 1.6 and 1.9 mg CGA173506/L) alongside a negative control and solvent control. Based on mean measured concentrations, the 48-hour EC_{50} was 0.90 mg CGA173506/L with 95 % confidence limits of 0.75 to 1.0 mg CGA173506/L.

Materials

Test Material	CGA173506 Technical
Lot/Batch #:	P.905004/5
Purity:	95.4 %
Description:	Fluffy white powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	16 April 1994

[16] 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.

Treatments

Test concentrations:	Negative control, solvent control and nominal concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg CGA173506/L (mean measured concentrations: 0.50, 0.83, 1.1, 1.6 and 1.9 mg CGA173506/L)
Solvent:	Acetone
Positive control:	None reported
Analysis of test concentrations:	Yes, 0 and 48 hours using HPLC analysis with a photo diode array detector

Test organisms

Species:	*Daphnia magna*
Age:	< 24 hours
Source:	Continuous laboratory cultures
Feeding:	None during test
Culture medium:	Filtered well water

Test design

Test vessels:	300 mL beakers (6.5 cm in diameter, 12 cm in height) with a Nytex® screen attached to an opening on each side, suspended in Teflon®-lined, 8-L polyethylene test chambers containing approximately 6.5 L of test medium, at a depth of 5.8 cm, connected to a diluter system
Test medium:	Filtered well water
Replication:	2 replicates of 10 daphnids
Exposure regime:	Flow-through
Duration:	48 hours

Environmental conditions

Test temperature:	19.7 – 20.0 °C
pH range:	8.0 – 8.3
Dissolved oxygen:	8.6 – 9.0 mg/L
Total hardness of dilution water:	136 – 148 mg/L as $CaCO_3$
Lighting:	16 hours light and 8 hours dark with a 30-minute transition period (light intensity: approx. 474 lux)

Study Design and Methods

Experimental dates: 23rd to 25th June 1992

A flow-through test system was employed. A primary stock solution with a nominal concentration of 5.2 mg a.s./mL was prepared by dissolving CGA173506 in acetone and mixed by inversion. Aliquots of the primary stock solution were diluted with acetone to prepare additional stock solutions at concentrations of 0.7, 1.1, 1.9 and 3.1 mg CGA173506/mL. The stock solutions were injected into the diluter mixing chambers where they were mixed with dilution water to achieve the test concentrations. Resultant test concentrations were adjusted for the purity of the active ingredient in the test substance. The flow of test water from each mixing chamber was split and allowed to flow into duplicate chambers. Each test chamber received approximately 28 volume additions every 24 hours. The negative control consisted of dilution water only, and the solvent control consisted of dilution water and 1.0 mL/L of acetone. Delivery of the test substance was initiated approximately 19 hours prior to the introduction of the daphnids to test water in order to achieve equilibrium of the test substance in the test chambers and daphnids were added to the test vessels.

The mortality and immobility of the daphnids was determined and the numbers of individuals exhibiting clinical signs of toxicity or abnormal behaviour after approximately 3.5, 24 and 48 hours of exposure.

The EC_{50} was calculated by the binomial probability with nonlinear interpolation method at 24 hours and probit analysis at 48 hours. The NOEC was determined by visual inspection of the data.

The pH and dissolved oxygen were measured at the start, after 24 hours and at the end of the test in alternate replicates of each test concentration and the control. The temperature was measured at the beginning and end of the test in each test chamber and continuous temperature monitoring was performed in one replicate of the dilution water control solution. Hardness, alkalinity, and conductivity was measured in the dilution water at test initiation.

The test concentrations were verified by chemical analysis of CGA173506 at 0 and 48 hours using HPLC analysis with a photo diode array detector. All samples were centrifuged prior to analysis.

Results and Discussion

At the start of the test, the mean measured concentrations were in the range 42 to 76 % of the nominal values in centrifuged samples and at the end of the test were in the range 34 to 77 % (see table below). The limit of quantification in this study was 0.1 mg CGA173506/L. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.4.1-1: Analytical results

Nominal concentrations (mg CGA173506/L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours	Mean measured concentrations (mg CGA173506/L)
Negative Control	n.a.	n.a.	n.a.
Solvent Control	n.a.	n.a.	n.a.
0.65	76	77	0.50
1.1	76	75	0.83
1.8	60	60	1.1
3.0	54	50	1.6
5.0	42	34	1.9

n.a. = not applicable

There was no immobility observed in the solvent control and one mortality in the negative control. Immobility and mortality data and estimated EC_{50} values are shown in the table below:

Table 8.2.4.1-2 Effects of CGA173506 on *Daphnia magna* following exposure for 48-hours in a flow-through test

Mean measured concentration (mg CGA173506/L)	Replicate	Immobilised and dead daphnids after 24 hours (n = 10 per replicate)		Immobilised and dead daphnids after 48 hours (n = 10 per replicate)		
		Immobilised (Number)	Dead (Number)	Immobilised (Number)	Dead (Number)	Immobilised and Dead (%)
Negative control	A	0	0	0	1	5
	B	0	0	0	0	
Solvent control	A	0	0	0	0	0
	B	0	0	0	0	
0.50	A	0	0	2	0	15

Mean measured concentration	Replicate	Immobilised and dead daphnids after 24 hours (n = 10 per replicate)		Immobilised and dead daphnids after 48 hours (n = 10 per replicate)		
	B	0	1	0	1	
0.83	A	0	0	1	7	45
	B	1	0	1	0	
1.1	A	3	0	6	0	60
	B	4	0	2	4	
1.6	A	1	0	9	0	80
	B	3	0	7	0	
1.9	A	3	1	7	3	100
	B	3	3	6	4	
EC_{50} mg CGA173506/L		> 0.83		0.90		
95% Confidence limits (mg CGA173506/L)		n.d.		0.75 – 1.0		
No Mortality Concentration (mg CGA173506/L)		n.d.		< 0.50		

n.d. = not determined

Validity Criteria

No validity criteria are reported.

Conclusions

Based on mean measured concentrations, the 48-hour EC_{50} for CGA173506 to *Daphnia magna* was 0.90 mg CGA173506/L. The 48-hour no mortality concentration was < 0.50 mg CGA173506/L.

(Holmes C.M., & Swigert J.P., 1993)

This study complies with the current validity criteria for acute toxicity testing with *Daphnia magna* (OECD 202; 2004). Therefore this study is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Holmes, Swigert, 1993, CGA173506/0259	US EPA FIFRA Guideline 72-2 (1982)	OECD No. 202 (2004)	The following are not reported: 1. If *Daphnia* used in the study were first brood progeny 2. If test vessels were covered The following deviation is noted: 1. Two groups of 10 daphnids per treatment (4 groups of 5 recommended)	While conditions of the study were not fully reported, the validity criteria were met: 1. Mortality in the negative control and solvent control was ≤ 10% (5% and 0% respectively) 2. DO ≥ 3 mg/L (8.6 to 9.0 mg/L) Mean measured concentrations ranged between 38% and 77% of nominal. CGA173506 has a solubility of 1.53 mg/L in water, thus the low mean measured concentrations in

				the highest treatment groups were unavoidable. However, the amount of test substance in solution was maximised by increasing the concentration of solvent (acetone) to 1.0 mL/L, and using high flow rates and vigorous mixing in the diluter. Results are based on mean measured concentrations. The solvent (acetone) concentration in the treatment and solvent control groups was 1.0 mL/L; this concentration is considered to be within the tolerance of *Daphnia* for acute toxicity testing (as confirmed by the absence of effects observed in the solvent control). The study is reliable and the endpoint can be used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.4 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-4 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Surprenant D.C., (1990). (CGA-173506 Technical) - Acute toxicity to daphnids (Daphnia magna) under flow-through conditions. Report number 89-05-2990, Springborn Laboratories, Inc., Environmental Sciences Division, 790 Main Street, Wareham, Massachusetts 02571, USA. (Syngenta File No. CGA173506/0059)*

Guidelines

US EPA/OPPTS, Guideline reference 72-2 (1985)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to *Daphnia magna* was determined under flow-through conditions. Daphnids were exposed to a range of nominal concentrations of 0.13, 0.22, 0.36, 0.60 and 1.0 mg a.s./L (mean measured concentrations of 0.12, 0.21, 0.31, 0.54 and 0.76 mg a.s./L), alongside a dilution water control and solvent control. Based on mean measured concentrations, the 48-hour EC_{50} was 0.40 mg a.s./L, with 95% confidence limits of 0.33 to 0.50 mg a.s./L.

Materials

Test Material CGA173506 technical

Lot/Batch #:	P805002
Purity:	97.5 %
Description:	Yellow powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	Not reported

Treatments

Test concentrations:	Dilution water control, solvent control and nominal concentrations of 0.13, 0.22, 0.36, 0.60 and 1.0 mg a.s./L (mean measured concentrations of 0.12, 0.21, 0.31, 0.54 and 0.76 mg a.s./L)
Solvent:	Acetone
Positive control:	None
Analysis of test concentrations:	Yes, analysis of CGA173506 at 0 and 48 hours using GC with a nitrogen phosphorus detector

Test organisms

Species:	*Daphnia magna*
Age:	≤ 24 hrs
Source:	Laboratory cultures
Feeding:	None during test
Culture medium:	Fortified well water

Test design

Test vessels:	2 L glass battery jar maintained with a constant test solution volume of approximately 1.8 L
Test medium:	Fortified well water
Replication:	2 replicates per treatment and control each containing 10 daphnids
Exposure regime:	Flow-through
Duration:	48 hours

Environmental conditions

Test temperature:	18 to 22°C
pH range:	8.1 to 8.2
Dissolved oxygen:	7.3 to 8.5 mg/L (no aeration)
Total hardness of dilution water:	170 to 180 mg/L $CaCO_3$
Lighting:	44 to 100 footcandles 16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 14th to 16th February 1989

A stock solution with a nominal concentration of 34.6 mg a.s./L was prepared by diluting 1.772 g of the test item (1.728 g active substance) in acetone to a total volume of 50 mL. An intermittent flow proportional diluter equipped with a 20-mL syringe delivered 0.0132 mL of the stock solution into 0.455 L of dilution water in the system's mixing chamber during each diluter cycle. This solution served as the highest treatment level and the remaining test solutions were obtained by further dilution (60 %). Approximately 50 mL of test solution was delivered to each test vessel during each cycle. The system cycled approximately 216 times each day, providing approximately 6 volume replacements in each test vessel every 24 hours. The solvent control solution contained the maximum amount of acetone present in

any treatment level (0.029 mL acetone/L). 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 recorded after 24 and 48 hours of exposure. Immobility was defined as the lack of movement except for minor appendages by the exposed organisms.

The EC_{50} was calculated using probit analysis at 24 hours and by moving average angle analysis at 48 hours. The NOEC was not determined.

The pH, temperature and dissolved oxygen were measured daily in each test concentration and the control.

The test concentrations were verified by chemical analysis of CGA173506 at 0 and 48 hours using gas chromatography with nitrogen phosphorus detection.

Results and Discussion

The measured concentrations ranged from 76 to 95 % of the nominal values (see table below). The minimal detectable concentration in this study was 0.00100 mg CGA173506 for 1000 mL sample. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.4.1-3: Analytical results

Nominal concentrations (mg a.s./L)	Replicate measured concentrations at 0 hours (mg a.s./L)		Replicate measured concentrations at 48 hours (mg a.s./L)		Mean measured concentrations (mg a.s./L)
	A	B	A	B	
Control	< 0.0090	< 0.0090	< 0.0088	< 0.0088	-
Solvent control	< 0.0090	< 0.0090	< 0.0088	< 0.0088	-
0.13	0.11	0.10	0.14	0.13	0.12
0.22	0.18	0.19	0.22	0.25	0.21
0.36	0.27	0.34	0.22	0.39	0.31
0.60	0.52	0.43	0.61	0.61	0.54
1.0	0.86	0.84	0.71	0.61	0.76

A NOEC could not unequivocally be identified due to the occurrence of slight mortality at the two lowest test concentrations, however based on the behaviour of the surviving daphnia and the possibility of incidental mortality in young daphnia, the daphnia were considered to be unaffected by exposure to the test material at the two lowest test concentrations. Immobility data and estimated EC_{50} values are shown in the table below:

Table 8.2.4.1-4: Effects of CGA173506 on *Daphnia magna* following exposure for 48-hours in a flow-through test

Mean measured concentration (mg a.s./L)	Mean % of immobilised daphnids after 24 hours	Mean % of immobilised daphnids after 48 hours
Control	0	5
Solvent control	0	0
0.12	0	15
0.21	0	10
0.31	5	25[a]

Mean measured concentration (mg a.s./L)	Mean % of immobilised daphnids after 24 hours	Mean % of immobilised daphnids after 48 hours
0.54	10[a]	60[ac]
0.76	50[ab]	95[ab]
EC_{50} mg a.s./L	0.82	0.40
95% Confidence interval	0.67 – 1.4	0.33 – 0.50
NOEC	-	Not determined

[a] Some or all surviving daphnids were lethargic
[b] One or several surviving daphnids were on the surface of the test solution
[c] Several surviving daphnids were on the bottom of the test vessel

Validity Criteria

No validity criteria are reported.

Conclusions

Based on nominal concentrations, the 48-hour EC_{50} for CGA173506 to *Daphnia magna* was 0.40 mg a.s./L with 95 % confidence limits of 0.33 to 0.50 mg a.s./L.

(Surprenant D.C., 1990)

This study complies with the current validity criteria for acute toxicity testing with *Daphnia magna* (OECD 202; 2004), but has some limitations as indicated in the table below.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Surprenant, 1990 CGA173506/0059	US EPA FIFRA Guideline 72-2 (1985)	OECD No. 202 (2004)	The following are not reported: 1. If *Daphnia* used in the study were first brood progeny 2. If test vessels were covered The following deviation is noted: 1. Two groups of 20 daphnids per treatment (4 groups of 5 recommended)	While conditions of the study were not fully reported, the validity criteria were met: 1. Mortality in the negative control and solvent control was ≤ 10% (5% and 0% respectively) 2. DO ≥ 3 mg/L (7.3 to 8.5 mg/L) The mean measured concentrations ranged from 76 to 95% of nominal, thus the results are based on the mean measured concentrations. The solvent (acetone) concentration in the treatment and solvent control groups was 0.029 mL/L; this concentration is considered to be within the tolerance of *Daphnia* for acute toxicity testing (as confirmed by the absence of effects observed

				in the solvent control). It is unclear whether the statistical analysis is a comparison between the treatments and solvent control, or negative control. A NOEC could not unequivocally be identified due to the occurrence of slight mortality at the two lowest test concentrations and in the negative control. This study provides supporting data for the risk assessment and is compliant with current guidance, but has some limitations as indicated above.

RMS comment: In the acute toxicity test on *Daphnia magna* conducted with fludioxonil (Surprenant, 1990, CGA173506/0059, KIlA 8.2.1/02), the amount of solvent is indicated to be the maximum amount present in any test concentration. It should be precised which test concentration has the same amount as in the solvent control. It is noted that no effect on immobility was observed in the solvent control. It is noted that no effect on immobility was observed in the solvent control.

Syngenta response:

Clarification of solvent control:

The test solution was prepared by creating a primary stock solution of active ingredient in the solvent acetone that was then quantitatively mixed with dilution water to achieve desired nominal treatment levels (see page 16 of 48). 1.772 g of material (equivalent to 1.728 g AI) was brought to a total volume of 50 using acetone (this is equivalent to 34.46 mg AI/mL of stock). 0.0132 ml of stock was delivered to the mixing cell, where it was diluted with 0.455 L of dilution water. Page 15 of 48 states "The solvent control solution contained the maximum amount of acetone (CAS# 67-64-1) present in any test concentration (0.029 mL/L). The 0.029 mL/L calculation can be performed by dividing 0.0132 ml of stock by 0.455 L of stock .

The concentration achieved in the mixing well is equivalent to the highest test concentration.

0.0132 mL of stock/diluter cycle x 34.46 mg AI/mL of stock = 0.454872 mg AI/diluter cycle

0.454872 mg AI/diluter cycle x 1 diluter cycle/0.455 L of dilution water = 0.9997 mg AI/L of dilution water

The amount of solvent per treatment decreased proportionally to the dilution factor (0.6). Therefore, the 1, 0.6, 0.36, 0.22, and 0.13 mg AI/L treatment contained 0.029 mL stock/L, 0.174 ml stock/L, 0.1044 mL stock/L, 0.06262 mL stock/L, and 0.37584 mL stock/L.

At the time of the study, it was very common for laboratories to only dilute the primary stock and therefore often the amount of solvent in each treatment decreases as treatment level decrease.

Statistical Comparisons:

Table 3 on page 29 of 48 shows that the cumulative percent immobilized organisms in the negative and solvent control were 5% and 0%, respectively. Overall, the EC50 value would be essentially the same regardless of whether data were pooled or only compared to one of the controls. Pooling the controls would likely have a greater impact on potentially defining the NOEC values. However, as noted on page 21 of 48 "A No Observed Effect Concentration (NOEC) could not unequivocally be identified due to the occurrence of slight mortality at the two lowest test concentrations. However, based on the behaviour of surviving daphnia at the 0.12 and 0.21 mg A.I./L levels, and the fact that some incidental mortality frequently occurs with young daphnia (as occurred in the negative control group) the daphnia are considered to be unaffected by exposure to the test material at the two lowest concentrations tested."

A summary of a study not previously reviewed is presented below:

Report:	Submitted to enable risk refinement: K-CA 8.2.4.1/01 Rufli H (1989e). Test for acute toxicity of CGA173506 technical to *Daphnia magna* Report number 881735, Ciba-Geigy Ltd., Basle, Switzerland. (Syngenta File No. CGA173506/0005).

Guidelines

EPA Guideline EG-1 (1982)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to *Daphnia magna* was determined under static conditions. Daphnids were exposed to a range of nominal concentrations of 0.032, 0.058, 0.10, 0.18, 0.32, 0.58, 1.0, 1.8, 3.2, 5.8, and 10 mg/L alongside a dilution water control. Based on nominal concentrations, the 48-hour EC_{50} was 1.1 mg/L.

Materials

Test Material	CGA173506 technical
Lot/Batch #:	805002
Purity:	97.5%
Description:	Beige solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	June 1992
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 0.032, 0.058, 0.10, 0.18, 0.32, 0.58, 1.0, 1.8, 3.2, 5.8, and 10 mg/L
Solvent:	Acetone

Positive control:	None
Analysis of test concentrations:	Yes, analysis at 0 and 48 hours using HPLC analysis
Test organisms	
Species:	*Daphnia magna* Straus 1820
Source:	Continuous laboratory cultures
Feeding:	None during test
Culture medium:	Reconstituted water
Test design	
Test vessels:	Glass beakers containing 250 mL, covered with glass
Test medium:	Reconstituted water
Replication:	2 replicates of 10 daphnids
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	21°C
pH range:	7.6 to 7.7
Dissolved oxygen:	96 to 98 % (no aeration).
Total hardness of dilution water:	240 mg/L $CaCO_3$.
Lighting:	1500 Lux 16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 31st January to 2nd February 1989

1.0g CGA173506 and 40 mg alkylphenol-polyglykol-ether were mixed and made up to 10 mL with acetone. This solution was diluted to 10 mg/L with water. Using this stock solution, the remaining nominal test concentrations as stated above were prepared by serial dilution. 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 3, 6, 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 CGA173506 at 0 and 48 hours using high performance liquid chromatography.

Results and Discussion

At the start of the test, the measured concentrations were in the range 81 to 96% of the nominal values and at the end of the test were in the range 20 to 87% (see table below). Nominal concentrations were used for the calculation and reporting of results.

Table 8.2.4.1-15: Analytical results

Nominal concentrations (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours
0.32	91	87
0.58	88	88
1.0	81	77
1.8	82	52
3.2	85	46
5.8	84	32
10	96	20

The EC_{50} values were calculated according to Berkson. There was no immobility observed in the dilution water control. Immobility data and estimated EC_{50} values are shown in the table below:

Table 8.2.4.1-26: Effects of CGA173506 on *Daphnia magna* following exposure for 48-hours in a static test

Nominal concentration (mg/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Dilution water control	0	0	0	0
Solvent control	0	0	1	5
0.032	0	0	4	20
0.058	0	0	1	5
0.10	0	0	2	10
0.18	0	0	2	10
0.32	0	0	0	0
0.58	0	0	5	25
1.0	0	0	6	30
1.8	6	30	12	60
3.2	9	45	15	75
5.8	10	50	18	90
10	13	65	20	100
EC_{50} mg/L	5.1		1.1	
95% Confidence limits	3.4 – 9.7		0.72 – 2.0	
NOEC	1.0		0.32	

Conclusions

Based on nominal concentrations, the 48-hour EC_{50} for CGA173506 to *Daphnia magna* was 1.1 mg a.s./L.

(Rufli H. 1989e)

At the request of the RMS the endpoint from Rufli 1989 (Syngenta file number CGA173506/0005) *Daphnia* study has been recalculated based on geometric mean measured concentrations. The resulting EC_{50} is 1.06 mg/L (95% confidence limits; 0.872 – 1.29 mg/L).

Report:	At request of RMS recalculated endpoints based on geometric mean measured concentrations: K-CA 8.2.4.1/02 Bebon, R., (2017), Fludioxonil- Statistical Re-analysis - Test for Acute Toxicity of CGA 173506 technical to Daphnia magna, Report Number 121341410, IBACON GmbH, Arheilger Weg 17, 63380 Rossdorf, Germany (Syngenta File No. CGA173506_12046)

Summary

The report from Ciba-Geigy Ltd., Test for Acute Toxicity of CGA 173506 technical to *Daphnia magna* (Rufli & Heinemann, 1989) did not provide estimates of the EC_{50} based on geometric mean measured test concentrations. Consequently the data generated have been re-analysed in an attempt to provide these values.

Statistical analyses revealed that the following EC_{50} value for immobile daphnids at 48 hours was reliably calculated:

EC_{50} [mg/L]	**1.06**
lower 95%-cl	0.782
upper 95%-cl	1.29

Statistical Analysis

For the calculation of parameters based on geometric mean measured concentrations, results for nominal concentrations of 0.032, 0.058, 0.10 and 0.18 were excluded from calculations since no measured concentrations were available. Geometric mean measured concentrations used in the reanalysis were as follows: 0.283, 0.508, 0.785, 1.180, 2.000, 3.010, 4.380 mg/L.

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 slope function (after Litchfield and Wilcoxon, 1949) was derived from the slope, b, of the linearised Probit function and computes as S = 10^(1/b); small values refer to steep concentration/response relationships and large ones to flat relationships. The results of the Probit analysis and their 95% confidence limits (based on normal distribution) were estimated.

Results

Statistical analyses revealed that the following EC_{50}, NOEC and LOEC values for immobile daphnids after 24 hours and at test end were reliably calculated:

Table 8.2.4.1-7: Recalculated endpoints based on geometric mean measured concentrations (Rufli 1989: Syngenta file number CGA173506/0005)

Parameter	**24 hours**	**48 hours**
	EC_{50}	**EC_{50}**
Value [mg/L]	2.72	1.06
lower 95%-cl	2.12	0.782
upper 95%-cl	3.50	1.29
NOEC	0.785	0.283
LOEC	1.18	0.508

Conclusion

Statistical analyses revealed that the following EC_{50} value for immobile daphnids at 48 hours (based on geometric mean measured concentrations) was reliably calculated:

EC_{50} [mg/L]	**1.06**
lower 95%-cl	0.782
upper 95%-cl	1.29

(Bebon 2017)

Aquatic invertebrate acute toxicity studies with metabolites of fludioxonil

Please refer to original EU review. The study previously submitted in the EU on CGA339833 by Grade 2000 (Syngenta file number: CGA339833/0004) was summarised in point B.9.2.4 of the DAR ~~therefore an additional study summary is not repeated here~~. ~~Summaries of studies conducted following the original EU review of fludioxonil are presented below~~. The endpoints are summarised in Table 8.2-5. At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.4 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-5 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Grade R, (2000). Acute toxicity of CGA 339833 (Metabolite of CGA 173506) to the Cladoceran Daphnia magna STRAUS in the Static System. Report number 2003512, Novartis Crop Protection AG, Product Portfolio Management, Environmental Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. CGA339833/0006)*

Guidelines

OECD Guideline No. 202: Daphnia, Acute Toxicity Test, Draft (1999)

US EPA Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, EPA 540/9-82-024, Guideline No. 72-2 (1989)

GLP: Yes

Executive Summary

The acute toxicity of CGA339833 (metabolite of CGA173506) 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 CGA339833/L alongside a dilution water control. Based on nominal concentrations, the 48-hour EC_{50} was > 100 mg CGA339833/L, the highest concentration tested.

Materials

Test Material	CGA339833
Parent:	CGA173506
Lot/Batch #:	KI 6119/6
Purity:	90 ± 2 %
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	May 2002

Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg CGA339833/L
Solvent:	None
Positive control:	None
Analysis of test concentrations:	Yes, analysis of CGA339833 at 0 and 48 hours using HPLC analysis with UV detection

Test organisms	
Species:	*Daphnia magna* STRAUS, Clone 5
Age:	< 24 hours
Source:	Laboratory cultures
Feeding:	None during test
Culture medium:	Elendt M4-Medium

Test design	
Test vessels:	150 mL glass vessel containing 100 mL of test medium
Test medium:	Elendt M4-Medium
Replication:	4 replicates of 5 daphnids
Exposure regime:	Static
Duration:	48 hours

Environmental conditions	
Test temperature:	21.5 °C
pH range:	Test start: 8.2 to 8.4 Test end: 8.2
Dissolved oxygen:	81 to 88 % (no aeration)
Total hardness of dilution water:	280 mg/L $CaCO_3$
Lighting:	Approximately 1100 Lux 16 hours light and 8 hours dark, with a 30 minute transition period

Study Design and Methods

Experimental dates: 16th to 19th May 2000

A stock solution was prepared by mixing 200.7 mg of the test item and making up to 2000 mL with dilution water. Appropriate volumes of the stock solution were diluted to give the test concentration series. The control consisted of dilution water only. Test solutions were added to the test vessels and the *Daphnia* added.

The immobility and other behavioural changes of the daphnids were recorded after 24 and 48 hours of exposure. Organisms unable to swim within 15 seconds after gentle agitation of the test container were considered to be immobile.

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

The test concentrations were verified by chemical analysis of CGA339833 at 0 and 48 hours using high performance liquid chromatography with ultra violet detection.

Results and Discussion

At the start of the test, the measured concentrations were in the range 99.6 to 102.4 % of the nominal values and at the end of the test were in the range 99.0 to 103.4 % (see table below). The limit of quantification in this study was 0.04 mg CGA339833/L. Nominal concentrations were used for the calculation and reporting of results.

Table 8.2.4.1-8: Analytical results

Nominal concentrations (mg CGA339833/L)	Mean measured concentrations at 0 hours (mg CGA339833/L)	% of nominal measured at 0 hours	Mean measured concentrations at 48 hours (mg CGA339833/L)	% of nominal measured at 48 hours
Control	< LOD	-	< LOD	-
6.25	6.4	102.4	6.4	102.4
12.5	12.6	100.8	12.7	101.6
25	24.9	99.6	25.4	101.6
50	50.2	100.4	51.7	103.4
100	101.1	101.1	99.0	99.0

LOD = 0.015 mg CGA339833/L

No immobilisation of daphnids was observed in the controls and at all test concentrations, therefore no statistical analysis was carried out.

Immobility data and estimated EC_{50} values are shown in the table below:

Table 8.2.4.1-9: Effects of CGA339833 on *Daphnia magna* following exposure for 48-hours in a static test

Nominal concentration (mg CGA339833/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Control	0	0	0	0
6.25	1	5	1	5
12.5	0	0	0	0
25	0	0	0	0
50	0	0	0	0
100	0	0	0	0
EC_{50} (mg CGA339833/L)	> 100		> 100	
NOEC	100		100	

Validity criteria

The following validity criterion for the test was met:

- Immobilisation in the control ≤ 10 % at the end of exposure (observed 0 %)

Conclusions

Based on nominal concentrations, the 48-hour EC_{50} for CGA339833 to *Daphnia magna* was >100 mg CGA339833/L. The 48-hour NOEC was 100 mg CGA339833/L, the highest concentration tested.

(Grade R, 2000)

This study complies with the current validity criteria for acute toxicity testing with *Daphnia magna* (OECD 202; 2004). Therefore this study is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Grade, 2000, CGA339833/0006	OECD No. 202 (draft, 1999)	OECD No. 202 (2004)	The following are not reported: 1. If *Daphnia* used in the study were first brood progeny 2. If test vessels were covered	While conditions of the study were not fully reported, the validity criteria were met: 1. Mortality in the negative control was ≤ 10% (0%) 2. DO ≥ 3 mg/L (81 to 88% ASV) The study is reliable and the endpoint can be used in the risk assessment.

The studies previously on CGA192155 (Volz 2005: Syngenta file number: CGA192155/0017) and CGA344623 (Volz 2004: Syngenta file number: CGA344623/0001) were summarised in the Final Addendum to the DAR (June 2007) ~~therefore additional study summaries are not repeated here~~. The endpoints are summarised in Table 8.2-5. At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.4 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-5 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Volz E, (2005). CGA192155 (Metabolite of Fludioxonil): Acute toxicity to Daphnia magna in a 48-hour immobilization test. Report Number 857598, RCC Ltd, Itingen, Switzerland. (Syngenta File No. CGA192155/0018)*

Guidelines

OECD (1984). OECD Guidelines for Testing of Chemical 202. *Daphnia sp.*, Acute Immobilization Test and Reproduction Test, Part I. Adopted 4 April 1984.

Official Journal of the European Communities, L 383 A, Part C.2, Acute Toxicity for Daphnia. 29 December 1992.

GLP: Yes

Executive Summary

Toxicity of CGA192155 (metabolite of fludioxonil) to *Daphnia magna* was determined. This study was run with a culture medium control and with nominal test item concentrations of 10, 18, 32, 56 and 100 mg CGA192155/L. The 48-hour EC_{50} was determined to be >100 mg/L based on nominal test item concentrations.

Materials

Test Material:	CGA192155
Description:	White solid
Lot/Batch #:	MLA-336/4
Purity:	96 %
Treatments:	
Test concentrations:	Dilution water control and nominal test item concentrations of 10, 18, 32, 56 and 100 mg CGA192155/L
Vehicle and/or positive control:	None / Potassium dichromate (tested once a year)
Analysis of test concentrations:	Yes, analysis of CGA192155 in the highest concentration at 0 and 48 hours
Test organism	
Species:	*Daphnia magna*
Source:	Continuous laboratory cultures originally obtained from the University of Sheffield, UK defined as Clone 5.
Feeding:	None during test
Test design	
Test vessels:	100 mL glass beakers containing 50 mL test solution
Dilution medium:	Reconstituted test water
Exposure regime:	Static
Aeration:	Prior to the start of the study (until oxygen saturation was reached).
Replication:	At each test concentration and for the control 20 daphnids were used divided into four replicates of five daphnids each.
Environmental conditions	
Test temperature:	21 °C
pH:	7.6 to 7.8 mg/L (after pH-adjustment in the highest concentrations of 56 and 100 mg/L)
Dissolved oxygen:	8.4 to 8.8 mg/L
Total hardness of dilution water:	250 mg/L ($CaCO_3$)
Lighting:	A 16-hour light to 8-hour dark photoperiod (with a 30 minute transition period). Light intensity during the light period was approximately within

	the range of 540 – 700 Lux
Length of test:	48 hours

Study Design and Methods

Experimental dates: 7th December 2004 to 22nd January 2005.

The test medium of the highest nominal test item concentration of 100 mg/L was prepared by dissolving 100.3 mg of the test item completely in 1000 mL of test water using ultrasonic treatment (30 minutes) and intense stirring (30 minutes at room temperature). This test medium was filtered through a membrane filter just before the preparation of the test media to avoid any small particles in the solution. This had no influence on the correct concentration of this solution. Adequate volumes of this filtrate were diluted with test water to prepare the test media with the lower test item concentrations. The test media were prepared just before the start of the test. The control consisted of the dilution water only. For evaluation of the quality of the daphnia clone and the experimental conditions, potassium dichromate is tested as a positive control at least once per year.

The test was performed in 100-mL glass beakers, covered with lids, filled with 50 mL of test medium. At each test concentration and for the control 20 daphnids were used, divided between four replicates with five daphnids each. The daphnids were randomly distributed among the test vessels at initiation of the test. The test was conducted under static conditions with no aeration. The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Those organisms not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The concentration of the CGA192155 in the test solutions from the highest treatment group was measured at 0 and 48 hours using a high performance liquid chromatography method. For the lower test concentrations no analytical measurements were performed since theses concentrations were below the 48-hour NOEC.

At the start and at the end of the test, the pH values, the dissolved oxygen concentrations, and the water temperature were determined in each test concentration and the control.

The appearance of the test media was visually recorded at the start of the test and after 24 and 48 hours.

Results and Discussion

The analytically determined test item concentration in the highest test medium concentration of 100 mg/L was 102 and 99% of the nominal value at the start and end of the test, respectively. The nominal test item concentrations were used for the calculation and reporting of the results.

At the beginning and the end of the test period, the pH ranged from 7.6 to 7.8 (after pH-adjustment in the highest concentrations of 56 and 100 mg/L). The water temperature was maintained at 21 °C throughout the exposure period. The mean light intensity was approximately 540 to 700 Lux. The water hardness was 250 mg/L as $CaCO_3$.

The EC_{50} value could not be determined due to the absence of a toxic effect of the test item in this test (Table 8.2.4.1-7).

Table 8.2.4.1-10 Effects of CGA192155 on the mobility of *Daphnia magna* following exposure for 48 hours in a static test

Nominal test item concentration (mg CGA192155/L)	No. of daphnids tested	Immobilized daphnids after 24 hours		Immobilized daphnids after 48 hours	
		No.	%	No.	%
Control	20	0	0	0	0
10	20	0	0	0	0
18	20	0	0	0	0
32	20	0	0	0	0
56	20	0	0	0	0
100	20	0	0	0	0

There was no inhibitory effect on mobility of *Daphnia magna* up to and including the concentration of 100 mg/L.

The 48-hour EC_{50} was >100 mg CGA192155/L (95% confidence limits: not determined).

Validity criteria

None reported.

Conclusions

Based on nominal test item concentrations, the 48-hour EC_{50} for CGA192155 was >100 mg CGA192155/L to the *Daphnia magna*.

(Volz E, 2005)

Reference	***Guidance followed***	***Current guidance in force***	***Differences from guidance in force***	***Critical assessment***
Volz 2005, Syngenta file number CGA192155/0018	*OECD 202 (1984)* *EU L383A Part C2 (1992)*	*OECD No. 201 (2006)* *or* *US EPA OPPTS 850.5400 (1996)*	*Compliant with both OECD and US EPA guidelines*	*Following critical validity criteria were met:* *1. Control immobility :≤10% or (actual: 0% control).* *This study is considered reliable.*

The following study was previously submitted in the EU and was summarised in point B.9.2.4 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-5 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Volz E, (2004). CGA344623 (Metabolite of Fludioxonil): Acute toxicity to Daphnia magna in a 48-hour immobilization test, Report Number 855699, RCC Ltd, Itingen, Switzerland (Syngenta File No. CGA344623/0001)*

Guidelines

OECD (1984). OECD Guidelines for Testing of Chemicals. Method 202, Part I *Daphnia* sp., Acute Immobilisation Test.

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

US EPA OPPTS 850.1010: Ecological Effects Test Guidelines, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids; Public Draft, April 1996.

GLP: Yes

Executive Summary

The acute toxicity of CGA344623 (metabolite of fludioxonil) to *Daphnia magna* was determined. This study was run with the single nominal concentration of 100 mg CGA344623/L together with a medium control.

No immobilisation was observed in either the control or 100 mg/L CGA344623 treatment group during the 48-hour exposure period. The 48-hour EC_{50} of CGA344623 to *Daphnia magna* was therefore concluded to be >100 mg/L.

Materials

Test Material:	CGA344623 (metabolite of fludioxonil)
Description:	White solid
Lot/Batch #:	KI 7182/2
Purity:	94 % (wt/wt)
Reanalysis date:	31 July 2006

Treatments:

Test concentration:	Dilution water control and the single nominal test item concentration of 100 mg CGA344623/L
Vehicle and/or positive control:	None / potassium dichromate tested once a year
Analysis of test concentration:	Yes, based on measurement of CGA344623 at 0 and 48 hours

Test animals:

Species:	*Daphnia magna* (6 – 24 hours old)
Source:	Continuous laboratory cultures originally obtained from the University of Sheffield, UK defined as Clone 5.
Treatment for disease:	None
Feeding:	None during test

Test design:

Test vessels:	100 mL glass beakers containing 50 mL test solution
Dilution medium:	Reconstituted water
Replication:	Four replicates, each containing 5 *Daphnia magna*
Exposure regime:	Static
Duration:	48 hours

Environmental conditions

Test temperature:	20 to 21 °C
pH:	7.2 to 7.9
Dissolved oxygen:	8.7 to 8.9 mg/L
Total hardness of dilution water:	250 mg/L $CaCO_3$
Lighting:	16 hours fluorescent light and 8 hours dark with 30 minute dawn and dusk transition periods

Study Design and Methods

Experimental dates: 8th to 16th November 2004

The test medium was prepared by dissolving 50.3 mg of test item completely in 500 mL of test water using ultrasonic treatment for 5 minutes and intense stirring for 10 minutes at room temperature. The control consisted of reconstituted test water only.

The test was performed in 100 mL glass beakers, covered with lids, filled with 50 mL of test medium. At the test concentration and for the control 20 daphnids were used, divided between four replicates with five daphnids each. The daphnids were randomly distributed among the test vessels at initiation of the test. The test was conducted under static conditions with no aeration.

The concentration of CGA344623 in the test solution was measured at 0 and 48 hours using a high performance liquid chromatography method.

The appearance of the test medium was visually recorded at the start of the test and after 24 and 48 hours.

The immobility of the daphnids was determined by visual observations after 24 and 48 hours of exposure. Those organisms not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile.

The median effect concentration (EC_{50}) was defined as the concentration resulting in 50% immobilisation of the *Daphnia* in the time period specified.

At the start and at the end of the test, the pH values, the dissolved oxygen concentrations, and the water temperature were determined in the single test concentration and the control.

Results and Discussion

The analytically measured test item concentration in the test medium samples from the start and the end of the test was 98% of the nominal value. Under the conditions of the test, the test item CGA344623 was stable during the test period of 48 hours. Therefore, all reported results are based on the nominal concentration of the test item.

At the beginning and end of the test period, the dissolved oxygen concentration in the test solutions ranged from 8.7 to 8.9, the pH ranged from 7.2 to 7.9 and the temperature ranged from 20 to 21 °C.

No immobilisations were observed in either the control or 100 mg/L CGA344623 treatment group during the 48-hour exposure period. The 48-hour EC_{50} of CGA344623 to *Daphnia magna* was therefore concluded to be >100 mg/L.

Table 8.2.4.1-11: Effects of CGA344623 on the response of *Daphnia magna* following exposure for 48 hours in a static test

Nominal test item concentration (mg CGA344623/L)	No. of daphnids tested	Immobilized daphnids after 24 hours		Immobilized daphnids after 48 hours	
		No.	%	No.	%
Control	20	0	0	0	0
100	20	0	0	0	0

Validity criteria

None reported.

Conclusions

It was concluded that the 48-hour EC_{50} for CGA344623 (metabolite of fludioxonil) to *Daphnia magna* is >100 mg CGA344623/L based on the nominal concentration of test item.

(Volz E, 2004)

No GLP definitive aquatic toxicity data is available for metabolite CGA265378 found at 7.9% in the soil photolysis study (see MCA Section 7, Miner 2009 (Syngenta file number CGA173506_11661)). This is due to the fact that it degrades very rapidly in water making it not possible to conduct studies. A study was requested during the original review and a statement was produced based on range finding data. The results of the work conducted are provided below in an OECD summary format combining both the information from the statement and from examination of the raw data (to confirm batch number, study duration, number of *Daphnia* etc.) and is presented below:

Due to the instability of the CGA265378 in water it was agreed with DEPA that a definitive study was not possible for this metabolite, and the risk in the environment was low due to instability.

Report:	Submitted at the request of ANSES during the pre-submission meeting in July 2015 to explain the on-going work conducted on the fludioxonil metabolite CGA265378: K-CA 8.2.4.1/~~0~~203 Volz E. (2005) CGA265378 (metabolite of fludioxonil): Statement of acute toxicity to *Daphnia magna* in a range finder immobilization test. Report Number 857600. RCC Ltd, Itingen, Switzerland (Syngenta File No. CGA265378/0013).

Guidelines

None cited

GLP: No

Executive Summary

A range-finding test was conducted with CGA265378 in a 48h acute *Daphnia magna* toxicity test. Concentrations used were 0.1, 1.0, 10 and 100 mg/L.

No biological effects occurred at up to and including the highest concentration tested.

Analysis of the test solutions revealed that the concentration of test substance was approximately 1% of nominal at the start of these test. Based on the measured concentrations then the EC_{50} would be >1 mg/L. Due to the instability of the CGA265378 in water it was agreed with DEPA that a definitive study was not possible for this metabolite, and the risk in the environment was low due to instability.

Materials

Test Material:	CGA265378 (metabolite of fludioxonil)
Batch number:	KI 6117/4
Reanalysis date:	September 2006
Appearance:	Yellow solid
Test concentration:	Dilution water control, 0.10, 1.0, 10 and 100 mg CGA265378/L
Vehicle and/or positive control:	None
Analysis of test concentration:	Yes (based on measurement of CGA265378)
Test animals	
Species:	*Daphnia magna*
Length of test:	48 hours

Study Design and Methods

Experimental date:9 May 2005

Test procedure and apparatus

A suspension of the highest test concentration of 100 mg/L CGA265376 was stirred for 26 hours in the dark and ultrasonic treatment was used for 30 minutes. The solution was then filtered through a 45 µm membrane. The test concentrations of 0.1, 1.0 and 10 mg/L were prepared by serial dilution of the 100 mg/L stock.

The test solutions were analysed using HPLC chromatography.

Results and Discussion

Analysis of the test solutions revealed that the concentration of test substance was approximately 1% of nominal at the start of these test. The test item had almost completely decomposed within 1 day.

Table 8.2.4.1-312: Effects of CGA265378 on the mobility of *Daphnia magna* following exposure for 48 hours in a static test (range finding results)

Nominal test item concentration (mg CGA265378/L)	No. of daphnids tested	Immobilized daphnids after 24 hours		Immobilized daphnids after 48 hours	
		No.	%	No.	%
Control	10	0	0	0	0
0.1	10	0	0	0	0
1.0	10	0	0	0	0
10	10	0	0	0	0
100	10	0	0	0	0

There was no inhibitory effect on mobility of *Daphnia magna* up to and including the concentration of 100 mg/L. Analysis of the test solutions revealed that the concentration of test substance was approximately 1% of nominal at the start of these test. The test item had almost completely decomposed within 1 day.

Conclusion

In a range-finding test which *D. magna* were exposed to CGA265378 at concentrations up to 100 mg/L no biological effects occurred at up to and including the highest concentration tested. Based on the measured concentrations then the EC_{50} would be >1 mg/L.

(Volz E, 2005)

Further clarification is provided at the request of the RMS to demonstrate the instability of CGA265378 in water:

An experiment was conducted to determine the stability of CGA265378 in Elendt M4 *Daphnia* media. The solution of CGA265378 was prepared by adding dissolving 8 mg of CGA265378 and dissolving in was dissolved in 80 µL of EtOH and thoroughly shaken. Then 20 µL was aliquoted into 3 separate vials and 20 mL of Elendt M4 Daphnia media was added to each vial. A further 10 µL of CGA265378 stock solution was added to a fourth vial, 10 mL of EtOH was added to form a control sample (this was added to stabilise further degradation at 0 hours (to demonstrate stability of CGA265378 in EtOH over a 24 hour period).

All samples were left to incubate at 20 °C under a 16h/8h light dark cycle. Then 1 mL aliquots were removed from the aqueous samples at 0, 2, 4 and 6 hours after application, and immediately diluted with ethanol (1mL) to prevent further degradation prior to analysis (however CGA265378 is so unstable that even this was not entirely successful at stopping further degradation). After 24 hours aqueous and control samples were aliquoted. Aliquots were diluted with 1 mL EtOH to prevent further reaction occurring and analysed in triplicate by High Performance Liquid Chromatography (HPLC) and UV detection.

Data for CGA265378 is presented below:

Incubation time (h)	Area under peak				% recovery compared to 0h time point
	Injection 1	Injection 2	Injection 3	Mean	
0	2395	2973.6	2105.5	2491	100
2	925.7	1426.6	804.5	1052	42.2
4	852.1	382.9	412.3	549	22
6	195.7	373.3	233.4	267	10.7
24	0	0	0	0	0
24 (control)	2460.5	2574.9	2597.4	2544	102.1

The data indicate that the CGA265378 solution was dosed correctly. Over the course of 24 hours CGA265378 degraded completely after 24 hrs, with 78% reduction after 4 hours. The DT_{50} and DT_{90} in water is therefore was estimated as 1.7 and 5.8 hours (SFO), respectively. Although this is a non-GLP investigation, and samples were difficult to stabilise (degrading even during the 1.5 hr time for HPLC analysis) it is indicative that CGA265378 degrades very rapidly in aquatic media.

Therefore it is not possible to test CGA265378 in an aquatic studies. The original RMS conclusion from the Praper meeting is correct that CGA265378 is a transient metabolite and that the aquatic risk assessment should focus on the more stable metabolites of CGA192155, CGA339833 and SYN545245.

A *Daphnia* acute toxicity study with the new soil metabolite SYN545245 ~~is currently being~~ has been conducted. The study ~~will be submitted and the dossier updated as agreed with the RMS. The study will be submitted~~ is summarised below and the dossier updated as agreed with the RMS.

Report:	Submitted at the request of ANSES during the pre-submission meeting in July 2015 as part of the top-up submission to include on-going on the fludioxonil metabolite SYN545245: K-CA 8.2.4.1/04 Sacker D. (2016), SYN545245 – Acute Toxicity to *Daphnia magna* in a 48-Hour Immobilization Test. Report number 41502654, Envigo Research Limited Shardlow Business Park, Shardlow Derbyshire DE72 2GD UK. (Syngenta File No. SYN545245_10005).

Guidelines

OECD Guideline 202: Daphnia sp., Acute Immobilisation Test (2004)

440/2008/EC, C.2 (2008)

GLP: Yes

Executive Summary

The acute toxicity of SYN545245 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/L alongside a dilution water control. Based on nominal concentrations, the 48-hour EC_{50} was >100 mg/L.

Materials

Test Material	SYN545245
Lot/Batch #:	MES 427/1
Purity:	98% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	31 October 2017
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L
Solvent:	None
Positive control:	Potassium dichromate used at least twice a year

Analysis of test concentrations:	Yes, analysis at 0 and 48 hours using HPLC analysis
Test organisms	
Species:	*Daphnia magna* Straus clone 5
Source:	Continuous laboratory cultures, originally obtained from the University of Sheffield, UK
Feeding:	None during test
Culture medium:	ISO reconstituted test water
Test design	
Test vessels:	150 mL glass beakers containing 100 mL
Test medium:	ISO reconstituted test water
Replication:	4 replicates of 5 daphnids
Exposure regime:	Static
Duration:	48 hours
Environmental conditions	
Test temperature:	21 to 22 °C
pH range:	6.6 to 8.0
Dissolved oxygen:	8.3 to 8.8 mg/L (no aeration).
Total hardness of dilution water:	250 mg/L $CaCO_3$
Lighting:	557 to 623 Lux 16 hours light and 8 hours dark, with a 20 minute dawn/dusk period

Study Design and Methods

Experimental dates: 14 December 2015 to 26 February 2016

A nominal amount of test item (200 mg) was dissolved in test water and the volume adjusted to 2 liters to give the 100 mg/L test concentration from which a series of dilutions was made to give further test concentrations of 50, 25, 12.5 and 6.25 mg/L. 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 and end of the test in each test concentration and the control.

The test concentrations were verified by chemical analysis of SYN545245 at 0 and 48 hours using high performance liquid chromatography with ultra violet-visible detection.

Results and Discussion

At the start of the test, the measured concentrations were in the range 103 to 112% of the nominal values and at the end of the test were in the range 93 to 104% (see table below). The limit of quantification in this study was 2.86. mg/L. Nominal concentrations were used for the calculation and reporting of results.

Table 8.2.4.1-13: Analytical results

Nominal concentrations (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 48 hours
6.25	112	104
12.5	111	97
25	104	100
50	103	93
100	106	96

An estimate of the EC_{50} values was given by inspection of the immobilization 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 8.2.4.1-14: Effects of SYN545245 on *Daphnia magna* following exposure for 48-hours in a static test

Nominal concentration (mg/L)	Immobilised daphnids after 24 hours		Immobilised daphnids after 48 hours	
	Number	%	Number	%
Dilution water control	0	0	0	0
6.25	0	0	0	0
12.5	0	0	0	0
25	0	0	0	0
50	0	0	0	0
100	0	0	0	0
EC_{50} mg/L	>100		>100	
95% Confidence limits	n.d.		n.d.	
NOEC	100		100	

n.d. – not determined

Conclusions

Based on nominal concentrations, the 48-hour EC_{50} for SYN545245 to Daphnia magna was 100 mg ai/L. The 48-hour NOEC was >100 mg/L.

(Sacker D., 2016)

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

Please refer to original EU review. No new data are provided. The endpoints for studies previously submitted in the EU were provided in point B.9.2.4 of the DAR, ~~therefore additional study summaries are not repeated here~~. The endpoints are summarised in Table 8.2-4. At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.4 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-4 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Holmes C.M. & Swigert J.P., (1993). CGA-173506 – A 96-hour flow through acute toxicity test with the saltwater mysid (Mysidopsis bahia). Report Number 108A-134. Wildlife International Ltd, 8598 Commerce Drive, Easton, MD 21601, USA. (Syngenta File No. CGA173506/0256)*

Guidelines

US EPA Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and aquatic organisms, EPA 540/9-82-024, Series 72-3 (1982)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to the saltwater mysid (*Mysidopsis bahia*) was determined under flow-through conditions. This study was run with mean measured concentrations of 0.075, 0.15, 0.24, 0.37 and 0.63 mg CGA173506/L together with a dilution water control and a solvent control.

The 96-hour LC_{50} was 0.27 mg CGA173506/L, based on mean measured concentrations.

Materials

Test material	CGA173506
Description:	Fluffy white powder
Lot/Batch #:	P905004/5
Purity:	95.4%
Stability of test compound:	Stable under test conditions
Water solubility:	1.53 mg/L at 20°C

Treatments

Test concentrations:	Nominal concentrations: 0.10, 0.17, 0.29, 0.48 and 0.80 mg CGA173506/L Mean measured concentrations: 0.075, 0.15, 0.24, 0.37 and 0.63 mg CGA173506/L
Dilution water:	Natural seawater (0.25 µm filtered) diluted with well water to obtain the appropriate salinity
Vehicle control:	Acetone vehicle (1.0 mL/L)
Analysis of test concentrations:	Yes, at 0 and 96 hours using HPLC analysis

Test organisms

Species:	Juvenile saltwater mysids (*Mysidopsis bahia*)
Source:	Wildlife International Ltd. Cultures 8651 Brookes Drive, Easton, Maryland 21601
Acclimatisation period:	Adult cultures maintained for at least 14 days before test initiation
Treatment for disease:	None
Life stage of test organism:	< 24 hours (juvenile)
Feeding:	Live brine shrimp (*Artemia* sp.) daily during test

Test design

Test vessels:	500 mL glass beakers with a Nytex screen attached to each side opening to allow flow-through. Beakers were suspended in 8 L Teflon-lined polyethylene aquaria filled with 6.5 L of test media to give a depth of 17 cm in each glass chamber.
Replication:	2 replicates, 10 mysids per replicate
Exposure regime:	Flow-through
Duration:	96 hours

Environmental conditions

Test temperature:	25 ± 1°C
pH range:	8.2 to 8.3 measured daily
Dissolved oxygen:	6.0 to 7.4 mg/L measured daily*
Salinity of dilution water:	25‰ at test start
Lighting:	16 hours fluorescent light and 8 hours dark daily, with 30 minute dawn and dusk transition periods. Light intensity ≈495 lux at water surface.

* A dissolved oxygen concentration of 4.3 mg/L represents 60% saturation at 25°C in saltwater with a salinity of 25‰

Study Design and Methods

Experimental dates: 12th to 16th June 1992

Test chambers were suspended in the aquaria which were filled with test water, giving a depth of 17 cm in each chamber. A continuous-flow diluter was used to deliver each concentration of the test substance, solvent control and dilution water control. The diluter was adjusted so that each chamber received 28 volume additions of test water every 24 hours. 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 aquaria were randomly positioned in a temperature-controlled water bath designed to maintain a temperature of 25 ± 1 °C. The water bath was enclosed in a plexiglass ventilation hood to minimise potential cross-contamination. 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 0.84 mg CGA173506/mL test material was prepared in acetone and following dilution with acetone yielded working stock solutions of 0.11, 0.18, 0.30 and 0.50 mg CGA173506/mL. The five test stocks were injected into the diluter mixing chambers where they were mixed with saltwater to achieve the desired test concentrations. The resultant test concentrations were adjusted for purity of the active ingredient in test substance. Acetone only was injected into the mixing chamber for the solvent control group. The concentration of acetone in the solvent control and treatment groups was 1.0 mL/L. The concentrations of test material in the test solutions were measured at the beginning and at 96 hours using high performance liquid chromatography (HPLC).

Observations were made for mortality and clinical symptoms of toxicity at approximately 4, 24, 48, 72 and 96 hours. The LC_{50} values were estimated using the binomial method.

Results and Discussion

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

Table 8.2.4.2-1: Analytical results

Nominal concentration (mg CGA173506/L)	Measured concentration (mg CGA173506/L) 0 hours	Measured concentration (mg CGA173506/L) 48 hours	Mean measured concentration (mg CGA173506/L)
Negative control	ND	ND	ND
Solvent control	ND	ND	ND
0.10	0.0575	0.0915	0.075
0.17	0.1541	0.1545	0.15
0.29	0.2539	0.2332	0.24
0.48	0.3692	0.3747	0.37
0.80	0.6310	0.6296	0.63

ND = not detected

With the exception of two incidental deaths in the solvent control, mysids in the negative and solvent control groups appeared healthy and normal throughout the study. Treatment related effects were observed in all mysids at test concentrations > 0.24 mg CGA173506/L.

Table 8.2.4.2-2 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 (mg CGA173506/L)	Cumulative percent mortality and treatment-related effects (n = 20)					
	R	4 hour	24 hour	48 hour	72 hour	96 hour
Dilution control	A	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)
	B	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)
Solvent control	A	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)
	B	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	20 (8AN)
0.075	A	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	10 (9AN)
	B	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)
0.15	A	0 (1C, 9AN)	20 (8AN)	20 (8AN)	20 (8AN)	20 (8AN)
	B	0 (1C, 9AN)	10 (9AN)	10 (9AN)	10 (9AN)	10 (9AN)
0.24	A	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)	0 (10AN)
	B	0 (10AN)	10 (9AN)	30 (7AN)	30 (7AN)	50 (2C, 3AN)
0.37	A	0 (10AN)	0 (10AN)	80 (2AN)	100	100
	B	0 (10AN)	0 (10AN)	80 (2AN)	100	100
0.63	A	0 (10AN)	80 (2C)	100	100	100
	B	0 (10AN)	60 (1A,1C, 2AN)	100	100	100
LC_{50} (mg CGA173506/L)	-	-	> 0.37	0.30	0.28	0.27
95% confidence level	-	-	N/A	0.24 - 0.37	0.24 - 0.37	0.24 - 0.37

[a] Ten mysids were exposed in each test vessel, two replicates per treatment.
Observed effects: A = surfacing; C = lethargy; AN = Appears normal

Validity Criteria

No validity criteria are reported.

Conclusions

The 96 hour LC_{50} for test material to the saltwater mysid (*Mysidopsis bahia*) was estimated to be 0.27 mg CGA173506/L, based on mean measured concentrations.

(Holmes C.M., & Swigert J.P., 1993)

This study complies with the current validity criteria for acute toxicity testing with the saltwater mysid. Therefore this study is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Holmes, Swigert, 1993, CGA173506/0256	US EPA FIFRA Guideline 72-3 (1982)	US EPA OCSPP 850.1035 (2016)	The following deviations are noted: 1. Saltwater with a salinity of 25‰ was used (salinity of 20 ± 2‰ is recommended). 2. The concentration of vehicle solvent used was 1.0 mL/L (should not exceed 0.1 mL/L)	The validity criteria were met: 1. Mortality in the negative control and solvent control was ≤ 10% (0% and 10% respectively). 2. Treatments and organisms were indiscriminately assigned. 3. All test vessels were identical. 4. A surfactant or dispersant was not used in the preparation of the stock/test solution. DO ≥ 60%; 4.3 mg/L represents 60% saturation at 25°C in saltwater with a salinity of 25‰ (6.0 to 7.4 mg/L). Mean measured concentrations ranged between 75% and 88% of nominal, thus results are based on mean measured concentrations. The solvent (acetone) concentration in the treatment and solvent control groups was 1.0 mL/L; this concentration is considered to be within the tolerance of Mysid for acute toxicity testing (as indicated by the acceptable level of mortality/effects in the solvent control). The study is reliable and the endpoint can be used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.4 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-4 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Surprenant D.C., (1990). (CGA-173506 Technical) – Acute Toxicity to Eastern Oysters (Crassostrea virginica) under Flow-Through Conditions. Report Number 89-5-2979, Ciba Plant Protection, Ciba-Geigy Corporation, Post Office Box 18300, Greensboro, NC 27419, USA. (Syngenta File No. CGA173506/0390)*

Guidelines

US EPA Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and aquatic organisms, EPA 540/9-82-024, Series 72-3 (1985)

ASTM Standard E 729-80. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians (1980)

GLP: Yes

Executive Summary

The acute toxicity of CGA173506 to eastern oyster *Crassostrea virginica* was determined under flow-through conditions. The study was run with mean measured concentrations of 93, 150, 220, 380 and 540 µg a.s./L, together with negative and solvent controls.

Based on mean measured concentrations, the 96-hour EC_{50} for CGA173506 was 370 µg a.s./L.

Materials

Test material	CGA173506 technical
Description:	Yellow powder
Lot/Batch #:	P85002
Purity:	97.5 %
Stability of test compound:	Not reported

Treatments

Test concentrations:	Negative (saltwater) control, solvent control, and nominal concentrations of 84, 140, 230, 390 and 650 µg a.s./L (mean measured: 93, 150, 220, 380 and 540 µg a.s./L)
Dilution water:	Natural unfiltered seawater
Vehicle and/or positive control:	Dilution water control and acetone control
Analysis of test concentrations:	Yes at 0 and 96 hours (based on analysis of CGA173506) using GC-NPD analysis

Test organisms

Species:	Eastern oysters (*Crassostrea virginica*)
Source:	Aquacultural Research Corporation, Dennis, Massachusetts, USA
Acclimatisation period:	Acclimatised 48 hours before test initiation
Treatment for disease:	None reported
Length of oysters at test start:	Not reported, mean valve height 42 mm (SD = 4)
Feeding:	180 mL of an algal suspension (10^7 cells/mL) of *Isochrysis galbana* and

	Tetraselmis maculata, three times daily

Test design

Test vessels:	Glass aquaria (60 x 30 x 30 cm) containing 18 L water with a 10 cm-high standpipe
Replication:	Two replicates per treatment and controls; 20 oysters per test vessel
Exposure regime:	Flow-through
Duration:	96 hours

Environmental conditions

Test temperature:	18 – 20 °C
pH:	7.7 – 8.0 measured
Dissolved oxygen:	6.5 – 7.9 mg/L
Salinity of dilution water:	31 – 33 ‰
Lighting:	16-hour fluorescent light

Study Design and Methods

Experimental dates: 9th to 13th February 1989

A continuous-flow diluter was used to deliver each concentration of the test substance, solvent control and negative control to each test chamber. The diluter was adjusted so that each chamber received approximately 75 mL/minute (equivalent to 6 volume replacements every 24 hours). A Nylon impeller pump was used to continuously recirculate the solution from one end of the aquarium to the other end. The flow rate of the recirculating test solution was 1.75 L/minute (equivalent to 5 L/oyster/hour). The test chambers were impartially positioned within a water bath to maintain temperature.

A diluter stock solution was prepared at 8.61 mg a.s./L by dissolving 2.6502 g of CGA173506 (2.5839 active substance) in a total volume of 300 mL acetone. The stock solution was delivered to the diluter at a rate of 0.0283 mL/minute along with 375 mL/minute of dilution seawater to produce the highest nominal test concentration. This was subsequently diluted (0.6 dilution factor) to produce the remaining nominal test concentrations. A control consisted of dilution seawater only, and a solvent control containing 75 µL acetone/L (the maximum amount present in each test concentration) were also tested.

The concentrations of CGA173506 in the test solutions were measured at 0 and 96 hours using a GC-nitrogen phosphorus detection (NPD) method.

Observations were made daily for mortality and symptoms of toxicity. At the end of the exposure period, mean shell growth was calculated for each control and treatment group by measuring microscopically to the nearest 0.1 mm using a calibrated micrometer.

The EC_{50} for reduced shell deposition was calculated by fitting the untransformed and transformed data to a best fit linear regression curve based on least squares, and then using the method of inverse prediction (Sokal and Rohlf, 1969). The NOEC was determined using Williams' Test coupled with either Bartlett's Test or Kruskal-Wallis Test for the determination of homogeneity of variances.

Results and Discussion

Measured concentrations ranged from 83 to 111 % of the nominal treatment levels. The limit of detection in this study was 0.00877 mg a.s./L for a 1000 mL sample. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.4.2-3: Analytical results

Nominal concentration (µg a.s./L)	Measured concentration at 0 hours (µg a.s./L)		Measured concentration at 96 hours (µg a.s./L)		Mean measured concentration (µg a.s./L) [a]
	Replicate A	Replicate B	Replicate A	Replicate B	-
Control	< 5.6	< 5.6	< 7.0	< 7.0	-
Solvent control	< 5.6	< 5.6	< 7.0	< 7.0	-
84	81	94	99	99	93
140	130	140	170	180	150
230	180	210	250	220	220
390	360	370	420	380	380
650	510	520	570	580	540

[a] Mean measured concentrations were calculated using the unrounded analytical results

No mortalities were observed among treatment and control groups. Oysters exposed to the highest test concentration (540 µg a.s./L) exhibited reduced feeding and faecal production after 72 hours of exposure. No abnormal behaviour was observed among *organisms* exposed to the remaining measured concentrations (≤ 380 µg a.s./L). Shell growth was reduced by 75, 54, 29, 13 and 8.3 % in the 540, 380, 220, 150 and 93 µg a.s./L treatment levels, respectively.

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

Table 8.2.4.2-4: Effects of CGA173506 on the shell deposition of the eastern oyster, *Crassostrea virginica* following exposure for 96 hours in a flow-through test

Mean measured concentration (µg a.s./L)	Shell deposition (mm) n = 40 [a]		Shell growth reduction[b] (%)
	Mean	Standard deviation	
Control	2.5	1.1	n.a.
Solvent control	2.4	1.2	n.a.
Pooled control	2.4	1.1	n.a.
93	2.2	1.1	8.3
150	2.1	1.0	13
220	1.7	1.0	29
380	1.1	0.8	54
540	0.6	0.5	75
EC_{50}	370 µg a.s./L		
95 % confidence limits	300 – 444 µg a.s./L		
NOEC	93 µg a.s./L		

[a] The mean shell deposition represents the measurements of 40 oysters per treatment and each control.
[b] % reduction of shell deposition as compared to pooled control oyster shell growth.
n.a. = not applicable

Validity Criteria

No validity criteria are reported.

Conclusions

Based on mean measured concentrations, the 96-hour EC_{50} for CGA173506 to eastern oyster *Crassostrea virginica* was 370 µg a.s./L with 95 % confidence limits of 300 – 444 µg a.s./L.

(Surprenant D.C., 1990)

This study complies with the current validity criteria for acute toxicity testing with the Eastern oyster. Therefore this study is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Surprenant, 1990, CGA173506/0390	US EPA FIFRA Guideline 72-3 (1985)	US EPA OCSPP 850.1025 (2016)	The study is compliant with US EPA OCSPP 850.1025 (2016)	The validity criteria were met: 1. Disease/stress in the negative control and solvent control was ≤ 10% (0%). 2. Treatments and organisms were indiscriminately assigned. 3. All test vessels were identical. 4. Overall mean ≥2 mm of new shell growth in negative control and solvent control (2.5 and 2.4 mm respectively). 5. A surfactant or dispersant was not used in the preparation of the stock/test solution. DO ≥ 60%; 7.7 mg/L represents 100% saturation at 19°C in saltwater with a salinity of 32‰ (6.5 to 7.9 mg/L). Mean measured concentrations ranged between 83% and 111% of nominal; the results are based on mean measured concentrations. The solvent (acetone) concentration in the treatment and solvent control groups was 75µL/L; this concentration is considered to be within the tolerance of Eastern oyster for acute toxicity testing. Negative control and solvent control growth results were not statistically significantly different (1-way ANOVA); therefore the controls were pooled for comparison with the treatments.

				The study is reliable and the endpoint can be used in the risk assessment.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

Please refer to original EU review. The endpoints for studies previously submitted in the EU are provided in point B.9.2.5 of the DAR**.** The endpoints are summarised in Table 8.2-6.

The RMS requested that a justification is provided for the robustness and the reliability of the NOEC values. Syngenta's response is below:

The *Daphnia* and Mysid chronic studies providing the proposed chronic invertebrate endpoints for fludioxonil, were all conducted according to the most recent versions of the internationally recognised test guidelines (OECD 211 (2012), US EPA OCSPP 850.1300 (1996), US EPA OCSPP 850.1350 (1996)). The replication and number of organisms proposed in these guidelines is in place to derive a reliable and robust NOEC.

While the chironomid study was conducted prior to the finalisation of the OECD guideline, there is nothing to call the study endpoint into question (EC50 and NOEC ≥highest test concentration).

EFSA have confirmed that assessing the statistical power of a NOEC from a lower tier laboratory test is not necessary (*EFSA; Outcome of the pesticides peer review meeting (133) on general recurring issues in ecotoxicology, 2015*).

The studies conducted by Rufli, 1989 (Syngenta file number CGA173506/0006) and Putt, 1991 (Syngenta file number CGA173506/0179) were considered to have major deficiencies. These are addressed under the 'Long-term invertebrate endpoint – *Daphnia magna*' section presented earlier in this document. For completeness updated OECD summaries have been provided at the request of ANSES during the pre-submission meeting on 14 July 2015.

Report:	Re-submitted the OECD summary at the request of ANSES for ease of dossier re-evaluation: K-CA 8.2.5.1/01 Rufli H (1989). Daphnia, Reproduction Test with CGA173506 technical, Report Number 88 17 36, CIBA-GEIGY, Ltd., CH-4002 Basle, Switzerland. (Syngenta File No. CGA173506/0006)

Guidelines

OECD Guidelines No. 202, Phase 2 (1984) modified according to EEC/OECD-ringtest (1985/1986)

GLP: Yes

Executive Summary

The effects of fludioxonil on the survival and reproduction of *Daphnia magna* were determined over 21 days under semi-static conditions. The study was run with a blank control, a solvent control and nominal concentrations of 0.00074, 0.0022, 0.0067, 0.020, 0.060 and 0.18 mg a.s./L (mean measured: 0.0004, 0.0009, 0.005, 0.01, 0.03 and 0.1 mg a.s./L). Based on mean measured concentrations, the 21-day EC_{50}

for reproduction (total cumulative number of young) was 0.03 – 0.1 mg a.s./L and the 21-day NOEC for reproduction (total cumulative number of young) was 0.03 mg a.s./L.

Materials

Test material	CGA 173506 technical
Lot/Batch #:	805002
Purity:	97.5 %
Description:	Beige solid
Reanalysis date:	30 June 1992
Treatments	
Test concentrations:	Blank control, vehicle control and nominal concentrations of 0.00074, 0.0022, 0.0067, 0.020, 0.060 and 0.18 mg a.s./L (mean measured concentrations: 0.0004, 0.0009, 0.005, 0.01, 0.03 and 0.1 mg a.s./L)
Solvent/Carrier:	0.04 mg alkylphenol-polyglykol-ether/L and 71.2 mg acetone/L water
Positive control:	None
Analysis of test concentrations:	Yes, analysis of CGA173506 on days 0, 7, 14 and 21 by HPLC analysis; analysis on day 7, 14 and 21 performed on old test solutions.
Test animals	
Species:	*Daphnia magna* Straus 1820 (neonates <24 hours old)
Source:	In-house laboratory culture
Feeding:	Daily, except on Sundays, with freshly grown green alga (*Scenedesmus subspicatus*) and a suspension of ground Tetramin® fish depending on age and condition of daphnids
Culture medium:	Reconstituted water; aerated with filtered air for at least 24 hrs before use.
Test design	
Test vessels:	100 mL glass beakers covered with watch glasses containing 50 mL medium
Test medium:	Reconstituted water; aerated with filtered air for at least 24 hrs before use.
Replication:	10 beakers for each control and test concentration (one organism per chamber); 15 daphnia from day 0 – 13 to account for the occurrence of males.
Exposure regime:	Semi-static; solution renewal each Monday, Wednesday and Friday
Duration:	21 days
Environmental conditions	
Test temperature:	20 ± 1 °C
pH range:	7.8 to 8.9
Dissolved oxygen:	95 to 133 % saturation (no aeration)
Lighting:	Approx. 1500 lux 16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 1st to 22nd March 1989

A stock solution was prepared by dissolving 0.2 g fludioxonil and 40 mg alkylphenol-polyglycol in 100 mL of acetone. The stock solution was then used to prepare a 0.2 mg a.s./L solution with water. Calculated amounts of the 0.2 mg a.s./L solution were used to prepare a range of solutions at lower concentrations. The blank control consisted of water only, while the vehicle control contained 71.2 mg acetone/L and 0.04 mg alkylphenol-polyglykol-ether/L.

The test was started by adding 1 neonate (less than 24 hours old) to each replicate vessel. The test vessels were held at 20 ± 1 °C. The *Daphnia* were fed daily except on Sundays with a green alga (*Scenedesmus subspicatus*) and a suspension of ground Tetramin® fish depending on age and condition of the daphnids. Three times each week the test medium was renewed.

The number of immobilised daphnids was determined on test days 1, 2, 4, 7, 14 and 21. The number of young produced was recorded on test days 7, 9, 12, 14, 16, 19 and 21.

The concentration of fludioxonil in the test solutions was measured in samples collected from the test vessels on day 0 in fresh solutions, and Days 7, 14 and 21 in old solutions. Samples were analysed using HPLC analysis

The temperature was continuously monitored, dissolved oxygen was measured thrice weekly immediately before the renewal of the test solution and pH was measured thrice weekly before and after each renewal.

For immobilisation, the EC_{50} was calculated using probit analysis using log(concentration) as the predictor. For the NOEC, the Cochran-Armitage trend test was used to assess increasing or decreasing number of immobilised daphnids with increasing concentration.

For the reproduction data for the NOEC, preliminary investigation of the data indicated that the data was non-normal with skewed and possibly heavy tail, therefore, a Jonckheere's test (Lehmann, 1975) was used to test for either increasing or decreasing number of young with increasing concentration and the NOEC was calculated.

For each mother that produced young, the fraction of dead young was taken as a measure of morbidity. EC_{50} values were estimated from a probit analysis using ln(concentration) as the predictor and ln(0.00038/3) in place of ln(0), which is undefined (Turkey *et al.* 1985). For the NOEC, the analysis was performed as per the reproduction data.

For the length of time for the appearance of the first brood, a trend test according to Mantel (1963) was applied, and the NOEC was calculated as previously described.

All statistical analysis was performed using a significance level of $\alpha = 0.05$.

Results and Discussion

The mean measured concentrations of the test item were in the range 41 to 78 % of the nominal values (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 (mg a.s./L)	Measured concentration (mg a.s./L)					Standard deviation[b]	Coefficient of variation[b] (%)	Percent of nominal (%)
	Day 0	Day 7 [a]	Day 14 [a]	Day 21 [a]	Mean measured concentration (mg a.s./L)			
Control	n.r.	n.r.	n.r.	n.r.	-	-	-	-

Solvent control	n.r.	n.r.	n.r.	n.r.	-	-	-	-
0.00074	0.00076	0.00042	0.00025	0.00047	0.00038	0.00018	48	51
0.0022	0.0020	0.0008	0.0009	0.0011	0.0009	0.00047	53	41
0.0067	0.0059	0.0063	0.0047	0.0047	0.0052	0.00071	14	78
0.020	0.018	0.0092	0.014	0.0096	0.0109	0.0036	33	55
0.060	0.052	0.036	0.039	0.025	0.033	0.0096	29	55
0.180	0.165	0.104	0.141	0.084	0.110	0.032	29	61

[a] values measured in 'old' test solutions (after 48 hours exposure) just prior to renewal
[b] calculated after report was produced
n.r. not reported
- not applicable

The number of immobilised daphnids and cumulative numbers of offspring are presented in the table below.

One immobile parent animal was observed in the blank control and solvent control and the 0.0009 mg a.s./L treatment at test termination. In the 0.0004, 0.005, 0.01 and 0.03 mg a.s./L treatment levels, 5, 2, 0 and 5 *Daphnia* were observed immobilised, respectively. At the highest concentration (0.1 mg a.s./L) 4 immobilised daphnia were observed.

The first brood juveniles were observed on day 9 in the controls and in the 0.0004, 0.0009, 0.005, and 0.01 mg a.s./L treatment levels. Daphnids in the 0.03 and 0.1 mg a.s./L treatments released first brood offspring between days 9 and 12.

The mean number of juveniles per surviving adult showed a statistically significant inhibitory effect on the reproduction of *D. magna* over 21 days at a concentration of 0.1 mg a.s./L (see table below).

Table 8.2.5.1-2: Effects of fludioxonil on *Daphnia* reproduction and immobilisation of the adult (P) generation

Nominal concentrations (mg a.s./L)	Mean measured concentrations (mg a.s./L)	Immobilised daphnids at Day 21 (n = 10)	Mean cumulative juveniles per female at Day 21	
			Dead and alive	Dead
Control	-	1	62	7
Solvent control	-	1	54	7
0.00074	0.00038	5	53	17
0.0022	0.0009	1	70	13
0.0067	0.0052	2	68	17
0.020	0.0109	0	59	20
0.060	0.033	5	52	12
0.18	0.110	4	7	3

Table 8.2.5.1-3: Effects of fludioxonil on time to appearance of first brood

Mean measured concentrations (mg a.s./L)	Number of *Daphnia* with first brood			
	Day 9 [b]	Day 12 [b]	Day 14 [b]	≥Day 14 [b]
Control	9	1	0	0
Solvent control	7	3	0	0
0.00038	7	3	0	0

0.0009	8	2	0	0
0.0052	6	4	0	0
0.0109	1	9	0	0
0.033	0	10	0	0
0.110	0	3	1	6 [a]

[a] Three of the daphnids had died before offspring production had started
[b] Neonates were not counted on the days occurring during the intervals

The results of the statistical analyses are summarised in the table below.

Table 8.2.5.1-4 Summary of the effects of fludioxonil on *Daphnia magna* after 21 days exposure

Endpoint	EC_{50} (mg a.s./L)	LOEC (mg a.s./L)	NOEC (mg a.s./L)
Immobilisation	> 0.1 [a]	> 0.1	0.1
Reproduction: Total cumulative number of young	0.03 – 0.1 [b]	0.1 [c]	0.03 [c]
Reproduction: Fraction of cumulative dead neonates	20 [d]	0.01	0.005 [e]
Appearance of first brood	-	0.01	0.005

The vehicle and the blank were not significantly different in respect to the above parameters
[a] Extrapolated EC_{50} = 18 mg/L (including all concentrations); extrapolated EC_{50} = 0.4 mg/L (excluding 0.0004 mg a.s./L where 50% parental mortality occurred by 21d); both estimates are unstable.
[b] EC_{50} could not be calculated reliably from the data, ranges are presented
[c] The low cumulative number of young for concentration 0.0004 mg a.s./L was not considered dose response compared to the two next higher concentrations of 0.0009 and 0.005 mg a.s./L.
[d] This estimate is unstable
[e] Not dose responsive (see Table 8.2.5.101)
- not applicable

Validity criteria

None reported

Conclusions

In a test in which the effects of fludioxonil on reproduction of *Daphnia magna* were investigated, the 21-day NOEC reproduction (total cumulative number of young) was 0.03 mg a.s./L. The NOEC for the other reproduction parameters measured, i.e. fraction of cumulative dead and time to appearance of first brood was 0.005 mg a.s./L.

It was concluded that the 21-day EC_{50} reproduction (total cumulative number of young) for fludioxonil to *Daphnia magna* was 0.03 – 0.1 mg a.s./L, based on the mean measured concentrations.

The 21-day NOEC reproduction (total cumulative number of young) was 0.03 mg a.s./L.

(Rufli H. 1989)

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

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.2.5.1/02 Taylor, S. & Allen M. (2016), Fludioxonil (CGA173506) – Report on the *Daphnia* Reproduction Test with CGA173506 technical, Statistical re-analysis. Report Number: CEA.1589, Reference Number CCE2068_002. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11865)

Summary

The report from CIBA GEIGY Ltd, study number 88 17 36 (Rufli, 1989, Syngenta file number CGA173506/0006) for the chronic toxicity of CGA173506 to *Daphnia magna* did not provide estimates of the EC_{10} and EC_{20} for the response variables evaluated as part of the original study. Additionally, an EC_{50} value was not presented for reproduction. Consequently the data generated in this study have been re-analysed in an attempt to provide these values.

Mean measured concentrations of CGA173506 ranged from 41 to 78% of the nominal values. As a result, and in line with the approach used in the original report, the results for this analysis are based on the mean measured concentrations of 0.0004, 0.0009, 0.005, 0.01, 0.03 and 0.1 mg/L.

The following parameters were analysed statistically:

- Immobilisation of parental *Daphnia* after 21 days
- Total number of live offspring per adult *Daphnia* after 21 days

Data were also available for the time to appearance of the first brood, however these were inappropriate for estimation of effect concentrations and were not analysed.

Statistical analyses of the available data for the number of offspring per parent on day 21 revealed that the following ECx values and 95% confidence limits were reliably calculated.

Table 8.2.5.1-5: Statistical analysis for EC_{10}, EC_{20} and EC_{50} endpoints for the fludioxonil *Daphnia magna* reproduction study (Rufli *et al.*, 1989; Syngenta file number CGA173506/0006)

Parameter	Number of Offspring per Parent (Day 21)		
	EC_{10}	EC_{20}	EC_{50}
Value [mg/L]	0.0276	0.0343	0.0517
lower 95%-cl	0.0069	0.0131	0.0161
upper 95%-cl	0.1108	0.0922	0.1706

cl: confidence limits

Statistical analyses of the available data for adult immobility parameter revealed that no reliable ECx values could be calculated.

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 for adult immobility.

All computations were carried out in ToxRat Professional version 3.1 (ToxRat Solutions GmbH, 2005).

Results

Adult immobility

The relationship between dose and response was not significant p(F) = 0.804 and as a result, no ECx values could be reliably calculated.

Mean total number of live young per adult *Daphnia* on Day 21

A significant amount of variance was explained by the regression model (p(F):Regression = 0.028). As a result, the EC_{10}, EC_{20} and EC_{50} values and the 95% confidence limits presented were reliably determined.

Conclusion

Mean measured concentrations of CGA173506 ranged from 41 to 78% of the nominal values. As a result and in line with the approach used in the original report, all reported biological results are based on the mean measured concentrations of 0.0004, 0.0009, 0.005, 0.01, 0.03 and 0.1 mg/L).

Statistical analyses of the available data for the number of offspring per parent on day 21 revealed that the following ECx values and 95% confidence limits were reliably calculated.

Table 8.2.5.1-6: Statistical analysis for EC_{10}, EC_{20} and EC_{50} endpoints for the fludioxonil *Daphnia magna* reproduction study (Rufli *et al.*, 1989; Syngenta file number CGA173506/0006)

Parameter	Number of Offspring per Parent (Day 21)		
	EC_{10}	EC_{20}	EC_{50}
Value [mg/L]	0.0276	0.0343	0.0517
lower 95%-cl	0.0069	0.0131	0.0161
upper 95%-cl	0.111	0.0922	0.171

cl: confidence limits

Statistical analyses of the available data for adult immobility parameter revealed that no reliable ECx values could be calculated.

(Taylor S, Allen M, 2016)

Report:	Re-submitted the OECD summary at the request of ANSES for ease of dossier evaluation: K-CA 8.2.5.1/03 Putt AE (1991). (CGA173506 technical) – Chronic Toxicity to Daphnids (*Daphnia* magna) under flow-through conditions, Report Number 91-2-3672, Springborn Laboratories, Inc., Environmental Sciences Division, 790 Main Street, Wareham, MA 02571. (Syngenta File No. CGA173506/0179)

Guidelines

US EPA. 1982. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. October 1982. EPA-540/-85-024 [FIFRA 72-4]

GLP: Yes

Executive Summary

The effects of fludioxonil (CGA173506) on the survival, reproduction and growth of *Daphnia magna* were determined over 21 days under flow-through conditions. The study was run with a dilution water

control, solvent control and nominal concentrations of 20, 40, 80, 160 and 320 µg a.s./L (19, 34, 62, 86 and 190 µg a.s./L, mean measured).

Based on mean measured concentrations the 21-day NOEC was 19 µg a.s./L and the 21-day LOEC was 34 µg a.s./L. The 21-day EC_{50} was empirically estimated to be > 190 µg a.s./L.

Materials

Test material	CGA173506 technical
Lot/Batch #:	P805002 FL # 881677
Purity:	97.5 %
Description:	Yellow powder
Stability of test compound:	Stable under test conditions
Reanalysis date:	Not reported
Treatments	
Test concentrations:	Dilution water control, solvent control and nominal concentrations of 20, 40, 80, 160 and 320 µg a.s./L (mean measured concentrations: 19, 34, 62, 86 and 190 µg a.s./L)
Solvent/Carrier:	Acetone
Positive control:	None
Analysis of test concentrations:	Yes, analysis of fludioxonil, in low, middle and high concentrations, twice prior to test start, and in all concentrations on days 0, 7, 14 and 21 by GC analysis with nitrogen phosphorus detection
Test animals	
Species:	*Daphnia magna* (<24 hours old)
Source:	Continuous laboratory cultures
Feeding:	Two- or three-times daily with trout food suspension, algal suspension (*Ankistrodesmus falcatus*) and Selco® food supplement
Culture medium:	Well water fortified to a total hardness of approx. 160 – 180 mg as $CaCO_3$
Test design	
Test vessels:	1.6 L glass jars, with a 2.0 cm hole approximately 15 cm from the bottom of the jar, containing 1.4 L of test medium
Test medium:	Fortified well water adjusted to hardness of 160 mg/L as $CaCO_3$, and filtered prior to test initiation
Replication:	Four replicate chambers for each control and test concentration (ten organisms per chamber)
Exposure regime:	Flow-through
Duration:	21 days
Environmental conditions	
Test temperature:	20 ± 1 °C
pH range:	7.6 to 8.1
Dissolved oxygen:	7.8 to 8.7 mg/L

Lighting:	24 to 50 footcandles (258 to 538 lux) 16 hours light and 8 hours dark

Study Design and Methods

Experimental dates: 12th November to 3rd December 1990

A diluter stock solution of 18.5 mg a.s./L was prepared by dissolving 0.947 g of CGA173506 with acetone to volume in a 50 mL volumetric flask. The flask was inverted several times to ensure that all of the test material had dissolved completely. An intermittent flow proportional diluter equipped with a 20-mL syringe delivered 6.6 µL of the stock solution into 382 mL of dilution water in the system's mixing chamber during each diluter cycle. This 382 mL solution served as the highest treatment level and the remaining test solutions were obtained by further dilution. The mixing chamber was partially submerged in an ultrasonicator throughout the study and the solution continuously stirred with a magnetic stirrer. Test solutions were delivered to the vessels at a rate of 6 test vessel volumes per 24-hour period in order to provide a 90 % test solution replacement rate of approximately 9 hours. The solvent control solutions contained the greatest amount of acetone present in any treatment level (17 µL/L).

The test was started by adding 10 daphnids (≤ 24 hours old) to each replicate vessel. The test vessels were held in a temperature controlled water bath at 20 ± 1 °C. The *Daphnia* were fed three times daily on weekdays and twice a day at weekends with a trout food suspension, algal suspension (*Ankistrodesmus falcatus*) and Selco® food supplement.

The number of surviving adults was determined on test days 1, 2, 4, 7, 9, 11, 14, 16, 18 and 21. Numbers of offspring were determined on test days 9, 11, 14, 16, 18 and 21 and at each observation interval offspring were removed, counted and discarded. At test termination the body lengths and dry weight of the surviving adult daphnids were also measured.

The concentration of CGA173506 in the test solutions was measured in samples collected from the test vessels on days 0, 7, 14 and 21. Samples were analysed using gas chromatography with nitrogen phosphorus detection.

The temperature was measured daily, dissolved oxygen was measured every weekday and water hardness, alkalinity, conductivity and pH were measured weekly. In addition temperature was continuously monitored in one replicate of the nominal 160 µg a.s./L treatment.

Significant differences in percent survival in the control and test item treatments were determined after arcsine square-root percentage transformation of the data. A Student's t-Test was performed for each endpoint to compare the control and solvent control organisms. Statistical comparison of the dilution water control and solvent control data established a significant difference between the two control groups. Therefore, comparisons to determine treatment level effects for all parameters were performed against the solvent control data.

The Chi-Square Goodness of Fit Test was conducted and compared the observed sample distribution with a normal distribution. The assumption that observations are normally distributed must be validated before subsequent analysis. The homogeneity of variance of normally distributed data was analysed using Bartlett's Test. The results of the each treatment were compared to the solvent control using the Williams' Test.

All statistical analysis was performed at the 95 % significance level except for the Chi-Square Goodness of Fit and Bartlett's Tests which were performed at the 99 % significance level.

Results and Discussion

The mean measured concentrations of the test item in the test media were determined to be 19, 34, 62, 86 and 190 µg a.s./L (see table below). Recovery of CGA173506 in water in the pre-test period was 75 % of the nominal values, and in the fortified quality control samples averaged 102 % of the nominal values. Mean measured concentrations were used for the calculation and reporting of the results.

Table 8.2.5.1-7: Analytical results

Nominal concentration (µg a.s./L)	Measured concentration (µg a.s./L)									Standard deviation	Coefficient of variation[e] (%)	Percent of nominal (%)
	Day 0 [a]		Day 7 [b]		Day 14 [a]		Day 21 [bc]		Mean [d]			
Replicates	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**-**	**-**	**-**	**-**
Control	<1.2	<1.2	<1.5	<1.5	<1.3	<1.3	<2.7	<2.7	-	-	-	-
Solvent control	<1.2	<1.2	<1.5	<1.5	<1.3	<1.3	<2.7	<2.7	-	-	-	-
20	17	18	18	19	18	19	18	24	19	2.2	12	95
40	33	25	35	38	36	30	35	45	34	5.7	17	85
80	57	28	69	67	41	57	87	92	62	22	35	78
160	52	51	95	100	26	78	170	110	86	45	52	54
320	170	130	140	110	150	150	280	420	190	103	54	59

[a] Test solutions from replicates A and B
[b] Test solutions from replicates C and D
[c] Higher than expected analytical results on Day 21 were believed to be due to undissolved test material that was present in the test solution at the time exposure solution samples were removed. Prior to sampling, exposure solutions had been stirred during the removal of test organisms.
[d] Mean measured concentrations were calculated using the actual analytical results and not the rounded values presented in this table.
[e] calculated after report was produced
- not applicable

Survival of the parent animals was 100 % in the solvent control and 93 % in the water control and survival in all test concentrations up to and including 86 µg a.s./L was within the range 95 – 100 %. At the highest test concentration (190 µg a.s./L) survival was 80 % which was significantly less ($p \leq 0.05$) than in the solvent control. At test termination sublethal effects, including lethargy, erratic swimming behaviour, daphnids on the surface of the test solution, and daphnids carrying or caught on organic matter, were observed at test concentrations ≥ 34 µg a.s./L.

First brood juveniles were observed in the controls on day 8. Organisms in the 19 and 34 µg a.s./L treatment levels released their offspring by Day 10, which was comparable to the controls. Daphnids in the 62 and 86 µg a.s./L treatments released first brood offspring between days 13 and 14. The delay in first brood release among these organisms was considered to be a treatment-related response. At 190 µg a.s./L no juveniles were produced throughout the test.

The mean number of juveniles per surviving adult showed a statistically significant inhibitory effect on the reproduction of *D. magna* over 21 days at concentrations of 34 µg a.s./L and above (see table below).

Statistical analysis established a significant reduction in total body length among surviving organisms exposed to concentrations of 34, 62 and 86 µg a.s./L, at test termination compared to the solvent control organisms. Dry weight of organisms exposed to CGA173506 showed a statistically significant reduction compared to the solvent control at concentrations of 62 and 86 µg a.s./L.

Table 8.2.5.1-8: Effects of CGA173506 on *Daphnia* reproduction, growth and survival of the adult (P) generation

Nominal concentrations (µg a.s./L)	Mean measured concentrations (µg a.s./L)	Percentage of surviving adults	Juveniles per surviving parent		Body length		Mean organism dry weight	
			Mean number	SD	Mean (mm)	SD	Mean (mg)	SD
Control	-	93	170	48	4.8	0.1	1.28	0.144
Solvent control	-	100	111	13	4.9	0.1	1.19	0.176
20	19	100	103	14	4.8	0.1	1.21	0.145
40	34	98	31*	8	4.6*	0.2	1.14	0.140
80	62	100	15*	9	4.0*	0.3	0.582*	0.0937
160	86	95	1*	1	3.4*	0.2	0.310*	0.0603
320	190	80*	0[a]	0	2.8[a]	0.2	0.150[a]	0.0481

* Significantly ($p \leq 0.05$) different compared to the solvent control organisms
[a] Due to an adverse effect on organism survival, reproduction and growth data for this treatment level were not statistically analysed for treatment effects

The results of the statistical analyses are summarised in the table below.

Table 8.2.5.1-9 Summary of the effects of CGA173506 on *Daphnia magna* after 21 days exposure

Endpoint	EC_{50} (µg a.s./L)	LOEC (µg a.s./L)	NOEC (µg a.s./L)
Immobilisation	> 190	n.r.	n.r.
Reproduction and length of adults	n.r.	34	19

n.r. not reported

Validity criteria

The validity criteria for the test were met:

- Survival in the control and solvent control ≥ 70 % (observed 93 % for control; 100 % for solvent control)
- Offspring per female in the control and solvent control ≥ 40 (observed 170 for control; 111 for solvent control)

Conclusions

In a test in which the effects of fludioxonil (CGA173506) on reproduction of *Daphnia magna* were investigated, based on mean measured concentrations, the 21-day NOEC was 19 µg CGA173506/L and the 21-day LOEC was 34 µg CGA173506/L. The 21-day EC_{50} was empirically estimated to be > 190 µg CGA-173506/L.

(Putt AE. 1991)

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

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.2.5.1/04 Taylor, S. & Radford F. (2016), Fludioxonil (CGA173506) – Chronic Toxicity to Daphnids (*Daphnia magna*) Under Flow-Through Conditions, Statistical re-analysis. Report Number: CEA.1590, Reference Number CCE2054_003. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11866)

Summary

Springborn Laboratories report number 91-2-3672 (Putt, 1991; Syngenta file number CGA173506/0179) for the *Daphnia magna* reproduction test with CGA173506 to 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.

The test used nominal concentrations of 20, 40, 80, 160 and 320 µg a.s./L. The mean measured concentrations over the duration of the test were 19, 34, 62, 86 and 190 µg a.s./L and in accordance with the approach used in the original study, the mean measured values have been used in this statistical analysis. In addition, the treatment data were compared to the solvent control data only as statistical differences were identified between the control groups in the original report.

The following parameters were analysed statistically:

- Survival of parental *Daphnia* at the end of the test
- The number of alive young per female surviving *Daphnia* at the end of the test (reproduction)
- The mean length and dry weight of the adult *Daphnia* surviving until the end of the test

Statistical analyses of the available data revealed that the following EC_{10} and EC_{20} values were reliably calculated:

Table 8.2.5.1-10: Statistical analysis for EC_{10} and EC_{20} endpoints for the fludioxonil *Daphnia magna* reproduction study (Putt, 1991; Syngenta file number CGA173506/0179)

Parameter	Parental Survival		Reproduction	
	EC_{10}	EC_{20}	EC_{10}	EC_{20}
Value [µg a.s./L]	124.20	200.82	18.92	21.77
lower 95%-cl	83.57	138.46	14.80	17.23
upper 95%-cl	204.15	546.84	24.19	27.44
Parameter	**Adult Length**		**Adult Weight**	
	EC_{10}	EC_{20}	EC_{10}	EC_{20}
Value [µg a.s./L]	37.66	70.01	35.58	43.43
lower 95%-cl	8.45	33.04	3.28	8.31
upper 95%-cl	60.36	99.54	48.83	55.59

cl: confidence limits

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 ToxRat Professional version 3.2 (ToxRat Solutions GmbH, 2015).

Results

Parental survival

The relationship between dose and response was significant, p(F) = 0.050 and the slope function was 4.058. The EC_{10} and EC_{20} values and their 95% confidence limits presented were reliably determined.

Reproduction

A significant amount of variance was explained by the regression model (p(F):Regression = <0.001) and there was no significant lack of fit (p(F):Lack of fit = 0.059). As a result, the EC_{10} and EC_{20} values and the 95% confidence limits presented were reliably determined.

Adult length

The relationship between dose and response was significant, p(F) = 0.012 and the slope function was 4.094. The EC_{10} and EC_{20} values and their 95% confidence limits presented were reliably determined.

Adult weight

The relationship between dose and response was significant, p(F) = 0.028 and the slope function was 1.573. The EC_{10} and EC_{20} values and their 95% confidence limits presented were reliably determined.

Conclusion

The test used nominal concentrations of 20, 40, 80, 160 and 320 µg a.s./L. Chemical analysis of the test solutions showed that the measured concentrations ranged from 16.3% to 131.3% of the nominal values. As a result, the mean measured concentrations of 19, 34, 62, 86 and 190 µg a.s./L have been used in this statistical analysis.

In addition, the treatment data were compared to the solvent control data only as statistical differences were identified between the control groups in the original report.

Statistical analyses of the available data revealed that the following EC_{10} and EC_{20} values were reliably calculated:

Table 8.2.5.1-11: Statistical analysis for EC_{10} and EC_{20} endpoints for the fludioxonil *Daphnia magna* reproduction study (Putt, 1991; Syngenta file number CGA173506/0179)

Parameter	Parental Survival		Reproduction	
	EC_{10}	EC_{20}	EC_{10}	EC_{20}
Value [µg a.s./L]	124.20	200.82	18.92	21.77
lower 95%-cl	83.57	138.46	14.80	17.23
upper 95%-cl	204.15	546.84	24.19	27.44
Parameter	**Adult Length**		**Adult Weight**	
	EC_{10}	EC_{20}	EC_{10}	EC_{20}
Value [µg a.s./L]	37.66	70.01	35.58	43.43
lower 95%-cl	8.45	33.04	3.28	8.31
upper 95%-cl	60.36	99.54	48.83	55.59

cl: confidence limits

(Taylor S, Radford F, 2016)

A summary of a study conducted since the original EU review of fludioxonil is presented below:

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.5.1/05 Fournier A. (2014) Fludioxonil – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna*, Under Static Renewal Conditions. Report Number 1781.6936. Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571-1037, USA (Syngenta File No. CGA173506_51057).

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 211: *Daphnia magna* Reproduction test (2012)

US EPA Ecological Effects Test Guidelines, OPPTS 850.1300: Daphnia Chronic Toxicity Test (1996)

GLP: Yes

Executive Summary

The effects of fludioxonil on the survival, growth and reproduction of *Daphnia magna* were determined over 21 days under semi-static-renewal conditions. Nominal concentrations for the treatment levels tested were 3.8, 7.5, 15, 30 and 60 µg a.s./L (2.2, 4.4, 8.9, 18 and 35 µg a.s./L as mean measured concentrations). Following 21 days of exposure, survival, growth (based on total body length and dry body weight) and reproduction were evaluated. Based on mean measured concentrations, the 21-day NOEC for survival, growth and reproduction was 35 µg/L and the 21-day EC_{50} and LOEC values for survival, growth and reproduction were > 35 µg/L.

No additional testing was required to further define the EC_{50} value since the highest concentration tested closely approximated the functional limit of solubility under testing conditions.

Materials

Test Material	Fludioxonil
Batch #:	612044
Purity:	95.5% (wt/wt), tested as 100%
Description:	Grey powder
Stability of test compound:	Stable under standard conditions
Recertification Date:	End of January 2014
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 3.8, 7.5, 15, 30 and 60 µg/L (2.2, 4.4, 8.9, 18 and 35 µg a.s./L mean measured)
Solvent:	None
Positive control:	Potassium dichromate used at least twice a year
Analysis of test concentrations:	Yes, analysis of fludioxonil in newly prepared exposure solutions on days 0, 2, 9, 16 and 19 and in aged exposure solutions on days 2, 5, 12, 19 and 21.
Test animals	
Species:	*Daphnia magna* Straus 1820
Source:	Continuous laboratory cultures
Feeding:	During the in-life test period, test organisms were fed daily 200 µL of unicellular green algae, *Ankistrodesmus falcatus* (4×10^7 cells/mL) in addition to a 50 µL suspension of YCT (yeast, cereal leaves and flaked fish food). In culture, test organisms were fed increasing amounts of food with increasing age.
Culture medium:	Fortified well water
Test design	
Test vessels:	100 mL glass beakers containing 80 mL medium
Test medium:	Fortified well water
Replication:	Ten replicate vessels for each control and test concentration (one parent organism per vessel)
Exposure regime:	Semi-static-renewal, renewal of test solutions three times per week during exposure
Duration:	21 days
Environmental conditions	
Test temperature:	19 to 21°C
pH range:	7.7 to 8.5
Dissolved oxygen:	7.0 to 8.7 mg/L (no aeration)
Water hardness:	130 to 170 mg/L $CaCO_3$
Total Alkalinity:	72 to 90 mg L $CaCO_3$
Conductivity:	570 to 660 µS/cm
Lighting:	9 to 17 $\mu E \cdot m^{-2} \cdot s^{-1}$ 16 hours light and 8 hours dark, with a 15 minute dawn/dusk period

Study Design and Methods

Experimental dates: 11th September to 2nd October 2013

At exposure initiation and every 2 to 4 days thereafter, a 1.0 mg/L primary stock solution was prepared by adding 0.0010 g of fludioxonil to 1.0 L of deionized water. The solution was observed to be clear and colourless with ultrafine, undissolved test substance and was ultrasonicated for approximately one hour. The solution was then mixed overnight using a magnetic stir plate and Teflon®-coated stir bar. The resulting solution was observed to be clear and colourless with no visible undissolved test substance. At exposure initiation and at each renewal interval, exposure solutions were prepared from the primary stock solution.

The test solutions were added to each test vessel and the test was started by adding one daphnid (less than 24 hours old) to each replicate vessel. The test vessels were held in a temperature-controlled environmental chamber at 20 ± 1°C. Three times each week the test solutions were renewed and the test organisms were transferred to freshly prepared test solution.

During the exposure, test organisms were fed daily 200 µL of unicellular green algae, *Ankistrodesmus falcatus* (4×10^7 cells/mL), in addition to a 50 µL suspension of YCT (yeast, cereal leaves and flaked fish food). This quantity of algal suspension (200 µL/daphnid/day) was equivalent to approximately 0.48 mg carbon/daphnid/day.

The number of immobilized adults and observations of abnormal behaviour were recorded daily. Immobilization was defined as when a daphnid was not able to swim within 15 seconds after gentle agitation of the test vessel. Numbers of offspring were determined upon the first brood release in any vessel and thereafter throughout the remainder the test. Offspring were removed, counted and discarded at each observation interval. In addition, the number of immobilized offspring and the time to first brood release were recorded for each treatment level and the control. Observations of physical characteristics of test solutions (e.g., precipitate, cloudy solution) were recorded whenever the test organisms were observed.

The concentrations of fludioxonil in the test solutions were measured in freshly prepared solutions on days 0, 2, 9, 16 and 19 and in the aged solutions on days 2, 5, 12, 19 and 21. A liquid chromatography with mass spectrometry (LC/MS/MS) method was used.

Dissolved oxygen, temperature and pH were measured in each test and control solution at the beginning (new solutions) and end (aged solutions) of each renewal period. In addition, the temperature of the environmental chamber was continuously monitored. Total hardness, alkalinity and conductivity were measured and recorded in the freshly prepared solutions of the highest nominal test concentration (60 µg/L) and the control at exposure initiation and weekly thereafter in the freshly prepared solutions.

Fisher's Exact Test with Bonferroni-Holm's Adjustment was used to determine the NOEC for survival and Dunnett's Multiple Comparison Test was used to determine the NOECs for growth (length and dry weight) and reproduction.

Since no concentration tested resulted in ≥ 50% inhibition of survival, growth or reproduction, the EC_{50} values were empirically estimated to be greater than the highest concentration tested.

Results and Discussion

The measured concentration of the test item in the new test solutions ranged from 49 to 79% of the nominal values and the measured concentrations in the aged test solutions ranged from 43 to 68% (see table below). Therefore, the test item was stable in the test medium over the renewal periods of 48 and 72 hours. Mean measured concentrations were used for the calculation and reporting of the results.

Table 8.2.5.1-12: Analytical results

Nominal concentration (µg a.s./L)	Measured concentration[a] (µg a.s./L)										Mean measured concentration[a] (µg a.s./L)
	Day 0	Day 2		Day 5	Day 9	Day 12	Day 16	Day 19		Day 21	
	(new)	(aged)	(new)	(aged)	(new)	(aged)	(new)	(aged)	(new)	(aged)	
Control	< 0.46[b] (NA)	< 0.50 (NA)	< 0.50 (NA)	< 0.45 (NA)	< 0.43 (NA)	< 0.51 (NA)	< 0.51 (NA)	< 0.51 (NA)	< 0.51 (NA)	< 0.50 (NA)	NA (NA)
3.8	1.9 (49)	2.0 (53)	2.9 (76)	2.5 (66)	2.3 (60)	2.1 (56)	2.0 (53)	1.7 (45)	2.4 (64)	2.1 (55)	2.2 (58)
7.5	4.7 (63)	4.2 (56)	5.9 (79)	5.1 (68)	3.9 (52)	3.7 (49)	4.1 (55)	3.5 (46)	4.8 (64)	4.3 (57)	4.4 (59)
15	8.2 (54)	8.6 (57)	10 (68)	10 (66)	9.2 (61)	9.0 (60)	7.5 (50)	6.4 (43)	11 (74)	8.4 (56)	8.9 (59)
30	17 (57)	15 (52)	21 (70)	20 (65)	18 (59)	17 (57)	19 (64)	14 (47)	18 (59)	18 (59)	18 (59)
60	35 (58)	32 (53)	40 (66)	41 (68)	36 (59)	37 (61)	33 (55)	28 (47)	36 (59)	35 (59)	35 (59)

[a] Each measured and mean measured concentration is presented with percent recovery (based on nominal concentration) in parentheses.

[b] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.

The minimum standard criteria established by OECD guidelines (i.e., ≥ 80% survival and ≥ 60 offspring per female in the control) were met. The dilution water control daphnids released their first brood of offspring on day 8. Time of the first brood release was within normal performance expectation for this species. Therefore, all of the above parameters were well within the acceptable ranges of the study guidelines.

Survival among organisms exposed to the control and all treatment levels tested (2.2, 4.4, 8.9, 18 and 35 µg/L) was 100%. First brood release occurred on day 9 for the 15 µg/L treatment level and on day 8 for the control and all other treatment levels tested.

No statistically significant inhibitory effect on the survival, growth or reproduction of *D. magna* was detected over 21 days (see table below).

Table 8.2.5.1-13: Effects of fludioxonil on *Daphnia magna* reproduction, growth and survival of the adult generation

Nominal concentration (µg/L)	Mean measured concentration (µg/L)	Mean percent survival (%)	Mean number of offspring released per female[a]	Mean total body length[a] (mm)	Mean dry weight[a] (mg)
Control	Control	100	82 (15)	4.32 (0.15)	0.84 (0.08)
3.8	2.2	100	89 (19)	4.39 (0.10)	0.89 (0.13)
7.5	4.4	100	85 (10)	4.33 (0.13)	0.93 (0.12)
15	8.9	100	87 (10)	4.39 (0.13)	0.93 (0.04)
30	18	100	79 (22)	4.36 (0.13)	0.85 (0.14)
60	35	100	84 (15)	4.45 (0.13)	0.84 (0.11)

[a] Standard deviations are presented in parentheses.

The results of the statistical analyses are summarised in the table below.

Table 8.2.5.1-14: Summary of the effects of fludioxonil on *Daphnia magna* after 21 days exposure

Endpoint	EC_{10} (µg/L)	EC_{20} (µg/L)	EC_{50} (µg/L)	95% Confidence limits (µg/L)	NOEC	LOEC
Adult survival	>35	>35	>35	NA[a]	35	>35
Reproduction	>35	>35	>35	NA	35	>35
Total Body Length	>35	>35	>35	NA	35	>35
Dry Body Weight	>35	>35	>35	NA	35	>35

[a] NA = Not Applicable. EC value was empirically estimated; therefore, corresponding 95% confidence limits could not be determined.

Conclusions

In a test in which the effects of fludioxonil on reproduction of *Daphnia magna* were investigated, based on mean measured concentrations, the 21-day NOEC for survival, growth and reproduction was 35 µg/L and the 21-day EC_{10}, EC_{20}, EC_{50} and LOEC values for survival, growth and reproduction were > 35 µg/L.

No additional testing was required to further define the EC_{50} value since the highest concentration tested closely-approximated the functional limit of solubility under testing conditions.

(Fournier A, 2014)

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

No data was previously submitted. Tests with mysid shrimp (*Americamysis bahia* also known as *Mysidopsis bahia*) have been conducted since the original EU review as part of a US EPA Data Call-In (DCI) and are summarised below:

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.5.2/01 Claude MB, Kendall .Z & Krueger .O (2011). Fludioxonil - A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid (*Americamysis bahia*). Report Number 528A-213A. Wildlife International Ltd, Easton, MD, USA (Syngenta File No. CGA173506_11519).

Guidelines

OPPTS 850.1350

GLP: Yes

Executive Summary

The chronic toxicity of fludioxonil to the saltwater mysid (*Americamysis bahia* also known as *Mysidopsis bahia*) was determined under flow-through conditions. This study was conducted with nominal concentrations of 5.0, 10, 20, 40 and 80 µg a.s./L (4.6, 9.1, 18, 36 and 70 µg a.s./L, mean measured) together with negative and solvent controls.

The 28 day NOEC, based on growth, was 18 µg a.s./L and the LOEC was 36 µg a.s./L based on mean measured concentrations.

Materials

Test material

Description:	CGA173506 Fludioxonil technical
Lot/Batch #:	Batch: 428860; Lot SSH2I135
Purity:	95.4%
Stability of test compound:	February 2012

Treatments

Test concentrations:	Nominal: 5.0, 10, 20, 40 and 80 µg a.s./L Mean measured: 4.6, 9.1, 18, 36 and 70 µg a.s./L
Dilution water:	Saltwater (0.25 µm filtered seawater)
Vehicle and/or positive control:	dimethylformamide (DMF)
Analysis of test concentrations:	Yes using HPLC analysis

Test organisms

Species:	Saltwater mysid (*Americamysis bahia*)
Source:	Test facility
Acclimatisation period:	Adults acclimated 14 days before collection of juveniles
Treatment for disease:	None
Life stage of test organism:	Juveniles (<24 hours)
Feeding:	Live brine shrimp (*Artemia* sp.) up to four times daily, supplemented once daily with a marine alga (*Skeletonema* sp.).

Test design

Test vessels:	Pre sexual maturity: 2 L glass containers, with nylon mesh covered holes on opposite sides of the container placed in 9 L glass aquaria containing approximately 2.5 L of test solution. Post sexual maturity: reproductive compartments were placed in 19 L glass aquaria filled with approximately 14.5 L of test solution
Replication:	4 replicates of 15 mysids
Exposure regime:	Flow-through
Duration:	96 hours

Environmental conditions

Test temperature:	24.8 to 25.1 °C
pH range:	7.9 to 8.1
Dissolved oxygen:	5.7 to 7.4 mg/L
Salinity of dilution water:	19 to 21‰ at test start
Lighting:	16 hours fluorescent light and 8 hours dark daily, with 30 minute dawn and dusk transition periods. Light intensity ≈105 lux at water surface.

Study Design and Methods

Experimental dates: 4th March to 26th April 2011

Test chambers were suspended in the aquaria which were filled with test water, giving a depth of 6.0 cm in each chamber. A continuous-flow diluter was used to deliver each concentration of the test substance, solvent control and negative control. The diluter was adjusted so that each chamber received at least 6 volume additions of test water every 24 hours. 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. 15 mysids were randomly allocated to each prepared test vessel, until maturity where male and female adults were paired in each treatment and control group, with a maximum of five reproductive pairs per replicate.

Individual stock solutions were prepared for each of the concentrations tested, and were prepared four times during the test. All test solutions were adjusted to 100% active ingredient during preparation, based on the test substance purity (95.4%). A primary stock solution was prepared by mixing a calculated amount of test substance into HPLC-grade dimethylformamide (DMF) at a nominal concentration of 800 µg a.s./mL. Four secondary stock solutions were prepared in DMF at nominal concentrations of 50, 100, 200 and 400 µg a.s./mL by proportional dilution of the primary stock.

Throughout the test, the five test substance stock solutions were injected into the diluter mixing chambers at a rate of 25 µL/minute where they were mixed with dilution water delivered at a rate of 250 mL/minute to achieve the desired test concentrations. The negative control received dilution water only. The solvent control was prepared by delivering HPLC-grade DMF to the mixing chamber for the solvent control. The concentration of DMF in the solvent control and all fludioxonil treatment groups was 0.1 mL/L.

The concentrations of test material in the test solutions were measured at the beginning of the test, approximately weekly during the test and at test termination. HPLC analysis was used.

Observations of the survival and behaviour of each first-generation mysid were made daily throughout the test. At pairing on Day 14, the sex and maturity of each mysid was determined by microscopic examination, and when possible, five male/female pairs were assigned to reproductive compartments in each replicate test chamber, with one pair per compartment. Any immature mysids or extra females were discarded at this time. Any sexually mature males remaining after pairing were maintained in a separate compartment within the respective replicate test chamber. If a male in a reproductive compartment died, it was replaced with a male from the pool of males maintained in the same replicate, if available.

Following pairing, reproductive females were observed daily for presence of eggs in the brood pouch. Second-generation mysids produced in each compartment were counted, recorded and removed daily. Second-generation mysids were also observed for abnormal development and abnormal behaviour. The test was terminated on Day 28, which was at least seven days past the median time of first brood release for the negative and solvent controls (Day 18). At test termination, the sex of each surviving first-generation mysid was confirmed and the total length of each mysid was measured using calipers. The mysids then were placed in two drying ovens, both at approximately 60 C, to obtain dry weight data. They mysids were dried for approximately 96 to 99 hours.

Measurements of temperature, dissolved oxygen, pH and salinity in the test chambers were made throughout the test.

Test endpoints analyzed statistically for first-generation mysids were survival, reproduction (the number of live young produced per reproductive day), and growth (total body length and dry weight). The results of the statistical analyses were used to aid in the determination of the NOEC and LOEC. However, scientific judgement was used to determine if statistical differences were biologically meaningful, and if the data followed a concentration-dependent response.

Data from the negative and solvent control groups for each parameter were compared using an

appropriate test. Since no differences were detected between the two control groups ($p > 0.05$) for survival, reproduction, male or female dry weight or female length data the control data were pooled for comparison among the treatment groups for these parameters. A statistical difference ($p < 0.05$) between the negative and solvent control groups was noted for the male length data. Therefore, the solvent control data was compared to the treatment group data for male length.

Survival data were considered to be discrete-variable data, while reproduction and growth data were considered to be continuous-variable data. Discrete-variable data were analyzed using Chi-square and Fisher's Exact tests to identify treatment groups that showed a statistically significant difference from the pooled control ($p \leq 0.05$). All continuous-variable data were evaluated for normality using the Shapiro-Wilk's test and for homogeneity of variance using Levene's test ($p = 0.01$). The data for all parameters passed the assumptions of normality and homogeneity of variance. Those treatment means that were significantly different from the control means were identified using Dunnett's test ($p \leq 0.05$). All statistical tests were performed using a personal computer with SAS (3) software.

Results and Discussion

The nominal and mean measured concentrations are shown in the table. The test concentrations were within 80 and 120% maintained throughout the study. However, mean measured concentrations were used for the calculation and reporting of results. The limit of quantification in this study was 3.00 µg a.s./L.

Table 8.2.5.2-1: Analytical results

Fludioxonil concentration (µg a.s./L)	Measured fludioxonil concentration (and % of nominal) (µg a.s./L) [a]						
	Pre-test	Day 0	Day 7	Day 14	Day 15	Day 21	Day 28
Negative control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Solvent control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
5.0	4.20 (84.1)	5.04 (101)	4.74 (94.8)	4.50 (89.9)	4.10 (82.0)	4.62 (92.4)	4.58 (91.5)
10	8.83 (88.3)	9.91 (99.1)	9.27 (92.7)	9.12 (91.2)	7.78 (77.8)	9.10 (91.0)	9.15 (91.5)
20	19.3 (96.5)	17.5 (87.4)	18.5 (92.6)	18.7 (93.7)	16.2 (80.8)	20.6 (103)	18.1 (90.4)
40	36.7 (91.7)	37.1 (92.6)	36.0 (90.0)	38.7 (96.7)	30.7 (76.7)	36.6 (91.6)	35.5 (88.7)
80	76.0 (95.1)	72.3 (90.4)	70.4 (88.0)	71.4 (89.2)	64.9 (81.2)	73.0 (91.2)	70.0 (87.5)

[a] the limit of quantification was 3.00 µg a.s./L

The survival and reproduction data are shown in the table below.

Table 8.2.5.2-2: Effects of fludioxonil on the survival and reproductive success of saltwater mysids (*Americamysis bahia*) after 28 days

Mean measured concentration (µg a.s./L)	Percent juvenile survival to pairing on day 14	Percent adult survival to test termination on day 28	Mean number of young produced per reproductive day ± SD	Percent of females producing young[a]	Average number of young per female[a]
Dilution water control	85.0	97.7	0.543 ± 0.299	90.0	7.6
Solvent control	91.7	96.1	0.336 ± 0.135	88.9	4.6
Pooled control	88.3	96.8	0.439 ± 0.242	89.5	6.1
4.6	100	85.5*[b]	0.238 ± 0.079	84.2	3.1
9.1	100	96.0	0.389 ± 0.121	80.0	5.5
18	96.7	97.7	0.552 ± 0.153	94.4	7.4
36	96.7	88.9	0.252 ± 0.048	75.0	3.4
70	75.0*	95.1	0.031 ± 0.018**	33.3	0.40

* Statistically significant decrease in survival in comparison to the pooled control using Fisher's Exact test ($p \leq 0.05$).
** Statistically significant decrease in reproduction in comparison to the pooled control using Dunnett's test ($p \leq 0.05$).
[a] No statistical analyses were conducted for this endpoint. Statistical evaluation for effects on reproduction are based upon mean number of young produced per reproductive day.
[b] While the decrease in survival was statistically significant in comparison to the pooled control, it was not considered to be treatment-related since the difference was slight and was not concentration-responsive.

After 14 days of exposure, there was a statistically significant decrease in survival in the 70 µg a.s./L treatment group when compared to the pooled controls ($p \leq 0.05$). The marked decrease in survival among mysids in the 70 µg a.s./L treatment group was considered to be treatment related. Consequently, the LOEC for juvenile survival (Days 0 –14) was 70 µg a.s./L, and the NOEC was 36 µg a.s./L.

At test termination, there was a statistically significant decrease in survival in the 4.6 µg a.s./L treatment group when compared to the pooled controls ($p \leq 0.05$). Average survival in the 4.6 µg a.s./L treatment group was 85.5% and ranged from 76.9 - 93.3%, while average survival in the controls and other treatments was >88.9%. While statistically significant, the decrease in survival in the 4.6 µg a.s./L treatment group was not considered to be treatment related, since the decrease was slight and was not concentration-responsive. Consequently, the NOEC for adult survival (Days 15 – 28) was 70 µg a.s./L, the highest concentration tested.

There was a statistically significant decrease in reproduction in the 70 µg a.s./L treatment group in comparison to the pooled controls ($p \leq 0.05$). Consequently, the LOEC for reproduction was 70 µg a.s./L and the NOEC was 36 µg a.s./L.

The effects of fludioxonil on the weight and length of the adult mysids at test termination are presented in the table below.

Table 8.2.5.2-3: Effects of fludioxonil on the growth of saltwater mysids (*Americamysis bahia*) after 28 days

Mean measured concentration (µg a.s./L)	Growth parameters at termination on day 28			
	Mean total length ± SD (mm)		Mean total weight ± SD (mg)	
	Males	Females	Males	Females
Dilution water control	8.35 ± 0.156	8.36 ± 0.149	1.08 ± 0.049	1.30 ± 0.103
Solvent control	8.14 ± 0.058	8.24 ± 0.023	1.03 ± 0.044	1.34 ± 0.020
Pooled control	8.25 ± 0.157	8.30 ± 0.119	1.06 ± 0.053	1.32 ± 0.072
4.6	8.01 ± 0.125	8.22 ± 0.169	0.99 ± 0.064	1.21 ± 0.113
9.1	8.04 ± 0.192	8.43 ± 0.126	1.00 ± 0.055	1.33 ± 0.076
18	8.13 ± 0.109	8.44 ± 0.130	0.98 ± 0.058	1.38 ± 0.100
36	8.04 ± 0.094	8.27 ± 0.190	0.93 ± 0.052**	1.13 ± 0.076**[a]
70	7.85 ± 0.176*	8.10 ± 0.247	0.91 ± 0.093**	1.21 ± 0.165

* Statistically significant decrease in comparison to the pooled control using Dunnett's test ($p \leq 0.05$).
** Statistically significant decrease in comparison to the pooled control using Dunnett's test ($p \leq 0.05$).
[a] While the decrease in dry weight was statistically significant in comparison to the pooled control, it was not considered to be treatment-related since the difference was slight and was not concentration-responsive.

When compared to the solvent control group, Dunnett's test indicated there was a statistically significant decrease in mean total length for males in the 70 µg a.s./L treatment group only ($p \leq 0.05$). Dunnett's test indicated there were statistically significant decreases in mean dry weight for males in the 36 µg a.s./L and 70 µg a.s./L treatment groups, in comparison to the pooled controls ($p \leq 0.05$).

There was a statistically significant ($p \leq 0.05$) decrease in mean dry weight for females in the 36 µg a.s./L treatment group when compared to the pooled control. However, this difference was slight and not dose-responsive, and was not considered to be treatment related.

Based on the effects on growth observed among males in the 36 µg a.s./L and 70 µg a.s./L treatment groups, the LOEC for growth (total length and dry weight) was 36 µg a.s./L and the NOEC was 18 µg a.s./L.

Conclusions

In a full life cycle test in which saltwater mysids (*Americamysis bahia*) were exposed to fludioxonil the NOEC, based on growth, was 18 µg a.s./L. The LOEC was 36 µg a.s./L.

(Claude M.B, Kendall TZ & Krueger HO, 2011)

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

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.2.5.2/02 Taylor, S. (2015a) Fludioxonil – A Flow-Through Life Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*) Statistical Re-analysis. Report Number CEA.1604. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11873).

Summary

Wildlife International Ltd. report number 528A-213A (Claude *et al*., 2011; Syngenta file number CGA173506_11519) for the chronic (life-cycle) flow-through toxicity test of fludioxonil to the saltwater mysid (*Mysidopsis bahia* also known as *Americamysis bahia*) did not provide estimates of the EC_{10}, EC_{20} and EC_{50} for the response variables evaluated as part of the original study. Consequently the data generated in this study have been re-analysed in order to provide these values.

The nominal test concentrations were 5, 10, 20, 40 and 80 µg a.s./L. The mean measured concentrations over the test period were 4.6, 9.1, 18, 36 and 70 µg a.s./L confirming the nominal concentrations were achieved. As a result, the nominal concentrations were used for this statistical analysis. In addition, all statistical comparisons were made against the pooled controls as statistical analysis in the original report confirmed there were no differences between them.

The following parameters were analysed statistically using the methods described in the Section 3.0 of this report:

- Juvenile survival to pairing (day 0 to day 14)
- Adult survival after pairing (day 15 to day 28)
- Mean number of young produced per reproductive day
- Mean adult dry weight for males and females
- Mean adult length for males and females

Statistical analyses of the available data revealed that the following EC_{10} and EC_{20} values were reliably calculated (no EC_{10}, EC_{20} or EC_{50} were able to be reliably calculated for the any of the remaining parameters due to the lack of suitable response functions):

Table 8.2.5.2-4: Statistical analysis for EC_{10} and EC_{20} endpoints for the fludioxonil Saltwater Mysid (*Mysidopsis bahia* Flow-Through Life Cycle Toxicity Test (Claude *et al.*, 2011; Syngenta file number CGA173506_11519)

Parameter	Pre-pairing survival	
	EC_{10}	EC_{20}
Value [µg a.s./L]	47.681	72.692
lower 95%-cl	34.306	56.341
upper 95%-cl	62.038	112.011

cl: confidence limits, nd: not determinable

Statistical Analysis

All computations were carried out in ToxRat Professional version 3.2 (ToxRat Solutions GmbH, 2015).

Where possible, non-linear regression was used to derive the ECx values. However, in cases where this approach did not provide reliable estimates and where the data were quantal, linear max likelihood regression was used in an attempt to derive these values.

Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

A 3-parameter normal cumulative distribution function (Bruce and Versteeg, 1992) was fitted to the original data and a non-linear regression without weighting was performed using the Levenberg-Marquardt algorithm to calculate the function parameters. Analysis of variance (ANOVA) and a test for

the lack of fit was then performed to determine the probability that the variance explained by the regression analysis was due to chance. Where p(F:Regression) <= 0.05, a significant amount of the variance is explained by the regression and where p(F:Lack of Fit) > 0.05, there is no significant lack of fit. If both of these criteria are met, the results of the regression analysis are accepted and the point estimates from the 3-parameter normal cumulative distribution function are meaningful.

Results

Pre-pairing survival (day 0 to day 14)

The relationship between concentration and response was significant, p(F) = 0.027 and the slope function was 2.608. Although the EC_{10} and EC_{20} values presented in Table 4 were reliably determined, no EC_{50} value could be calculated due to the lack of suitable response functions.

Post-pairing survival (day 14 to day 28)

The relationship between concentration and response was not significant, p(F) = 0.334 and as a result, no reliable EC_{10}, EC_{20} and EC_{50} values could be calculated.

Reproduction

The relationship between concentration and response was not significant, p(F) = 0.232 and as a result, no reliable EC_{10}, EC_{20} and EC_{50} values could be calculated.

Mean adult male dry weight

The relationship between concentration and response was significant, p(F) = 0.022 and the slope function was 156.54. Although the EC_{20} and EC_{50} could not be determined due to the lack of suitable response functions, the EC_{10} presented in Table 14 was determined. However, the 95% confidence limits span more than two test concentrations and as a result, the reported EC_{10} value is considered unreliable.

Mean adult male length

The relationship between concentration and response was not significant, p(F) = 0.294 and as a result, no reliable EC_{10}, EC_{20} and EC_{50} values could be calculated.

Mean adult female dry weight

The relationship between concentration and response was not significant, p(F) = 0.495 and as a result, no reliable EC_{10}, EC_{20} and EC_{50} values could be calculated.

Mean adult female dry length

The relationship between concentration and response was not significant, p(F) = 0.255 and as a result, no reliable EC_{10}, EC_{20} and EC_{50} values could be calculated.

Conclusion

Statistical analyses of the available data revealed that the following EC_{10} and EC_{20} values were reliably calculated (no EC_{10}, EC_{20} or EC_{50} values were able to be reliably calculated for any of the remaining parameters due to the lack of suitable response functions):

Table 8.2.5.2-5: Statistical analysis for EC_{10} and EC_{20} endpoints for the fludioxonil Saltwater Mysid (*Mysidopsis bahia* Flow-Through Life Cycle Toxicity Test (Claude *et al.*, 2011; Syngenta file number CGA173506_11519)

Parameter	Pre-pairing survival	
	EC_{10}	EC_{20}
Value [µg a.s./L]	47.681	72.692
lower 95%-cl	34.306	56.341
upper 95%-cl	62.038	112.011

cl: confidence limits, nd: not determinable

(Taylor S, 2015a)

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.5.2/03 Marini JP (2014a). Fludioxonil − Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*). Report Number 1781.6969. Smithers Viscient 790 Main Street, Wareham, Massachusetts 02571-1037 USA (Syngenta File No. CGA173506_51333).

Guidelines

US EPA Ecological Effects Test Guidelines, OCSPP 850.1350 (public draft): Mysid Chronic Toxicity Test (1996)

GLP: Yes

Executive Summary

The chronic (full life-cycle) toxicity of fludioxonil to mysids (*Americamysis bahia* also known as *Mysidopsis bahia*) was determined under flow-through conditions for a period of 28 days. Mysids were exposed to nominal concentrations of 2.5, 5.0, 10, 20, 40 and 80 µg/L (3.2, 5.5, 12, 24, 44 and 96 µg/L time-weighted mean), alongside a dilution water control. Following the 28-day exposure, first generation (F_0) data for survival, reproductive success and growth (total body length and dry weight) as well as second generation (F_1) data for survival were evaluated. Based on time-weighted mean concentrations of fludioxonil and male length (the most sensitive indicator of toxicity), the No-Observed-Effect Concentration (NOEC) was determined to be 24 µg/L. The Lowest-Observed-Effect Concentration (LOEC) for mysids was determined to be 44 µg/L.

Materials

Test material	Fludioxonil
Lot/Batch #:	SMO3J0464
Purity:	97.7% (w/w); tested as 100%
Description:	Brownish powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	End of October 2017
Treatments	
Test concentrations:	Dilution water control and nominal concentrations of 2.5, 5.0, 10, 20, 40 and 80 µg/L (3.2, 5.5, 12, 24, 44 and 96 µg/L time-weighted mean)
Solvent:	None
Analysis of test concentrations:	Yes, analysis of fludioxonil on days 0, 7, 14, 21, 22 and 28

Test organisms	
Species:	Mysid, *Americamysis bahia*
Source:	Continuous laboratory cultures, brood stock originated from MBL Aquaculture, Sarasota, Florida
Acclimatisation period:	None; culture organisms maintained at similar test conditions
Age at exposure initiation:	<24 hours
Treatment for disease:	None
Feeding:	Throughout the test, mysids were fed live brine shrimp (*Artemia salina*) nauplii, approximately 48 hours old (post-hydration), twice daily. At least one of these feedings was with brine shrimp nauplii enriched with Selco®, a substance high in saturated fatty acids. F_0 retention chambers (containing 20 mysids each) received approximately 90 nauplii/mysid on test days 0 to 3, 135 nauplii/mysid on test days 4 to 6, 180 nauplii/mysid on test days 7 to 9 and 225 nauplii/mysid on test day 10 to pairing. From day of pairing until test termination, the F_0 pairing chambers were fed approximately 450 nauplii/mysid and the retention chambers were fed approximately 3600 nauplii/chamber. After F_1 chambers were initiated, chambers were fed a ration of approximately 90 nauplii/mysid.
Test design	
Test vessels:	Glass aquaria measuring 30 x 15 x 10 cm with a 10-cm high side drain that maintained a constant exposure solution volume of approximately 4.5 L.
Test medium:	Dilute, filtered, natural seawater
Replication:	4 replicate aquaria
No of -mysids per aquaria:	20
Exposure regime:	Flow-through, exposure system consisted of an intermittent-flow proportional diluter, a temperature-controlled water bath and a set of 28 exposure aquaria
Duration:	28 days
Environmental conditions	
Test temperature:	26 to 27° C
pH:	7.7 to 8.2
Dissolved oxygen:	4.92 to 7.21 mg/L; 68.8 – 99.9% saturation
Salinity:	20 to 21‰
Lighting:	360 to 690 lux 16 hours fluorescent light and 8 hours dark; 30-minute transition period

Study Design and Methods

Experimental dates: 3rd to 31st July 2014

Based on the solubility and chemical properties of the test substance, a glass wool saturator column was used to deliver fludioxonil to the exposure system. The columns were designed to provide a constant flow of saturated aqueous solutions (1.3 mg/L) of the test substance to the diluter system without the use of a carrier solvent. Columns were constructed entirely of chemically inert materials (glass and Teflon®). To coat a column, approximately 9 g of fludioxonil was diluted with 50 mL of acetone (CAS No. 67-64-1) and the solution was poured into the glass column. New saturator columns were prepared approximately every two weeks during testing.

Prior to exposure initiation, an FMI Pump was calibrated to deliver 0.12 L/cycle of the 1.3 mg/L stock solution to the diluter's mixing chamber which also received 1.85 L of dilution water per cycle. The solution contained in the mixing chamber constituted the highest nominal test concentration (79 µg/L) and was subsequently diluted (50%) to provide the remaining nominal exposure concentrations (40, 20, 10, 5.0 and 2.5 µg/L).A set of control vessels was also established which contained the same dilution

water and was maintained under the same conditions as the treatment level vessels, but contained no fludioxonil.

The flow-through exposure system consisted of an intermittent-flow proportional diluter, a temperature-controlled water bath and a set of 28 exposure aquaria. Flow-splitting cells were employed to equally distribute the solutions to the replicate vessels at a rate of 250 mL of control and test solution per vessel per cycle. The diluter delivered the control and test solutions to the exposure aquaria at a rate sufficient to provide approximately 7.7 aquarium volumes per 24-hour period, with a 90% replacement time of approximately seven hours.

At the start of the exposure, 20 mysids (<24 hours old) were allocated to each replicate aquaria. For the first 12 days of exposure, each exposure aquarium contained one retention chamber (used to retain sexually immature mysids).

Once all mysids appeared to be sexually mature (test day 13), male and female pairs were transferred to separate pairing chambers (used to retain sexually mature male and female mysids). One mature male and one mature female were randomly assigned to each of the pairing chambers (with a maximum of five male/female pairs per replicate). Unpaired mysids were pooled and maintained in the initial retention chambers until they were paired or until test termination. When three or fewer unpaired mysids remained in a retention chamber, they were transferred to a separate pairing chamber to reduce the amount of total surface area within the exposure tank. Male mysids from this pool were used to replace dead males from the paired (male/female) groups. Females that died in the pairing chambers before the onset of reproduction were replaced. Daily observations were made for mysid survival, behaviour and appearance.

During the reproductive phase, groups of 10 offspring per replicate, 40 per treatment (if 10 offspring could not be collected, a reduced number of young was collected and evaluated; $n > 5$) were removed from mysid chambers in each replicate vessel, pooled and placed in a separate pairing chamber in that replicate. One F_1 group was established and monitored for each replicate vessel. The chambers with F_1 mysids were established to monitor survival 96 hours post-release. Observations of stress, abnormal behaviour and survival were made.

At test termination, all mysids were euthanized by immersion in cold, deionized water. The mysids were carefully removed, blotted dry on absorbent paper, and separated into male and female groups for each replicate exposure level. A digital photograph was then taken of each mysid using a binocular dissection microscope for individual body length measurements. Male and female mysids were then transferred to aluminium pans, dried in an oven at approximately 100 °C for 23 hours, cooled and individually weighed to determine dry weight. Reproductive success was calculated for each replicate aquarium (treatments and the control) as the total number of offspring produced per female. In addition, the percentage of actively reproducing females in each replicate of each treatment and the control was determined.

At exposure initiation, dissolved oxygen concentration, pH, temperature and salinity were measured in each replicate aquarium. For the remainder of the exposure, one replicate of each concentration and control was measured for these parameters.

The test concentrations were verified by chemical analysis of fludioxonil at days 0 (experimental initiation), 7, 14, 21, 22 and 28 (experimental termination) using liquid chromatography/mass spectrometry (LC/MS/MS).

Data for the survival endpoints were analysed using Fisher's Exact Test with Bonferroni-Holm's Adjustment to establish exposure level effects. Data for growth and reproduction endpoints were analysed using Dunnett's Multiple Comparison Test to establish exposure level effects.

Results and Discussion

The limit of quantification (LOQ) for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. In this study, the LOQ ranged from 0.46 to 0.58 µg/L. Since the sampling intervals were not spaced equitably, a time-weighted mean concentration was calculated for each treatment level and was used for the calculation and reporting of results.

Time-weighted average fludioxonil concentrations ranged from 110 to 130% of nominal concentrations and defined the treatment levels tested as follows: 3.2, 5.5, 12, 24, 44 and 96 µg/L.

Table 8.2.5.2-6: Analytical results

Nominal concentration (µg/L)	Measured Concentration (µg/L)							Percent Nominal (%)
	Day 0	Day 7	Day 14	Day 21	Day 22[a]	Day 28	Time-Weighted Mean	
Control	< LOQ[b]	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA[c]	NA
2.5	3.3	3.3	3.0	3.5	3.8	2.0	3.2	130
5.0	6.4	6.9	6.4	1.3	6.8	5.1	5.5	110
10	13	13	12	12	14	6.7	12	120
20	26	26	24	23	25	16	24	120
40	46	46	45	43	49	32	44	110
80	97	100	95	97	89	82	96	120

[a] Samples were taken on Day 22 to confirm that the diluter malfunction was resolved at the 5.0 µg/L nominal dose. Samples were also taken from the control and other treatments to demonstrate proper diluter function throughout the system.
[b] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. LOQ concentrations for this testing ranged from 0.46 to 0.58 µg/L.
[c] NA = Not Applicable.

The effects observed and the endpoint summaries are shown in the tables below:

Table 8.2.5.2-7: Survival and reproductive effects of fludioxonil on *Americamysis bahia*

Time-weighted mean concentration (µg/L)	F_0 survival[a]				F_0 reproduction[a]	
	Male[b] (%)	Female[b] (%)	Post-pairing (%)	28-Day (%)	Females producing young (%)	Number of offspring per female
Control	79 (11)	83 (12)	80 (11)	74 (10)	100 (0)	18.3 (3.8)
3.2	84 (12)	93 (9)	89 (5)	85 (5)	100 (0)	19.6 (4.6)
5.5	91 (11)	89 (3)	88 (5)	82 (11)	95 (10)	20.2 (3.0)
12	82 (14)	82 (19)	81 (14)	75 (14)	100 (0)	16.5 (4.2)
24	83 (19)	96 (8)	89 (10)	76 (15)	100 (0)	20.5 (5.9)
44	85 (12)	86 (5)	86 (5)	82 (7)	95 (10)	12.5 (3.2)
96	80 (4)	88 (11)	83 (11)	77 (15)	95 (10)	15.2 (4.4)
Endpoint Summary (µg/L)						
NOEC	96	96	--	96	--	96
LOEC	>96	>96	--	>96	--	>96

[a] Mean values are presented with standard deviations (SD) in parentheses.
[b] Calculations of male and female survival began after pairing.

Table 8.2.5.2-8: Growth effects of fludioxonil on *Americamysis bahia*

Time-Weighted Mean Concentration (µg/L)	F_0 Total Body Length[a]		F_0 Dry Weight[a]	
	Male (mm)	Female (mm)	Male (mg)	Female (mg)
Control	7.52 (0.38)	7.64 (0.28)	0.92 (0.02)	1.26 (0.10)
3.2	7.40 (0.25)	7.70 (0.23)	0.89 (0.05)	1.22 (0.09)
5.5	7.32 (0.13)	7.53 (0.13)	0.87 (0.04)	1.17 (0.10)
12	7.15[b] (0.26)	7.32 (0.20)	0.89 (0.07)	1.16 (0.04)
24	7.35 (0.11)	7.64 (0.16)	0.89 (0.08)	1.22 (0.09)
44	7.03[c] (0.14)	7.36 (0.26)	0.81 (0.05)	1.07 (0.12)
96	7.05[c] (0.11)	7.37 (0.12)	0.79[c] (0.06)	1.10 (0.10)
Endpoint Summary (µg/L)				
NOEC	24	96	44	96
LOEC	44	>96	96	>96

[a] Mean values are presented with standard deviations (SD) in parentheses.
[b] Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test. However, due to the lack of a concentration response in the 24 µg/L level, the effect observed at this treatment level was not considered to be toxicant related.
[c] Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test.

Table 8.2.5.2-9: F_1 survival effects of fludioxonil on *Americamysis bahia*

Time-Weighted Mean Concentration (µg/L)	96-Hour F_1 Survival[a]
Control	98 (5)
3.2	98 (5)
5.5	100 (0)
12	98 (5)
24	100 (0)
44	97 (6)
96	90 (8)
NOEC	96
LOEC	>96

[a] Mean values are presented with standard deviations (SD) in parentheses.

Neither EC_{10} nor EC_{20} values were derived from these data. It is however clear that a clear concentration response does not exist for any of the biological parameters measured in the test.

The biological performance of the control organisms met the minimum guideline acceptability criteria since F_0 post-paring survival in the control was greater than 70%, greater than75% of females were reproductively active and control reproductive performance was greater than 3 offspring per female.

Conclusions

In a full life cycle test in which saltwater mysids (*Americamysis bahia*) were exposed to fludioxonil based on time-weighted mean concentrations of fludioxonil and male length (the most sensitive indicator of toxicity), the No-Observed-Effect Concentration (NOEC) was determined to be 24 µg/L. The Lowest-Observed-Effect Concentration (LOEC) for mysids was determined to be 44 µg/L.

(Marini J, 2014a)

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

The study previously submitted in the EU for fludioxonil (Grade, 1998, Syngenta File No. CGA173506/1274) was summarised in point B.9.2.7 of the DAR, ~~therefore an additional study summary is not repeated here.~~ The endpoints are summarised in Table 8.2-6. At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.7 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2 -6 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.5.3 Grade R, (1998). Toxicity Test of CGA 173506 tech. on Sediment-Dwelling Chironomus riparius (syn. Chironomus thummi) under Static Conditions. Report Number 983752, Novartis Crop Protection AG, Product Portfolio Management, Environmental Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. CGA173506/1274)*

Guidelines

OECD Guidelines for the Testing of Chemicals: Proposal for toxicity test with Chironomidae (1997)

Proposal for a BBA-Guideline: Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system (1995)

GLP: Yes

Executive Summary

The effects of CGA173506 on the development of *Chironomus riparius* were determined. Organisms were exposed to nominal concentrations of 5.0, 10, 20, 40, 80 and 160 mg a.s./kg dry weight of sediment, alongside an aged sediment control and a solvent control.

Based on nominal concentrations of CGA173506, the 28-day EC_{50} for emergence rate and development rate were >160 mg a.s./kg of dry sediment, the highest concentration tested. The corresponding NOECs were 160 mg a.s./kg of dry sediment.

Materials

Test Material

Description:	CGA173506
Lot/Batch #:	P.910007
Purity:	97.5 %
Description:	Brown powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	March 1999

Treatments

Test concentrations:	Aged sediment control, aged sediment with solvent, and nominal concentrations of 5.0, 10, 20, 40, 80 and 160 mg a.s./kg dry weight of sediment
Solvent:	Acetone

Analysis of test concentrations:	Yes. Samples collected from water phase of all treatments on days 0, 2, 7, 14, 28 days. Samples collected from sediment phase of highest and lowest treatment groups on days 0, 7, 28 days. All samples were analysed for CGA173506 using HPLC-UV
Test organism	
Species:	*Chironomus riparius*, first instar (2 – 3 days old)
Source:	Continuous laboratory cultures, originally obtained from Dr. F. Heimbach, Bayer AG (Pflanzenschutz Umweltforschung, Institut für Oekobiologie, D-5090 Leverkusen)
Feeding:	Fish food (Tetramin) suspension (1 g Tetramin in 20 mL medium) at a rate of about 1.0 mg fish food per larvae per day until day 26
Test design	
Test vessels:	1 L glass beakers (9 cm diameter) covered with parafilm with small holes and containing 1.6 cm of artificial sediment (160 g moist weight sediment) and 8 cm of overlaying reconstituted water (6 cm at the end of exposure)
Test medium:	Reconstituted water (M4 – medium)
Artificial Sediment:	5.5 % sphagnum peat 20 % kaolin clay 74.5 % industrial sand (> 50 % of the particles between 50 and 200 µm) Calcium carbonate (to adjust to pH 6.3) The organic carbon content of the final sediment mixture was 1.7 %
Sediment moisture content:	Not reported
Replication:	3 replicate test vessels, 20 larvae per vessel
Duration:	28 days
Environmental conditions	
Test temperature:	18.4 to 21.3 °C
pH range of overlying water:	7.1 to 9.2
Dissolved oxygen of overlying water:	5.9 to 8.9 mg/L, vessels were gently aerated through a glass Pasteur pipette fixed at approximately 2-3 cm above the sediment layer
Total Hardness:	244 to 245 mg/L $CaCO_3$
Conductivity:	660 to 670 µS/cm
Lighting:	16 hours light (800 – 1000 lux) and 8 hours dark, 30 minute transition period

Study Design and Methods

Experimental dates: 22nd June to 22nd July 1998

A primary stock solution was prepared by mixing 316.8 mg of test material and making up to 5 mL with acetone (to give a nominal concentration of 63.36 mg CGA173506/L). Using this stock solution, the remaining five stock solutions were prepared by serial dilution. Then, 2 mL of the relevant stock solution was applied to 15.5 of sand, and the solvent was allowed to evaporate. The dry sand was then added to the aged sediment (1100 g sediment, equivalent to 775.84 g dry sediment) to achieve the required test concentrations, and thoroughly mixed for approximately 30 minutes.

Larvae of *Chironomus riparius* were exposed for 28 days to the test item in glass beakers filled with sediment and test medium. The test vessels were prepared 47 hours before the larvae were introduced and a gentle aeration was carried out for 1 hour before larvae were introduced. After the addition of the larvae, aeration was stopped for the following 24 hours. Throughout the test the larvae were fed at a rate

of about 1.0 mg fish food per larvae per day until day 26 and the vessels were gently aerated after 24 hours of the exposure start.

Visual assessments for behaviour, mortalities and emergence were made daily (with the exception of test days 3, 4, 10, 11, 18, 24 and 25). The number, time and sex of emerged adults was recorded. Once emerged, the adults were discarded. Any signs of chemical effect on the larvae, or emerged midges were recorded. The weight of larvae was measured after 9 days of exposure, but no statistical analysis was performed as no replicates were used for measuring the weight of larvae. The emergence rate, the gender rate, and the development rate were calculated from the total numbers of emerged male and female adults, and from the mean time of emergence.

NOEC and LOEC values were calculated using the Dunnett's test (α = 5 %), following the arcsine-squareroot transformation of emergence rate and gender rate. The 28-day EC_x and 95 % confidence limits for emergence rate and development rate were calculated by Probit analysis, but EC_x estimation was found to be not possible. Male and female results were pooled for statistical analyses since no different sensitivities of sexes were found.

At the start and at the end of the test and on a weekly basis the pH and dissolved oxygen were measured in each test vessel. Water temperature was recorded continuously. Water conductivity and hardness were measured after freshly preparing the M4-medium.

The concentrations of test material were determined at Days 0, 2, 7, 14 and 28 in the overlying water of all treatments, and at Days 0, 7, and 28 in the sediment, of the highest and lowest treatment group, using an HPLC-UV method. In addition, separate sediment chemistry chambers were set up and sampled at Days 0 and 7.

Results and Discussion

On Day 0, the distribution of test material between sediment and water was of 99 and 1 % of total measured concentration in the sediment and in the water, respectively. After 28 days of the test, the distribution of test material measured in the sediment were in the range of 98 – 99 % and were between 1 and 2 % of total in the water (see table below). The recovery rates ranged from 108 to 109 % at Day 0, and from 101 to 92 % at Day 28. The Limit of Quantification (LOQ) for sediment and water analyses was 0.017 mg CGA173506/kg. Biological results are based on nominal concentrations of CGA173506 in the sediment.

Table 8.2.5.3-1: Analytical results

Nominal concentrations (mg CGA173506/kg dry sediment)	% of total measured in water		Measured concentration in water (mg CGA173506/L)		% of total measured in sediment	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
80	1	2	0.25	0.36	99	98
160	1	1	0.22	0.43	99	99

The effects of CGA173506 on *C. riparius* emergence and development, based on nominal concentrations are given in the table below:

Table 8.2.5.3-2 Effects of CGA173506 on emergence and development of *Chironomus riparius* after 28 days exposure

Nominal concentration (mg CGA173506/kg dry sediment)	Emergence rate (ER[a])	Gender rate (GR[b])	Mean development rate per vessel (male/females pooled)
Control	0.90	0.42	0.066
Solvent control	0.90	0.49	0.066
5.0	0.90	0.49	0.063
10.0	0.98	0.59	0.061
20.0	0.92	0.60	0.066
40.0	0.92	0.46	0.061
80.0	0.95	0.57	0.059
160.0	0.92	0.58	0.060

[a] ER = No. adults emerged / No. larvae introduced (i.e. 20).
[b] GR = No. male adults emerged / No. adults emerged

Emerged midges showed severe effect at the two highest test concentrations of 80 and 160 mg CGA173506/kg of dry sediment, where most midges were dead and thin and located on the surface of the water. No symptoms were observed at test concentration of 40 mg CGA173506/kg of dry sediment and below. The NOEC, LOEC and EC_{50} data are tabulated below:

Table 8.2.5.3-3 Summary of the effects of CGA 173506 on *Chironomus riparius* after 28 days exposure

Endpoint	EC_{50} (mg CGA173506/kg)*	95 % Confidence limits (mg CGA173506/kg)*	NOEC (mg CGA173506/kg)*	LOEC (mg CGA173506/kg)*
Emergence rate	> 160	-	160	> 160
Development rate (pooled)	> 160	-	160	> 160

*Results expressed as mg CGA173506/kg of dry sediment

Validity Criteria

The validity criteria for the test were met:

- The emergence of the controls must be greater than 80 % (measured range 85 to 95 %)
- The mean development time for larvae must be less than 23 days after application

Conclusions

Based on nominal concentrations of CGA173506, the 28-day EC_{50} for emergence rate and development rate were > 160 mg CGA173506/kg of dry sediment, the highest concentration tested. The corresponding NOECs were 160 mg CGA173506/kg of dry sediment.

(Grade R, 1998)

This study complies with the current validity criteria for toxicity testing with *Chironomus riparius*. Despite some test conditions not being reported and minor deviations, the study is reliable and still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Grade, 1998 CGA173506/1274	OECD Proposal for Toxicity Test with Chironomidae, November (1997) Proposal for a BBA-Guideline: Effects of Plant Protection Products on the Development of Sediment-Dwelling Larvae of *Chironomus riparius* in a Water-Sediment System (1995)	OECD No. 218 (2004)	The following is not reported: 1. Reference test The following deviations are noted: 1. Water temp varied by >1°C (18.4-21.3 °C) 2. Sediment contained 5.5% peat (4-5% recommended) 3. Equilibrium period for sediment less than the recommended minimum (47hrs; 48hrs recommended) 4. Only 3 replicates (4 recommended)	Conditions of the study were not fully reported and there are deviations from the recommended test design. Most of the validity criteria were met: 1. Emergence must be at least 70% at the end of the test in the negative and solvent controls (>85%) 2. Emergence to adults from control vessels should occur between 12 and 23 days (≤23 days) 3. At the end of the test, DO should be at least 60% of the air saturation value (7.6 to 8.9 mg/L) 4. pH of overlying water should be in the 6-9 range in all test vessels (pH 7.1-9.2) 5. Water temperature should not differ by more than 1.0°C (18.4-21.3°C; 2.9°C difference) The minor exceedances in pH and temperature do not impact the reliability of the study. Results from the solvent control are used for statistical comparison with the treatments; no statistical comparison of the negative control and solvent control is presented. The study is reliable and the endpoint can be used in the risk assessment.

At the request of the RMS the analytical determinations wer clarified versus guideline requirements:

It is recommended in OECD 218 that, as a minimum, samples of the overlying water, the pore water and the sediment be analysed at the start and at the end of the test, at the highest concentration and a lower one.

In the Grade 1998 study (Syngenta file number CGA173506/1274), analytical samples were taken of the sediment, pore water and overlying water at days 0, 7 and 28 days. The test item was measured in the two highest test concentrations 80 and 160 mg/kg dw sed and the concentrations in the sediment were 99% at the start of the study and between 98-99% of nominal concentrations at the end of the study after 28 days. These indicate that both test concentrations were correctly dosed and that there was no dissipation of fludioxonil once spiked into the sediment over the 28 days.

All sediment samples were prepared in the same way. Examination of how the spiked sediment was prepared indicates that each sediment was dosed with a specific solvent stock solution, and each solvent stock was then added to sand before combining with the sediment.

Therefore the analytical sampling regime used fulfils the current OECD 218 requirements and based on the data from the two highest test concentrations. Therefore this is indicative that the level of fludioxonil in 40 mg/kg dose in the sediment was consistent and stable during the 28d study.

RMS comment: Regarding the chronic toxicity study on C. riparius conducted with fludioxonil (Grade, 1998, CGA173506/1274, KIlA 8.2.7.1/01), it is necessary to provide some details regarding: the preparation of the vehicle control: There is no indication on how the vehicle control was prepared. It should be demonstrated that the amount of solvent in the vehicle control is identical to the one in all tested concentrations. It is also necessary to precise whether the solution containing the solvent for the vehicle control group was added to sand after evaporation of acetone as done for test concentrations.

Syngenta response: The same amount of acetone (2.0 mL) from acetone stock solutions 1 to 5) were added to 15.5 g of sand. The sand was left until the acetone had evaporated off and then the dry sand was mixed into 775.83 g of dry sediment. The solvent control was prepared in the same manor with no test item. Therefore the amount of solvent used in the solvent control and each test concentration was the same. As the acetone is left to evaporate this would be negligible, but as added equates to 2% acetone in each test concentration and solvent control, before evaporation.

In accordance with **Commission Regulation (EU) No 283/2013**, the EC_{10} and EC_{20} values have been estimated for this 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 8.2.5.3/01 Taylor S. & Howells A. (2015a) Fludioxonil (CGA173506) - Toxicity Test of CGA173506 tech. on Sediment-Dwelling Chironomus riparius (syn. *Chironomus thummi*) under Static Conditions. Statistical re-analysis. Report Number: CEA.1605. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11840).

Summary

The report from Novartis Crop Protection AG study number 983752 (Grade, 1998; Syngenta file number CGA173506/1274) for the toxicity of CGA173506 on sediment-dwelling *Chironomus riparius* in a water-sediment system under static conditions did not provide estimates of the EC_{10} and EC_{20} for the response variables evaluated as part of the original study. As a result, the data generated in this study were intended to be re-analysed in an attempt to provide these values.

No treatment related effects were observed in the original study and the 28 day endpoints (EC_{50}, NOEC and the LOEC for emergence and development rate) were all reported as at least >160 mg/kg (the highest nominal concentration). As a result, no reliable EC_x values can be calculated.

Results

No treatment related effects were observed in the original study and the 28 day endpoints in the original study report (EC_{50}, NOEC and the LOEC for emergence and development rate) were all reported as at

least >160 mg/kg (the highest nominal concentration). As a result, no reliable EC_x values can be calculated.

Conclusion

No concentration related responses were observed in the original study and the 28 day endpoints reported in the original study report (EC_{50}, NOEC and the LOEC for emergence and development rate) were all reported as at least >160 mg/kg (the highest nominal concentration). As a result, no reliable EC_x values can be calculated.

(Tayor S, Howells A, 2015a)

CA 8.2.5.4 Sediment dwelling organisms

No studies were submitted previously. Tests with other sediment dwelling organisms have been conducted after the original EU review as part of a US EPA Data Call-In and are summarised below. The endpoints are summarised in Table 8.2-4.

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.5.4/01 Bradley MJ (2014). 10-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to Fludioxonil TGAI Applied to Sediment Under Static-Renewal Conditions. Report Number 1781.6944. Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571-1037, USA (Syngenta File No. CGA173506_51325).

Guidelines

US EPA Ecological Effects Test Guidelines, OCSPP Draft Guideline 850.1735. Whole Sediment Acute Toxicity Invertebrates, Freshwater (1996)

GLP: Yes

Executive Summary

The 10-day acute toxicity of fludioxonil to *Hyalella azteca* was determined under static-renewal conditions. Amphipods (eight days old) were exposed to nominal concentrations of 6.3, 13, 25, 50 and 100 mg/kg sediment dry weight (5.2, 11, 21, 41 and 84 mg/kg sediment dry weight, respectively, mean measured) alongside a dilution water control and a solvent (acetone) control. Following 10 days of exposure, amphipod survival and growth (final dry weight per amphipod) were evaluated. Based on mean measured concentrations, the 10-day LC_{50} and EC_{50} values were empirically estimated to be >84 mg/kg sediment dry weight. The NOEC and LOEC values based on survival and growth were determined to be 84 and >84 mg/kg sediment dry weight, respectively.

Materials

Test Material:	Fludioxonil
Lot/Batch #:	SMO3J0464
Purity:	97.7% (w/w); tested as 100%
Description:	Brownish powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	End of October 2017

Treatments

Test concentrations:	Dilution water control, solvent (acetone) control and nominal concentrations of 6.3, 13, 25, 50 and 100 mg/kg sediment dry weight
Solvent:	Acetone (CAS No. 67-64-1)
Analysis of test concentrations:	Yes, analysis of fludioxonil at 0 and 10 days

Test organisms:

Species:	*Hyalella azteca*
Age:	Eight-day old amphipods
Source:	Continuous laboratory cultures, originally obtained from Environmental Testing & Consulting, Superior, Wisconsin
Feeding:	During holding and acclimation, the freshwater amphipods were fed a combination of yeast, cereal leaves and flaked fish food suspension (YCT) on a daily basis. The day the test population was isolated, a small amount of 100 mg/mL flaked fish food suspension and *Ankistrodesmus falcatus*, a unicellular green algae, were added to the isolation vessels as a supplemental food source. During the 10-day exposure, each replicate test vessel received 1.0 mL of YCT daily.
Culture medium:	Laboratory well water

Test design:

Test vessels:	300 mL glass vessels with two openings on the top edge covered with 40-mesh Nitex® screen for drainage. Approximately 100 mL (4-cm layer) of sediment (equivalent to 50.0 g dry weight per vessel) and 175 mL of overlying water. Total overlying/sediment volume of approximately 275 mL
Test medium:	Sediment with overlying laboratory well water
Sediment:	Natural freshwater sediment collected from Glen Charlie Pond, Wareham, Massachusetts. Prior to use and characterization, the sediment was pressed through a 2.0-mm sieve to remove large particles and indigenous organisms. 89% sand, 7% silt, 4% clay Organic Carbon: 5.0% pH: 5.6 % Solids: 36.64% Porewater ammonia: 6.2 mg N/L
Sediment moisture content:	63.36%
Replication:	8 replicates of 10 amphipods
Exposure regime:	Static-renewal Overlying water was renewed using an intermittent delivery system in combination with a calibrated water-distribution system. The water delivery system cycled approximately 7 times per day, providing approximately 350 mL per vessel every 24 hours. Due to an observed trend of decreasing dissolved oxygen measurements in the test vessels on test day 3, the flow rate in the system was doubled so that the water delivery system cycled approximately 14 times per day, providing approximately 700 mL per vessel every 24 hours (i.e., approximately four overlying volume replacements per vessel per day).
Duration:	10 days

Environmental conditions

Test temperature:	22 to 24°C
pH range:	6.4 to 6.9

Dissolved oxygen:	3.4 to 8.0 mg/L[17] (no aeration).
Total hardness:	56 to 72 mg/L $CaCO_3$
Total alkalinity:	16 to 20 mg/L $CaCO_3$
Conductivity:	400 to 460 µmhos/cm
Ammonia:	Day 0: 0.56 to 0.97 mg N/L Day 10: ≤0.10 to 0.76 mg N/L
Lighting:	16 hours light (510 to 800 lux) and 8 hours dark, ; sudden transitions of light to dark and dark to light were avoided

Study Design and Methods

Experimental dates: 26th August to 5th September 2014

A 10-mL volume of each dosing stock solution was applied to 0.050 kg of fine silica sand and mixed. Following evaporation of the solvent, the entire sand/test substance mixture was added to 2.0 kg of wet sediment (0.7828 kg total dry weight based on 36.64% solids and including the aforementioned 0.050 kg of fine silica sand) in individual glass jars.

The test substance was applied to the sediment by adding 10 mL of the appropriate dosing stock solution to 0.050 kg of fine silica sand, allowing the acetone to evaporate, and then mixing with the remaining part of the wet sediment using a jar-rolling technique to give the nominal concentrations of 6.3, 13, 25, 50 and 100 mg/kg sediment dry weight. The spiked sediments were allowed to equilibrate for a 27 day period in the refrigerator with weekly mixing on a rolling mill. One day before the amphipods were introduced, the sediment was added to the test vessels. The wet sediment was filled into each test beaker to give a layer of approximately 100 mL (4 cm depth), corresponding to 125 g wet weight or 50.0 g dry weight. The sediment was then covered with 175 mL of overlying water for a total overlying/ sediment volume of approximately 275 mL. A turbulence reducer, consisting of a modified plastic disk, was used to minimize the disruption of the sediment layer during the introduction of overlying water.

The test vessels were left to stand overnight under test conditions, after which the amphipods were randomly distributed amongst the test vessels (Day 0). Throughout the exposure, the amphipods were fed 1.0 mL of YCT daily. Following the addition of amphipods, all vessels were examined at exposure initiation and daily thereafter, until test termination (day 10). Observations of mortality and abnormal behaviour were made and the physical characteristics of the test solutions were recorded. At test termination (day 10), the remaining sediment was sieved using fine mesh fish nets (approximately 0.25 mm) to remove all surviving amphipods and the total number and growth (expressed as dry weight) of surviving amphipods was determined for each test vessel. Growth of the surviving amphipods was determined by pooling all surviving amphipods from each replicate and drying at approximately 63 to 64 ºC for 24 hours. The pooled amphipods were then weighed on a calibrated analytical balance to the nearest 0.01 mg.

At exposure initiation and termination, temperature, dissolved oxygen concentration and pH were measured for each treatment level and control. In addition, the temperature was continuously monitored in an auxiliary vessel in the temperature controlled water bath. Total hardness, alkalinity, conductivity and ammonia concentration of the overlying water were monitored at exposure initiation and test termination in each treatment level and control.

[17] The guideline states that dissolved oxygen should be greater than 40% of the air saturation value for the duration of the exposure. During this study, dissolved oxygen in the solvent control and the 13 mg/kg (nominal) treatment level decreased to 39% of saturation on test day 10. Since this level is within the tolerance of *H. azteca* and because the deviation occurred at test termination, this deviation did not have a negative impact on the results or interpretation of the study.

Sediment, pore water and overlying water samples were analysed for fludioxonil on days 0 and 10 , using liquid chromatography/mass spectrometry (LC/MS/MS).

Wilcoxon's Rank Sum Test and an Equal Variance Two-Sample t-Test were conducted for survival and growth (dry weight) data, respectively, to compare the performance of the dilution water control organisms with that of the solvent control organisms. Neither test indicated a significant difference between the two control groups for either endpoint. Survival and growth (dry weight) data from relevant dose levels were compared to the negative control data to determine treatment level effects per current EPA guidance. For determination of the LOEC and NOEC values, Dunnett's Multiple Comparison Test and Steel's Many-One Rank Sum Test were used to identify significant differences in the amphipod growth (dry weight) and survival, respectively, compared to the dilution water control. The 10-day LC_{50} and EC_{50} values were empirically estimated.

Results and Discussion

The limit of quantification (LOQ) for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. In this study, the LOQ for the sediment analysis at 0 and 10 days was 0.24 and 0.25 mg/L, respectively. Mean measured concentrations were used for the calculation and reporting of results.

Measured porewater concentrations in the 6.3, 13, 25, 50 and 100 mg/kg treatment levels were 0.076, 0.19, 0.47, 0.90 and 1.7 mg/L on day 0 and 0.050, 0.091, 0.27, 0.53 and 1.1 mg/L on day 10, respectively. Measured overlying water concentrations in the 6.3, 13, 25, 50 and 100 mg/kg treatment levels were 0.0024, 0.0050, 0.0088, 0.020, 0.044 mg/L on day 0 and 0.00069, 0.0035, 0.0020, 0.0049 and 0.0083 mg/L on day 10, respectively.

Table 8.2.5.4-1: Analytical results

Nominal concentration (mg/kg sediment dry weight)	Measured sediment concentration (mg/kg sediment dry weight)			Mean percent recovery
	Day 0	Day 10	Mean (SD)	
Control	< 0.24[a]	< 0.25	NA[b]	NA
Solvent Control	< 0.24	< 0.25	NA	NA
6.3	4.8	5.7	5.2 (0.59)	83
13	11	11	11 (0.12)	84
25	21	21	21 (0.044)	86
50	44	38	41 (4.3)	82
100	79	88	84 (6.7)	84

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.

[b] NA = Not Applicable.

Amphipod survival and growth observed in both the negative control and solvent control during this period met the minimum standard criteria established by the draft OCSPP Guideline (i.e., 80% control survival and measurable growth in the controls).

The effects of fludioxonil on amphipod survival and growth are summarised in the following table.

Table 8.2.5.4-2: Effects of fludioxonil on the response of *Hyalella azteca* following a 10-day static-renewal exposure

Mean measured concentration (mg/kg sediment dry weight)	Test day 10	
	Mean percent survival (SD[a])	Mean dry weight per amphipod in mg (SD)
Control	99 (4)	0.21 (0.012)
Solvent Control	96 (5)	0.20 (0.014)
5.2	94 (5)	0.21 (0.011)
11	99 (4)	0.19 (0.020)
21	99 (4)	0.20 (0.0073)
41	94 (7)	0.19 (0.016)
84	96 (5)	0.19 (0.015)
Endpoint summary (mg/kg dw)		
LC_{50}/EC_{50}	>84	>84
95% Confidence limits	NA[b]	NA
NOEC	84	84
LOEC	>84	>84

[a] SD = Standard Deviation.
[b] NA = Not Applicable. LC/EC50 value was empirically estimated; therefore, corresponding 95% confidence intervals could not be determined.

Conclusions

In a 10-day acute toxicity test in which *Hyalella azteca* were exposed to fludioxonil applied to the sediment based on mean measured concentrations, the 10-day LC_{50} and EC_{50} values were empirically estimated to be >84 mg/kg sediment dry weight. The NOEC and LOEC values based on survival and growth were determined to be 84 and >84 mg/kg sediment dry weight.

(Bradley M, 2014)

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.5.4/02 Bradley MJ. (2014a) 10-Day Toxicity Test Exposing Estuarine Amphipods (*Leptocheirus plumulosus*) to Fludioxonil Technical Applied to Sediment under Static Conditions. Report Number 1781.6945. Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571-1037, USA (Syngenta File No. CGA173506_51327).

Guidelines

US EPA Ecological Effects Test Guidelines, OCSPP Draft Guideline 850.1740. Whole Sediment Acute Toxicity Invertebrates, Marine (1996)

GLP: Yes

Executive Summary

The 10-day acute toxicity of fludioxonil to *Leptocheirus plumulosus* was determined under static conditions. Juvenile amphipods were exposed to nominal concentrations of 6.3, 13, 25, 50 and 100 mg/kg sediment dry weight (5.7, 11, 23, 45 and 90 mg/kg sediment dry weight, mean measured) alongside a dilution water control and a solvent (acetone) control. Following 10 days of exposure, amphipod survival was evaluated. Based on mean measured concentrations, the 10-day LC_{50} value for survival was empirically estimated to be >90 mg/kg sediment dry weight. The NOEC and LOEC values for amphipod survival were determined to be 90 and > 90 mg/kg sediment dry weight.

Materials

Test Material:	Fludioxonil
Lot/Batch #:	SMO3J0464
Purity:	97.7% (w/w); tested as 100%
Description:	Brownish powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	End of October 2017

Treatments

Test concentrations:	Dilution water control, solvent (acetone) control and nominal concentrations of 6.3, 13, 25, 50 and 100 mg/kg sediment dry weight (5.7, 11, 23, 45 and 90 mg/kg sediment dry weight, mean measured)
Solvent:	Acetone (CAS No. 67-64-1)
Analysis of test concentrations:	Yes, analysis of fludioxonil at 0 and 10 days

Test organisms:

Species:	*Leptocheirus plumulosus*
Age:	The marine amphipods used during this study were approximately the same size and age (juvenile amphipods that pass through a 1.0-mm sieve and are retained on a 0.50-mm sieve).
Source:	Chesapeake Cultures, Hayes, Virginia
Feeding:	During the holding and acclimation period prior to testing, amphipods in each holding vessel were fed 100 mg of flaked fish food suspended in laboratory well water, daily. Amphipods were not fed during the exposure.
Culture medium:	20‰ natural, filtered seawater

Test design:

Test vessels:	1000mL glass beakers. Approximately 175 mL (2 cm layer) of sediment (equivalent to 62.5 g dry weight per vessel) and 725 mL of overlying water. Total overlying/sediment volume of approximately 900 mL
Test medium:	Sediment with overlying natural, filtered seawater
Sediment:	Marine sediment collected from Sequim Bay, Sequim, Washington 35% sand, 40% silt, 25% clay Organic Carbon: 3.9% pH: 7.7 % Solids: 30.55%
Sediment moisture content:	69.45%
Replication:	5 replicates of 20 amphipods

Exposure regime:	Static
Duration:	10 days

Environmental conditions

Test temperature:	Overlying water/Auxiliary vessel in water bath: 24 to 26°C
pH range:	Overlying water: 7.8 to 8.4 Porewater: 7.2 to 7.4
Dissolved oxygen:	Overlying water: 5.9 to 8.2 mg/L (no aeration).
Salinity	Overlying water: 21 to 22‰ Porewater: 21 to 26‰
Ammonia:	Overlying water: 2.2 mg N/L (day 0), ≤ 0.10 to 1.6 mg N/L (day 10) Porewater: 8.4 to 9.9 mg N/L (day 0), 1.0 to 1.9 mg N/L (day 10)
Lighting:	Continuous illumination at 770 to 940 lux

Study Design and Methods

Experimental dates: 12th to 22nd September 2014

A 10 mL volume of each dosing stock solution was applied to 0.050 kg of fine silica sand and mixed. Following evaporation of the solvent, the entire sand/test substance mixture was added to 2.75 kg of wet sediment (0.8901 kg total dry weight based on 30.55% solids and including the aforementioned 0.050 kg of fine silica sand) in individual glass jars.

The test substance was applied to the sediment by adding 10 mL of the appropriate dosing stock solution to 0.050 kg of fine silica sand, allowing the acetone to evaporate, and then mixing with the remaining part of the wet sediment using a jar-rolling technique to give the nominal concentrations of 6.3, 13, 25, 50 and 100 mg/kg sediment dry weight. The spiked sediments were allowed to equilibrate for a 27-day period in the refrigerator with weekly mixing on a rolling mill. One day before the amphipods were introduced, the sediment was added to the test vessels. The wet sediment was filled into each test beaker to give a layer of approximately 175 mL (2-cm depth), corresponding to 205 g wet weight or 62.5 g dry weight. The sediment was then covered with 725 mL of overlying water for a total overlying/ sediment volume of approximately 900 mL. Overlying water was added gently to prevent suspension of the sediment layer in the water column and each vessel was then randomly placed in the water bath used to house the exposure. Each test vessel was covered with a plastic plate and aeration was supplied with a constant trickle flow of bubbles from a 1-mL glass pipette.

The test vessels were left to stand overnight under test conditions, after which the amphipods were randomly distributed amongst the test vessels (Day 0). Following the addition of amphipods, all vessels were examined at exposure initiation and daily thereafter, until test termination (Day 10). All vessels were examined at exposure initiation and at daily intervals thereafter, until test termination (Day 10). Observations of organism mortality and abnormal behaviour were made and the physical characteristics of the sediment:water system were recorded. At test termination (day 10), the total number of surviving amphipods was determined in each test vessel by sieving the sediment through a fine mesh net or sieve (≤ 0.50 mm opening).

At exposure initiation and termination, dissolved oxygen concentration, salinity, temperature and pH were measured in the overlying water of each treatment level and control. In addition, the temperature was continuously monitored in an auxiliary vessel in the temperature-controlled water bath. Ammonia concentration (as nitrogen) of the overlying water was monitored at exposure initiation and termination in each treatment level and control. At exposure initiation and termination, salinity, pH, temperature and ammonia concentration (as nitrogen) were also measured in a porewater sample of each treatment level and control.

Sediment, pore water and overlying water samples were analysed for fludioxonil on day 0 and 10, using liquid chromatography/mass spectrometry (LC/MS/MS).

Wilcoxon's Rank Sum Test was conducted for survival data to compare the performance of the dilution water control organisms with that of the solvent control organisms. No significant difference between negative control and solvent control survival data was detected. Survival data from relevant dose levels were compared to the negative control data to determine treatment level effects per current EPA guidance. For determination of the LOEC and NOEC values, Dunnett's Multiple Comparison Test was used to identify significant differences in survival compared to the dilution water control. The 10-day LC_{50} value was empirically estimated.

Results and Discussion

The limit of quantification (LOQ) for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. In this study, the LOQ for the sediment analysis at 0 and 10 days was 0.26 and 0.24 mg/kg, respectively. Mean measured concentrations were used for the calculation and reporting of results.

Measured porewater concentrations in the 6.3, 13, 25, 50 and 100 mg/kg treatment levels were 0.042, 0.10, 0.20, 0.35 and 0.53 mg/L on day 0, respectively and 0.036, 0.082, 0.19, 0.33 and 0.58 mg/L on day 10, respectively. Measured concentrations on in the 6.3, 13, 25, 50 and 100 mg/kg treatment levels were 0.0081, 0.019, 0.042, 0.064 and 0.11 mg/L on day 0, respectively and 0.022, 0.048, 0.098, 0.18 and 0.29 mg/L on day 10, respectively.

Table 8.2.5.4-3: Analytical results

Nominal concentration (mg/kg sediment dry weight)	Measured sediment concentration (mg/kg sediment dry weight)			Mean percent recovery
	Day 0	Day 10	Mean (SD)	
Control	< 0.26[a]	< 0.24	NA[b]	NA
Solvent Control	< 0.26	< 0.24	NA	NA
6.3	6.1	5.2	5.7 (0.67)	90
13	13	9.7	11 (2.3)	87
25	26	21	23 (3.3)	93
50	47	43	45 (2.6)	90
100	96	84	90 (8.6)	90

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.

[b] NA = Not Applicable.

Table 8.2.5.4-4: Effects of fludioxonil on the response of *Leptocheirus plumulosus* following a 10-day static exposure

Mean measured concentration (mg/kg sediment dry weight)	Test day 10
	Mean percent survival (SD[a])
Control	99 (2)
Solvent Control	99 (2)
5.7	98 (3)
11	98 (3)
23	99 (2)
45	96 (4)
90	96 (2)
Endpoint summary (mg/kg dw)	
LC_{50} (mg/kg dw)	>90
NOEC	90
LOEC	>90

[a] SD = Standard Deviation.

Amphipod survival observed in the control during this period met the minimum standard criteria established by the EPA test method (i.e., ≥ 90% mean survival). The effects of fludioxonil on amphipod survival are summarised in the following table.

Conclusions

In a 10-day acute toxicity test in which *Hyalella azteca* were exposed to fludioxonil applied to the sediment based on mean measured concentrations, the 10-day LC_{50} value for survival was empirically estimated to be >90 mg/kg sediment dry weight. The NOEC and LOEC values for amphipod survival were determined to be 90 and > 90 mg/kg sediment dry weight.

(Bradley M, 2014a)

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: KCA 8.2.5.4/03 Bradley, MJ (2014b) 10-Day Toxicity Test Exposing Midge (*Chironomus dilutus*) to Fludioxonil TGAI Applied to Sediment Under Static-Renewal Conditions, Report Number 1781.6942, Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571-1037 USA, (Syngenta File No. CGA173506_51329)

Guidelines

US EPA Ecological Effects Test Guidelines, OCSPP Draft Guideline 850.1735. Whole Sediment Acute Toxicity Invertebrates, Freshwater (1996)

GLP: Yes

Executive Summary

The acute toxicity of fludioxonil to *Chironomus dilutus* was determined under static-renewal conditions. Third instar larvae (10 days old) were exposed to nominal concentrations of 0.80, 2.0, 5.0, 13, 31 and 100 mg/kg sediment dry weight (0.85, 2.0, 5.3, 13, 31 and 100 mg/kg sediment dry weight, mean measured) alongside a dilution water control and a solvent (acetone) control.

Following 10 days of exposure, larval survival and growth (mean ash-free dry weight per midge larvae) were evaluated. Based on mean measured concentrations, the 10-day LC_{50} value for survival was empirically estimated to be >100 mg/kg sediment dry weight. The NOEC and LOEC values based on survival were determined to be 100 and >100 mg/kg sediment dry weight, respectively. The EC_{50} value for growth was calculated to be 46 mg/kg sediment dry weight. The NOEC and LOEC values based on growth were determined to be 2.0 and 5.3 mg/kg sediment dry weight, respectively.

Materials

Test Material:	Fludioxonil
Lot/Batch #:	SMO3J0464
Purity:	97.7% (w/w); tested as 100%
Description:	Brownish powder
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	End of October 2017
Treatments	
Test concentrations:	Dilution water control, solvent (acetone) control and nominal concentrations of 0.80, 2.0, 5.0, 13, 31 and 100 mg/kg sediment dry weight (0.85, 2.0, 5.3, 13, 31 and 100 mg/kg sediment dry weight, mean measured)
Solvent:	Acetone (CAS No. 67-64-1)
Analysis of test concentrations:	Yes, analysis of fludioxonil at 0 and 10 days
Test organisms:	
Species:	*Chironomus dilutus*
Age:	Third instar (10-day old larvae)
Source:	Continuous laboratory cultures
Feeding:	The midge larvae were fed a diet consisting of a finely ground flaked fish food suspended in laboratory well water (4.0 mg/mL). During the exposure, the food was introduced daily at a rate of 1.5 mL of flaked fish food suspension per test vessel.
Culture medium:	Laboratory well water
Test design:	
Test vessels:	300 mL glass vessels with two openings on the top edge covered with 40-mesh Nitex® screen for drainage. Approximately 100 mL (4 cm layer) of sediment (equivalent to 97.6 g dry weight per vessel) and 175 mL of overlying water. Total overlying/sediment volume of approximately 275 mL
Test medium:	Sediment with overlying laboratory well water
Sediment:	Artificial sediment prepared according to OECD Guideline No. 218 79% sand, 4% silt, 17% clay Organic Carbon: 2.4% pH: 6.5 % Solids: 60.73%

Sediment moisture content:	39.27%
Replication:	8 replicates of 10 midge larvae
Exposure regime:	Static-renewal Overlying water was renewed using an intermittent delivery system in combination with a calibrated water-distribution system. The water delivery system cycled approximately 7 times per day, providing approximately 350 mL per vessel every 24 hours. Due to an observed trend of decreasing dissolved oxygen measurements in the test vessels on test day 3, the flow rate in the system was doubled so that the water delivery system cycled approximately 14 times per day, providing approximately 700 mL per vessel every 24 hours (i.e., approximately four overlying volume replacements per vessel per day).
Duration:	10 days
Environmental conditions	
Test temperature:	22 to 24°C
pH range:	6.4 to 7.3
Dissolved oxygen:	3.0 to 8.1 mg/L[18]
Total hardness:	64 to 80 mg/L $CaCO_3$
Total alkalinity:	18 to 24 mg/L $CaCO_3$
Conductivity:	380 to 460 µmhos/cm
Ammonia:	≤ 0.10 to 0.81 mg N/L
Lighting:	16 hours light (510 to 870 lux) and 8 hours dark, ; sudden transitions of light to dark and dark to light were avoided

Study Design and Methods

Experimental dates: 9th to 19th September 2014

A 15 mg/mL primary stock solution was prepared by bringing 0.7504 g of fludioxonil to a volume of 50 mL with acetone. A 10 mL volume of each dosing stock solution was applied to 0.050 kg of fine silica sand and mixed. Following evaporation of the solvent, the entire sand/test substance mixture was added to 2.0 kg of wet sediment (0.7828 kg total dry weight based on 60.73% solids and including the aforementioned 0.050 kg of fine silica sand) in individual glass jars.

The test substance was applied to the sediment by adding 10 mL of the appropriate dosing stock solution to 0.050 kg of fine silica sand, allowing the acetone to evaporate, and then mixing with the remaining part of the wet sediment using a jar-rolling technique to give the nominal concentrations of 0.80, 2.0, 5.0, 13, 31 and 100 mg/kg sediment dry weight. The spiked sediments were allowed to equilibrate for a 27-day period in the refrigerator with weekly mixing on a rolling mill. One day before the larvae were introduced, the sediment was added to the test vessels. The wet sediment was filled into each test beaker

[18] The guideline states that dissolved oxygen should be greater than 40% of the air saturation value for the duration of the exposure. During this study, dissolved oxygen in the negative control, solvent control, 0.80, 2.0 and 100 mg/kg (nominal) treatment levels fell below 40% of saturation on test day 10. The air saturation value observed among the deviated test vessels in these treatments ranged from 34 to 39%. Since these levels are within the tolerance of *C. dilutus* and since the deviation occurred at test termination, this deviation did not have a negative impact on the results or interpretation of the study.

to give a layer of approximately 100 mL (4-cm depth), corresponding to 161 g wet weight or 97.6 g dry weight. The sediment was then covered with 175 mL of overlying water for a total overlying/ sediment volume of approximately 275 mL. A turbulence reducer, consisting of a modified plastic disk, was used to minimize the disruption of the sediment layer during the introduction of overlying water.

The larvae were reared under static conditions with gentle, oil-free aeration. Midge larvae used in the toxicity test were 10 days old at exposure initiation (third instar). The head capsule width of a second subset of twenty midge larvae was measured at exposure initiation to confirm the instar of the larvae used. The head capsule width ranged from 0.30 to 0.40 mm, with a mean of 0.35 mm. All measurements were consistent with expected 3rd instar characteristics.

The test vessels were left to stand overnight under test conditions, after which the larvae were randomly distributed amongst the test vessels (Day 0). During the exposure, the food was introduced daily at a rate of 1.5 mL of flaked fish food suspension (4.0 mg/mL) per test vessel. Following the addition of larvae, all vessels were examined at exposure initiation and daily thereafter, until test termination (day 10). Observations of mortality and abnormal behaviour were made and the physical characteristics of the test solutions were recorded. At test termination (Day 10), the remaining sediment was sieved using fine mesh fish nets (approximately 0.25 mm) to remove all surviving midge and the total number and growth (expressed as ash-free dry weight) of surviving midge was determined for each test vessel. Growth was determined by pooling all surviving midge larvae within each replicate in ash weighing tins and drying at 62 to 64°C for approximately 24 hours. After drying, the weighing tins containing the pooled dried larvae were weighed on an analytical balance to the nearest 0.01 mg. The weighing tins containing the pooled dried larvae were then ashed at 550 ºC in a furnace for approximately two hours. The pooled, ashed midge larvae were then weighed on an analytical balance to the nearest 0.01 mg.

At exposure initiation and termination, temperature, dissolved oxygen concentration and pH were measured for each treatment level and control. In addition, the temperature was continuously monitored in an auxiliary vessel in the temperature controlled water bath. Total hardness, alkalinity, conductivity and ammonia concentration of the overlying water were monitored at exposure initiation and test termination in each treatment level and control.

Sediment, pore water and overlying water samples were analysed for fludioxonil on days 0 and 10, using liquid chromatography/mass spectrometry (LC/MS/MS).

Wilcoxon's Rank Sum Test and an Equal Variance Two-Sample t-Test were conducted for survival and growth (ash-free dry weight) data, respectively, to compare the performance of the dilution water control organisms with that of the solvent control organisms. Wilcoxon's Rank Sum Test indicated no significant difference between negative control and solvent control survival data. The Equal Variance Two-Sample t-Test did however determine a significant difference between negative control and solvent control growth (ash-free dry weight) data. Given the dosing procedure, the observed difference is not perceived as being a function of solvent interference and is instead related to natural variability commonly observed for *Chironomus dilutus* larval growth. Survival and growth (ash-free dry weight) data from relevant dose levels were compared to the negative control data to determine treatment level effects per current EPA guidance. For determination of the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values, Dunnett's Multiple Comparison Test and Steel's Many-One Rank Sum Test were used to identify significant differences in the midge growth (ash-free dry weight) and survival, respectively, compared to the dilution water control. The 10-day LC_{50} value (defined as the concentration resulting in 50% mortality) was empirically estimated and the 10-day EC_{50} values (defined as the concentration resulting in 50% inhibition of growth) was calculated using CETIS™ Version 1.8.

Results and Discussion

The limit of quantification (LOQ) for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. In this study, the LOQ for the sediment analysis at 0 and 10 days was 0.040 and 0.037 mg/kg, respectively. Mean measured concentrations were used for the calculation and reporting of results.

Measured porewater concentrations in the 0.80, 2.0, 5.0, 13, 31 and 100 mg/kg treatment levels were 0.0055, 0.015, 0.044, 0.16, 0.44 and 0.95 mg/L on day 0 and 0.0039, 0.012, 0.037, 0.13, 0.32 and 0.89 mg/L on day 10, respectively. Measured overlying water concentrations in the 0.80, 2.0, 5.0, 13, 31 and 100 mg/kg treatment levels were 0.0020, 0.0035, 0.021, 0.047, 0.13 and 0.45 mg/L on day 0 and < 0.00048, 0.0020, 0.0040, 0.0045, 0.033 and 0.10 mg/L on day 10, respectively.

Table 8.2.5.4-5: Analytical results

Nominal concentration (mg/kg sediment dry weight)	Measured sediment concentration (mg/kg sediment dry weight)			Mean percent recovery
	Day 0	Day 10	Mean (SD)	
Control	< 0.040[a]	< 0.037	NA[b]	NA
Solvent Control	< 0.037	< 0.037	NA	NA
0.80	0.77	0.92	0.85 (0.11)	110
2.0	1.9	2.0	2.0 (0.052)	99
5.0	5.4	5.2	5.3 (0.083)	110
13	13	14	13 (0.88)	100
31	30	32	31 (1.9)	100
100	110	89	100 (17)	100

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.

[b] NA = Not Applicable.

Midge survival and growth observed in both the negative control and solvent control during this period met the minimum standard criteria established by the OCSPP Draft Guideline (i.e., 70% survival and 0.60 mg ash-free dry weight per midge larvae).

The effects of fludioxonil on midge survival and growth are summarised in the following table.

Table 8.2.5.4-6: Effects of fludioxonil on the response of *Chironomus dilutus* following a 10-day static-renewal exposure

Mean measured concentration (mg/kg sediment dry weight)	Test day 10	
	Mean percent survival (SD[a])	Mean ash-free dry weight per midge larvae in mg (SD)
Control	98 (5)	1.46 (0.21)
Solvent Control	98 (5)	1.89 (0.47)
0.85	95 (8)	1.91 (0.26)
2.0	100 (0)	1.94 (0.39)
5.3	95 (11)	1.03[b] (0.20)
13	99 (4)	1.15[b] (0.37)
31	98 (5)	1.15[c] (0.16)
100	90 (8)	0.42[b] (0.10)
Endpoint summary (mg/kg dw)		
LC_{50}/EC_{50}	>100	46
95% Confidence limits	NA[d]	39 – 53
NOEC	100	2.0
LOEC	>100	5.3

[a] SD = Standard Deviation.
[b] Significantly reduced compared to the control, based on Dunnett's Multiple Comparison Test.
[c] Although no significant reduction was observed, this statistical result was perceived as being out of trend, given the observed statistical reductions at both lower and high treatment levels. Consequently, this result was not considered to be representative of a true no-effect concentration.
[d] NA = Not Applicable. LC/EC_{50} value was empirically estimated; therefore, corresponding 95% confidence intervals could not be determined.

Conclusions

In a 10-day acute toxicity in which *Chironomus dilutus larvae* were exposed to fludioxonil applied to the sediment, based on mean measured concentrations, the 10-day LC_{50} value for survival was empirically estimated to be >100 mg/kg sediment dry weight. The NOEC and LOEC values based on survival were determined to be 100 and >100 mg/kg sediment dry weight, respectively. The EC_{50} value for growth was calculated to be 46 mg/kg sediment dry weight. The NOEC and LOEC values based on growth were determined to be 2.0 and 5.3 mg/kg sediment dry weight, respectively.

(Bradley M, 2014b)

CA 8.2.6 Effects on algal growth

At the request of the RMS a point of clarificiation has been added regarding the pH variation in algal studies (applies to all algal studies):

pH variation:

The pH variation during an algal study frequently shows variation during the studysometimes more than the 1.5 untis noted in OECD 201. The duration of an algal study affects the final pH. The longer the study, the higher the pH tat the end of the study. If flasks are shaken enough, or aerated, this will reduce the pH rise. However, some algal studies were conducted over 5 days which is significantly longer than the 72-h OECD 201 test. Even the US OCSPP test is 96 hour and notably in that test guideline there is no corresponding no statement regarding pH change during the test. In studies which use a high initial inoculum (e.g. 10,000 cells/mL) this can also result in high final pHs. The high pH reached at the end

such studies is expected and is indicative of strong, healthy algal growth. The pH change during such tests result from the algae absorbing the dissolved CO_2 (carbonic acid), and releasing oxygen into the test medium. As the CO_2 is absorbed and oxygen released by the photosynthesising algae, the pH of the medium rises. The stronger the algal growth the greater the pH rise. A study by Dubinsky & Rotem 1974[19] examines the effect of pH on algae and found that algae grew well between pH 5 and 12, while sterile controls grew under pH 4 to 10. Therefore the rise in pH during the studies presented here is not considered to be of any consequence for the validity of these studies, and the absence of this criteria from the OCSPP guidelines validates this statement.

CA 8.2.6.1 Effects on growth of green algae

Studies conducted by Rufli, 1989 (Syngenta file number CGA173506/0007) and Hoberg, 1992 (Syngenta file number CGA173506/0243) which were previously submitted in the EU for fludioxonil were summarised in point B. 9.2.6 of the DAR ~~therefore additional study summaries are not repeated here~~. The endpoints are summarised in Tables 8.2-7.

At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.6 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoints are summarised in Table 8.2-7 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.5.3 Hoberg JR, (1992). CGA-173506 technical – toxicity to the freshwater green alga Selenastrum capricornutum. Report Number #92-9-4399, Springborn Laboratories Inc., Environmental Sciences Division, 790 Main Street, Wareham, Massachusetts 02571, USA (Syngenta File No. CGA173506/0243).* *Hoberg JR, (2005). Supplemental report (Syngenta File No. CGA173506/6590).*

Guideline

FIFRA Guideline Ref. 122-2 and 123-2, December 1989

GLP: Yes

Executive Summary

The toxicity of CGA173506 to the green alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) was determined. Algae were exposed to nominal concentrations of 0.026, 0.050, 0.10, 0.20, 0.40 and 0.80 mg a.s./L (geometric mean measured concentrations were 0.014, 0.020, 0.027, 0.085, 0.22 and 0.51 mg a.s./L) alongside a culture medium control and a solvent control. Based on geometric mean measured concentrations, the 72-, 96- and 120-hour E_rC_{50} values were 0.41, 0.31 and 0.33 mg a.s./L, respectively. E_bC_{50} values for the same time points were determined to be 0.029, 0.024 and 0.024 mg a.s./L, respectively.

[19] Dubinsky, Z. and Rotem, J. (1974) Relations between algal populations and the pH of their media. Oecologia (Berl.)16: 53-60.

Materials

Test Material	CGA173506
Lot/Batch #:	P905004/5
Purity:	94.5%
Description:	White powder
Stability of test compound:	Stable under test conditions
Reanalysis date:	16 April 1994

Treatments

Test concentrations:	Culture medium control and nominal concentrations of 0.026, 0.050, 0.10, 0.20, 0.40 and 0.80 mg CGA173506/L (geometric mean measured concentrations were 0.014, 0.020, 0.027, 0.085, 0.22 and 0.51 mg a.s./L)
Solvent:	Acetone
Positive control:	None reported
Analysis of test concentrations:	Yes, analysis of CGA173506 at 0 and 120 hours, using HPLC analysis with UV detection

Test organism

Species:	*Selenastrum capricornutum*, strain 1648 (= *Pseudokirchneriella subcapitata*, SAG 61.81)
Source:	Initially obtained from the University of Texas, Austin, Texas, USA, and was maintained in stock culture at the Test Facility.

Test design

Test vessels:	125 mL flasks containing 50 mL of media covered with stainless steel caps which permitted gas exchange
Test medium:	Marine Biological Laboratory (MBL) medium prepared with distilled water
Replication:	3
Starting cell density:	111 x 10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	120 hours

Environmental conditions

Test temperature:	24 to 25 °C
pH:	test start: 7.6 to 7.8
	test end: 9.2 to 10.6
Lighting:	Continuous cold white fluorescent illumination at 3200 to 5400 lux

Study Design and Methods

Experimental dates: 24th to 29th June 1992

A 8 mg a.s./mL stock solution was prepared by dissolving 0.4193 mg of test item in 50 mL acetone. A 50 µL aliquot of the stock was diluted to 500 mL with sterile MBL medium to create the highest nominal test concentration of 0.80 mg a.s./L. Appropriate volumes of the stock solution were diluted in 10 mL acetone to give the test concentration series. The solvent control contained 0.10 mL acetone/L, which was equivalent to the same concentration of acetone in each of the test solutions.

Ten minutes after preparation of the test solutions 0.14 mL of the test algae at a density of 111 x 10^4 cells/mL was introduced to the test flasks to give a starting cell density of approximately 0.3 x 10^4

cells/mL. Test solutions were constantly shaken at 100 rpm and were held at 24 to 25 °C under continuous illumination.

Algal cell counts were conducted every 24 hours using a haemocytometer (Neubauer improved). The 72-, 96- and 120-hour E_rC_{50} and E_bC_{50} values were determined by linear regression of response vs exposure concentration. A computer program, TOXSTAT® (Gulley et al., 1996), was used to determine the EC values and 95% confidence intervals.

The pH was measured at 0 and 120 hours, and the water temperature was measured continuously in the room and daily in water in a separate test vessel.

The test concentrations were verified by chemical analysis of CGA173506 at 0 and 120 hours, using high performance liquid chromatography (HPLC) with ultra-violet detection. For sampling at the end of the test, the test media of the treatment replicates was pooled.

Results and Discussion

At the start of the test, the measured concentrations were in the range 53 to 124 % of the nominal values and at the end of the test were below the limit of quantification for the lowest 3 concentrations and ranged from 18 to 77% for the top three (see table below). The limit of quantification (LOQ) in this study was 0.013 mg CGA173506/L. Geometric mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.6.1-1: Analytical results

Nominal concentrations (mg a.s./L)	Measured concentrations at 0 hours (mg a.s./L /L)	% of nominal at 0 hours	Measured concentrations at 120 hours (mg a.s./L)	% of nominal at 120 hours	Geometric mean measured concentrations (mg a.s./L)	% of nominal
Control	< LOQ	NA	< LOQ	NA	NA	NA
Solvent control	< LOQ	NA	< LOQ	NA	NA	NA
0.026	0.028	109	< LOQ	NA	0.014	52
0.050	0.062	124	< LOQ	NA	0.020	40
0.10	0.11	108	< LOQ	NA	0.027	27
0.20	0.20	99	0.036	18	0.085	42
0.40	0.36	89	0.13	33	0.22	54
0.80	0.43	53	0.61	77	0.51	64

LOQ = 0.013 mg CGA173506/L

Growth rates

The growth rate was calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 8.2.6.1-2: Mean values at each concentration of CGA339833 for the growth rate at 72-, 96- and 120-hours for *Selenastrum capricornutum*

Geometric mean measured concentration (mg CGA173506/L)	Mean growth rate, 72 hrs (s.d.)	Mean growth rate, 96 hrs (s.d.)	Mean growth rate, 120 hrs (s.d.)	Percentage inhibition [a,b]
Control	1.46 (0.02)	1.33 (0.02)	1.24 (0.01)	NA [c]
Solvent control	1.45 (0.03)	1.28 (0.03)	1.20 (0.01)	NA

Pooled control	1.46 (0.02)	1.31 (0.03)	NA	NA
0.014	1.38 (0.01)	1.31 (0.03)	1.19 (0.02)	1
0.020	1.32 (0.02)	1.16 (0.03) [d]	1.11 (0.01) [f]	7
0.027	1.23 (0.03)	1.08 (0.01) [d]	1.01 (0.03) [f]	16
0.085	1.07 (0.03)	1.01 (0.02) [d]	0.88 (0.03) [f]	27
0.22	0.84 (0.06) [e]	0.86 (0.02) [d]	0.77 (0.01) [f]	36
0.51	0.66 (0.14) [e]	0.21 (0.08) [d]	0.33 (0.05) [f]	73
E_rC_{50} values (mg a.s./L) [95% C.L.]	0.41 [0.32 – 0.48]	0.31 [0.30 – 0.33]	0.33 [0.32 – 0.34]	-
NOEC (mg a.s./L)	0.014	0.014	0.014	-

[a] : percent inhibition relative to the solvent control
[b] : mean, standard deviation (s.d.) and percent inhibition and percent inhibition were calculated from original raw data, not from the rounded data presented in this table
[c] : NA = Not applicable
[d] : Significantly reduced relative to the pooled control, based on Williams' test
[e] : Significantly reduced relative to the pooled control, based on Kruskal-Wallis' test
[f] : Significantly reduced relative to the solvent control, based on Williams' test

Area under the growth curve

The area under the growth curve was calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 8.2.6.1-3: Mean values at each concentration of CGA173506 for area under the growth curve at 72-, 96- and 120-hours for *Selenastrum capricornutum*

Geometric mean measured concentration (mg CGA173506/L)	72 hour total biomass (cells x 10^4) (s.d.)	96 hour total biomass (cells x 10^4) (s.d.)	120 hour total biomass (cells x 10^4) (s.d.)	120 hour percent inhibition [a, b]
Control	27.30 (3.34)	77.16 (6.70)	163.28 (8.65)	NA [c]
Solvent control	22.67 (3.97)	66.28 (6.34)	138.08 (10.98)	NA
Pooled control	24.99 (4.14)	71.72 (8.34)	NA	NA
0.014	26.89 (5.81)	71.59 (9.38)	142.83 (13.67)	-3
0.020	14.32 (0.70) [d]	40.96 (1.66) [d]	84.77 (4.66) [e]	39
0.027	13.07 (1.08) [d]	32.73 (0.78) [d]	61.71 (3.02) [e]	55
0.085	6.66 (0.61) [d]	20.32 (1.13) [d]	37.57 (3.46) [e]	73
0.22	4.19 (1.52) [d]	11.34 (1.34) [d]	20.92 (1.08) [e]	85
0.51	1.97 (0.09) [d]	3.30 (0.64) [d]	4.03 (0.87) [e]	97
E_bC_{50} values (mg a.s./L) [95% C.L.]	0.029 [0.20 – 0.041]	0.024 [0.022 – 0.026]	0.024 [0.023 – 0.026]	-
NOEC (mg a.s./L)	0.014	0.014	0.014	-

[a] : percent inhibition relative to the solvent control
[b] : mean, standard deviation (s.d.) and percent inhibition and percent inhibition were calculated from original raw data, not from the rounded data presented in this table
[c] : NA = Not applicable
[d] : Significantly reduced relative to the pooled control, based on Williams' test
[e] : Significantly reduced relative to the solvent control, based on Williams' test

Validity Criteria

No validity criteria are reported.

Conclusions

In an algal test in which *Pseudokirchneriella subcapitata* were exposed to CGA173506, based on geometric mean measured concentrations, the 72-, 96- and 120-hour E_rC_{50} values were 0.41, 0.31 and 0.33 mg a.s./L, respectively. E_bC_{50} values for the same time points were determined to be 0.029, 0.024 and 0.024 mg a.s./L, respectively.

(Hoberg J.R., 1992)

RMS comment:

It seems that the percentage of inhibition of biomass and growth rate EC50 are based on comparison with solvent control as a difference exists between the blank and the solvent controls. This is agreed but the statistics to determine significant differences from the control seems to be based on the pooled controls in view of the Table 3 and 5 of Hoberg (2005, CGA 173506/6950, KIlA 8.2.6/05). Thus the NOEC should be re-calculated using the solvent control only.

Syngenta response:

In Hoberg, 2005, it is stated on Page 6 that a t-Test was used to compare the control data and if they were similar, they were pooled for comparison with the treatments. If the data were not similar, the solvent control was used for comparison. This was done at each time point; e.g. 48, 72, 96 and 120 hours. In Tables 3 and 5 of this report, the footnotes describe which control was used for comparison with the treatments. It is clear that at 48, 72 and 96 hours, the pooled control was used (as there was no difference between controls) and then at 120 hours endpoints for the inhibition data AND the statistically significant differences were derived by comparing to the solvent control (as there was a difference between controls). Therefore no recalculation of EC50s or NOEC values is necessary.

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

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.2.6.1/01 Priestly S. & Allen M. (2015) Fludioxonil (CGA173506) Technical – Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum*. Statistical Re-analysis. Report Number: CEA.1594. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11886).

Summary

The original report from Springborn Smithers Laboratories (study number 1781.6180, report number 92-9-4399, Hoberg, 1992; Syngenta file number CGA173506/0243) and the supplemental report (Hoberg, 2005; Syngenta file number CGA173506/6950) for the toxicity of fludioxonil technical to the green alga *Selenastrum capricornutum* (subsequently renamed *Pseudokirchneriella subcapitata*) did not provide estimates of the EC_{10} and EC_{20} values. Consequently the data generated in this study have been re-analysed in an attempt to provide these values.

The test used nominal concentrations of 0.026, 0.050, 0.10, 0.20, 0.40, 0.80 mg a.s./L. Chemical analysis of the test solutions showed that the measured concentrations ranged from 53% to 124% of the nominal values at the start of the test and 18% to 77% at the end of the test. As a result, the geometric mean

measured concentrations of 0.014, 0.020, 0.027, 0.085, 0.22 and 0.51 mg a.s./L (used in the estimation of EC_{50} values in the Hoberg [2005] supplemental report) have been used in this statistical analysis. In addition, in keeping with the approach used in the original report (number: 92-9-4399), the control data were found to have a statistically significant difference and the treatment groups were compared to the solvent control.

The validity of the study was evaluated against the study guideline (OECD 201) with the following results:

Table 8.2.6.1-14: Validity criteria for the fludioxonil toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Hoberg, 1992; Syngenta file number CGA173506/0243)

Criterion	Required	Achieved	Valid
Increase in biomass 0 – 72 h (solvent control)	16	87.2	Yes
Mean growth rate 0 – 72 h (solvent control)	0.920/ d	1.188/ d	Yes
CV mean growth rate 0 – 72 h (solvent control)	7.0	2.0	Yes
Mean CV for sectional growth rate (solvent control)	35.0	21.6	Yes

CV: coefficient of variation

As the study is valid, the following parameters were analysed statistically using the methods described in this report:

- Yield (*Y*)
- Average specific growth rate (μ)

Statistical analyses of the available data for yield revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated:

Table 8.2.6.1-25: Statistical analysis of yield for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Hoberg, 1992; Syngenta file number CGA173506/0243 using mean measured concentrations from Hoberg, 2005; Syngenta file number CGA173506/6950)

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_{20}	E_yC_{20}	E_yC_{10}	E_yC_{20}
Value [mg a.s./L]	0.015	0.017	0.0093	0.0175	0.0097	0.0118	0.0085	0.0129
lower 95%-cl	0.015	0.016	0.0005	0.0023	0.0090	0.0104	0.0078	0.0122
upper 95%-cl	0.015	0.017	0.0211	0.0340	0.0104	0.0132	0.0091	0.0135

cl: confidence limits

No E_yC_{10} values at 72 or 96 hours could be reliably determined.

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:

Table 8.2.6.1-36: Statistical analysis of average specific growth rate for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Hoberg, 1992; Syngenta file number CGA173506/0243 using mean measured concentrations from Hoberg, 2005; Syngenta file number CGA173506/6950)

Parameter	72 hours		96 hours		120 hours	
	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}

Value [mg a.s./L]	0.017	0.047	0.0286	0.0610	0.0456	0.0809
lower 95%-cl	0.009	0.025	0.0262	0.0574	0.0406	0.0744
upper 95%-cl	0.032	0.091	0.0311	0.0646	0.0505	0.0872

cl: confidence limits

No E_rC_x values at 24 or 48 hours could be reliably determined.

Statistical Analysis

Where possible, non-linear regression was used to derive the EC_x values. However, in cases where this approach did not provide reliable estimates, linear max likelihood regression was used in an attempt to derive these values.

For each parameter, a Students t-test for homogenous variances was used to determine if there was any significant difference between the negative control and solvent control using pair-wise comparisons.

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.

A 3-parameter normal cumulative distribution function (Bruce and Versteeg, 1992) was fitted to the original data and a non-linear regression without weighting was performed using the Levenberg-Marquardt algorithm to calculate the function parameters.

Results

Yield after 24 hours

A significant amount of variance was explained by the regression model (p(F):Regression = <0.001) and there was no significant lack of fit (p(F):Lack of fit = 0.082). As a result, the E_yC_{10} and E_yC_{20} values and the 95% confidence limits presented were reliably determined.

Yield after 48 hours

The relationship between concentration and response was significant, p(F) = <0.001 and the slope function was 4.223. The E_yC_{10} and E_yC_{20} values and their 95% confidence limits presented were reliably determined.

Yield after 72 hours

The relationship between concentration and response was significant, p(F) = <0.001 and the slope function was 4.947. The E_yC_{20} values and their 95% confidence limits presented were reliably determined, however, no E_yC_{10} values were determined.

Yield after 96 hours

The relationship between concentration and response was significant, p(F) = <0.001 and the slope function was 3.970. The E_yC_{20} values and their 95% confidence limits presented were reliably determined, however, no E_yC_{10} values were determined.

Yield after 120 hours

The relationship between concentration and response was significant, p(F) = <0.001 and the slope function was 2.593. The E_yC_{10} and E_yC_{20} values and their 95% confidence limits presented were reliably determined.

Average specific growth rate after 24 hours

A significant amount of variance was explained by the regression model (p(F):Regression = 0.004) and there was no significant lack of fit (p(F):Lack of fit = 0.081). Although the E_rC_{10} and E_rC_{20} values and the 95% confidence limits were determined, the confidence limits are too wide to enable reliable EC_x determination.

Average specific growth rate after 48 hours

A significant amount of variance was explained by the regression model (p(F):Regression = <0.001) and there was no significant lack of fit (p(F):Lack of fit = 0.052). Although the E_rC_{10} and E_rC_{20} values and the 95% confidence limits were determined, the confidence limits are too wide to enable reliable EC_x determination.

Average specific growth rate after 72 hours

A significant amount of variance was explained by the regression model (p(F):Regression = <0.001) and there was no significant lack of fit (p(F):Lack of fit = 0.682). As a result, the E_rC_{10} and E_rC_{20} values and the 95% confidence limits were reliably determined.

Average specific growth rate after 96 hours

The relationship between concentration and response was significant, p(F) = <0.001 and the slope function was 3.687. The E_rC_{10} and E_rC_{20} values and their 95% confidence limits presented were reliably determined.

Average specific growth rate after 120 hours

The relationship between concentration and response was significant, p(F) = <0.001 and the slope function was 5.588. The E_rC_{10} and E_rC_{20} values and their 95% confidence limits presented were reliably determined.

Conclusion

The test used nominal concentrations of 0.026, 0.050, 0.10, 0.20, 0.40, 0.80 mg a.s./L. Chemical analysis of the test solutions showed that the measured concentrations ranged from 53% to 124% of the nominal values at the start of the test and 18% to 77% at the end of the test. As a result, the geometric mean measured concentrations of 0.014, 0.020, 0.027, 0.085, 0.22 and 0.51 mg a.s./L have been used in this statistical analysis. In addition, in keeping with the approach used in the original report (number: 92-9-4399), the control data were found to have a statistically significant difference, therefore the treatment groups were compared to the solvent control.

The following parameters were analysed statistically:

- Yield (*Y*)
- Average specific growth rate (μ)

Statistical analyses of the available data for yield revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated:

Table 8.2.6.1-47: Statistical analysis of yield for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Hoberg, 1992; Syngenta file number CGA173506/0243 using mean measured concentrations from Hoberg, 2005; Syngenta file number CGA173506/6950)

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_{20}	E_yC_{20}	E_yC_{10}	E_yC_{20}
Value [mg a.s./L]	0.015	0.017	0.0093	0.0175	0.0097	0.0118	0.0085	0.0129
lower 95%-cl	0.015	0.016	0.0005	0.0023	0.0090	0.0104	0.0078	0.0122
upper 95%-cl	0.015	0.017	0.0211	0.0340	0.0104	0.0132	0.0091	0.0135

cl: confidence limits

No E_yC_{10} values at 72 or 96 hours could be reliably determined.

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:

Table 8.2.6.1-58: Statistical analysis of average specific growth rate for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Hoberg, 1992; Syngenta file number CGA173506/0243 using mean measured concentrations from Hoberg, 2005; Syngenta file number CGA173506/6950)

Parameter	72 hours		96 hours		120 hours	
	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}	E_rC_{10}	E_rC_{20}
Value [mg a.s./L]	0.017	0.047	0.0286	0.0610	0.0456	0.0809
lower 95%-cl	0.009	0.025	0.0262	0.0574	0.0406	0.0744
upper 95%-cl	0.032	0.091	0.0311	0.0646	0.0505	0.0872

cl: confidence limits

No E_rC_x values at 24 or 48 hours could be reliably determined.

(Priestly S, Allen M, 2015)

The following statistical re-calculation was deleted as it erroneously used values for cells/sample instead of cells/ml for calculations. The correct statistical summary is repeated below the OECD summary added for the original study.

~~**Report:**~~	~~Submitted for purposes of renewal due to change in data requirements guidance~~ ~~K-CA 8.2.6.1/02 Taylor, S. and Howells, A. (2016), Fludioxonil (CGA173506) – Alga, Growth Inhibition Test of CGA173506 technical to Green Algae (*Scenedesmus subspicatus*). Statistical Re-analysis. Report Number: CEA.1595. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11910).~~

~~**Summary**~~

~~The report from CIBA-GEIGY Ltd. study number 881737 (Rufli, 1989, Syngenta file number CGA173506/0007) for the toxicity of CGA173506 to *Scenedesmus subspicatus* 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.~~

The validity of the study was evaluated against the study guideline (OECD 201) with the following results:

Table 8.2.6.1-6: Validity criteria for the fludioxonil toxicity to Freshwater Green Alga, *Scenedesmus subspicatus* (Rufli, 1989; Syngenta file number CGA173506/0007)

Criterion	Required	Achieved	Valid
Increase in biomass 0 – 72 h (control)	16	955.1	Yes
Mean growth rate 0 – 72 h (control)	0.920/ d	2.285/ d	Yes
CV mean growth rate 0 – 72 h (control)	7.0	21.9	Yes
Mean CV for sectional growth rate (control)	35.0	46.3	No

CV: coefficient of variation

Although the reported mean coefficient of variation for sectional growth rate in the control was higher than the limit stated in the study guideline, the data suggested that the test was reliable and the following parameters were analysed statistically:

- Yield (*Y*)
- Average specific growth rate (μ)

Statistical analyses of the available data for yield and growth rate revealed that the following E_yC_x and E_rC_x values were reliably calculated:

Table 8.2.6.1-7: Statistical analysis of yield and growth rate for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Scenedesmus subspicatus* (Rufli, 1989; Syngenta file number CGA173506/0007)

Parameter	72 hours			72 hours
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_rC_{10}
Value [mg/L]	0.108	0.211	0.750	0.740
lower 95%-cl	0.039	0.109	0.549	0.518
upper 95%-cl	0.178	0.302	1.183	1.181

cl: confidence limits

No EC_{10}, EC_{20} or EC_{50} values were able to be calculated for any other time point due to the absence of suitable data.

Statistical Analysis

Where possible, non-linear regression was used to derive the EC_x values. However, in cases where this approach did not provide reliable estimates, linear max-likelihood regression was used in an attempt to derive these values.

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.

Results

Yield after 24 hours

Although statistically significant differences were observed between the treatment groups and the control (p(F) = 0.001), no E_yC_{10}, E_yC_{20} or E_yC_{50} values could be reliably calculated due to the lack of a suitable responses to treatment.

Yield after 48 hours

Although statistically significant differences were observed between the treatment groups and the control (p(F) = 0.002), no E_yC_{10}, E_yC_{20} or E_yC_{50} values could be reliably calculated due to the lack of suitable responses to treatment.

Yield after 72 hours

The relationship between dose and response was significant, p(F) = <0.001 and the slope function was 4.518. The E_yC_{10}, E_yC_{20} and E_yC_{50} values and the 95% confidence limits presented were reliably determined.

Average specific growth rate after 24 hours

Although statistically significant differences were observed between the treatment groups and the control (p(F) = <0.001), no E_rC_{10}, E_rC_{20} or E_rC_{50} values could be calculated due to the lack of suitable responses to treatment.

Average specific growth rate after 48 hours

Although statistically significant differences were observed between the treatment groups and the control (p(F) = <0.001), no E_rC_{10}, E_rC_{20} or E_rC_{50} values could be calculated due to the lack of suitable responses to treatment.

Average specific growth rate after 72 hours

The E_rC_{10} value and the 95% confidence limits presented were reliably determined. Despite this, no E_rC_{20} or E_rC_{50} values could be reliably calculated due to the lack of suitable responses to treatment.

Conclusion

The test was conducted at nominal concentrations of 0.063, 0.13, 0.25, 0.5 and 1.0 mg/L. Measured concentrations of CGA173506 ranged from 62 to 133% of the nominal values at the start of the test and from 20 to 92% of the nominal values at the end of the test. In line with the approach recommended by the test guideline (OECD 201, 2011), the results from this analysis have been based on the geometric mean measured concentrations of 0.057, 0.114, 0.0879, 0.512 and 0.926 mg/L.

Statistical analyses of the available data for yield and growth rate revealed that the following E_yC_x and E_rC_x values were reliably calculated:

Table 8.2.6.1-8: Statistical analysis of yield and growth rate for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Scenedesmus subspicatus* (Rufli, 1989; Syngenta file number CGA173506/0007)

Parameter	72 hours			72 hours
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_rC_{10}
Value [mg/L]	0.108	0.211	0.750	0.740
lower 95%-cl	0.039	0.109	0.549	0.518
upper 95%-cl	0.178	0.302	1.183	1.181

cl: confidence limits

~~No EC_{10}, EC_{20} or EC_{50} values were able to be calculated for any other time point due to the absence of suitable data.~~

~~(Taylor and Howells 2016)~~

The following study was previously submitted in the EU and was summarised in point B.9.2.6 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoints are summarised in Table 8.2-7 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.5.3 Rufli H, (1989). Alga, growth inhibition of CGA 173506 technical to green algae (Scenedesmus subspicatus). Report Number 881737, CIBA-GEIGY, CH-4002 Basle, Switzerland, Ecotoxicology (Syngenta File No. CGA173506/0007).*

Guideline

OECD guideline 201, Paris 1984

GLP: Yes

Executive Summary

The toxicity of CGA173506 to the green alga *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) was determined. Algae were exposed to nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L (mean measured concentrations were 0.039, 0.081, 0.050, 0.46 and 0.71 mg a.s./L) alongside a culture medium control. Based on mean measured concentrations, the 72-hour E_bC_{50} value was 0.93 mg a.s./L (95% confidence of 0.72 to 1.3 mg a.s./L).

Materials

Test Material	CGA173506
Lot/Batch #:	805002
Purity:	97.5%
Description:	Beige solid
Stability of test compound:	Stable under test conditions
Reanalysis date:	June 1992

Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg CGA173506/L (mean measured concentrations were 0.039, 0.081, 0.050, 0.46 and 0.71 mg a.s./L)
Solvent:	None
Positive control:	None reported
Analysis of test concentrations:	Yes, analysis of CGA173506 at 0 and 72 hours, using HPLC analysis with UV detection

Test organism	
Species:	*Scenedesmus subspicatus*, strain Cambridge 276/20 (= *Desmodesmus*

	subspicatus)
Source:	Not reported

Test design

Test vessels:	100 mL Erlenmeyer flasks containing 50 mL of media covered with aluminium caps which permitted gas exchange
Test medium:	Composition according to OECD 201
Replication:	6 replicates for the control, 3 for test substance
Starting cell density:	1.3 x 10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	72 hours

Environmental conditions

Test temperature:	24 ± 2 °C
pH:	test start: 7.4 to 7.5 test end: 9.0 to 9.4
Lighting:	Continuous cold white fluorescent illumination at approximately 8000 lux

Study Design and Methods

Experimental dates: 30th January to 2nd February 1989

A 1.0 mg/L stock solution was prepared and the required test concentrations were subsequently prepared in water from this. Algae were added to the flask to give a starting density of 1.3 x 10^4 cells/mL. Test solutions were placed on a laboratory shaker and were held at 24 ± 2°C under continuous illumination.

Algal cell counts were conducted every 24 hours using a TOA cell counter. The 72-hour E_bC_{50} value was calculated according to the maximum likelihood method using the probit model. The NOEC was calculated according to Dunnett.

The pH was measured at 0 and 72 hours, and the water temperature was measured continuously in the room and daily in water in a separate test vessel.

The test concentrations were verified by chemical analysis of CGA173506 at 0 and 72 hours, using high performance liquid chromatography (HPLC) with ultra-violet detection.

Results and Discussion

At the start of the test, the measured concentrations were in the range 62 to 133 % of the nominal values and at the end of the test were in the range 20 to 92 % (see table below). Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.6.2.1-9: Analytical results

Nominal concentrations (mg CGA173506/L)	Measured concentrations at 0 hours (mg CGA173506/L)	Measured concentrations at 0 hours (% of nominal)	Measured concentrations at 72 hours (mg CGA173506/L)	Measured concentrations at 72 hours (% of nominal)
0.063	0.0836	133	0.0386	61
0.13	0.161	124	0.0809	62
0.25	0.156	62	0.0495	20
0.50	0.573	115	0.458	92

1.0	1.21	121	0.709	71

Cell density and area under the growth curve

The cell density and area under the growth curve values are shown below:

Table 8.2.6.2.1-10: Mean values at each concentration of CGA173506 for area under the growth curve at 72-hours for *Desmodesmus subspicatus*

Mean measured concentration (mg CGA173506/L)	Cell density at 72 hours (x 10^4)	Mean area under the growth curve at 72 h (A) (x 10^4)	% Inhibition 0 – 72 h (IA)
Control	186.25	3488	-
0.039	182.67	3266	6.4
0.081	173.67	3140	10.0
0.050	170.33	3228	7.4
0.46	103.00	2310	33.8
0.71	89.50	1863	46.6

The 72-hour E_bC_{50} value was estimated to be 0.93 mg a.s./L (95% confidence of 0.72 to 1.3 mg a.s./L).

Validity Criteria

No validity criteria are reported.

Conclusions

In an algal test in which *Desmodesmus subspicatus* were exposed to CGA173506, based on measured concentrations, the 72-hour E_bC_{50} value was 0.93 mg a.s./L.

(Rufli H 1989)

The following report is a statistical recalculation of the above study (Rufli 1989) and was amended to rectify a mistake in the data used to calculate the endpoints and validity criteria (cells/sample previously used rather than cells/ml).

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.2.6.1/02 Pickering and Allen. (2017), Fludioxonil (CGA173506) – Alga, Growth Inhibition Test of CGA173506 technical to Green Algae (*Scenedesmus subspicatus*). Statistical Re-analysis. Report Number: CEA.1595. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11910).

Summary

The report from CIBA-GEIGY Ltd. study number 881737 (Rufli, 1989, Syngenta file number CGA173506/0007) for the toxicity of CGA173506 to *Scenedesmus subspicatus* 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.

The validity of the study was evaluated against the study guideline (OECD 201) with the following results:

Table 8.2.6.1-11: Validity criteria for the fludioxonil toxicity to Freshwater Green Alga, *Scenedesmus subspicatus* (Rufli, 1989; Syngenta file number CGA173506/0007)

Criterion	Required	Achieved	Valid
Increase in biomass 0 – 72 h (control)	16	143.3	Yes
Mean growth rate 0 – 72 h (control)	0.920/ d	1.652/ d	Yes
CV mean growth rate 0 – 72 h (control)	7.0	2.6	Yes
Mean CV for sectional growth rate (control)	35.0	25.4	Yes

CV: coefficient of variation

Although the reported mean coefficient of variation for sectional growth rate in the control was higher than the limit stated in the study guideline, the data suggested that the test was reliable and the following parameters were analysed statistically:

- Yield (*Y*)
- Average specific growth rate (μ)

Statistical analyses of the available data for yield and growth rate revealed that the following E_yC_x and E_rC_x values were reliably calculated:

Table 8.2.6.1-12: Statistical analysis of yield and growth rate for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Scenedesmus subspicatus* (Rufli, 1989; Syngenta file number CGA173506/0007)

Parameter	72 hours			72 hours
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_rC_{10}
Value [mg/L]	0.108	0.210	0.746	0.483
lower 95%-cl	0.039	0.109	0.546	0.269
upper 95%-cl	0.178	0.302	1.176	0.675

cl: confidence limits

No EC_{10}, EC_{20} or EC_{50} values were able to be calculated for any other time point due to the absence of suitable data.

Statistical Analysis

Where possible, non-linear regression was used to derive the EC_x values. However, in cases where this approach did not provide reliable estimates, linear max likelihood regression was used in an attempt to derive these values.

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

Yield after 24 hours

Although statistically significant differences were observed between the treatment groups and the control (p(F) = 0.001), no E_vC_{10}, E_yC_{20} or E_yC_{50} values could be reliably calculated due to the lack of a suitable responses to treatment.

Yield after 48 hours

Although statistically significant differences were observed between the treatment groups and the control (p(F) = 0.002), no E_vC_{10}, E_yC_{20} or E_yC_{50} values could be reliably calculated due to the lack of suitable responses to treatment.

Yield after 72 hours

The relationship between dose and response was significant, p(F) = <0.001 and the slope function was 4.518. The E_yC_{10}, E_yC_{20} and E_yC_{50} values and the 95% confidence limits presented were reliably determined.

Average specific growth rate after 24 hours

Although statistically significant differences were observed between the treatment groups and the control (p(F) = <0.001), no E_rC_{10}, E_rC_{20} or E_rC_{50} values could be calculated due to the lack of suitable responses to treatment.

Average specific growth rate after 48 hours

Although statistically significant differences were observed between the treatment groups and the control (p(F) = <0.001), no E_rC_{10}, E_rC_{20} or E_rC_{50} values could be calculated due to the lack of suitable responses to treatment.

Average specific growth rate after 72 hours

The E_rC_{10} value and the 95% confidence limits presented were reliably determined. Despite this, no E_rC_{20} or E_rC_{50} values could be reliably calculated due to the lack of suitable responses to treatment.

Conclusion

The test was conducted at nominal concentrations of 0.063, 0.13, 0.25, 0.5 and 1.0 mg/L. Measured concentrations of CGA173506 ranged from 62 to 133% of the nominal values at the start of the test and from 20 to 92% of the nominal values at the end of the test. In line with the approach recommended by the test guideline (OECD 201, 2011), the results from this analysis have been based on the geometric mean measured concentrations of 0.057, 0.114, 0.0879, 0.512 and 0.926 mg/L.

Statistical analyses of the available data for yield and growth rate revealed that the following E_yC_x and E_rC_x values were reliably calculated:

Table 8.2.6.1-13: Statistical analysis of yield and growth rate for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Scenedesmus subspicatus* (Rufli, 1989; Syngenta file number CGA173506/0007)

Parameter	72 hours			72 hours
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_rC_{10}
Value [mg/L]	0.108	0.210	0.746	0.483
lower 95%-cl	0.039	0.109	0.546	0.269
upper 95%-cl	0.178	0.302	1.176	0.675

cl: confidence limits

No EC_{10}, EC_{20} or EC_{50} values were able to be calculated for any other time point due to the absence of suitable data.

(Pickering, F., Allen, M., 2017)

Summaries of studies not previously reviewed are included below.

Fludioxonil studies conducted with green algae

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.6.1/03 Porch J.R., Kendall T.Z. & Krueger H.O. (2011) Fludioxonil - A 96-Hour Toxicity Test with the Freshwater Alga (*Pseudokirchneriella subcapitata*). Report Number 528A-212. Wildlife International, Ltd., 8598 Commerce Drive, Easton, MD 21601 USA (Syngenta File No. CGA173506_11518).

Guidelines

OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)

OPPTS 850.5400: Algal Toxicity, Tiers I and II, (1996)

GLP: Yes

Executive Summary

The toxicity of CGA173506 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations 5.1, 13, 32, 80, 200 and 500 µg a.s./L (2.6, 4.3, 14, 37, 132 and 442 µg a.s./L, mean measured concentrations) alongside a culture medium control. Based on mean measured concentrations, the 72-hour E_rC_{50} was >442 mg/L, the E_yC_{50} was 244 mg/L and the E_bC_{50} was 251 mg/L. The 96-hour E_rC_{50} was >442 mg/L, the E_yC_{50} was 301 mg/L and the E_bC_{50} was 302 mg/L.

Materials

Test Material	CGA173506 Fludioxonil
Lot/Batch #:	428860
Purity:	95.4% (w/w)
Description:	Brown powder
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	February 2012

Treatments

Test concentrations:	Culture medium control and nominal concentrations of 0.051, 0.13, 0.32, 0.80 and 2.0 mg a.s./mL 5.1, 13, 32, 80, 200 and 500 µg a.s./L (2.6, 4.3, 14, 37, 132 and 442 µg a.s./L, mean measured concentrations)
Solvent:	DMF
Positive control:	None
Analysis of test concentrations:	Yes, with HPLC analysis

Test organism

Species:	*Pseudokirchneriella subcapitata*

Source:	Continuous laboratory cultures
Test design	
Test vessels:	250 mL glass Erlenmeyer flasks containing 100 mL of media plugged with foam stoppers
Test medium:	AAP algal medium
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	5000 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours
Environmental conditions	
Test temperature:	22.3 to 24.8 °C
pH:	test start: 7.4 to 7.5 test end: 8.4 to 8.6
Lighting:	Continuous illumination at 3930 to 4670Lux

Study Design and Methods

Experimental dates: 4th to 12th May 2011

A stock solution with a nominal concentration of 5.0 µg/L was prepared by dissolving 0.0524 g of the test item completely in 10 mL of dimethyl formamide (DMF). The primary stock solution was serially diluted with DMF to prepare the five additional working stocks at nominal concentrations of 0.051, 0.13, 0.32, 0.80 and 2.0 mg a.s./mL. Six test solutions were prepared by diluting 0.05 mL of each working stock to a final volume of 500 mL with freshwater algal medium.

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 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 an electronic particle counter. In addition, after 96 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 CGA173506 at 0 and 96 hours, using high performance liquid chromatography.

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 and 96-hour E_bC_{50}, E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were calculated using non-linear regression analysis. For determination of the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values, a Shapiro-Wilk's and Levene's test was used to evaluate normality and homogeneity of variance.

Results and Discussion

At the start of the test, the measured concentrations were in the range 91.3 to 97.3% of the nominal values and at the end of the test were in the range 20.0 to 81.1% (see table below). The limit of quantification in this study was 1.5 µg a.s./L. Geometric mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.6.1-914: Analytical results

Nominal concentrations (µg/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours	Geometric Mean measured concentration (µg a.s./L)
5.1	91.3	<LOQ	2.6
13	96.0	<LOQ	4.3
32	97.3	20.0	14
80	96.1	21.8	37
200	97.1	45.0	132
500	96.4	81.1	442

At test termination, cells in the 442 µg a.s./L group appeared to be enlarged, otherwise there were no noticeable changes in cell morphology in any treatment group or the solvent control when compared to the negative control.

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, alongside the estimated EC_{50} values.

Table 8.2.6.1-1015: Mean values at each concentration of CGA173506 for the growth rate at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Geometric Mean Measured concentrations (µg/L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition	Mean growth rate (1/day) 0 – 96 hrs	Percentage inhibition
Negative control	0.0668	--	0.0645	--
Solvent control	0.0637	--	0.0614	--
2.6	0.0644	-1	0.0637	-4
4.3	0.0617	3	0.0615	0
14	0.0618	3	0.0611	0
37	0.0605	5	0.0611	0
132	0.0580	9	0.0603	2
442	0.0498*	22	0.0495*	19
E_rC_{50} mg /L (95% confidence limits)	>442 (n.a.)		>442 (n.a.)	
NOEC	132		132	
LOEC	442		442	

Percent inhibition was calculated relative to the solvent control mean. Negative values indicate an increase relative to the control mean.
* Statistically significant difference from the solvent control mean using Dunnett's t-test (*p* 0.05). Determined at 72- and 96-hour intervals only

Yield

The yield 0 to 72 hours and 0 to 96 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~~16: Mean values at each concentration of CGA173506 for the yield at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Geometric Mean Measured concentrations (µg/L)	Mean yield (cells/mL) 0 – 72 hrs	Percentage inhibition	Mean yield (cells/mL) 0 – 96 hrs	Percentage inhibition
Negative control	626,390	--	2,473,005	--
Solvent control	493,982	--	1,821,971	--
2.6	522,879	-6	2,276,496	-25
4.3	419,981	15	1,834,164	-1
14	455,382	8	1,775,113	3
37	386,520	22	1,763,463	3
132	321,596	35	1,632,298	10
442	179,562*	64	590,671*	68
E_yC_{50} mg/L (95% confidence limits)	244 (109 to >442)		301 (231 to 393)	
NOEC	132		132	
LOEC	442		442	

Percent inhibition was calculated relative to the solvent control mean. Negative values indicate an increase relative to the control mean.
* Statistically significant difference from the solvent control mean using Dunnett's t-test (*p* 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, alongside the estimated EC_{50} values.

Table 8.2.6.1-~~12~~17: Mean values at each concentration of CGA173506 for the biomass integral (area under the growth curve) at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Geometric Mean Measured concentrations (µg/L)	Mean cell density (cells/mL) 0 – 72 hrs	Percentage inhibition	Mean cell density (cells/mL) 0 – 96 hrs	Percentage inhibition
Negative control	631,390	--	2,478,005	--
Solvent control	498,982	--	1,826,971	--
2.6	527,879	-6	2,281,496	-25
4.3	424,981	15	1,839,164	-1
14	460,382	8	1,780,113	3
37	391,520	22	1,768,463	3
132	326,596	35	1,637,298	10
442	184,562*	63	595,671*	67
E_bC_{50} mg/L (95% confidence limits)	251 (112 to >442)		302 (232 to 394)	
NOEC	132		132	
LOEC	442		442	

Percent inhibition was calculated relative to the solvent control mean. Negative values indicate an increase relative to the control mean.
* Statistically significant difference from the solvent control mean using Dunnett's t-test (p 0.05).

Validity criteria

The validity criteria were met since:

- The mean cell density in the control flasks must have increased by a factor of at least 16 within three days (actual increase *in solvent control replicates* was a factor of 100).
- The coefficient of variation (CV) of average specific growth rate in the control replicates during the whole test period should not exceed 7% (actual CV was 2.64% *in solvent control replicates*).
- The mean percent coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control replicates must not exceed 35% (actual was 35% *in solvent control replicates*).

Conclusions

Based on geometric mean measured concentrations, the 72-hour E_rC_{50} for CGA173506 to *Pseudokirchneriella subcapitata* was >442 mg/L, the E_yC_{50} was 244 mg/L and the E_bC_{50} was 251 mg/L. The 96-hour E_rC_{50} was >442 mg/L, the E_yC_{50} was 301 mg/L and the E_bC_{50} was 302 mg/L.

The Lowest Observed Effect Concentration at 72 and 96 hours, based on growth rate, yield and biomass integral, was 442 mg/L, and the No Observed Effect Concentration was 132 mg/L.

(Porch JR, Kendall TZ & Krueger HO, 2011)

RMS comment:

For the study of Porch, Kendall & Krueger (2011, CGA 173506_11518, K-CA 8.2.6.1/03) conducted with fludioxonil on Pseudokirchneriella subcapitata, the data indicated that there is a difference between the negative control and the solvent control. The determination of EC10, EC50 and NOEC can be based on solvent data only if the solvent concentration is identical in the control and in each tested concentrations. As requested for other studies, this should be demonstrated. In case the solvent concentration is identical in the control and each tested concentration, the reliability of the study should anyway be discussed as the coefficient of variation for the solvent control is 35.3% at 72 hours.

Syngenta response:

Archived data scans of the raw data were examined and the amount of solvent in the solvent control and all test concentrations were checked and verified.

Dimethylformamide solvent was added to the solvent control and every test concentration at 0.1 mL/L. As a significant difference was calculated between the control and the solvent control the solvent control was used for statistical comparison with the test concentrations for calculation of the endpoints.

The results were based on geometric mean measured concentrations.

The following criteria were used to judge the validity of the test:

1. The mean cell density in the control flasks must have increased by a factor of at least 16 within three days. The mean solvent control cell density increased by a factor of 100 within three days.

2. The coefficient of variation (CV) of average specific growth rate in the control replicates during the whole test period should not exceed 7%. The CV for average specific growth rate in the solvent control replicates during the test was 2.64%.

3. The mean percent coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control replicates must not exceed 35%. The mean coefficient of variation for section-by-section specific growth rates in the solvent control was 35% at 72 hours (35.3% is acceptably rounded to 35%).

Based on these criteria, the test was considered valid.

In accordance with **Commission Regulation (EU) No 283/2013**, the 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 8.2.6.1/04 Taylor S. & Howells A. (2016a) Fludioxonil – A 96 – Hour Toxicity Test with the Freshwater Alga (*Pseudokirchneriella subcapitata*) Statistical Re-analysis. Report Number: CEA.1603. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11897).

Summary

The report from Wildlife International, Ltd. study number 528A-212 (Porch *et al*, 2011; Syngenta file number CGA173506_11518) for the toxicity of fludioxonil to *Pseudokirchneriella subcapitata* did not provide estimates of the EC_{10} and EC_{20} for the response variables evaluated as part of the original study. Additionally, EC_{50} values were not provided for all the observation intervals. Consequently the data generated in this study have been re-analysed in an attempt to provide these values.

The test used nominal concentrations of 5.1, 13, 32, 80, 200 and 500 µg a.s./L. Chemical analysis of the test solutions showed that the measured concentrations ranged from 20% to 97% of the nominal values. As a result, the geometric mean measured concentrations of 2.6, 4.4, 14, 37, 132 and 442 µg a.s./L have been used in this statistical analysis.

The following parameters were analysed statistically:

- Yield (*Y*)
- Average specific growth rate (µ)

Statistical analyses of the available data for yield revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated:

Table 8.2.6.1-~~13~~18: Statistical analysis of yield for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Porch et al., 2011; Syngenta file number CGA173506_11518)

Parameter	72 Hours		96 Hours	
	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}
Value [µg a.s./L]	12.448	34.869	111.500	157.300
lower 95%-cl	0.428	4.466	54.052	79.451
upper 95%-cl	34.795	75.983	230.006	313.041

cl: confidence limits

No E_yC_x values at any other time point could be reliably calculated.

Statistical analyses of the available data for average specific growth rate revealed that the following E_rC_{10} values were reliably calculated:

Table 8.2.6.1-1419: Statistical analysis of average specific growth rate for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Porch et al., 2011; Syngenta file number CGA173506_11518)

Parameter	48 Hours	72 Hours	96 Hours
	E_rC_{10}	E_rC_{10}	E_rC_{10}
Value [µg a.s./L]	422.125	255.297	343.844
lower 95%-cl	169.652	136.733	281.951
upper 95%-cl	2466.077	476.669	419.322

cl: confidence limits

No E_rC_x values at any other time point could be reliably calculated.

Statistical Analysis

Where possible, non-linear regression was used to derive the EC_x values. However, in cases where this approach did not provide reliable estimates, linear max likelihood regression was used in an attempt to derive these values.

For each parameter, a Students t-test for homogenous variances was used to determine if there was any significant difference between the negative control and solvent control using pair-wise comparisons.

A 3-parameter normal cumulative distribution function (Bruce and Versteeg, 1992) was fitted to the original data and a non-linear regression without weighting was performed using the Levenberg-Marquardt algorithm to calculate the function parameters.

Results

Yield after 24 hours

Although the relationship between dose and response was significant, p(F) = 0.014, no E_yC_{10}, E_yC_{20} and E_yC_{50} values were reliably determined due to the wide ranging confidence limits.

Yield after 48 hours

Although the relationship between dose and response was significant, p(F) = <0.001, no E_yC_{10}, E_yC_{20} and E_yC_{50} values were reliably determined as no confidence limits were able to be calculated due to the lack of suitable response functions.

Yield after 72 hours

The relationship between dose and response was significant, p(F) = 0.006 and the slope function was 10.396. The E_yC_{10} and E_yC_{20} values and their 95% confidence limits presented were reliably determined.

Yield after 96 hours

A significant amount of variance was explained by the regression model (p(F):Regression = <0.001) and there was no significant lack of fit (p(F):Lack of fit = 0.098). The E_yC_{10} and E_yC_{20} values and their 95% confidence limits presented were reliably determined.

Average specific growth rate after 24 hours

Although the relationship between dose and response was significant, p(F) = 0.005, no E_rC_{10}, E_rC_{20} and E_rC_{50} values were reliably determined due to the wide ranging confidence limits.

Average specific growth rate after 48 hours

Although the E_rC_{10} value and the 95% confidence limits presented were reliably determined, no E_rC_{20} and E_rC_{50} values were able to be calculated due to the lack of suitable response functions.

Average specific growth rate after 72 hours

Although the E_rC_{10} value and the 95% confidence limits presented were reliably determined, no E_rC_{20} and E_rC_{50} values were able to be calculated due to the lack of suitable response functions.

Average specific growth rate after 96 hours

Although the E_rC_{10} value and the 95% confidence limits presented were reliably determined, no E_rC_{20} and E_rC_{50} values were able to be calculated due to the lack of suitable response functions.

Conclusion

The following parameters were analysed statistically:

- Yield (*Y*)
- Average specific growth rate (μ)

Statistical analyses of the available data for yield revealed that the following E_yC_{10} and E_yC_{20} values were reliably calculated:

Table 8.2.6.1-~~15~~20: Statistical analysis of yield for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Porch et al., 2011; Syngenta file number CGA173506_11518)

Parameter	72 Hours		96 Hours	
	E_yC_{10}	E_yC_{20}	E_yC_{10}	E_yC_{20}
Value [µg a.s./L]	12.448	34.869	111.500	157.300
lower 95%-cl	0.428	4.466	54.052	79.451
upper 95%-cl	34.795	75.983	230.006	313.041

cl: confidence limits

No E_yC_x values at any other time point could be reliably calculated.

Statistical analyses of the available data for average specific growth rate revealed that the following E_rC_{10} values were reliably calculated:

Table 8.2.6.1-~~16~~21: Statistical analysis of average specific growth rate for EC_{10} and EC_{20} endpoints for the fludioxonil toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Porch et al., 2011; Syngenta file number CGA173506_11518)

Parameter	48 Hours	72 Hours	96 Hours
	E_rC_{10}	E_rC_{10}	E_rC_{10}
Value [µg a.s./L]	422.125	255.297	343.844
lower 95%-cl	169.652	136.733	281.951
upper 95%-cl	2466.077	476.669	419.322

cl: confidence limits

No E_rC_x values at any other time point could be reliably calculated.

(Taylor S, Howells A, 2016a)

Algae studies with metabolites of fludioxonil conducted with green algae

The study conducted with CGA339833 by Grade 2000 (Syngenta file number CGA339833/0004) which was previously submitted in the EU for fludioxonil metabolites was summarised in the DAR ~~therefore an additional study summary is not repeated here~~. The endpoint is summarised in Table 8.2-8.

For the study conducted with CGA192155 (Volz, 2005, Syngenta file number CGA192155/0017) a summary of this was presented in the Final Addendum to the DAR (June 2007) ~~therefore an additional study summary is not repeated here~~. The endpoint is summarised in Table 8.2-8.

At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.6 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-8 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Volz E, (2005). CGA192155 (Metabolite of Fludioxonil): A 96-hour algal growth inhibition test with Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum), Report Number 857594, RCC Ltd, Itingen, Switzerland. (Syngenta File No. CGA192155/0017)*

Guidelines

OECD (1984). OECD Guidelines for Testing of Chemical 201. Alga, Growth Inhibition Test. Adopted 7 June 1984.

Official Journal of the European Communities, L 383 A, Part C.3, Algal inhibition test. 29 December 1992.

GLP: Yes

Executive Summary

Toxicity of CGA192155 (metabolite of fludioxonil) to the green alga species *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was determined. This study was run with a culture medium control and with nominal test item concentrations of 6.3, 13, 25, 50 and 100 mg CGA192155/L. The 72-hour E_bC_{50} and E_rC_{50} values were determined to be >100 mg/L based on nominal test item concentrations.

Materials

Test Material:	CGA192155
Description:	White solid
Lot/Batch #:	MLA-336/4
Purity:	96%
Treatments:	
Test concentrations:	Culture medium control and nominal test item concentrations of 6.3, 13, 25, 50 and 100 mg CGA192155/L
Vehicle and/or positive control:	None / Potassium dichromate (tested once a year)
Analysis of test concentrations:	Yes, analysis of CGA192155 concentration at 0 and 96 hours
Test organism	
Species:	*Pseudokirchneriella subcapitata,* Strain No. 61.81 SAG
Source:	Collection of Algal Cultures (SAG, Institute for Plant Physiology, University of Göttingen, Germany). The algae are cultivated in the RCC laboratories under standardized conditions according to the test guidelines.
Test design	
Test vessels:	50 mL Erlenmeyer flasks containing 15 mL test solution
Test medium:	Reconstituted water prepared according to guideline
Exposure regime:	Static
Aeration:	No
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	1.0×10^4 cells/mL 2.0
Environmental conditions	
Test temperature:	23 – 24 °C
pH:	test start: 7.9 to 8.2 (after pH-adjustment in the highest test concentration) test end: 8.3 and 8.6
Lighting:	7370 to 9100 Lux (mean 8280 Lux)
Length of test:	96 hours

Study Design and Methods

Experimental dates: 26th November to 10th December 2004.

The test medium of the highest nominal test item concentration of 100 mg/L was prepared by dissolving 100.0 mg of the test item completely in 1000 mL of test water using ultrasonic treatment (30 minutes) and intense stirring (30 minutes at room temperature). Adequate volumes of this intensively mixed test

medium were diluted with test water to prepare the test media with the lower test item concentrations. The pH of the test medium with the nominal concentration of 100 mg/L was 6.3. It was adjusted to 8.1 with some drops of a 1 M sodium hydroxide solution. The control consisted of culture medium only. For evaluation of the algal quality and the experimental conditions, potassium dichromate is tested as a positive control at least once per year.

The test vessels were 50 mL Erlenmeyer flasks covered with glass dishes. Each flask contained 15 mL of algal suspension. Six replicate cultures of the control and triplicate cultures of each test concentration were employed. The test solutions were continuously stirred by magnetic stirrers. The test was started by inoculation of 10,000 algal cells per mL of test medium. They were incubated in a temperature controlled water bath at a temperature of 23 to 24 °C and continuously illuminated at 8280 Lux.

Small volumes of all test concentrations and the control (0.4 - 1.0 mL) were taken from all test flasks after 24, 48, 72 and 96 hours of exposure. The algal cell densities in the samples were determined by counting with an electronic particle counter. In addition, after 96 hours exposure, a sample was taken from the control and from the highest test concentration (nominal 100 mg/L). The shape of the algal cells was examined microscopically in these samples. For determination of the LOEC and NOEC values, a Dunnett's test was used to identify significant differences in the calculated mean biomass and mean growth rate at the test concentrations compared to the control.

The concentrations of the CGA192155 in the test solutions were measured at 0 and 96 hours using a high performance liquid chromatography method. The analytical measurements were performed in the test medium of nominal 100 mg/L. For the lower test concentrations no analytical measurements were performed since theses concentrations were below the 96-hour NOEC.

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.

Results and Discussion

The analytically determined test item concentration in the highest test medium concentration of 100 mg/L was 101 and 102% of the nominal value at the start and end of the test, respectively. The nominal test item concentrations were used for the calculation and reporting of the results.

At the start of the test, the pH ranged from 7.9 to 8.2 and at the end of the test the range was from 8.3 to 8.6. The water temperature was 23 to 24°C. The mean light intensity was 8280 Lux. The hardness of the dilution medium was 24 mg/L as $CaCO_3$.

The algal cell densities were measured at 24, 48, 72 and 96 hours and the means of these values were calculated. The E_bC_{50} and E_rC_{50} values could not be determined due to the absence of toxicity of CGA192155 at the test concentration of 100 mg/L.

Areas under the growth curve

The area under the growth curve 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture (Table 8.2.6.1-22).

Table 8.2.6.1-22: Mean values at each concentration of CGA192155 for the area under the growth curve at 72 and 96 hours for *Pseudokirchneriella subcapitata*

Nominal Test concentrations (mg CGA192155/L)	Mean area under growth curve (0 – 72 hrs)	Percentage of control	Mean area under growth curve (0 – 96 hrs)	Percentage of control
Control	2620	0.0	7841	0.0
6.3	2777	-6.0	8014	-2.2
13	2586	1.3	7918	-1.0
25	2330	11.1	7248	7.6
50	2831	-8.1	8245	-5.2
100	2668	-1.8	7927	-1.1

* Significant difference (P=0.05) from the control (Here in no case a significant difference was noted)
- % inhibition: increase in growth relative to that of the control

There was no statistically significant inhibitory effect on the biomass of *P. subcapitata* up to and including the concentration of 100 mg/L.

The 72- and 96-hour E_bC50 were >100 mg CGA192155/L (95% confidence limits: not determined).

Growth rates

The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture (Table 8.2.6.1-23).

Table 8.2.6.1-23: Mean values at each concentration of CGA192155 for the growth rate at 72 and 96 hours for *Pseudokirchneriella subcapitata*

Nominal Test concentrations (mg CGA192155/L)	Mean growth rate (0 – 72 hrs)	Percentage of control	Mean growth rate (0 – 96 hrs)	Percentage of control
Control	1.66	0.0	1.42	0.0
6.3	1.69	-1.6	1.41	0.6
13	1.67	-0.4	1.42	-0.4
25	1.62	2.6	1.41	0.4
50	1.70	-2.3	1.42	0.0
100	1.69	-1.4	1.41	0.4

* Significant difference (P=0.05) from the control (Here in no case a significant difference was noted)
- % inhibition: increase in growth relative to that of the control

There was no statistically significant inhibitory effect on the growth rate of *P. subcapitata* up to and including the concentration of 100 mg/L.

The 72- and 96-hour E_rC50 were >100 mg CGA192155/L (95% confidence limits: not determined).

Validity criteria

None reported.

Conclusions

Based on nominal test item concentrations, the 72-hour E_bC50 and the 72-hour E_rC_{50} values for CGA192155 were >100 mg CGA192155/L to the green alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*).

(Volz E, 2006)

Reference	***Guidance followed***	***Current guidance in force***	***Differences from guidance in force***	***Critical assessment***
Volz 2005, Syngenta file number CGA192155/0017	*OECD 201 (1984)* *EU 92/69/EEC C.3 (1992)*	*OECD No. 201 (2006)* *or* *US EPA OPPTS 850.5400 (1996)*	*Compliant with both OECD and US EPA guidelines*	*Following validity criteria were met:* *3. Increase in biomass required :>16 (actual: 148 control).* *4. CV mean growth rate 0-72h required ≤7% (actual: 3.2%)* *5. CV mean section-by section growth rate 0-72h required ≤35% (actual: 18%)* *This study is considered reliable.*

In accordance with **Commission Regulation (EU) No 283/2013**, the 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 8.2.5.1/05 Priestly S. & Howells A. (2015), CGA192155 (metabolite of fludioxonil) – A 96-Hour Algal Inhibition Test with *Pseudokirchneriella subcapitata* (Formerly *Selenastrum capricornutum*). Statistical Re-analysis. Report Number: CEA.1602. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA192155_11294).

Summary

The report from RCC Ltd. study number 857594 (Volz, 2005; Syngenta file number CGA192155/0017) for the toxicity of CGA192155 to *Pseudokirchneriella subcapitata* 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.
However, no inhibition was observed in the original study and the 96 hour endpoints (EC_{50} for growth rate and yield and the No Observed and Lowest Observed Effect Concentration) were all reported as at least 100 mg/L (the highest nominal concentration tested).

It can be concluded that no reliable dose response and therefore EC_x values could be determined and no additional statistical analysis was performed on any of the parameters.

Results

No inhibition was observed in the original study and the 96 hour endpoints (EC_{50} for growth rate and yield and the No Observed and Lowest Observed Effect Concentration) were all reported as at least 100 mg/L (the highest nominal concentration tested). As a result, it can be concluded that no reliable dose response and therefore EC_x values could be determined and no additional statistical analysis was performed on any of the parameters.

Conclusion

It can be concluded that no reliable dose response and therefore ECx values could be determined and no additional statistical analysis was performed on any of the parameters.

(Priestly S, Howells F, 2015)

The study conducted with CGA339833 (Grade, 2000, Syngenta file number CGA339833/0004) was previously submitted in the EU and was summarised in point B.9.2.6 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-8 above.~~a summary of this was presented in the DAR.~~

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Grade R, (2000). Growth Inhibition of CGA 339833 (Metabolite of CGA 173506) to Green Algae (Selenastrum capricornutum) under Static Conditions. Report Number 2003514, Novartis Crop Protection AG, Product Portfolio Management, Environmental Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. CGA339833/0004)*

Guidelines

OECD Guideline No. 201 (1984)

Guideline 92/69/EEC, C.3

FIFRA Guideline Ref. 122-2 and 123-2, December 1989

GLP: Yes

Executive Summary

The toxicity of CGA339833 (metabolite of CGA173506) to the green alga *Selenastrum capricornutum* was determined. Algae were exposed to nominal concentrations 6.25, 12.5, 25, 50 and 100 mg CGA 39833/L alongside a culture medium control. Based on nominal concentrations, the 72-hour E_rC_{50} and E_bC_{50} were determined to be 104.7 and 95.8 mg CGA339833/L, respectively.

Materials

Test Material	CGA339833
Parent:	CGA173506
Lot/Batch #:	KI 6119/6
Purity:	90 ± 2 %
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/expiry date:	May 2002
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg CGA339833/L
Solvent:	None
Positive control:	Potassium dichromate, last tested 24 June 1999
Analysis of test concentrations:	Yes, analysis of CGA339833 at 0 and 72 hours, using HPLC analysis with UV detection
Test organism	
Species:	*Selenastrum capricornutum*, ATCC 22662 (= *Pseudokirchneriella subcapitata*, SAG 61.81)
Source:	Collection of algae cultures, Pflanzenphysiologisches Institut University, Nikolausberger Weg 180, D-3400 Göttingen, Germany
Test design	
Test vessels:	100 mL Erlenmeyer flasks containing 30 mL of media covered with cotton stoppers
Test medium:	Reconstituted water prepared according to guideline
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	10,490 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	72 hours
Environmental conditions	
Test temperature:	24 ± 1 °C
pH:	test start: 7.6 to 7.9 test end: 8.1 to 8.2
Lighting:	Continuous cold white fluorescent illumination at 122 µE/m^2 sec

Study Design and Methods

Experimental dates: 15th to 20th May 2000

A stock solution was prepared by mixing 104.17 mg of test item with OECD medium and making up to 1000 mL. 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 10,490 algal cells per mL of test medium. Test solutions were constantly shaken at 150 rpm and were held at 24 ± 1 °C under continuous illumination.

The algal cell densities at 24, 48 and 72 hours of exposure were determined by fluorescence measurement. The algal cell densities were measured at 24, 48 and 72 hours and the growth rate and area under the growth curves calculated. The 72-hour E_rC_{50} and E_bC_{50} values were calculated using logit analysis. For determination of the NOEC, a Dunnett's Multiple Comparison Test was used. The program package ECOS was used to analyse data according to the 1996 OECD draft guideline for the Anabaena toxicity test.

The pH was measured at 0 and 72 hours, and the water temperature was measured continuously in the room and daily in water in a separate test vessel.

The test concentrations were verified by chemical analysis of CGA339833 at 0 and 72 hours, using high performance liquid chromatography with ultra-violet detection. For sampling at the end of the test, the test media of the treatment replicates was pooled.

Results and Discussion

At the start of the test, the measured concentrations were in the range 96.8 to 100.8 % of the nominal values and at the end of the test were in the range 100.0 to 104.0 % (see table below). The limit of quantification (LOQ) in this study was 0.02 mg CGA339833/L. Nominal concentrations were used for the calculation and reporting of results.

Table 8.2.6.2.1-24: Analytical results

Nominal concentrations (mg CGA339833/L)	Measured concentrations at 0 hours (mg CGA339833/L)	% of nominal measured at 0 hours	Measured concentrations at 72 hours (mg CGA339833/L)	% of nominal measured at 72 hours
Control	< LOD	-	< LOD	-
6.25	6.3	100.8	6.5	104.0
12.5	12.1	96.8	12.5	100.0
25	25.1	100.4	25.3	101.2
50	49.9	99.8	51.2	102.4
100	98.4	98.4	102.4	102.4

LOD = 0.009 mg CGA339833/L

Growth rates

The growth rate (72 hours) was calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} value.

Table 8.2.6.2.1-25: Mean values at each concentration of CGA339833 for the growth rate 72 hours for *Selenastrum capricornutum* and relevant endpoints

Nominal concentrations (mg CGA339833/L)	Mean growth rate, 72 hrs (cells/hr)	Percentage inhibition
Control	0.0665	0

6.25	0.0699	0
12.5	0.0679	0
25	0.0684	0
50	0.0680	0
100	0.0439*	34.08
E_rC_{50} (mg CGA339833/L)	104.7	
95 % confidence limit	none	
NOEC (mg CGA339833/L)	50	

* Significant at 0.1 % significance level

Area under the growth curve

The area under the growth curve (72 hours) was calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} value.

Table 8.2.6.2.1-26: Mean values at each concentration of CGA339833 for area under the growth curve at 72 hours for *Selenastrum capricornutum* and relevant endpoints

Nominal concentrations (mg CGA339833/L)	Mean areas under the growth curves, 72 hrs	Percentage inhibition
Control	13997.0968	0
6.25	16427.0968	0
12.5	15579.0968	0
25	15019.0968	0
50	16095.0968	0
100	4611.0968*	67.06
E_bC_{50} (mg CGA339833/L)	95.8	
95 % confidence limit	none	
NOEC (mg CGA339833/L)	50	

* Significant at 1 % significance level

Validity Criteria

No validity criteria are reported.

Conclusions

Based on nominal concentrations, the 72-hour E_rC_{50} and E_bC_{50} to *Selenastrum capricornutum* were determined to be 104.7 and 95.8 mg CGA339833/L, respectively.

The NOECat 72 hours, based on growth rate and area under the growth curve, was 50 mg CGA339833/L.

(Grade R, 2000)

Whilst there are minor exceedances of the 2011 validity criteria for algal studies, this study (which pre-dates the criteria) is considered valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Grade, 2000 CGA339833/0004	OECD No. 201 (1984)	OECD No. 201 (2011)	The following are not reported: 1. If test flasks were randomly positioned in incubator. 2. If growth measured in inoculum culture prior to test. 3. Section-by-section specific growth rates (these calculations were not a requirement at the time of reporting, but have now been calculated and are reported in Syngenta File No: GA339833_10011).	While conditions of the study were not fully reported and there were minor deviations from the current guidelines, the following validity criteria was met: 1. Control biomass increased by a factor of at least 16 (156), corresponding to a specific growth rate of > 0.92 day^{-1} (1.67 day^{-1}). Minor exceedances occurred in the following current validity criteria: 1. The mean coefficient of variation for section-by-section specific growth must not exceed 35%. (37.9%) 2. The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% (7.8%). Whilst there are minor exceedances of the 2011 validity criteria for algal studies, this study (which pre-dates the criteria) is considered valid for use in the risk assessment.

In accordance with **Commission Regulation (EU) No 283/2013**, the 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 8.2.6.1/06 Taylor, S. & Radford F. (2015a) CGA339833 (metabolite of fludioxonil) – Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum*. Statistical Re-analysis. Report Number: CEA.1599. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA339833_10011).

Summary

The report from Novartis Ltd. study number 2003512 (Grade 2000, Syngenta file number CGA339833/0004) for the toxicity of CGA339833 to *Pseudokirchneriella subcapitata* 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.

The test used nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L. Chemical analysis of the test solutions showed that the measured concentrations ranged from 96.8 to 100.8% of the nominal values at the start of the test and from 100.0% to 104.0% of the nominal values at the end of the test. As a result, the nominal concentrations have been used in this statistical analysis.

The validity of the study was evaluated against the study guideline (OECD 201) with the following results:

Table 8.2.6.1-~~17~~27: Validity criteria for the fludioxonil toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Grade 2000; Syngenta file number CGA339833/0004)

Criterion	Required	Achieved	Valid
Increase in biomass 0 – 72 h (control)	16	156.1	Yes
Mean growth rate 0 – 72 h (control)	0.920/ d	1.665/ d	Yes
CV mean growth rate 0 – 72 h (control)	7.0	7.8	No
Mean CV for sectional growth rate (control)	35.0	37.9	No

CV: coefficient of variation

Despite the CV for mean growth rate and sectional growth rate being slightly higher than the stated criteria, the study was still considered to be reliable and the following parameters were analysed statistically using the methods described in this report:

- Yield (*Y*)
- Average specific growth rate (μ)

Statistical analyses of the available data for yield revealed that no E_yC_x values were reliably determined at 24 and 48 hours. In addition, the lower confidence limits are too far from the E_yC_x values to enable a reliable determination at 72 hours.

Statistical analyses of the available data for average specific growth rate revealed that the following E_rC_{10} value was reliably calculated (the E_rC_{50} for this time-point was already reported so it is not provided here):

Table 8.2.6.1-~~18~~28: Statistical analysis of average specific growth rate for EC_{10} and EC_{20} endpoints for the CGA339833 toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Grade 2000; Syngenta file number CGA339833/0004)

Parameter	72 hours	
	E_rC_{10}	E_rC_{20}
Value [mg/L]	36.588	79.045
lower 95%-cl	27.580	59.015
upper 95%-cl	48.028	121.210

cl: confidence limits

No EC_x values were reliably determined at 24 and 48 hours.

Statistical Analysis

Where possible, non-linear regression was used to derive the ECx values. However, in cases where this approach did not provide reliable estimates, linear max likelihood regression was used in an attempt to derive these values.

Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

A 3-parameter normal cumulative distribution function (Bruce and Versteeg, 1992) was fitted to the original data and a non-linear regression without weighting was performed using the Levenberg-Marquardt algorithm to calculate the function parameters.

Results

Yield after 24 hours

No statistically significant differences were observed between the treatment groups and the control (p(F) = 0.604), therefore no E_yC_{10}, E_yC_{20} or E_yC_{50} values could be reliably calculated.

Yield after 48 hours

No statistically significant differences were observed between the treatment groups and the control (p(F) = 0.699), therefore no E_yC_{10}, E_yC_{20} or E_yC_{50} values could be reliably calculated.

Yield after 72 hours

The relationship between dose and response was significant, p(F) <0.001 and the slope function was 1.371. Although the E_yC_{10} and E_yC_{20} values and their 95% confidence limits presented were determined, the lower confidence limits are too wide to enable a reliable determination of the EC_x values.

Average specific growth rate after 24 hours

No statistically significant differences were observed between the treatment groups and the control (p(F) = 0.420), therefore no E_rC_{10}, E_rC_{20} or E_rC_{50} values could be reliably calculated.

Average specific growth rate after 48 hours

The relationship between dose and response was significant, p(F) <0.001 and the slope function was 34.017. Although E_rC_{10} values and their 95% confidence limits were determined, the upper confidence limits are too wide to enable a reliable calculation of the EC_x value. In addition, no E_rC_{20} or E_rC_{50} values were reliably determined due to the lack of suitable responses to treatment.

Average specific growth rate after 72 hours

The relationship between dose and response was significant, p(F) <0.001 and the slope function was 5.760. As a result, the E_rC_{10} and E_rC_{20} values and their 95% confidence limits presented were reliably determined.

Conclusion

The test used nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L. Chemical analysis of the test solutions showed that the measured concentrations ranged from 96.8 to 100.8% of the nominal values at the start of the test and from 100.0% to 104.0% of the nominal values at the end of the test. As a result, the nominal concentrations have been used in this statistical analysis.

The following parameters were analysed statistically using the methods described in this report:

- Yield (*Y*)
- Average specific growth rate (μ)

Statistical analyses of the available data for yield revealed that no E_yC_x values were reliably determined at 24 and 48 hours. In addition, the lower confidence limits are too far from the E_yC_x values to enable a reliable determination at 72 hours.

Statistical analyses of the available data for average specific growth rate revealed that the following E_rC_{10} value was reliably calculated (the E_rC_{50} for this time-point was already reported so it is not provided here):

Table 8.2.6.1-~~19~~29: Statistical analysis of average specific growth rate for EC_{10} and EC_{20} endpoints for the CGA339833 toxicity to Freshwater Green Alga, *Pseudokirchneriella subcapitata* (Grade 2000; Syngenta file number CGA339833/0004)

Parameter	72 hours	
	E_rC_{10}	E_rC_{20}
Value [mg/L]	36.588	79.045
lower 95%-cl	27.580	59.015
upper 95%-cl	48.028	121.210

cl: confidence limits

No EC_x values were reliably determined at 24 and 48 hours.

(Taylor S, Radford F, 2015a)

Following RMS France commenting the following table was added for clarity:

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Grade 2000, Syngenta file number CGA339833/0004	OECD 201 (1984) EU 92/69/EEC C.3 (1992) FIFRA Guideline 122-2 and 123-2 (1989)	OECD No. 201 (2006) or US EPA OPPTS 850.5400 (1996)	Compliant with both OECD and US EPA guidelines	Following critical deviations and the validity criteria were not met: 2. Increase in biomass required :>16 (actual: 156 control). 3. CV mean growth rate 0-72h required ≤7% (actual: 7.8% control) 4. CV mean section-by section growth rate 0-72h required ≤35% (actual: 37.9% control) The validity criteria are slightly exceeded as conducted to guidelines prior to 2006. However, the study shows little effect on algae as the EC_{50} = 95.8 mg/L. The endpoint is not drivng the risk assessment and aqequately demonstrates that the metabolite is not toxic

				to algae. This study is considered reliable.

An algae toxicity study with the new soil metabolite SYN545245 ~~is currently being~~ has been conducted. The study ~~will be submitted~~ is summarised below and the dossier updated as agreed with the RMS.

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.2.6.1/07 Vryenhoef H., 2016, SYN545245 – Toxicity to *Pseudokirchneriella subcapitata* in a 96 Hour Algal Growth Inhibition Test, Report Number 41502653, Envigo Research Limited Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK, (Syngenta File No. SYN545245_10001).

Guidelines

OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011)

761/2009 EC, C.3 (2009)

GLP: Yes

Executive Summary

The toxicity of SYN545245 to the green alga *Pseudokirchneriella subcapitata* was determined. Algae were exposed to nominal concentrations of 10, 18, 32, 56 and 100 mg/L alongside a culture medium control. Based on geometric mean measured concentrations, the 72-hour E_rC_{50} was 43 mg/L, the E_vC_{50} was 41 mg/L and the E_bC_{50} was 30 mg/L. The 96-hour E_rC_{50} was 44 mg/L, the E_yC_{50} was 43 mg/L and the E_bC_{50} was 40 mg/L.

Materials

Test Material	SYN545245
Lot/Batch #:	MES 427/1
Purity:	98% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	31 October 2017
Treatments	
Test concentrations:	Culture medium control and nominal concentrations of 10, 18, 32, 56 and 100 mg/L
Solvent:	None
Positive control:	Potassium dichromate is used at least twice a year
Analysis of test concentrations:	Yes, analysis at 0 and 96 hours

Test organism

Species:	*Pseudokirchneriella subcapitata*, Strain No. CCAP 278/4
Source:	Continuous laboratory cultures, originally obtained from Culture Collection of Algae and Protozoa (CCAP), SAMS Research Services Ltd, Scottish Marine Institute, Oban, Argyll, Scotland.

Test design

Test vessels:	250 mL glass Erlenmeyer flasks containing 100 mL of media plugged with a polyurethane foam bung
Test medium:	ASTM algal medium
Replication:	Six vessels for the control and three vessels for each test concentration
Starting cell density:	5000 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours

Environmental conditions

Test temperature:	24 ± 1 ºC
pH:	test start: 4.6 to 8.0 test end: 4.1 to 8.6
Lighting:	Continuous illumination at 6850 to 7180 Lux

Study Design and Methods

Experimental dates: 11th December 2015 to 2nd February 2016

A stock solution with a nominal concentration of 100 mg/L was prepared by dissolving 200 mg of the test item completely in 2000 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 5,000 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 and 96 hours of exposure. The algal cell densities in these samples were determined by counting with an electronic particle counter. In addition, after 96 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 SYN545245 at 0 and 96 hours, using high performance liquid chromatography with tandem quadrapole mass spectrometery.

Results and Discussion

At the start of the test, the measured concentrations were in the range 88 to 98% of the nominal values and at the end of the test were in the range 67 to 91% (see table below). The limit of quantification in this

study was 0.00048 mg/L. Geometric mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.6.1-30: Analytical results

Nominal concentrations (mg/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours	Geometric mean measured concentration (mg ai/L)
10	88	91	9.0
18	90	91	16
32	91	67	25
56	98	85	51
100	94	71	87

For each individual test vessel (mean values for yield), percentage inhibition (arithmetic axis) was plotted against test concentration (logarithmic axis) and a line fitted by computerized interpolation using the Xlfit software package (IDBS). ECx values were then determined from the equation for the fitted line. Where appropriate 95% confidence limits for the EC50 values were calculated, using the simplified method of evaluating dose-effect experiments of Litchfield and Wilcoxon (1949).

One way analysis of variance incorporating Bartlett's test for homogeneity of variance (Sokal and Rohlf, 1981) and Dunnett's multiple comparison procedure for comparing several treatments with a control (Dunnett, 1955) was carried out on the growth rate, yield and biomass integral (AUC) data after 72 and 96 hours for the control and all test concentrations to determine any statistically significant differences between the test and control groups. All statistical analyses were performed using the SAS computer software package (SAS, 1999 - 2001).

All test and control cultures were inspected microscopically at 96 hours. There were no abnormalities detected in the control or test cultures at 9.0, 16 and 25 mg/L. No intact cells were observed to be present at 51 and 87 mg/L.

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, alongside the estimated EC_{50} values.

Table 8.2.6.1-31: Mean values at each concentration of SYN545245 for the growth rate at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Geometric mean measured concentration (mg ai/L)	Mean growth rate (cells/mL/hour) 0 – 72 hrs	Percentage inhibition	Mean growth rate (cells/mL/hour) 0 – 96 hrs	Percentage inhibition
Control	0.070	-	0.062	-
9.0	0.070	1	0.062	1
16	0.070	0	0.062	0
25	0.070	0	0.063	-1
51	0.008	89	0.017	73
87	0.001	99	0.006	90
E_rC_{50} mg /L (95% confidence limits)	43 n.d.		44 41 – 47	

ErC_{20} mg /L (95% confidence limits)	39 n.d.	36 n.d.
ErC_{10} mg /L (95% confidence limits)	36 n.d.	32 n.d.
NOEC	25	25
LOEC	51	51

Negative values = Increase in growth compared to the control
n.d. – not determined

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 8.2.6.1-32: Mean values at each concentration of SYN545245 for the yield at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Geometric mean measured concentration (mg ai/L)	Mean yield (cells/mL) 0 – 72 hrs	Percentage inhibition	Mean yield (cells/mL) 0 – 96 hrs	Percentage inhibition
Control	7.83E+05	-	1.99E+06	-
9.0	7.56E+05	3	1.91E+06	-4
16	7.60E+05	3	1.96E+06	1
25	7.80E+05	0	2.04E+06	-2
51	4.45E+03	99	2.99E+04	98
87	1.47E+02	100	4.15E+03	100
E_yC_{50} mg/L (95% confidence limits)	41 n.d.		43 n.d.	
EyC_{20} mg/L (95% confidence limits)	39 n.d.		41 n.d.	
EyC_{10} mg/L (95% confidence limits)	37 n.d.		39 n.d.	
NOEC	25		25	
LOEC	51		51	

Negative values = Increase in growth compared to the control
n.d. – not determined

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, alongside the estimated EC_{50} values.

Table 8.2.6.1-33: Mean values at each concentration of SYN545245 for the biomass integral (area under the growth curve) at 72 and 96 hours for *Pseudokirchneriella subcapitata* and relevant endpoints

Geometric mean measured concentration (mg ai/L)	Mean biomass integral (cells/mL) 0 – 72 hrs	Percentage inhibition	Mean biomass integral (cells/mL) 0 – 96 hrs	Percentage inhibition
Control	1.35E+07	-	4.68E+07	-
9.0	1.33E+07	1	4.54E+07	3
16	1.31E+07	3	4.58E+07	2

25	1.33E+07	1	4.72E+07	-1
51	-8.84E+04	101	3.24E+05	99
87	-1.55E+05	101	-1.04E+05	100
E_bC_{50} mg/L (95% confidence limits)	30 n.d.		40 n.d.	
EbC_{20} mg/L (95% confidence limits)	29 n.d.		38 n.d.	
EbC_{10} mg/L (95% confidence limits)	28 n.d.		37 n.d.	
NOEC	25		25	
LOEC	51		51	

Negative values = Increase in growth compared to the control
n.d. – not determined

Validity criteria

- The algal biomass in the control increased by a factor of 158 over 72 hours (must be least 16).
- The mean coefficient of variation of the daily growth rates in the control cultures was 7% over 96 hours (must be ≤ 35%).
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 1 % (must be <7%).

Therefore, all validity criteria were met.

Conclusions

Based on geometric mean measured concentrations, the 72-hour E_rC_{50} for SYN545245 to *Pseudokirchneriella subcapitata* was 43 mg/L, the E_vC_{50} was 41 mg/L and the E_bC_{50} was 30 mg/L. The 96-hour E_rC_{50} was 44 mg/L, the E_yC_{50} was 43 mg/L and the E_bC_{50} was 40 mg/L.

The Lowest Observed Effect Concentration at 72 and 96 hours, based on growth rate, yield and biomass integral, was 51 mg/L, and the No Observed Effect Concentration was 25 mg/L.

(Vryenhoef H., 2016)

CA 8.2.6.2 Effects on growth of an additional algal species

The studies previously submitted in the EU were summarised in the previous EU review, therefore they are not repeated here. The data are summarised in Table 8.2-7.

At the request of the RMS the application of OECD 201 algal validity criteria concerning average specific growth rate and the section-by-section growth rates are discussed below in relation to their application to other test species and studies which last >72 hours:

Validity criteria for species of non- green species of algae (RMS request):

The applicability of the validity criteria set in the OECD Test Guideline 201 (2006) has been investigated in a paper by Crop Life International 2008. It is unclear what scientific basis and/or data were used for establishing the numerical values of these criteria. This position paper summarizes analysis of control data for the algal species, *Navicula pelliculosa,* and *Anabaena flos-aquae and Skeletonema costatum* to determine the impact of the validity criteria. Control data from 9 laboratories and 79 algal studies were analyzed against the OECD 201 validity criteria for the mean coefficient of variation for section-by-section specific growth rates (SSGR) and coefficient of variation for average specific growth rates (ASGR). The results of these analyses, based on the OECD201 validity criteria indicate a:

- 69% to 88% failure rate using the validity criteria mean coefficient of variation for section-by-section specific growth rate across the three algal species and
- 21% to 75% failure rate using average specific growth rate across all three species.

Although the criteria for average specific growth rate is a smaller number and apparently easier to achieve (10%), up to 75% of the studies on non-green algal species studies would potentially be rejected using this criteria. This difference between green and non-green algae may be attributable to their natural pattern of growth which often involves a longer "lag-phase" and/or a filamentous growth form. Regardless, regulators should not be strictly using these criteria to reject studies done on non-green algal species.

The results of this analysis show that the likelihood of meeting the proposed validity criteria for non-green algae is limited, especially for the blue-green algae (*Anabaena flos-aquae*). Table 1 below shows that 88% fail the SSGR.

Algal species-specific percent of studies not meeting the validity criteria based on mean section-by-section coefficient of variation < 35% and average specific growth rate of <10%

Validity Criteria	*Skeletonema costatum*	*Anabaena flos-aquae*	*Navicula pelliculosa*
% Failure for C.V. section-by-section growth rate (Number of studies)	69% (16)	88% (36)	71% (31)
% Failure for C.V. average specific growth rate (Number of studies)	58% (16)	75% (36)	21% (28)

Crop Life International believes that if a study done on non-green algal species meets the first validity criterion (i.e. 16X growth over the duration of the study), then the study should be considered valid. The SSGR and ASGR criteria should not be applied to non-green algal species until a ring-test demonstrates that these validity criteria are truly achievable. Given that this study was conducted recently (2013) by a highly experienced and world leading laboratory, then this suggests that these criteria remain unattainable to date.

New studies conducted since the original EU review are summarised below.

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.6.2/01Soucy KL (2013). Fludioxonil – 96-Hour Toxicity Test with the Cyanobacterium, *Anabaena flos-aquae*, Report Number 1781.6935, Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571-1037, USA, (Syngenta File No. CGA173506_51018

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)

US EPA Ecological Effects Test Guidelines, OCSPP 850.4550: Algal Toxicity (2012)

GLP: Yes

Executive Summary

The toxicity of fludioxonil to the cyanobacterium *Anabaena flos-aquae* was determined. Algae were exposed to nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L (0.095, 0.15, 0.30, 0.62 and 1.3 mg/L as mean measured concentrations) alongside a culture medium control and a solvent (dimethylformamide, DMF) control. Based on mean measured concentrations, the 72-hour E_rC_{50}, E_yC_{50} and E_bC_{50} values were >1.3 mg/L,. The 96-hour E_rC_{50} and E_bC_{50} values were >1.3 mg/L and the 96-hour E_yC_{50} was 1.1 mg/L. For all endpoints evaluation, the 72-hour NOEC and LOEC values were determined to be 1.3 and >1.3 mg/L, respectively and the 96-hour NOEC and LOEC values were determined to be 0.62 and 1.3 mg/L, respectively.

The results of the subculture used to determine algistatic/algicidal effects of the test substance determined that fludioxonil has an algistatic effect on *A. flos-aquae*.

Materials

Test Material	Fludioxonil
Batch #:	612044
Purity:	95.5% (wt/wt), tested as 100%
Description:	Grey powder
Stability of test compound:	Stable under standard conditions
Recertification Date:	End of January 2014
Treatments	
Test concentrations:	Culture medium control, solvent (dimethylformamide, DMF) control and nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L (0.095, 0.15, 0.30, 0.62 and 1.3 mg/L as mean measured concentrations)
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Positive control:	Potassium dichromate is used at least once a year
Analysis of test concentrations:	Yes, analysis of fludioxonil at 0 and 96 hours
Test organism	

Species:	*Anabaena flos-aquae,* strain 1444, Class Cyanophyceae
Source:	Continuous laboratory cultures, originally obtained from the Culture Collection of Algae at The University of Texas (UTEX), Austin, Texas
Test design	
Test vessels:	250 mL glass flasks containing 100 mL of media covered with stainless steel caps which permitted gas exchange
Test medium:	Algal Assay Procedure (AAP) medium
Replication:	Eight vessels for the solvent control and four vessels for the culture medium control and each test concentration
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours
Environmental conditions	
Test temperature:	24 to 25°C
pH:	test start: 7.2 to 7.5 test end: 8.8 to 9.2
Lighting:	Continuous illumination at 1800 to 2200 lux

Study Design and Methods

Experimental dates: 19th to 27th August 2013 (including recovery period)

A 20 mg/mL primary stock solution was prepared prior to exposure initiation by placing 0.2001 g of fludioxonil in a 10-mL volumetric flask and bringing it to volume with DMF. The 2.0 mg/L test solution was prepared by adding 0.20 mL of the 20 mg/mL primary stock solution to 2000 mL of AAP medium and mixing using a magnetic stir plate and Teflon®-coated stir bar for approximately one hour. Due to the presence of undissolved material, the 2.0 mg/L test solution was filtered through a 0.45-µm membrane filter. Appropriate volumes of the 2.0 mg/L stock solution were diluted with AAP medium to give the test concentration series. The control consisted of culture medium only. The solvent control was prepared by adding DMF at a concentration of 0.10 mL/L to AAP medium. An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 1.0×10^4 algal cells per mL of test medium. Test solutions were hand-shaken daily and were held in a temperature controlled environmental chamber under continuous illumination.

Small volumes of all test concentrations and the control 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 haemocytometer. Observations of the health of the algal cells and test solutions (i.e., precipitation, clarity, material adhering to the sides of the test vessels) were made at each 24-hour interval.

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 measured daily in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of fludioxonil at 0 and 96 hours, using liquid chromatography/mass spectrometry (LC/MS/MS).

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 and 96-hour E_bC_{50}, E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were calculated using linear interpolation of response using the ICp method. For determination of the LOEC and NOEC values, Dunnett's Multiple Comparison Test was used to identify significant differences in the calculated mean biomass, growth rate and yield at the test item treatments compared to the pooled control.

Results and Discussion

The limit of quantification (LOQ) for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. In this study, the LOQ was 0.0061 mg/L at 0 and 96 hours. Mean measured concentrations were used for the calculation and reporting of results.

Table 8.2.6.2-1: Analytical results

Nominal concentrations (mg/L)	Measured Concentration (mg/L)		Mean measured concentration (mg/L)	Percent of Nominal (%)
	0 Hour	96 Hour		
Control	<0.0061[a]	<0.0061	NA[b]	NA
Solvent Control	<0.0061	<0.0061	NA	NA
0.13	0.12	0.070	0.095	73
0.25	0.15	0.15	0.15	60
0.50[c]	0.30	0.29	0.30	59
1.0	0.64	0.61	0.62	62
2.0	1.4	1.3	1.3	66

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.

[b] NA = Not Applicable.

[c] An additional 0.50 mg/L (nominal) sample was prepared and was not inoculated with algae. At 96-hours, this sample had a measured concentration of 0.31 mg/L. These results indicated that the presence of algae may have had a slight impact on the concentration of fludioxonil in the test solution.

Cells exposed to all treatment levels tested and the controls were observed to be normal throughout the exposure period.

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, alongside the estimated EC_{50} values.

Table 8.2.6.2-2: Mean values at each concentration of fludioxonil for the growth rate at 72 and 96 hours for *Anabaena flos-aquae* and relevant endpoints

Mean measured concentrations (mg/L)	Mean growth rate (1/day) 0 – 72 hrs (SD)	Percentage inhibition (%)	Mean growth rate (1/day) 0 – 96 hrs (SD)	Percentage inhibition (%)
0 (pooled control)	1.44 (0.12)	NA	1.40 (0.09)	NA
0.095	1.41 (0.21)	2	1.41 (0.05)	-1
0.15	1.57 (0.09)	-9	1.37 (0.05)	2
0.30	1.48 (0.09)	-3	1.3 (0.03)	7
0.62	1.25 (0.35)	13	1.31 (0.07)	6
1.3	1.41 (0.15)	2	1.2 (0.09)[a]	14
E_rC_{10} mg /L (95% confidence limits)	>1.3 (NA[b])		>1.3 (NA)	
E_rC_{20} mg /L (95% confidence limits)	>1.3 (NA[b])		>1.3 (NA)	
E_rC_{50} mg /L (95% confidence limits)	>1.3 (NA[b])		>1.3 (NA)	
NOEC	1.3		0.62	
LOEC	>1.3		1.3	

[a] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test.
[b] NA = Not Applicable. E_rC_{50} value was empirically estimated; therefore, corresponding 95% confidence intervals could not be determined.

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 8.2.6.2-3: Mean values at each concentration of fludioxonil for the yield at 72 and 96 hours for *Anabaena flos-aquae* and relevant endpoints

Nominal concentrations (mg/L)	Mean yield (x 10^4 cells/mL) 0 – 72 hrs (SD)	Percentage inhibition (%)	Mean yield (x 10^4 cells/mL) 0 – 96 hrs (SD)	Percentage inhibition (%)
0 (pooled control)	65.69 (26.68)	NA	242.89 (100.42)	
0.095	63.44 (30.04)	3	240.41 (46.22)	1
0.15	93.19 (27.69)	-42	208.10 (39.99)	14
0.30	71.19 (20.96)	-8	155.57 (19.25)	36
0.62	53.34 (60.58)	19	164.86 (40.95)	32
1.3	60.03 (24.01)	9	106.50 (29.87)[a]	56
E_yC_{50} mg /L (95% confidence limits)	>1.3 (NA[b])		1.1 (0.19 – ND[c])	
E_yC_{20} mg /L (95% confidence limits)	0.56 (0.070 – ND[c])		ND	
E_yC_{10} mg /L (95% confidence limits)	0.39 ND		ND	
NOEC	1.3		0.62	
LOEC	>1.3		1.3	

[a] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test.
[b] NA = Not Applicable. E_rC_{50} value was empirically estimated; therefore, corresponding 95% confidence intervals could not be determined.
[c] ND = Not Determined. A corresponding upper 95% confidence limit could not be determined.

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, alongside the estimated EC_{50} values.

Table 8.2.6.2-4: Mean values at each concentration of fludioxonil for the biomass integral (area under the growth curve) at 72 and 96 hours for *Anabaena flos-aquae* and relevant endpoints

Nominal concentrations (mg/L)	Mean biomass integral (x 10^4) 0 – 72 hrs (SD)	Percentage inhibition (%)	Mean biomass integral (x 10^4) 0 – 96 hrs	Percentage inhibition (%)
0 (pooled control)	63.86 (20.46)	NA	218.69 (46.36)	NA
0.095	63.4 (4.06)	1	215.85 (29.45)	1
0.15	69.99 (23.22)	-10	221.16 (42.15)	-1
0.30	50.13 (11.09)	22	163.9 (12.78)	25
0.62	62.02 (30.93)	3	171.5 (54.31)	22
1.3	51.29 (15.61)	20	134.84 (15.71)[a]	38
E_bC_{50} mg /L (95% confidence limits)	>1.3 (NA[b])		>1.3 (NA)	
E_bC_{20} mg /L (95% confidence limits)	1.1 ND		ND	
E_bC_{10} mg /L (95% confidence limits)	0.25 ND		ND	
NOEC	1.3		0.62	
LOEC	>1.3		1.3	

[a] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test.
[b] NA = Not Applicable. E_rC_{50} value was empirically estimated; therefore, corresponding 95% confidence intervals could not be determined.

Validity criteria

The following criteria are required per the OECD 201 guideline:

- The mean cell growth in the pooled control must increase by more than 16 times after 96 hours of growth (actual increase was 240-fold)
- The mean coefficient of variation for section-by-section specific growth rates (e.g., day 0 to 1, 1 to 2, 2 to 3 and 3 to 4) in the pooled control replicates should not exceed 35% (actual CV was 72%)
- The coefficient of variation for average specific growth rate of the pooled control for the entire test period (e.g., 0 to 96 hours) should not exceed 10% (actual CV was 6.4%)

Although one of the validity criterion was not met, cell counts for *A. flos-aquae* are typically more variable than single cell green algae due to its growth in chains and groups of cells. Toxicity observed with *A. flos-aquae* is consistent with the other algal species tested (*N. pelliculosa* and *S. costatum*), therefore, these results are considered acceptable and clearly define the toxicity of fludioxonil to *A. flos-aquae.*

Conclusions

The toxicity of fludioxonil to the cyanobacterium *Anabaena flos-aquae* was determined. Based on mean measured concentrations, the 72-hour E_rC_{50}, E_yC_{50} and E_bC_{50} values were >1.3 mg/L. The 96-hour E_rC_{50} and E_bC_{50} values were and the 96-hour E_yC_{50} was 1.1 mg/L. For all endpoints evaluation, the 72-hour NOEC and LOEC values were determined to be 1.3 and >1.3 mg/L, respectively and the 96-hour NOEC and LOEC values were determined to be 0.62 and 1.3 mg/L, respectively.

(Soucy K, 2013)

RMS comment:

For the study of Soucy (2013, CGA173506_51018, K-CA 8.2.6.2/01) conducted with fludioxoni l on Anabaena f/os-aquae, the reliability of the study should be justified as:

- it is unclear if the amount of solvent in the positive control and the test concentrations is identical.

- The pH varies by more than 1.5 in the controls and some of the tested concentrations.

- The validity criteria according to OECD 201 (2011) for the coefficient of variation for sectional growth rate is of 72% for the pooled control.

Syngenta response:

Solvent use details:

A 20 mg/mL primary stock solution was prepared prior to exposure initiation by placing 0.2001 g of fludioxonil in a 10-mL volumetric flask and bringing it to volume with dimethylformamide (DMF, CAS No. 68-12-2). The resulting stock solution was observed to be clear and brown in color with no visible undissolved test substance following multiple shakes and inversions of the flask and approximately five minutes of sonication. The 2.0 mg/L test solution was prepared by adding 0.20 mL of the 20 mg/mL stock solution to 2000 mL of AAP medium was mixed using a magnetic stir plate and Teflon®-coated stir bar for approximately one hour. After mixing, the 2.0 mg/L test solution was observed to be clear and colorless with a large amount of undissolved material at the surface of the solution. Due to the presence of undissolved material this test solution was then filtered using a 0.45-µm membrane filter. The filtrate was observed to be clear and colourless with no visible undissolved material and was used to prepare the remaining Exposure solutions as noted below:

Filtrate Solution (mg/L)	Volume Filtrate Solution Used (mL)	Diluted to (mL) with Sterile AAP Medium	Nominal Concentration (mg/L)
2.0	400	NA	2.0
2.0	500	1000	1.0
2.0	250	1000	0.50
2.0	125	1000	0.25
2.0	65	1000	0.13

All test solutions were observed to be clear and colorless with no visible undissolved test substance following preparation.

A solvent (DMF) solution was prepared by adding DMF at a concentration of 0.10 mL/L to AAP medium. This solution was used to establish eight replicate flasks for the solvent control. Additional untreated AAP medium was used to prepare the control.

pH variation:

The pH variation during an algal study frequently shows variation during the study. The duration of an algal study affects the final pH. The longer the study, the higher the pH tends to go. If flasks are shaken enough, or aerated, this will reduce the pH rise. However some algal studies were conducted over 5 days

which is significantly longer than the 72-h OECD test. Even the US OCSPP test is 96 hour; in that test there is no corresponding no statement regarding pH change during the test. In studies which use a high initial inoculum (e.g. 10,000 cells/mL) this can also result in high final pHs. The high pH reached at the end such studies is expected and is indicative of strong, healthy algal growth. pH change during such tests result from the algae absorbing the dissolved CO2 (carbonic acid), and releasing oxygen into the test medium. As the CO2 is absorbed and oxygen released by the photosynthesising algae the pH of the medium rises. The stronger the algal growth the greater the pH rise. A study by Dubinsky & Rotem 1974* examines the effect of pH on algae and found that algae grew well between pH 5 and 12, while sterile controls grew under pH 4 to 10. Therefore the rise in pH during the studies presented here is not considered to be of any consequence for the validity of these studies, and the absence of this criteria from the OCSPP guidelines validates this statement.

* Dubinsky, Z. and Rotem, J. (1974) Relations between algal populations and the pH of their media. Oecologia (Berl.)16: 53-60.

Validity criteria for species of non- green species of algae:

The applicability of the validity criteria set in the OECD Test Guideline 201 (2006) has been investigated in a paper by Crop Life International 2008. It is unclear what scientific basis and/or data were used for establishing the numerical values of these criteria. This position paper summarizes analysis of control data for the algal species, *Navicula pelliculosa,* and *Anabaena flos-aquae and Skeletonema costatum* to determine the impact of the validity criteria. Control data from 9 laboratories and 79 algal studies were analyzed against the OECD 201 validity criteria for the mean coefficient of variation for section-by-section specific growth rates (SSGR) and coefficient of variation for average specific growth rates (ASGR). The results of these analyses, based on the OECD201 validity criteria indicate a:

- 69% to 88% failure rate using the validity criteria mean coefficient of variation for section-by-section specific growth rate across the three algal species and
- 21% to 75% failure rate using average specific growth rate across all three species.

Although the criteria for average specific growth rate is a smaller number and apparently easier to achieve (10%), up to 75% of the studies on non-green algal species studies would potentially be rejected using this criteria. This difference between green and non-green algae may be attributable to their natural pattern of growth which often involves a longer "lag-phase" and/or a filamentous growth form. Regardless, regulators should not be strictly using these criteria to reject studies done on non-green algal species.

The results of this analysis show that the likelihood of meeting the proposed validity criteria for non-green algae is limited, especially for the blue-green algae (*Anabaena flos-aquae*). table below shows that 88% fail the SSGR.

Algal species-specific percent of studies not meeting the validity criteria based on mean section-by-section coefficient of variation < 35% and average specific growth rate of <10%

Validity Criteria	***Skeletonema costatum***	***Anabaena flos-aquae***	***Navicula pelliculosa***
% Failure for C.V. section-by-section growth rate (Number of studies)	69% (16)	88% (36)	71% (31)
% Failure for C.V. average specific growth rate (Number of studies)	58% (16)	75% (36)	21% (28)

Crop Life International believes that if a study done on non-green algal species meets the first validity criterion (i.e. 16X growth over the duration of the study), then the study should be considered valid. The SSGR and ASGR criteria should not be applied to non-green algal species until a ring-test demonstrates that these validity criteria are truly achievable. Given that this study was conducted recently (2013) by a highly experienced and world leading laboratory, then this suggests that these criteria remain unattainable to date.

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.6.2/02 Soucy KL (2013a). Fludioxonil – 96-Hour Toxicity Test with the Freshwater Diatom, *Navicula pelliculosa*, Report Number 1781.6933, Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571-1037 USA, (Syngenta File No. CGA173506_51014)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)

US EPA Ecological Effects Test Guidelines, OCSPP 850.4500: Algal Toxicity (2012)

GLP: Yes

Executive Summary

The toxicity of fludioxonil to the freshwater diatom, *Navicula pelliculosa,* was determined. Algae were exposed to nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L (0.074, 0.14, 0.30, 0.63 and 1.2 mg/L as mean measured concentrations) alongside a culture medium control and a solvent (dimethylformamide, DMF) control. Based on mean measured concentrations, the 72-hour E_rC_{50} was >1.2 mg/L, the 72-hour E_yC_{50} was 0.86 mg/L and the 72-hour E_bC_{50} was 0.88 mg/L. The 96-hour E_rC_{50} was >1.2 mg/L, the 96-hour E_yC_{50} was 0.85 mg/L and the 96-hour E_bC_{50} was 0.86 mg/L. For the 72- and 96-hour growth rate and 96-hour yield endpoints, the NOEC and LOEC values were determined to be 0.63 and 1.2 mg/L, respectively. For the 72-hour yield and 72- and 96-hour biomass integral, the NOEC and LOEC values were determined to be 0.30 and 0.63 mg/L, respectively.

Materials

Test Material	Fludioxonil
Batch #:	612044
Purity:	95.5% (wt/wt), tested as 100%
Description:	Grey powder
Stability of test compound:	Stable under standard conditions
Recertification Date:	End of January 2014
Treatments	
Test concentrations:	Culture medium control, solvent (dimethylformamide, DMF) control and nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L (0.074, 0.14, 0.30, 0.63 and 1.2 mg/L as mean measured concentrations)
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)

Positive control:	Potassium dichromate is used at least once a year
Analysis of test concentrations:	Yes, analysis of fludioxonil at 0 and 96 hours
Test organism	
Species:	*Navicula pelliculosa,* strain 661, Class Bacillariophyceae
Source:	Continuous laboratory cultures, originally obtained from the Culture Collection of Algae at The University of Texas (UTEX), Austin, Texas
Test design	
Test vessels:	250- mL glass flasks containing 100 mL of media covered with stainless steel caps which permitted gas exchange
Test medium:	Algal Assay Procedure (AAP) medium
Replication:	Eight vessels for the solvent control and four vessels for the culture medium control and each test concentration
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours
Environmental conditions	
Test temperature:	25 to 26°C
pH:	test start: 7.1 to 7.4 test end: 7.5 to 8.9
Lighting:	Continuous illumination at 3700 to 4400 lux

Study Design and Methods

Experimental dates: 12th to 20th August 2013 (including recovery period)

A 20 mg/mL primary stock solution was prepared prior to exposure initiation by placing 0.2001 g of fludioxonil in a 10 mL volumetric flask and bringing it to volume with DMF. The 2.0 mg/L test solution was prepared by adding 0.20 mL of the 20 mg/mL primary stock solution to 2000 mL of AAP medium and mixing using a magnetic stir plate and Teflon®-coated stir bar for approximately one hour. Due to the presence of undissolved material, the 2.0 mg/L test solution was filtered through a 0.45-µm membrane filter. Appropriate volumes of the 2.0 mg/L stock solution were diluted with AAP medium to give the test concentration series. The control consisted of culture medium only. The solvent control was prepared by adding DMF at a concentration of 0.10 mL/L to AAP medium. An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 1.0×10^4 algal cells per mL of test medium. Test solutions were hand-shaken daily and were held in a temperature controlled environmental chamber under continuous illumination.

Small volumes of all test concentrations and the control 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 haemocytometer. Observations of the health of the algal cells and test solutions (i.e., precipitation, clarity, material adhering to the sides of the test vessels) were made at each 24-hour interval.

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 measured daily in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of fludioxonil at 0 and 96 hours, using liquid chromatography/mass spectrometry (LC/MS/MS).

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 and 96-hour E_bC_{50}, E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were calculated using linear interpolation of response using the ICp method. For determination of the LOEC and NOEC values, Dunnett's Multiple Comparison Test was used to identify significant differences in the calculated mean biomass, growth rate and yield at the test item treatments compared to the pooled control.

Results and Discussion

The limit of quantification (LOQ) for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. In this study, the LOQ at 0 and 96 hours was 0.0060 and 0.0058 mg/L, respectively. Mean measured concentrations were used for the calculation and reporting of results.

The mean measured concentrations ranged from 57 to 63% of nominal concentrations and defined the treatment levels tested as 0.074, 0.14, 0.30, 0.63 and 1.2 mg/L.

Table 8.2.6.2-5: Analytical results

Nominal concentrations (mg/L)	Measured Concentration (mg/L)		Mean measured concentration (mg/L)	Percent of Nominal (%)
	0 Hour	96 Hour		
Control	<0.0060[a]	<0.0058	NA[b]	NA
Solvent Control	<0.0060	<0.0058	NA	NA
0.13	0.082	0.065	0.074	57
0.25	0.16	0.13	0.14	57
0.50	0.34	0.26/0.32 [c]	0.30	60
1.0	0.68	0.57	0.63	63
2.0	1.3	1.2	1.2	62

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.
[b] NA = Not Applicable.
[c] An additional 0.50 mg/L (nominal) sample was prepared and was not inoculated with algae. At 96-hours, this sample had a measured concentration of 0.32 mg/L. These results indicated that the presence of algae may have had a slight impact on the concentration of fludioxonil in the test solution.

Cells exposed to all treatment levels tested and the controls were observed to be normal throughout the exposure period.

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, alongside the estimated EC_{50} values.

Table 8.2.6.2-6: Mean values at each concentration of fludioxonil for the growth rate at 72 and 96 hours for *Navicula pelliculosa* and relevant endpoints

Mean measured concentrations (mg/L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition (%)	Mean growth rate (1/day) 0 – 96 hrs	Percentage inhibition (%)
0 (pooled control)	1.41 (0.05)	NA	1.29 (0.04)	NA
0.074	1.42 (0.05)	-1	1.30 (0.05)	-1
0.14	1.41 (0.05)	0	1.30 (0.04)	-1
0.30	1.40 (0.05)	0	1.27 (0.03)	1
0.63	1.33 (0.06)	5	1.24 (0.03)	4
1.2	0.71 (0.10)[a]	50	0.72 (0.07)[a]	44
E_rC_{50} mg /L (95% confidence limits)	>1.2 (NA[b])		>1.2 (NA)	
E_rC_{20} mg /L (95% confidence limits)	0.80 (0.74 – 0.86)		ND	
E_rC_{10} mg /L (95% confidence limits)	0.68 (0.61 – 0.73)		ND	
NOEC	0.63		0.63	
LOEC	1.2		1.2	

[a] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test.
[b] NA = Not Applicable. E_rC_{50} value was empirically estimated; therefore, corresponding 95% confidence intervals could not be determined.

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 8.2.6.2-7: Mean values at each concentration of fludioxonil for the yield at 72 and 96 hours for *Navicula pelliculosa* and relevant endpoints

Nominal concentrations (mg/L)	Mean yield (x 10^4 cells/mL) 0 – 72 hrs (SD)	Percentage inhibition (%)	Mean yield (x 10^4 cells/mL) 0 – 96 hrs (SD)	Percentage inhibition (%)
0 (pooled control)	57.98 (8.27)	NA	162.42 (25.60)	NA
0.074	59.56 (8.14)	-3	168.58 (30.47)	-4
0.14	58.56 (7.69)	-1	168.08 (24.54)	-3
0.30	57.63 (8.37)	1	150.92 (20.58)	7
0.63	46.88 (7.65)[a]	19	130.60 (16.68)	20
1.2	7.00 (2.56)[a]	88	16.69 (4.81)[a]	90
E_yC_{50} mg /L (95% confidence limits)	0.86 (0.75 – 0.93)		0.85 (0.76 – 0.92)	
E_yC_{20} mg /L (95% confidence limits)	0.63 (0.33 – 0.74)		ND	
E_yC_{10} mg /L (95% confidence limits)	0.44 (ND [b] – 0.75)		ND	
NOEC	0.30		0.63	
LOEC	0.63		1.2	

[a] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test.
[b] ND – Not determined. The lower 95% confidence limit could not be determined.

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, alongside the estimated EC_{50} values.

Table 8.2.6.2-8: Mean values at each concentration of fludioxonil for the biomass integral (area under the growth curve) at 72 and 96 hours for *Navicula pelliculosa* and relevant endpoints

Nominal concentrations (mg/L)	Mean biomass integral (x 10^4) 0 – 72 hrs (SD)	Percentage inhibition (%)	Mean biomass integral (x 10^4) 0 – 96 hrs	Percentage inhibition (%)
0 (pooled control)	46.17 (5.54)	NA	160.96 (17.83)	NA
0.074	47.89 (3.52)	-4	166.72 (19.86)	-4
0.14	47.45 (4.98)	-3	165.49 (20.65)	-3
0.30	43.75 (5.51)	5	152.37 (18.52)	5
0.63	37.52 (5.79)[a]	19	129.96 (17.37)[a]	19
1.2	8.42 (0.89)[a]	82	20.75 (4.18)[a]	87
E_bC_{50} mg /L (95% confidence limits)	0.88 (0.77 – 0.95)		0.86 (0.76 – 0.92)	
E_bC_{20} mg /L (95% confidence limits)	0.62 (0.22 – 0.73)		ND	
E_bC_{10} mg /L (95% confidence limits)	0.36 (0.11 – 0.67)		ND	
NOEC	0.30		0.30	
LOEC	0.63		0.63	

[a] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test.
ND = not determined

Validity criteria

The following criteria as per the OECD 201 guideline were met in that:

- The mean cell growth in the pooled control must increase by more than 16 times after 96 hours of growth (actual increase was 163-fold).
- The mean coefficient of variation for section-by-section specific growth rates (e.g., day 0 to 1, 1 to 2, 2 to 3 and 3 to 4) in the pooled control replicates should not exceed 35% (actual CV was 22%).
- The coefficient of variation for average specific growth rate of the pooled control for the entire test period (e.g., 0 to 96 hours) should not exceed 10% (actual CV was 2.8%).

Conclusions

The toxicity of fludioxonil to the freshwater diatom, *Navicula pelliculosa,* was determined. Based on mean measured concentrations, the 72-hour E_rC_{50} was >1.2 mg/L, the 72-hour E_yC_{50} was 0.86 mg/L and the 72-hour E_bC_{50} was 0.88 mg/L. The 96-hour E_rC_{50} was >1.2 mg/L, the 96-hour E_yC_{50} was 0.85 mg/L and the 96-hour E_bC_{50} was 0.86 mg/L. For the 72- and 96-hour growth rate and 96-hour yield endpoints, the NOEC and LOEC values were determined to be 0.63 and 1.2 mg/L, respectively. For the 72-hour yield and 72- and 96-hour biomass integral, the NOEC and LOEC values were determined to be 0.30 and 0.63 mg/L, respectively.

The results of the subculture used to determine algistatic/algicidal effects of the test substance determined that fludioxonil has an algistatic effect on *N. pelliculosa*.

(Soucy K, 2013a)

RMS comment:

For the study of Soucy (2013, CGA173506_51014, K-CA 8.2.6.2/02) conducted with fludioxonil on Navicula pelliculosa, the reliability of the study should be justified as:

- it is unclear if the amount of solvent in the positive control and the test concentrations is identical.
- The pH varies by more than 1.5 in the controls and some of the tested concentrations.

Syngenta response:

Solvent use details:

A 20 mg/mL primary stock solution was prepared prior to exposure initiation by placing 0.2001 g of fludioxonil in a 10-mL volumetric flask and bringing it to volume with dimethylformamide (DMF, CAS No. 68-12-2). The resulting stock solution was observed to be clear and brown in color with no visible undissolved test substance following multiple shakes and inversions of the flask and approximately five minutes of sonication. The 2.0 mg/L test solution was prepared by adding 0.20 mL of the 20 mg/mL primary stock solution to 2000 mL of AAP medium and mixing using a magnetic stir plate and Teflon®-coated stir bar for approximately one hour. After thorough mixing, the test solution was observed to be clear and colorless with a large amount of undissolved test substance present on the surface of the solution. Due to the presence of undissolved material, this test solution was then filtered using a 0.45-µm membrane filter. The filtrate was observed to be clear and colorless with no visible undissolved test substance and was used to prepare the remaining exposure solutions as follows:

Filtrate Solution (mg/L)	Volume Filtrate Solution Used (mL)	Diluted to (mL) with Sterile AAP Medium	Nominal Concentration (mg/L)
2.0	400	NA[a]	2.0
2.0	500	1000	1.0
2.0	250	1000	0.50
2.0	125	1000	0.25
2.0	65	1000	0.13

[a] NA = Not Applicable.

All test solutions were observed to be clear and colorless with no visible undissolved test substance following preparation. A solvent (DMF) solution was prepared by adding DMF at a concentration of 0.10 mL/L to AAP medium. This solution was used to establish eight replicate flasks for the solvent control and used for dilutions of the four lower test concentrations. Additional untreated AAP medium was used to prepare the control.

pH variation:

The pH variation during an algal study frequently shows variation during the study. The duration of an algal study affects the final pH. The longer the study, the higher the pH tends to go. If flasks are shaken enough, or aerated, this will reduce the pH rise. However some algal studies were conducted over 5 days which is significantly longer than the 72-h OECD test. Even the US OCSPP test is 96 hour; in that test there is no corresponding no statement regarding pH change during the test. In studies which use a high initial inoculum (e.g. 10,000 cells/mL) this can also result in high final pHs. The high pH reached at the end such studies is expected and is indicative of strong, healthy algal growth. pH change during such tests result from the algae absorbing the dissolved CO_2 (carbonic acid), and releasing oxygen into the test medium. As the CO_2 is absorbed and oxygen released by the photosynthesising algae the pH of the medium rises. The stronger the algal growth the greater the pH rise. A study by Dubinsky & Rotem 1974* examines the effect of pH on algae and found that algae grew well between pH 5 and 12, while sterile controls grew under pH 4 to 10. Therefore the rise in pH during the studies presented here is not considered to be of any consequence for the validity of these studies, and the absence of this criteria from the OCSPP guidelines validates this statement.

* Dubinsky, Z. and Rotem, J. (1974) Relations between algal populations and the pH of their media. Oecologia (Berl.)16: 53-60.

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.6.2/03 Soucy KL (2013b). Fludioxonil – 96-Hour Toxicity Test with the Marine Diatom, *Skeletonema costatum*, Report Number 1781.6934, Smithers Viscient, 790 Main Street, Wareham, Massachusetts 02571 USA, (Syngenta File No. CGA173506_51017)

Guidelines

OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)

US EPA Ecological Effects Test Guidelines, OCSPP 850.4500: Algal Toxicity (2012)

GLP: Yes

Executive Summary

The toxicity of fludioxonil to the marine diatom *Skeletonema costatum* was determined. Algae were exposed to nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L (0.074, 0.14, 0.31, 0.62 and 1.3 mg/L as mean measured concentrations) alongside a culture medium control and a solvent (dimethylformamide, DMF) control. Based on mean measured concentrations, the 72-hour E_rC_{50} was 0.37 mg/L, the 72-hour E_yC_{50} was 0.26 mg/L and the 72-hour E_bC_{50} was 0.25 mg/L. The 96-hour E_rC_{50} was 0.43 mg/L, the 96-hour E_yC_{50} was 0.28 mg/L and the 96-hour E_bC_{50} was 0.26 mg/L. For all endpoints evaluated, the 72- and 96-hour NOEC values were determined to be 0.14 mg/L and the 72- and 96-hour LOEC values were determined to be 0.31 mg/L.

Materials

Test Material	Fludioxonil
Batch #:	612044

Purity:	95.5% (wt/wt), tested as 100%
Description:	Grey powder
Stability of test compound:	Stable under standard conditions
Recertification Date:	End of January 2014
Treatments	
Test concentrations:	Culture medium control, solvent (dimethylformamide, DMF) control and nominal concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L (0.074, 0.14, 0.31, 0.62 and 1.3 mg/L as mean measured concentrations)
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Positive control:	Potassium dichromate is used at least once a year
Analysis of test concentrations:	Yes, analysis of fludioxonil at 0 and 96 hours
Test organism	
Species:	*Skeletonema costatum,* strain CCMP 1332, Class Bacillariophyceae
Source:	Continuous laboratory cultures, originally obtained from Bigelow Laboratories, West Boothbay Harbour, Maine
Test design	
Test vessels:	250- mL glass flasks containing 100 mL of media covered with stainless steel caps which permitted gas exchange
Test medium:	Artificially-Enriched Seawater (AES) medium
Replication:	Eight vessels for the solvent control and four vessels for the culture medium control and each test concentration
Starting cell density:	1.0×10^4 cells/mL
Exposure regime:	Static
Aeration:	No
Duration:	96 hours
Environmental conditions	
Test temperature:	20 to 21°C
pH:	test start: 7.7 to 8.0 test end: 7.8 to 8.7
Lighting:	Continuous illumination at 3900 to 4800 lux

Study Design and Methods

Experimental dates: 12th to 20th August 2013 (including recovery period)

A 20 mg/mL primary stock solution was prepared prior to exposure initiation by placing 0.2001 g of fludioxonil in a 10 mL volumetric flask and bringing it to volume with DMF. The 2.0 mg/L test solution was prepared by adding 0.20 mL of the 20 mg/mL primary stock solution to 2000 mL of AES medium and mixing using a magnetic stir plate and Teflon®-coated stir bar for approximately one hour. Due to the presence of undissolved material, this test solution was then filtered using a 2.7-µm membrane filter. Appropriate volumes of the 2.0 mg/L stock solution were diluted with AES medium to give the test concentration series. The control consisted of culture medium only. The solvent control was prepared by

adding DMF at a concentration of 0.10 mL/L to AES medium. An aliquot of test solution was placed into each test vessel and the test was started by inoculation of 1.0×10^4 algal cells per mL of test medium. Test solutions were hand-shaken daily and were held in a temperature controlled environmental chamber under continuous illumination.

Small volumes of all test concentrations and the control 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 haemocytometer. Observations of the health of the algal cells and test solutions (i.e., precipitation, clarity, material adhering to the sides of the test vessels) were made at each 24-hour interval.

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 measured daily in a flask incubated under the same conditions as the test flasks.

The test concentrations were verified by chemical analysis of fludioxonil at 0 and 96 hours, using liquid chromatography/mass spectrometry (LC/MS/MS).

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 and 96-hour E_bC_{50}, E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were calculated using linear interpolation of response using the ICp method. For determination of the LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) values, either Williams' Multiple Comparison Test, Dunnett's T3 Multiple Comparison Test or Jonckheere-Terpstra Step-Down Test was used to identify significant differences in the calculated mean biomass, growth rate and yield at the test item treatments compared to the pooled control.

Results and Discussion

The limit of quantification (LOQ) for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. In this study, the LOQ at 0 and 96 hours was 0.0055 and 0.0074 mg/L, respectively. Mean measured concentrations were used for the calculation and reporting of results.

The mean measured concentrations ranged from 55 to 63% of nominal concentrations and defined the treatment levels tested as 0.074, 0.14, 0.31, 0.62 and 1.3 mg/L.

Table 8.2.6.2-9: Analytical results

Nominal concentrations (mg/L)	Measured Concentration (mg/L)		Mean measured concentration (mg/L)	Percent of Nominal (%)
	0 Hour	96 Hour		
Control	<0.0055[a]	<0.0074	NA[b]	NA
Solvent Control	<0.0055	<0.0074	NA	NA
0.13	0.087	0.061	0.074	57
0.25	0.17	0.11	0.14	55
0.50	0.34	0.27/0.32[c]	0.31	62
1.0	0.66	0.58	0.62	62
2.0	1.4	1.1	1.3	63

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls.

[b] NA = Not Applicable.

[c] An additional 0.50 mg/L (nominal) sample was prepared and was not inoculated with algae. At 96-hours, this sample had a measured concentration of 0.32 mg/L. These results indicated that the presence of algae may have had a slight impact on the concentration of fludioxonil in the test solution.

At the 72- and 96-hour observation intervals, cells exposed to the 1.3 mg/L (mean measured) treatment level were observed to have thin cell walls compared to the control. Cells exposed to all remaining treatment levels tested (0.074, 0.14, 0.31 and 0.62 mg/L, mean measured) and the controls were observed to be normal throughout the exposure period.

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, alongside the estimated EC_{50} values.

Table 8.2.6.2-10: Mean values at each concentration of fludioxonil for the growth rate at 72 and 96 hours for *Skeletonema costatum* and relevant endpoints

Mean measured concentrations (mg/L)	Mean growth rate (1/day) 0 – 72 hrs (SD)	Inhibition (%)	Mean growth rate (1/day) 0 – 96 hrs	Inhibition (%)
0 (pooled control)	1.00 (0.08)	NA	0.98 (0.06)	NA
0.074	1.00 (0.10)	0	0.93 (0.07)	5
0.14	0.97 (0.06)	4	0.95 (0.11)	3
0.31	0.66 (0.08)[a]	34	0.76 (0.12)[a]	22
0.62	-0.08 (0.27)[a]	108	0.09 (0.20)[a]	90
1.3	-0.18 (0.23)[a]	118	-0.09 (0.18)[a]	109
E_rC_{50} mg /L (95% confidence limits)	0.37 (0.33 – 0.41)		0.43 (0.36 – 0.49)	
E_rC_{20} mg /L (95% confidence limits)	0.23 (0.18 – 0.26)		ND	
E_rC_{10} mg /L (95% confidence limits)	0.17 (0.12 – 0.20)		ND	
NOEC	0.14		0.14	
LOEC	0.31		0.31	

[a] Significantly reduced compared to the pooled control, based on Williams' Multiple Comparison Test.

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table 8.2.6.2-11: Mean values at each concentration of fludioxonil for the yield at 72 and 96 hours for *Skeletonema costatum* and relevant endpoints

Nominal concentrations (mg/L)	Mean yield (x 10^4 cells/mL) 0 – 72 hrs (SD)	Inhibition (%)	Mean yield (x 10^4 cells/mL) 0 – 96 hrs (SD)	Inhibition (%)
0 (pooled control)	17.75 (4.37)	NA	45.52 (11.93)	
0.074	17.69 (6.18)	0	37.63 (10.08)	17
0.14	15.69 (3.20)	12	42.38 (18.18)	7
0.31	5.88 (1.49)[a]	7	19.63 (7.43)[b]	57
0.62	-0.06 (0.52)[a]	100	0.75 (1.06)[b]	98
1.3	-0.81 (0.24)[a]	105	-0.94 (0.13)[b]	102
E_yC_{50} mg /L (95% confidence limits)	0.26 (0.19 – 0.29)		0.28 (0.18 – 0.38)	
E_yC_{20} mg /L (95% confidence limits)	0.16 (ND [c] – 0.21)		ND	
E_yC_{10} mg /L (95% confidence limits)	0.13 (ND [c] – 0.19)		ND	
NOEC	0.14		0.14	
LOEC	0.31		0.31	

[a] Significantly reduced compared to the pooled control, based on Jonckheere-Terpstra Step-Down Test.
[b] Significantly reduced compared to the pooled control, based on Dunnett's T3 Multiple Comparison Test.
[c] ND – Not determined. The lower 95% confidence limit could not be determined.

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, alongside the estimated EC_{50} values.

Table 8.2.6.2-12: Mean values at each concentration of fludioxonil for the biomass integral (area under the growth curve) at 72 and 96 hours for *Skeletonema costatum* and relevant endpoints

Nominal concentrations (mg/L)	Mean biomass integral (x 10^4) 0 – 72 hrs (SD)	Inhibition (%)	Mean biomass integral (x 10^4) 0 – 96 hrs (SD)	Inhibition (%)
0 (pooled control)	15.85 (2.45)	NA	47.49 (7.20)	NA
0.074	15.46 (2.47)	2	43.12 (8.41)	9
0.14	14.43 (2.40)	9	43.46 (11.71)	8
0.31	4.24 (1.40)[a]	73	16.99 (5.36)[b]	64
0.62	-0.68 (1.01)[a]	104	-0.34 (0.85)[b]	101
1.3	-1.18 (0.63)[a]	107	-2.06 (0.59)[b]	104
E_bC_{50} mg /L (95% confidence limits)	0.25 (0.21 – 0.27)		0.26 (0.22 – 0.31)	
E_bC_{20} mg /L (95% confidence limits)	0.17 (0.11 – 0.20)		ND	
E_bC_{10} mg /L (95% confidence limits)	0.14 (ND [c] – 0.18)		ND	
NOEC	0.14		0.14	
LOEC	0.31		0.31	

[a] Significantly reduced compared to the pooled control, based on Williams' Multiple Comparison Test.
[b] Significantly reduced compared to the pooled control, based on Dunnett's T3 Multiple Comparison Test.
[c] ND – Not determined. The lower 95% confidence limit could not be determined.

Validity criteria

The following criteria are required per the OECD 201 guideline:

- The mean cell growth in the pooled control must increase by more than 16 times after 96 hours of growth (actual increase was 47-fold).
- The mean coefficient of variation for section-by-section specific growth rates (e.g., day 0 to 1, 1 to 2, 2 to 3 and 3 to 4) in the pooled control replicates should not exceed 35% (actual CV was 83%).
- The coefficient of variation for average specific growth rate of the pooled control for the entire test period (e.g., 0 to 96 hours) should not exceed 10% (actual CV was 6.4%).

As presented in the second bullet point, during this study, the pooled control coefficient of variation for section-by-section specific growth rates was 83%, which exceeds the criterion of 35%. Cell counts for *S. costatum* are typically more variable than single-cell green algae due to its growth in chains and groups of cells. OECD Guideline #201 does not specifically require testing with marine algae or diatoms, and as such, the acceptance criteria stated in the protocol were based on freshwater algal species mentioned in the guideline. *Skeletonema costatum* and diatoms in general, reproduce more slowly than most other algal species. Therefore, the criteria proposed in the test guideline and protocol may not be appropriate for this test organism. Based on this laboratory's experience as well as additional testing laboratories, a failure rate of 69% (sample of 16 tests) has been noted within a position paper describing the OECD acceptance criteria applied to non-green algal species (see appendix). Therefore, this result is considered acceptable and clearly defines the toxicity of fludioxonil to *S. costatum*.

Conclusions

The toxicity of fludioxonil to the marine diatom *Skeletonema costatum* was determined. Based on mean measured concentrations, the 72-hour E_rC_{50} was 0.37 mg/L, the 72-hour E_yC_{50} was 0.26 mg/L and the 72-

hour E_bC_{50} was 0.25 mg/L. The 96-hour E_rC_{50} was 0.43 mg/L, the 96-hour E_yC_{50} was 0.28 mg/L and the 96-hour E_bC_{50} was 0.26 mg/L. For all endpoints evaluated, the 72- and 96-hour NOEC values were determined to be 0.14 mg/L and the 72- and 96-hour LOEC values were determined to be 0.31 mg/L.

(Soucy K, 2013b)

Following RMS commenting the following table was added for clarity:

Reference	***Guidance followed***	***Current guidance in force***	***Differences from guidance in force***	***Critical assessment***
Soucy 2013, Syngenta file number CGA173506_51017	*OECD 201: (2006) US EPA OCSPP 850.4500: Algal Toxicity (2012)*	*OECD 201: (2006) US EPA OCSPP 850.4500: Algal Toxicity (2012)*	*Compliant with both OECD and US EPA guidelines with caveat for non-green algal species with respect to adherence to validity criteria.*	*Following critical deviations and the validity criteria were not met:* *5. Increase in biomass required :>16 (actual: 47 pooled control).* *6. CV mean growth rate 0-72h required ≤10% (actual: 6.4% pooled control)* *7. CV mean section-by section growth rate 0-72h required ≤35% (actual: 83% pooled control)* *Cell counts for S. costatum are typically more variable than single-cell green algae due to its growth in chains and groups of cells. OECD Guideline 201 does not specifically require testing with marine algae or diatoms, and as such, the acceptance criteria stated in the protocol were based on freshwater algal species mentioned in the guideline. Skeletonema costatum, and diatoms in general, reproduce more slowly than most other algal species. Therefore, the criteria proposed in the test guideline and protocol may not be appropriate for this test organism. Based on this laboratory's experience as well as additional testing laboratories, a failure rate of 69% (sample of 16 tests) has been noted within a position paper describing the OECD acceptance criteria applied to non-green algal species (see appendix in report). Therefore, this result is considered acceptable and clearly defines the toxicity of fludioxonil to S. costatum.* *This study is considered reliable.*

RMS comment:

For the study of Soucy (2013, CGA173506_51017, K-CA 8.2.6.2/03) conducted with fludioxonil on Skeletonema costatum, the reliability of the study should be justified as it is unclear if the amount of solvent in the positive control and the test concentrations is identical.

Syngenta response:

Solvent use details:

A 20 mg/mL primary stock solution was prepared prior to exposure initiation by placing 0.2001 g of fludioxonil in a 10-mL volumetric flask and bringing it to volume with dimethylformamide (DMF, CAS No. 68-12-2). The resulting stock solution was observed to be clear and brown in color with no visible undissolved test substance following multiple shakes and inversions of the flask and approximately five minutes of sonication. The 2.0 mg/L test solution was prepared by adding 0.20 mL of the 20 mg/mL primary stock solution to 2000 mL of AES medium and mixing using a magnetic stir plate and Teflon®-coated stir bar for approximately one hour. After mixing, the 2.0 mg/L test solution was observed to be clear and light yellow in color with a significant amount of fine particulates dispersed throughout the

solution. Due to the presence of undissolved material, this test solution was then filtered using a 2.7-µm membrane filter. This particular size membrane filter was chosen in an attempt to avoid nutrient depletion of the AES medium, which can occur if a smaller pore size, e.g., 0.45-µm membrane filter is used with this medium. The filtrate was observed to be clear and colorless with no visible undissolved test substance and was used to prepare the remaining exposure solutions as noted below:

Filtrate Solution (mg/L)	Volume Filtrate Solution Used (mL)	Diluted to (mL) with Sterile AES Medium	Nominal Concentration (mg/L)
2.0	400	NA[a]	2.0
2.0	500	1000	1.0
2.0	250	1000	0.50
2.0	125	1000	0.25
2.0	65	1000	0.13

[a] NA = Not Applicable.

A solvent (DMF) solution was prepared by adding DMF at a concentration of 0.10 mL/L to AES medium. This solution was used to establish eight replicate flasks for the solvent control. Additional untreated AES medium was used to prepare the control.

In addition, 0.10 mL/L of DMF was added to the AES medium used to dilute the filtrate during preparation of all exposure concentrations. This step maintained a constant solvent concentration (0.10 mL DMF/L) in each test solution.

All test solutions were observed to be clear and light yellow in color with no visible undissolved test substance following preparation with the exception of the 2.0 mg/L test solution, which was observed to be clear and colorless with no visible undissolved test substance.

CA 8.2.7 Effects on aquatic macrophytes

The study previously submitted in the EU was for the Switch formulation A9219B and was summarised in point B.9.2.8 of the DAR from the previous EU review, therefore it is not repeated here.

A new study was conducted on fludioxonil but not submitted during the original EU review as it was not required; it is summarised below.

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints: K-CA 8.2.7/01 Hoberg J (1997). CGA173506 – 14 day toxicity test with duckweed *Lemna gibba*. Report Number 02571-1075. Springborn laboratories Inc. Health and Environmental Sciences, 790 Main street, Wareham, Massachusetts. (Syngenta File No. 66723).

Guidelines

FIFRA Guideline 122-2 and 123-2

GLP: Yes

Executive Summary

The toxicity of CGA173506 to the aquatic plant *Lemna gibba* was determined in a 14-day static test. The *Lemna* were exposed to nominal concentrations of 0.094, 0.19, 0.38, 0.75, and 1.5 mg a.s./L (0.095, 0.16, 0.34, 0.61 and 1 mg a.s./L, initial measured concentrations) alongside a dilution water control.

Based on biomass, the 14 day EC_{50} was determined to be >1.0 mg a.s./L, the highest concentration tested. For biomass The 14 day NOEC was determined to be 1.0 mg a.s./L.

Materials

Test Material	CGA173506 technical
Purity:	95.4%
Description:	Off-white powder
Stability of test compound:	Stable under standard conditions
Batch number:	FL-891552
Treatments	
Test concentrations:	Dilution water control; nominal concentration of 0.094, 0.38, 0.75, and 1.5 mg a.s./L (0.095, 0.16, 0.34, 0.61 and 1 mg a.s./L, initial measured concentrations).
Solvent:	Acetone
Vehicle and/or positive control:	None
Analysis of test concentrations:	Yes, analysis on days 0 and 14
Test organisms	
Species:	*Lemna gibba*
Source:	In-house cultures, originally obtained from University of California, Los Angeles, California.
Test design	
Test vessels:	270 mL crystalizing dishes covered with a petri dish
Test medium:	Hoagland's medium
Replication:	Three vessels for the control and each test concentration. Each vessel was inoculated with five plants each bearing three fronds.
Exposure regime:	Static
Duration:	14 days
Environmental conditions	
Temperature:	25 °C
pH:	4.9 -5.0 at test initiation; 6.1 – 6.3 at test termination
Lighting:	Continuous illumination, 4000 - 5400 Lux

Study Design and Methods

Experimental dates: 4th to 21st March 1997

A stock solution with a nominal concentration of 15 mg ai/L was prepared by dissolving 1.5743 g of the test item completely in 100 mL of acetone. Appropriate volumes of the stock solution were diluted in the test medium to give the test concentration series. The control consisted of culture medium only.

The test solutions were transferred into 270 mL crystalizing dishes and inoculated with five *Lemna gibba* plants each bearing three fronds. Cultures were then transferred to a temperature-controlled room where they were maintained under the conditions indicated above.

At each 3 day interval (i.e. days, 3, 6, 9 and 12) and at test termination (day 14), fronds were counted and observations made. Following test termination, frond densities for each replicate were determined. Fronds were dried at 90ºC for three days prior to dry weight determination.

The test concentrations were verified by chemical analysis of CGA173506 at days 0 and 14, using high performance liquid chromatography with ultra violet-visible detection.

A t-test (Sokal and Rohlf, 1982) was conducted to statically compare the 14 day control frond density and biomass with solvent control data. If the control data were not significantly different, date from the two control sets were pooled. The No observed effect concentration (NOEC), was determined using Williams' test. The data was first checked for normality using Shapiro-Wilk's test, and for homogeneity of variance using Bartlett's test.

The 14 day ECx values were calculated based on frond density and biomass after 14 days of exposure. The ECx values and their 95% confidence intervals were determined by linear regression of response vs. initial measured concentration.

Results and Discussion

At the start of the test, the concentrations of the test item were found to be in the range 70 to 100 % of the nominal values and at the end of the test could not be determined as the values were below the limit of quantification (LOQ =0.025 mg a.s./L. Initial measured concentrations were used for the calculation and reporting of results. Geometric mean measured concentrations have been calculated using a 14d expired concentration of 0.0125 mg a.s./L (half LOQ) value for all test concentrations .

Table 8.2.7-1: Analytical results

Nominal concentrations (mg a.s./L)	Measured concentrations on Day 0 (mg a.s./L)	% of nominal measured at Day 0	Measured concentrations on Day 14 (mg a.s./L)	Geometric mean measured concentrations (mg a.s./L)[a]
0.094	0.095	100	0.0125	0.034
0.19	0.16	83	0.0125	0.045
0.38	0.34	89	0.0125	0.065
0.75	0.61	81	0.0125	0.087
1.5	1.0	70	0.0125	0.11

[a] Not presented in original report. Calculated from data presented in the report.

Mean frond numbers are presented below along with the biomass and respective inhibition values, alongside estimated EC_{50} values:

Table 8.2.7-2: Effect of CGA173506 on biomass (frond number) of *Lemna gibba*

Initial measured concentration (mg/L)	Based on frond number at Day 14			
	Mean no. of fronds per replicate (SD)	Inhibition of frond number (%)	Biomass (g) (SD)	Inhibition of Biomass (%)
Pooled control	379 (37)	-	0.0786 (0.011)	-
0.095	377 (37)	0.53	0.0773 (0.011)	2.2
0.16	339 (27)	10	0.0667 (0.0047)	16
0.34	327 (28)	14	0.0740 (0.0038)	6.3
0.61	360 (16)	4.8	0.0787 (0.0066)	0.38
1.0	400 (11)	-5.7	0.0845 (0.0055)	-7.0
EC_{50} mg/L	>1.0		>1.0 [a]	
NOEC	1.0		1.0 [a]	
LOEC	>1.0		>1.0 [a]	

Inoculum = 12 fronds
inhibition – negative value indicates an increase relative to the control
[a] Endpoints are ≥0.11 mg a.s./L if based on geometric mean measured values.

For the test to be deemed valid there should be a seven-fold increase in seven days in the frond number in the control treatment. In this test there were 379 fronds in the control treatment at 14 days, which represents an increase of approximately 25-fold (starting frond number was 15).

Conclusions

In a test in which *Lemna gibba* were exposed to fludioxonil, based on initial measured for frond number, the 14-day EC_{50} for CGA173506 to *Lemna gibba* was >1.0 mg/L. For biomass, the 14-day EC_{50} was >1.0 mg/L. Based on geometric mean measured concentrations these endpoints are >0.11 mg a.s./L.

The 14 day NOEC was determined to be 1.0 mg/L and the 14-day LOEC was determined to be >1.0 mg/L. Based on geometric mean measured concentrations these endpoints are ≥0.11 mg a.s./L.

(Hoberg J. 1997)

Following RMS commenting the following table was added for clarity:

Reference	***Guidance followed***	***Current guidance in force***	***Differences from guidance in force***	***Critical assessment***
Hoberg 1997 66723	*FIFRA Guideline 122-2 and 123-2*	*OECD No. 221 (2006) or US EPA OPPTS 850.4400 (1996)*	*Not compliant with both OECD and US EPA guidelines*	*Following critical deviations and the validity criteria were not met:* *8. Doubling time required :>2.5d (actual: 15 – 70 over 6d and 14d).* *9. Static test, no media renewal (nutrient deficiency, test concentrations not maintained as <LOQ 0.025 mg/L after 14d)* *Not reliable for risk*

				assessment. Supplementary study to indicate little sensitivity to Lemna at 0.11 mg/L.

RMS comment:

For the static toxicity test with Lemna gibba (Hoberg, 1997, KCA 8.2.7/01): For completeness, it is necessary to provide a calculation of EC50, EC10 and NOEC based on growth rate. In order to fully validate the study, a calculation of the doubling time of frond number in the control as shown in paragraph 49 of the OECD guideline 221 would be helpful.

Syngenta response:

There was no effect at the highest initial measured test concentration of 1 mg/L in the study and the statistical re-analysis by Taylor & Howells (2015b; CGA173506_11839) attempted the calculation of ECx values but was unsuccessful due to lack of inhibition.

The study did fulfil the validity criteria of the time (7-fold increase of frond number in 7 days; 25-fold achieved). It does not comply with current guidance validity criteria:

Does not pass the ≤2.5d doubling time validity criteria.

Control 6d = 30

Control 14d = 15

S. Control 6d = 20

S. control 14d = 70

Pooled control 6d = 19

Poole control 14d = 22

Further calculation is not warranted.

In accordance with **Commission Regulation (EU) No 283/2013**, the 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 8.2.7/02 Taylor S. & Howells A. (2015b) Fludioxonil (CGA173506): 14-Day Toxicity Test with Duckweed, *Lemna gibba* Statistical re-analysis. Report Number: CEA.1607, Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11839)

Summary

The report from Springborn Laboratories, Inc. study number 1781.0197.6548.410 (Hoberg, 1997; Syngenta file number 66723) for the toxicity of CGA173506 to *Lemna gibba* did not provide estimates of the EC_{10} and EC_{20} for the response variables evaluated as part of the original study. Additionally, EC_{50} values were not provided for all the observation intervals. Consequently the data generated in this study was intended to be re-analysed in an attempt to provide these values.

No concentration related effect was observed at any of the time points for yield, growth rate or biomass in the original study and no statistically significant treatments related effects ($P < 0.05$) were reported for any of the parameters evaluated. As a result, no EC_x values could be reliably calculated.

Results

No concentration related effect was observed at any of the time points for yield, growth rate or biomass in the original study and no statistically significant treatments related effects ($P < 0.05$) were reported for any of the parameters evaluated. As a result, no EC_x values could be reliably calculated.

Conclusion

The test used nominal concentrations of 0.094, 0.19, 0.38, 0.75 and 1.5 mg a.s./L. Chemical analysis of the test solutions showed that the measured concentrations at the start of the test ranged from 70% to 100% of the nominal values. All measured concentrations at test termination decreased to below the detectable limit (<0.025 mg a.s./L) therefore initial measured concentrations (0.095, 0.16, 0.34, 0.61 and 1.0 mg a.s./L) were used to define the treatment concentrations.

No concentration related effect was observed at any of the time points for yield, growth rate or biomass in the original study and no statistically significant treatments related effects ($P < 0.05$) were reported for any of the parameters evaluated. As a result, no EC_x values can be reliably calculated.

(Taylor S, Howells A, 2015b)

CA 8.2.8 Further testing on aquatic organisms

No further testing has been conducted. An updated OECD summary has been provided at the request of ANSES during the pre-submission meeting.

Report:	Re-submitted OECD summary at the request of ANSES for ease of dossier re-evaluation: K-CA 8.2.8/01 Giddings, J.M. (1993): CGA173506 Outdoor aquatic microcosm study of the environmental fate and ecological effects, Springborn Laboratories Inc., Wareham, MA, United States, unpublished report No. 92-12-4548, August 13, 1993. (Syngenta File No. CGA173506/0346).

Guidelines: None cited

Deviations: None

GLP: Yes (certified laboratory)

Test Material	Fludioxonil (CGA173506)
Description:	White powder
Lot/Batch No.:	P.905004/5
Purity:	95.4%

Test concentrations:	3.0, 8.2, 16.4 and 32.8 µg a.s./L and a deionised water control, five applications over a period of eight weeks with carryover between treatments
Vehicle and/or positive control:	Deionised water / Acetone
Analysis of test concentrations:	Water and sediment for residue analysis were sampled periodically during and after treatment period. Phytoplankton and periphyton were sampled at four-week intervals. Zooplankton was collected weekly for two weeks before and five weeks after the first treatment and biweekly thereafter. Artificial benthic substrate samples and emergent insect samples were collected at two-week intervals. Sediment core samples were collected three times during the study: 2 days after the 1st application, 1 day after the 3rd application and 26 days after the final application. Effects on fish and aquatic plants were measured at the end of the study.
Test systems:	Twenty-four outdoor aquatic microcosms were established in flat-bottomed fiberglass tanks. Each tank was 3.2 m in diameter (8.0 m^2 in surface area) and 1.5 m deep. When filled to nominal depth of 1.4 m, each tank contained 11.2 m^3 of water from local ponds. Each tank contained 10-cm layer of sediment containing native invertebrate and macrophyte communities. The sediment in each tank covered an area of 4.0 m^2, or approximately one-half (northern half) of the total area of the tank. The southern half of the tank contained no sediment except the settled material that originated within each tank. Each tank was stocked with 40 juvenile bluegill sunfish (*Lepomis macrochirus*) having a mean length of 2.4 cm (range 2.1 - 3.0 cm) and a mean weight of 0.13 g (range 0.07 - 0.32 g), resulting in an initial loading rate of 0.46 g fish/m^3 water. This is well below maximum recommended rates of 2 g – 3 g fish/m^3 (Touart 1988[20], SETAC-Resolve 1992[21], SETAC-Europe 1992[22])
Test design:	
Exposure regime:	Five applications at two-week intervals, on 26 May, 9 June, 23 June, 7 July and 21 July 1992. Water concentrations were measured at regular intervals, and actual measured exposure patterns with five peaks were well in line with the predicted exposure. The test material for Treatment levels 1 to 4 was applied to the microcosms in acetone based solutions. The control microcosms were treated the same way as Treatment levels 1 - 4, except that no test material was added to the acetone.
Treatment levels:	Four treated levels were tested, at nominal initial concentrations of 3.0, 8.2, 16.4 and 32.8 µg a.s./L, respectively, and controls.
Replication:	Five replicates were set up for each treatment level including controls, the highest concentration was tested only in four replicates (total n = 24).

Findings:

WATER QUALITY

[20] Touart, L W (1988) Aquatic mesocosm tests to support pesticide registrations. Hazard Evaluation Division Technical Guidance Document. U.S. Environmental Protection Agency, Washington, DC, Report no. EPA-540/09-88-035.

[21] SETAC-Resolve (1992) Proceedings of a workshop on aquatic microcosms for ecological assessment of pesticides (Wintergreen, Virginia, US, October 1991) SETAC Foundation for Environmental Education and the RESOLVE Program of the World Wildlife Fund, 56 pp.

[22] SETAC-Europe (1992) Guidance document on testing procedures for pesticides in freshwater mesocosms. Society of Environmental Toxicology and Chemistry-Europe, 46 pp.

Mean water temperature during sample week 2 (around first application) ranged from 17.2 to 18.0°C, increasing to 25°C during sample week 11, and then gradually decreasing to 23°C at the end of the study.

Mean dissolved oxygen concentrations ranged between 8.2 and 14.1 mg/L throughout the study.

Mean pH ranged between 8.3 and 8.8 during May through June (weeks 2-7) and generally remained above 8.9 from June (week 11) throughout the remainder of the study. Mean pH peaked during mid-August and early September (weeks 17 and 19), ranging from 9.5 to 9.7.

RESIDUE ANALYSIS

Water

Measured concentrations of fludioxonil in microcosm water collected on the day of the first application were very close to the nominal concentrations (97.1% to 108.9% of nominal), details see Table 8.2.8-1 and Figure 8.2.8-1. Mean maximum concentrations of fludioxonil were 4.2, 11.1, 23.5 and 48.1 µg a.s./L on Day 56, just after the final application (135 – 147% of nominal). The last analytical samples were collected on Day 112, thereafter fell below the limit of quantification (LOQ) at nominal concentrations of 3.0 and 8.2 µg a.s./L. Measured concentrations were very low (<1.6 µg a.s./L) at nominal concentrations of 16.4 and 32.8 µg a.s./L by the end of the study. Measured mean concentrations are listed in Table 8.2.8-1.

Fludioxonil was below the LOQ in all control samples, except for one sample on Day 0. The concentration detected in this sample was 0.34 µg a.s./L, just above the LOQ (<0.26 µg a.s./L). Detection in this control sample may have been due to sample contamination or to an interfering peak, which occurred near the fludioxonil retention time in samples early in the study.

Table 8.2.8-1: Summary of water concentrations (µg a.s./L) over time following exposure to fludioxonil

No. of Appl.[ns]	Days after appl.[n]	Treatment level							
		1		2		3		4	
		Nominal concentrations							
		3.0 µg a.s./L		8.2 µg a.s./L		16.4 µg a.s./L		32.8 µg a.s./L	
		Mean measured concentration (µg a.s./L)	SD	Mean measured concentration (µg a.s./L)	SD	Mean measured concentration (µg a.s./L)	SD	Mean measured concentration (µg a.s./L)	SD
1st	0.1	3.267	0.46	7.985	0.36	15.92	0.56	32.49	2.6
-	1	2.599	0.14	6.692	0.19	13.282	0.33	26.528	0.77
-	7	1.796	0.14	4.643	30.0	9.705	0.63	20.288	1.10
-	14	0.996	0.05	2.665	0.24	5.809	0.41	12.373	0.76
2nd	14.1	3.185	0.53	9.458	0.85	19.528	0.47	39.113	1.40
3rd	28	3.752	0.07	9.999	0.44	20.81	1.10	43.603	1.00
4th	42	4.028	0.10	10.672	0.59	22.46	1.90	47.175	2.50
5th	56	4.229	0.18	11.095	0.79	23.446	1.50	48.128	3.20
-	70	1.502	0.41	3.988	0.90	8.952	1.50	18.863	2.90
-	84	0.626	0.13	1.439	0.62	3.396	0.72	7.526	1.50
-	112	<LOQ	-	<LOQ	-	0.564	0.14	1.213	0.33

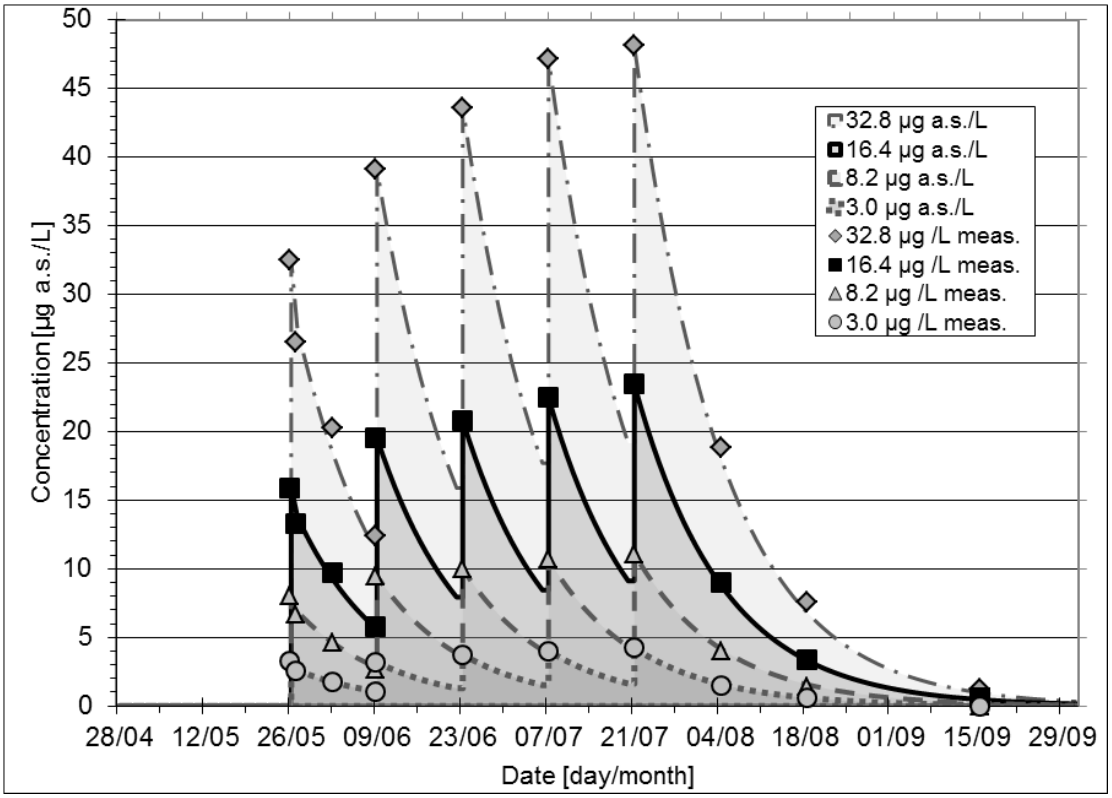


Figure 8.2.8-1: Exposure pattern based on measured concentrations [µg a.s./L]

Measured concentrations (meas.) are displayed as point markers, and interpolated exposure pattern displayed as curves with filled area under the curve, considering first-order degradation with an average DT_{50} of 10 days. Based on this degradation rate measured and interpolated concentrations were well in agreement. The regulatory relevant level (NOEAEC) is treatment level 3, nominally 16.4 µg a.s/L (marked with black squares).

The half-life of fludioxonil in water was calculated from the slope of the linear regression of ln(concentration) vs. time. Calculated half-lives for days 0-14 and days 56-84 averaged 9.8 and 10 days, respectively. With a half-life of 10 days, approximately 38% of the initial concentration of fludioxonil would remain in the water column after 14 days. Repeated applications of fludioxonil to the microcosms at 14-day intervals therefore resulted in some accumulation of fludioxonil in the water, with concentration maxima (immediately after each application) reaching about 130% to 160% of the nominal concentrations.

The regulatory relevant level (NOAEC) is considered to be 16.4 µg a.s./L (see black squares in Figure 8.2.8-1). Considering a maximum of two or three applications are likely in practice, the microcosm represents a worst case application scenario.

Sediment

Sediment samples were collected after each application (Days 0, 14, 28, 42 and 56), as well as on Days 7, 70, 84 and 112, and measured concentrations of fludioxonil expressed per dry weight of sediment.

Fludioxonil sediment concentrations increased throughout the study but accounted for a small fraction of the total added to a microcosm. Concentrations peaked between Days 70 and 84, with maximum concentrations of 65, 172, 390 and 669 µg a.s./kg for concentrations 3.0 to 32.8 µg a.s./L, respectively. Thereafter concentrations decreased, and on Day 112 mean concentrations were 44, 135, 323 and 477 µg

a.s./kg for concentrations 3.0, 8.2, 16.4, and 32.8 µg a.s./L, respectively. No fludioxonil was detected in any control sediment sample.

Half-life calculations for fludioxonil in sediment ranged from 51 to 154 days.

Fludioxonil in the sediment matrix accounts for only a small fraction (2.9% to 3.4%) of the amount added to each microcosm. This indicates that sediment sorption is unlikely to have played a major role in the fate of fludioxonil in the microcosms.

PHYTOPLANKTON

Endpoints

The following phytoplankton endpoints were assessed in detail in the report: Phytoplankton - chlorophyll-*a* concentrations, phaeophytin concentrations, total biomass (ash-free dry weight) , autotrophic index (chlorophyll/ash-free dw), total (cell densities), Cyanophyceae, Chrysophyceae, Chlorophyceae, Bacillariophyceae (diatoms). Fluctuations over time and between seasons were observed, but overall did not prevent conclusive assessment of potential effects.

Treatment-related effects

Significantly lower chlorophyll-a concentrations were observed at 32.8 µg a.s./L during week 9(June ~ 3rd application), which can be regarded as treatment-related. At this same concentration a non-significant reduction of total phytoplankton densities and Chrysophyceae occurred in weeks 5 and 9; a negative impact on chrysophytes was concluded. As there was no dose-response relation at lower levels, the differences observed at 3.0 and 8.2 µg a.s./L towards the end of the study are not regarded to be treatment-related.

Bacillariophyceae cell densities were significantly lower than control cell densities at 32.8 µg a.s./L during week 9, and are regarded as treatment-related. No effects were seen in the other two major classes of phytoplankton, green algae (Chlorophyceae) and blue green algae (Cyanophyceae). The diversity (taxonomic richness) remained relatively consistent throughout the study, with the number of taxa differentiated per tank ranging between 6 and 13.2 taxa; no treatment-related differences were observed.

PERIPHYTON

Endpoints

The following periphyton endpoints were assessed in detail and presented in tables and figures in the report: periphyton - chlorophyll-*a* concentrations, phaeophytin concentrations, total biomass (ash-free dry weight) , autotrophic index (chlorophyll/ash-free dw), total (cell densities), Cyanophyceae, Chrysophyceae, Chlorophyceae, Bacillariophyceae (diatoms), diversity (taxonomic richness). Fluctuations over time and between seasons were observed, but overall did not prevent conclusive assessment of potential effects. However, exceptionally high control values observed in week 13 together with normal values in all four concentrations, these differences were statistically significant yet are not necessarily treatment related.

Treatment-related effects

Periphyton chlorophyll-*a* concentrations in the microcosms were variable, and there was some uncertainty if any of the observed differences between concentrations and controls were treatment-related. A significant reduction in periphyton chlorophyll-*a* concentrations was apparent in week 13 (July); however exceptionally high chlorophyll-*a* concentrations occurred in the controls (16.4 µg/cm^2) and coincided with normal (but much lower) chlorophyll-*a* concentrations (< 4 µg/cm^2) in all four treatment groups; all

treatment groups were significantly lower than the controls. This effect was not present in preceding or following sampling times. It is not clear if the significant differences were treatment related, or due to a random peak in the controls after the last application. There was no difference four weeks later by week 17 (August). This was reported as a treatment-related effect (now would be classified as Effect Class 2 (short-term effect with recovery).

Periphyton phaeophytin concentrations were variable, and no clear dose-response relation was observed at any time.

Exceptionally high control values were also observed in the cell counts, in particular Chlorophyceae and Cyanophyceae, and also total periphyton cell counts and taxa richness indicated significant deviations between controls and all treatments in week 13, but at none of the adjacent sampling dates. The taxon that contributed most significantly to the pattern at week 13 was Cyanophyceae, significant deviations compared to the controls were observed at 32.8 µg a.s./L. All differences had however disappeared within four weeks. The observed changes at 32.8 µg a.s./L are regarded to be Effect Class 3A.

Conclusion

Periphyton densities were highly variable, which is often observed due to their natural succession patterns on the submersed surfaces; diatom densities were the most consistent, and were unaffected by fludioxonil. The original report considers some weight of evidence for fludioxonil having caused a temporary reduction in periphyton abundance near the end of the treatment period at all treatment levels, as a conservative approach. However, the observed difference coincided with exceptionally high control abundances, and four weeks later all abundances had normalised in all treatment groups and no more dose-related effects could be observed.

MACROPHYTES

Five macrophyte taxa were differentiated, Najas, Chara, two Potamogeton morphotypes and other plants; the two former were the dominant groups, with *Chara* sp being slightly more abundant. Abundance and total weight were similar between all microcosms at the end of the study and no treatment-related effects were observed.

ZOOPLANKTON

Endpoints

Zooplankton samples were taken at weekly intervals during the initial application period and biweekly until the end of the study. The following zooplankton endpoints were assessed in detail and presented in tables and figures in the report: Zooplankton – total abundance, Rotifera, Synchaetidae, *Polyarthra*, *Keratella*, Brachionidae, Ploima, Flosculariaceae, Crustacea, Copepoda, Nauplii (Copepoda), Diaptomidae, Copepodits, Cladocera, Bosmidae, Chydoridae, *Chydorus* spp., and diversity (taxonomic richness). Fluctuations over time and between seasons were observed, but overall did not prevent conclusive assessment of potential effects.

Treatment-related effects

Rotifers were dominant in spring and early summer, whereas crustaceans took over late summer and remained dominant until study termination. *Keratella*, the dominant genus of Brachionidae, was significantly reduced at 32.8 µg a.s./L week 9 (June), see Table 8.2.8-2. Ploima showed a similar pattern, and the effect was also detectable at higher taxonomic levels, i.e. for the family Brachionidae, the Phylum Rotifera and the total zooplankton. In contrast, exposed rotifers of the Order Flosculariaceae did not deviate from control populations.

Table 8.2.8-2: Summary of zooplankton abundance in microcosms following exposure to fludioxonil ~ *Keratella* sp. (individuals/L); geometric mean of all replicates.

Nominal concentrations (µg a.s./L)	Sample event												
	Week 3 5/13	Week 4 5/20	Week5 5/27	Week 6 6/3	Week 7 6/10	Week 8 6/17	Week 9 6/24	Week 11 7/8	Week 13 7/22	Week 15 8/5	Week 17 8/19	Week 19 9/2	Week 21 9/16
Control	2741	207	45	38	88	340	346	386	13	5	5	6	2.5
3.0	2661	117	101	49	90	414	373	528	41	17	2.4	2.0	1.2
8.2	2063	180	78	109	192	961	193	420	34	13	4.2	1.8	0.2
16.4	3140	116	30	72	92	252	285	542	43	4.5	2.1	2.4	0.2
32.8	2244	464	92	67	97	84	60*	41	12	6	2.7	8	1.9

* Significant effect ($p<0.05$), shaded

Crustaceans generally increased throughout the study period, reaching densities of 200 to 400 individuals/L and dominating the zooplankton from the last application (week 13) to the end of the study. Diaptomidae numbers were significantly lower than controls at two occasions, at 3.0 µg a.s./L during week 15 (2 weeks after the last application) and at 32.8 µg a.s./L in week 17 (1 month after the last application). The effect at 32.8 µg a.s./L could be treatment-related but no effects were evident on adjacent sampling dates, also it occurred a long time after last application therefore it is unlikely to be caused by the exposure to fludioxonil. No other significant and dose-response-related differences between control and treated crustacean populations were observed for any taxon at any time.

Conclusion

The zooplankton community was not substantially affected by fludioxonil treatment. However, *Keratella* and some higher taxa were observed to undergo clear short-term treatment-related effects at 32.8 µg a.s./L but recovered completely within eight weeks after the first and four weeks after the last application.

ARTIFICIAL BENTHIC SUBSTRATES

Endpoints

Artificial benthic substrate (ABS) samplers were deployed for four-week periods, except during sample week 9, on 26 June, when the samplers were retrieved after only two weeks.

The following groups were assessed in detail: , Chironomidae , Diptera, Tanypodinae, Chironominae, Pentaneurini, Tanytarsini, Chironomini, *Psectrocladius*, Orthocladiinae, Insecta, Ceratopogonidae, Ephemeroptera, Gastropoda, Total invertebrates, and diversity (taxonomic richness). Insects were the dominant group except for week 9 (late June), when Gastropods dominated. Among insects the order Diptera was most abundant, and Ephemeroptera were also present in substantial numbers on several occasions. Total invertebrate densities were greatest during the last sample week, with densities ranging from 50 to 200 individuals/sampler.

Treatment-related effects

Among Diptera, the Chironomidae were most abundant in the ABS samplers. Densities were highest in week 7 (June) and September, with densities remaining below 21 individuals/sampler (mean of replicates) throughout the study (see Table 8.2.8-4). Taxonomic groups differentiated were Tanypodinae, Chironominae and Orthocladiinae, and individual counts are presented in the report. Pentaneurini was the dominant tribe of Tanypodinae, Chironomini and Tanytarsini were differentiated among Chironominae, and *Psectrocladius* sp. most abundant among the Orthocladiini and Orthocladiinae. While single instances of significant differences to controls were observed for individual concentrations on single sampling dates, they generally did not follow a dose-response pattern and also were not consistent between sampling dates; see Tables 8.2.8-3 to 8.2.8-7 for details.

Table 8.2.8-3: Summary of total benthic abundance (individuals/samplers) on artificial benthic substrates (ABS) following exposure to fludioxonil (geometric means)

Nominal concentrations (µg a.s./L)	Sample event								
	Week5	Week 7	Week 9	Week 11	Week 13	Week 15	Week 17	Week 19	Week 21
Control	10.2	28.2	57.0	32.9	24.0	41.7	66.6	54.6	151.9
3.0	5.0	15.0	35.5	37.5	31.1	20.1	45.3	57.5	194.0
8.2	12.0	24.3	63.7	35.2	35.6	36.0	50.4	56.1	114.2
16.4	22.2	16.2	70.0	47.9	30.1	46.3	95.3	55.0	180.4
32.8	11.7	25.2	32.3	34.6	46.9	16.4*	47.0	49.9	48.3

* Significant effect ($p<0.05$), shaded

Table 8.2.8-4: Summary of Chironominae (individuals/samplers) on artificial benthic substrates (ABS) following exposure to fludioxonil (geometric means)

Nominal concentrations (µg a.s./L)	Sample event								
	Week5	Week 7	Week 9	Week 11	Week 13	Week 15	Week 17	Week 19	Week 21
Control	2.8	6.0	6.4	3	4.9	1.7	3.5	3.3	17.6
3.0	1.3	4.9	1.5	5.8	4.7	2.6	3.8	3.1	14.3
8.2	1.6	4.4	6.2	4.1	3.8	1.4	1.9	4.2	12.1
16.4	5.4	3.8	3.4	9.0	2.7	1.0	2.2	5.7	20.7
32.8	2.1	3.9	2.4	4.6	2.0	1.9	1.9	4.9	2.9

Table 8.2.8-5: Summary of Ephemeroptera (individuals/samplers) on artificial benthic substrates (ABS) following exposure to fludioxonil (geometric means)

Nominal concentrations (µg a.s./L)	Sample event								
	Week5	Week 7	Week 9	Week 11	Week 13	Week 15	Week 17	Week 19	Week 21
Control	0	1.2	2.1	9.0	4.6	7.3	10.6	9.2	33.2
3.0	0	0.1	2.0	8.2	8.4	3.6	10.3	11.3	59.5
8.2	0	0.5	2.8	6.7	5.1	2.4	9.2	14.2	15.2
16.4	0	0.1	2.2	15.5	6.8	10.5	22.3	10.0	30.8
32.8	0	0.7	2.3	5.6	3.7	1.9	9.9	14.3	11.6

Table 8.2.8-6: Summary of Gastropoda (individuals/samplers) on artificial benthic substrates (ABS) following exposure to fludioxonil (geometric means)

Nominal concentrations (µg a.s./L)	Sample event								
	Week5	Week 7	Week 9	Week 11	Week 13	Week 15	Week 17	Week 19	Week 21
Control	1.7	4.3	29.9	10.0	5.2	17.1	27.9	14.5	37.7
3.0	1.9	3.0	20.6	5.4	6.7	6.1*	10.5	12.9	41.3
8.2	6.1	8.5	36.9	15.3	14.8	24.5	14	8.0	40.5
16.4	8.9	4.9	49.4	40.9	7.9	20.6	24.3	11.1	59.1
32.8	2.4	4.0	10	6.6	32.5	6.0	12.6	6.6	17.9

* Significant effect ($p<0.05$), shaded

Table 8.2.8-7: Summary of invertebrate taxonomic richness (taxa per mesocosm) on artificial benthic substrates (ABS) following exposure to fludioxonil (geometric means)

Nominal concentrations (µg a.s./L)	Sample event								
	Week5	Week 7	Week 9	Week 11	Week 13	Week 15	Week 17	Week 19	Week 21
Control	4.0	7.6	7.6	8.3	7.4	8.4	9.8	11.0	14.4
3.0	3.2	6.4	5.8	8.0	8.4	6.8	9.2	10.4	15.2
8.2	3.8	7.2	8.4	7.0	8.8	7.0	10.0	10.6	14.2
16.4	4.8	5.8	7.4	8.6	8.4	8.6	12.4	11.8	15.8
32.8	4.0	7.8	7.3	7.3	8.3	7.3	10.0	10.3	10.3

No significant treatment-related effects were observed for any of these taxa. Also Ceratopogonidae and Ephemeroptera were not affected by fludioxonil.

Snails (class Gastropoda) were another major group of macroinvertebrates present in ABS samples. On day 15 mean gastropod densities in 32.8 µg a.s./L were lower than those in 3.0 µg a.s./L, but not significantly different from the controls. Gastropoda numbers were significantly lower than in the controls in 3.0 µg a.s./L in August (two weeks after the last application). Gastropoda densities were consistently less than 50% of control over the last six weeks in 32.8 µg a.s./L, although this was not significant. The combination of low Gastropoda numbers and the slightly lower numbers of some Diptera and Ephemeroptera resulted in a significant reduction of total macroinvertebrates in 32.8 µg a.s./L during week 15 (see Table 8.2.8-3).

Taxonomic richness

Taxonomic richness gradually increased over the study period, with densities ranging from 3.2 to 4.8 taxa per microcosm in May to 10.3 to 15.8 taxa per microcosm in September. Taxonomic richness in ABS was not affected by exposure to fludioxonil.

SEDIMENT CORES

Endpoints

Sediment cores were collected only on three occasions. The following groups were assessed in detail: total invertebrates, Insecta, Diptera, Chironomidae, Tanypodinae, Chironominae, Orthocladiinae, Ephemeroptera, Trichoptera, and diversity (taxonomic richness).

Treatment-related effects

No significant effects were observed for any of the taxa assessed. Gastropoda occurred only at low numbers in the sediment cores (<25 individuals/m^2) throughout the study so could not be evaluated in detail in sediment cores (however they were also resent in the ABS samples). Taxonomic richness in sediment cores showed no significant effects from fludioxonil.

EMERGENT INSECTS

Endpoints

Emergence was collected in biweekly intervals. The following groups were assessed in detail: Emerging insects – Insecta, Diptera, Chironomidae, Chironominae, Tanytarsini, Chironomini, Tanypodinae, Orthocladiinae, Podonominae, Ceratopogonidae, Ephemeroptera and diversity (taxonomic richness).

Diptera were by far the most dominant insect group, whereas Ephemeroptera and Trichoptera were present at low numbers.

Treatment-related effects

No significant treatment-related effects were observed in Chironomidae, Diptera or class Insecta. Orthocladiinae and Chironominae were the dominant subfamilies of Chironomidae. Orthocladiinae occurred in lower numbers than in the controls end of June (Week 9); this was not significantly different from controls. Ephemeroptera emergence of 3.0, 8.2 and 32.8 µg a.s./L were significantly lower than control numbers in August, but at 16.4 µg a.s./L so not treatment related.

Conclusion: for Orthocladiinae, Tanytarsini and Chironominae a slight effect could be apparent at 32.8 µg a.s./L; however it is minor and occurred on one sampling occasion..

Conclusion macro-zoo benthos (ABS, sediment cores and emerging insects)

The response of the macroinvertebrate communities to treatment with fludioxonil was most pronounced in the artificial benthic substrate (ABS) data. Significant deviations between treatments and controls were observed only at 32.8 µg a.s./L.

FISH

Survival and Growth

There was no significant effect on the number, weight, length or total biomass of bluegill recovered at the end of the study.

OVERALL CONCLUSION

Measured concentrations were close to nominal concentrations after first application. The DT_{50} calculated as 10 days and remained constant over time. As fludioxonil was applied 5 times at 14d intervals there was some accumulation in the water and concentrations of the penultimate and last application were up to 147% of nominal. Only a small fraction (2.9% to 3.4%) of fludioxonil was found in sediment samples.

Phytoplankton, periphyton and zooplankton showed no pronounced effects up to and including 16.4 µg a.s./L. At 32.8 µg a.s./L significant differences were observed for different taxa / measured parameters at individual sampling dates. Periphyton data were affected by an extreme peak occurring only in the controls in Week 13 (end July). The resulting statistically significant differences may be regarded as potentially treatment-related, but were not supported by preceding or subsequent samples.

The response of the macroinvertebrate communities were assessed by means of ABS, sediment cores and traps for emergent insects. Effects were most pronounced in the artificial benthic substrate (ABS) data. Near the end of the study, densities of many ABS taxa increased in the controls but failed to increase (or increased more slowly) at 32.8 µg a.s./L. This trend occurred in both major Dipteran families (Chironomidae and Ceratopogonidae), within the three chironomid subfamilies (Tanypodinae, Chironominae and Orthocladiinae), and in the two tribes within the Chironominae (Chironomini and Tanytarsini). Ephemeroptera and gastropods were also reduced at 32.8 µg a.s./L near the end of the study. Total macroinvertebrate abundance followed the same trend. Although these differences were not statistically significant (except for total invertebrates on Week 15), the weight of evidence potentially suggests a treatment related effect between temporarily lower abundances of macroinvertebrates in ABS samples at 32.8 µg/L. This assessment is also supported by temporarily lower numbers of emerging insects (in particular some tribes of Chironomidae, but not Ceratopogonidae). For the lower concentrations effects on these taxa can be ruled out. Sediment core samples showed no significant effects on benthic taxa.

The number of surviving bluegill sunfish, length and weight, and total biomass, were not affected directly by fludioxonil, nor indirectly via reductions in zooplankton and macroinvertebrates.

Overall, the pond communities assessed were largely unaffected by the fludioxonil treatment. Only at the 32.8 µg a.s./L concentrations were significantly lower numbers evident for several taxa at several sampling dates, and effects were followed by complete recovery.

Overview of effects

Table 8.2.8-8: Overview of effects - Summary of results at each treatment level following exposure to fludioxonil

Nominal concentration (µg a.s./L)	Relevant peak concentrations (µg a.s./L)	**Response** *(Control: Summary of parameters assessed)* **Effect classes [a] acc. to the EFSA aquatic guidance document 2013[21]**
Control	0	*(Diverse zooplankton community; frequent sampling - at least biweekly)* *(Diverse phytoplankton & periphyton community, 4-wk sampling intervals)* *(Moderately diverse macrozoobenthos community, evaluated via ABS (2-wk sampling intervals) and sediment cores (total of three sampling dates)* *(Emerging insects, several taxa differentiated, 2-wk sampling intervals)* *(Macrophytes; 5 groups differentiated, final harvest),* *(Survival and growth and fish (bluegill), loading rate sufficiently low, final harvest)* *(Physico-chemical parameters)* *(Analytical verification of exposure in water and in sediment) (all treatment levels)*
3.0	4.028, 4.229	Phytoplankton: No significant changes (Effect class 1) Possibly slight effects on periphyton chlorophyll, phaeophytin concentrations; short-term effects (one sampling date only, Effect class 2-3A [b]) No effects on other groups (Effect class 1, overall NOEC)
8.2	10.672, 11.095	Phytoplankton: No significant changes (Effect class 1) Possibly effects on periphyton chlorophyll, phaeophytin concentrations; short-term effects (one sampling date only, Effect class 2-3A [b]) No effects on other groups
16.4	22.460, 23.446	Phytoplankton: No significant changes (Effect class 1) Possibly effects on periphyton chlorophyll, phaeophytin concentrations; short-term effects (one sampling date only, Effect class 2-3A [b]) No effects on other groups (overall NOAEC)
32.8	47.175, 48.128	Phytoplankton chlorophyll-*a* concentrations, Short-term effects (Effect class 3A [b]) Phytoplankton Chrysophyceae, Bacillariophyceae (diatoms): Short-term effects (Effect class 3A) No significant effects on phytoplankton-Chlorophyceae and Cyanophyceae Periphyton chlorophyll and phaeophytin concentrations and Cyanophyceae densities reduced, (one sampling date only, Effect class 2-3A [b]) No significant effects on periphyton-Chlorophyceae and Cyanophyceae Zooplankton: Keratella and family Brachionidae, Ploima, Rotifera, total Zooplankton: pronounced effects; recovery within 56 days after the first and within 28 days after last application, i.e. Effect Class 3A Macrozoobenthos: Distinctly lower numbers of macroinvertebrates in ABS samples (Ephemeroptera, Chironomini, Gastropoda) in week 15. Effects somewhat uncertain as not occurring in preceding and succeeding samples; recovery within 70 days after the first and within 56 days after last application, i.e. Effect Class 3B. Emerging insects (Orthocladiinae, Tanytarsini and Chironominae) Effect Class 1-2 Overall LOAEC. No effects on macrophytes (Chara, Najas, Potamogeton) Effect Class 1 No effects on macrozoobenthos in sediment cores - Effect Class 1 No effects on emerging insects (all taxa except possibly Orthocladiinae, Tanytarsini and Chironominae) - Effect Class 1 No effects on bluegill sunfish (survival, length, individual weight, and total bluegill biomass per t - Effect Class 1

[a] Effect classes in brief, for details see EFSA aquatic guidance document (2013) [21]

Effect class 0: Treatment related effects cannot be evaluated (e.g. insufficient replications, very low numbers, MDD >100%).
Effect class 1: no treatment-related effects demonstrated
Effect class 2 (slight effects) Effects short-term (single sampling dates), quantitatively restricted responses
Effect class 3A: pronounced short-term effects (< 8 weeks), followed by recovery).
Effect class 3B: pronounced effects (> 8 weeks, recovery within 8 weeks after last application).
Effect class 4: Pronounced effect in short-term study; study is too short to demonstrate complete recovery.
Effect class 5A (pronounced long-term effects (> 8 weeks, (recovery > 8weeks after *last* application).
Effect class 5B (pronounced long-term effects without recovery)

[b] Sampling intervals were too long to be certain about the duration of the effects i.e. of Effect class 2 or 3A (see EFSA aquatic guidance document (2013) [21]). However, the significant differences could also be due to a random peak in the controls that occurred after the last application but receded to normal levels within the next four weeks. There were no long lasting effects.

General conclusion

1. After 1st dosing fludioxonil concentrations in the water were 97.1% to 108.9% of nominal and subsequently declined with a DT_{50} of 10 days.

2. Water concentrations accumulated slightly between applications reaching a maximum of 147% of nominal at the 16.4 µg a.s./L concentration.

3. Sorption of fludioxonil to sediments was minimal (3%).

4. Chrysophytes and diatoms in the phytoplankton were affected at 32.8 µg a.s./L. Green and blue green algae were not affected.

5. Periphyton abundance and taxonomic richness were significantly reduced at all treatment levels on one occasion near the end of the treatment period. However, periphyton data were highly variable, and effects did not appear on previous or subsequent sampling events.

6. Among zooplankton, some higher taxonomic units and in particular the rotifer *Keratella* showed consistent effects at 32.8 µg a.s./L.

7. Macroinvertebrates may have been reduced at 32.8 µg a.s./L near the end of the study, but there were few statistically significant differences in macroinvertebrate abundance.

8. Bluegill sunfish survival and growth were unaffected by fludioxonil treatment.

9. Overall, the impact of fludioxonil on the microcosm communities was minor, and occurred mainly at the lowest trophic level (phytoplankton and periphyton) at 32.8 µg a.s./L. Secondary effects may have occurred on the rotifer *Keratella*. Densities of benthic macroinvertebrates might have been reduced near the end of the study at 32.8 µg a.s./L. Fish were unaffected.

10. Based on the data presented the 'no observed ecologically-adverse effect concentration' (NOAEC, see **[23]**) for fludioxonil was nominally 16.4 µg a.s./L. This is a conservative approach, as the measured concentrations peaked at ≥22.46 µg a.s./L and five applications were applied to the microcosms.

[23] 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

An external review of the fludioxonil mesocosm data was conducted. It is summarized below:

Report:	Submitted for purposes of renewal to support mesocosm study: K-CA 8.2.8/02 Brock, T.C.M. (2006): CGA-173506: Outdoor aquatic mesocosm study of the ecological effects, Alterra, Wageningen University and Research centre Centre for Water and Climate, P.O. Box 47, 6700 AA Wageningen, The Netherlands, unpublished report, August 25, 2006. (Syngenta file No. CGA173506_11665).

Guidelines: No official guideline followed

GLP: No

Executive Summary

An unbiased and independent opinion of the experimental pond study described in the report "J.M. Giddings (1993): CGA-173506: Outdoor aquatic microcosm study of the environmental fate and ecological effects", was performed. The opinion paper is based on the fludioxonil data then available.

Single –species toxicity tests

First-tier toxicity data

The "regulatory acceptable concentrations" calculated on basis of the first-tier toxicity data indicate that algae, aquatic invertebrates and fish may be potentially at risk at peak exposure concentrations in water higher than 2.8 – 6.0 µg a.s./L. Potential risks to aquatic invertebrates might occur at long-term exposure concentrations higher than 0.97 µg a.s./L, while risks to fish might occur above long-term concentrations of 4 µg a.s./L. The reported toxicity value for *Chironomus riparius* suggests low risks to sediment-dwelling organisms at fludioxonil concentrations lower than 4 mg/kg dry weight sediment.

Additional toxicity tests

Table 8.2.8-91: Acute and chronic laboratory toxicity data reported* for the fungicide fludioxonil and standard and additional freshwater and saltwater species

	Acute EC_{50} or LC_{50} (µg a.s./L)	Chronic NOEC (µg a.s./L)
Oncorhynchus mykiss (freshwater fish)	329	40
Lepomis macrochirus (freshwater fish)	740	-
Cyprinodon variegatus (saltwater fish)	1200	-
Pimephales promelas (freshwater fish)	-*	39
Daphnia magna (freshwater crustacean)	600	9.7
Mysidopsis bahia (saltwater crustacean)	270	-
Crassostrea virginica (saltwater mollusc)	370	-
Scenedesmus subspicatus (freshwater alga)	930	50
Selenastrum capricornutum (freshwater alga)	28	-

* data available at the time of the evaluation in 2006.

On basis of the four chronic toxicity values mentioned above the calculated median HC_5 and 90% confidence interval is 7.5 (0.6 – 16.9) µg a.s./L. It should be noted, however, that for the application of the Species Sensitivity Distribution (SSD) approach the HARAP guidance document (Campbell et al. 1999) recommends a minimum of 8 toxicity data for different taxonomic groups in case of a plant protection product with a biocidal mode-of-action. The SSD based value of 7.5 µg a.s./L is approximately

a factor of 8 higher than the first-tier “regulatory acceptable concentration” for long-term exposure (0.97 µg a.s./L).
The SSD approach does not take into account the recoverability potential of affected populations as well as possible indirect effects. These aspects can be investigated in micro/mesocosm studies, such as the experimental pond study provided for the fungicide fludioxonil.

Experimental design and "validity" of the fludioxonil experimental pond study

It is concluded that, despite some shortcomings, the outdoor experimental pond study can be used for the ecological risk assessment of fludioxonil to populations of aquatic invertebrates and fish. Due to relatively long intervals between samplings of phytoplankton and periphyton, and the dynamic nature of these short-lived organisms, the experimental pond study does not provide a detailed picture of treatment-related effects on populations of algae. Zooplankton populations, however, usually become affected indirectly if phytoplankton productivity is reduced for longer periods; and these were sampled at shorter intervals so should capture pronounced effects on phytoplankton productivity if there are such.

The design and validity of the test followed a set of criteria which have subsequently been adopted in several guidance documents (e.g. De Jong *et al* 2008 (Dutch Platform doc) and EFSA Aquatic guidance document (2013), point 2.1.6, p. 23), therefore Brock’s assessment of the study is still up to date.

Treatment-related effects observed in the experimental pond study

Nominal treatment levels of 3.0 – 16.4 µg *a.s./L*

The treatment-related responses observed in experimental ponds that received the nominal treatment levels of 3.0, 8.2 and 16.4 µg a.s./L were very similar. In all these treatment levels a statistically significant lower biomass (expressed in pigment concentration and ash-free dry weight per cm2) than in the controls was observed for the periphyton community on glass slides in week 13. This was accompanied by a statistically significant lower taxonomic richness of periphyton in week 13 and a non-significant trend of a lower abundance of blue-green algae in the periphyton on week 13. However, the control samples contained exceptionally high pigment concentrations compared to earlier and later samples, which may also have contributed to the observed statistical differences. Brock regarded the significant deviations to be treatment-related; considering this is a worst-case approach, which may though be justified in view of the long sampling intervals (phytoplankton and periphyton only).

Consistent treatment-related effects on phytoplankton measurement endpoints that showed a clear concentration-response pattern could not be demonstrated in the experimental ponds treated with 3.0, 8.2 and 16.4 µg a.s./L. However, in contrast to the 16.4 µg a.s./L treatment the 3.0 and 8.2 µg a.s./L treatments showed statistically significant reductions in phytoplankton chlorophyll-a and phaeophytin concentrations in week 17. In week 17 a non-significant trend of lower Chrysophyceae densities in the phytoplankton was also observed in the 3.0 and 8.2 µg a.s./L treatments in contrast to the 16.4 µg a.s./L treatment.

Nominal treatment level of 32.8 µg *a.s./L*

Again, at the highest treatment level a statistically significant reduction in biomass (expressed in pigment concentration and ash-free dry weight per cm^2) was observed for the periphyton community on glass slides in week 13, also accompanied by a statistically significant lower taxonomic richness of periphyton in week 13, and a significantly lower abundance of blue-green algae in the periphyton on week 13.

In the 32.8 µg a.s./L test systems, clear treatment-related effects on the phytoplankton community became apparent. A statistically significant lower phytoplankton chl-a biomass was observed on sampling week 9, when also a statistically significant reduction of diatoms, and a non-significant trend of lower densities of total phytoplankton cells could be observed. Although statistically significant reductions in

Chrysophyceae densities could only be demonstrated in week 17, a clear trend of a reduced abundance was observed in the period week 5 – week 17.

At the highest treatment-level statistically significant responses that lasted longer than a single sampling date and that also showed a clear concentration response relationship could not be demonstrated for macro-invertebrate endpoints. However, a few non-significant trends of effects can be mentioned. On artificial substrates the densities of total macro-invertebrates, Chironomidae, Diptera, Tanypodinae, Pentaneurini, and total insects were relatively low compared to controls and other treatments on the last sampling day.

Again, at the highest treatment level no effects on survival, mean individual weight, mean length and total biomass of bluegill sunfish could be observed.

Overall classification of treatment-related effects, and the $NOEC_{community}$ and NOEAEC that can be derived from the experimental pond study

On basis of the univariate analysis of the data sets, an overall $NOEC_{community}$ for periphyton and phytoplankton cannot be derived from the provided experimental pond study, since the lowest treatment level (nominal 3.0 µg a.s./L) resulted in short-term effects on periphytic algae. In case short-term effects that last less than 8 weeks post first application are considered acceptable, the NOEAEC for periphyton and phytoplankton in the experimental pond study can be set at the nominal concentration of 16.4 µg a.s./L. The $NOEC_{community}$ for zooplankton and macro-invertebrates that can be derived from the experimental pond study is the nominal treatment level of 16.4 µg a.s./L. The NOEC for juvenile bluegill sunfish is ≥ 32.8 µg a.s./L (nominal).

Overall risk assessment on basis of the available lower and higher tier information and possible approaches to refine the risk assessment

On basis of the variability in treatment-related responses observed between different micro/mesocosm experiments performed with the same plant protection product, and when applying the Community Recovery Principle as the protection goal, Brock et al. (2006) suggested to apply an assessment factor of 3 for the spatio-temporal extrapolation of Effect-class 3A effects if only one micro/mesocosm experiment is available. On basis of the same data set, Brock et al. (2006) concluded that ecological threshold levels are remarkably consistent between different micro/mesocosm studies characterised by the same exposure regime. When applying these rules of thumb several "regulatory acceptable concentrations" for short- and long-term exposure can be derived from the fludioxonil experimental pond study.

It appears that the differences in RAC values between different tiers are relatively small. On the basis of the data presented in Table 8.2.8-2 Brock considers that a peak concentration of approximately 8 µg a.s./L can be used as the "regulatory acceptable concentration" for water organisms and short-term exposure, even under repeated pulse exposures. Measurement endpoints related to bluegill sunfish resulted in the lowest "regulatory acceptable concentration" for long-term exposure (>3.29 µg g a.s./L). Note that in the experimental pond study bluegill-sunfish did not show an effect at all and that the observed NOEC on which the long-term RAC is based is a "greater than" value. Furthermore, the first-tier RAC for fish and long-term exposure is 3.9 – 4.0 µg a.s./L.

Brock pointed out that it was possible that an additional semi-field experiment with e.g. juvenile rainbow trout, and in which higher concentration levels are tested, could result in a higher Regulatory Affect Concentrations (RAC) for long-term exposure. The RAC value for long-term exposure based on the responses of algae in the experimental ponds, however, is not much higher (6.6 µg a.s./L) than the first-tier long-term RAC for fish (3.9 – 4.0 µg a.s./L). This value of 6.6 µg a.s./L is based on a more or less conservative interpretation of the responses of periphytic and planktonic algae in the experimental ponds, because of the infrequent sampling regime. Furthermore Brock considered that perhaps an additional

microcosm study with a focus on the responses of phytoplankton and periphyton populations and communities could help to demonstrate that algae are not at risk at realistic exposure concentrations, and that an indoor microcosm experiment may be sufficient for that purpose. Alternatively, Brock suggested that the long-term risk assessment may be refined by performing chronic laboratory toxicity tests with at least 4 additional taxa of taxonomic groups not yet tested (e.g. a diatom, a chrysophycean, a rotifer, an insect) but that might be sensitive on basis of the responses observed in the experimental pond study. Since in the experimental pond study macro-crustaceans, Turbellaria, Hirudinea and Oligochaeta did not occur, it might be wise to perform a screening toxicity test to demonstrate that the results of the experimental pond study are also representative for these taxonomic groups.

Table 8.2.8-102: Overview of "regulatory acceptable concentrations" that can be derived from the different tiers of risk assessment.

	Short-term exposure (Peak concentrations µg a.s./L)	Long-term exposure (TWA concentrations µg a.s./L)
First-tier		
- Alga	2.8	2.8
- Daphnia	6.0	0.97
- Fish	3.29	3.9 – 4.0
SSD-approach		
- All species	8.9	(7.5)*
Experimental pond		
- Primary producers	7.8	6.6
- Invertebrates	23.4	15.8
- Fish	-	≥3.29

* based on 4 chronic NOEC values only

Conclusions

The "regulatory acceptable concentrations" calculated on basis of the first-tier acute toxicity data indicate that algae, aquatic invertebrates and fish may be potentially at risk at peak exposure concentrations in water higher than 2.8 – 6.0 µg a.s/L.

The first-tier chronic toxicity data suggest that potential risks to aquatic invertebrates and fish might occur at long-term exposure concentrations higher than approximately 1 µg a.s./L and 4 µg a.s./L, respectively.

The reported toxicity value for *Chironomus riparius* suggests low risks to sediment-dwelling organisms at fludioxonil concentrations lower than 4 mg/kg dry weight sediment.

A risk assessment on basis of the Species Sensitivity Distribution approach and 8 available acute toxicity values for freshwater and marine species results in a median HC_5 and its 90% confidence interval of 50.5 (8.9 – 123.7) µg a.s./L. The lower limit HC_5 value (in this case 8.9 µg a.s./L) is considered a "regulatory acceptable concentration" for short-term exposure, even under repeated pulse exposure conditions.

Despite some shortcomings, the outdoor experimental pond study can be used for the ecological risk assessment of the test compound to populations of aquatic invertebrates and the introduced 40 juvenile bluegill sunfish. Because of the relatively low frequency of sampling of phytoplankton and periphyton, and the dynamic nature of these short-lived organisms, the experimental pond study does not provide a detailed picture of treatment-related effects on populations of algae. However, the fact that community metabolism (DO, pH) and zooplankton endpoints were not affected at nominal treatment levels up to 16.4 µg/L indicates that the impact on primary producers did not result in pronounced indirect effects.

On basis of the univariate analysis of the data sets, an overall $NOEC_{community}$ for periphyton and periphyton cannot be derived from the provided experimental pond study, since the lowest treatment level (nominal 3.0 µg a.s./L) resulted in short-term effects on periphytic algae. The $NOEC_{community}$ for zooplankton and macro-invertebrates that can be derived from the experimental pond study is the nominal treatment level of 16.4 µg a.s./L. The NOEC for juvenile Bluegill sunfish is ≥ 32.8 µg a.s./L (nominal).

In case short-term effects that last less than 8 weeks post first application are considered acceptable, the NOEAEC for periphyton and phytoplankton in the experimental pond study can be set at the nominal treatment level of 16.4 µg a.s./L.

Brock considered that the differences in "regulatory acceptable concentrations = RAC" between different tiers were relatively small. On basis of the data presented, it seems than the lower limit HC_5 value based on acute toxicity data of all available aquatic species (= 8.9 µg a.s./L) can be used as the "regulatory acceptable concentration" for water organisms and short-term exposure (even under repeated pulse exposures). Measurement endpoints related to fish resulted in the lowest "regulatory acceptable concentration" for long-term exposure of 3.9 – 4.0 µg a.s./L.

Brock furthermore suggested that an additional semi-field experiment with e.g. juvenile rainbow trout, and in which higher concentration levels are tested, might result in an even higher RAC for long-term exposure. The RAC value for long-term exposure based on the responses of algae in the experimental ponds, however, is not much higher (6.6 µg a.s./L) than the first-tier RAC value for fish (3.9 – 4.0 µg a.s./L). This value of 6.6 µg a.s./L is based on a more or less conservative interpretation of the responses of periphytic and planktonic algae in the experimental ponds, because of the infrequent sampling regime. Brock also considered that an additional microcosm study with a focus on the responses of phytoplankton and periphyton populations and communities could be useful to demonstrate that algae are not at risk at realistic exposure concentrations, at least if a higher RAC value for fish can be demonstrated, and that an indoor microcosm experiment may be sufficient for that purpose.

Brock also noted that no populations of macro-crustaceans, Turbellaria, Hirudinea or Oligochaeta occurred in the experimental pond study, and wondered if further screening toxicity tests should be performed to demonstrate that the results of the experimental pond study are also representative for these taxonomic groups.

(Brock T.C.M., 2006)

Report:	Submitted for the purposes of renewal due to a change in data requirements and as requested by the RMS: K-CA 8.2.8/03 Stegger, P., *(2017)*. Statistical evaluation of a Mesocosm Study: Minimum detectable difference (MDD) and calculation and evaluation of (effect classes) NOEC at population level via Williams-test: CGA173506: Outdoor aquatic microcosm study of environmental fate and ecological effects. Institut für Gewaesserschutz MESOCOSM GmbH, Germany. TK0258203. (Syngenta File No CGA173506_12051)

The aim of the report was to evaluate the statistical power of the microcosm study with the test item CGA173506 (Fludioxonil) (Study No: 1781-0392-6303-310: Giddings, J.M. (1993): CGA 173506: Outdoor aquatic microcosm study of the environmental fate and ecological effects, Springborn Laboratories Inc., Wareham, United States, unpublished report No. 92-12-4548, August 13, 1993. Syngenta file No. CGA173506/0346) by calculating the minimum detectable differences (MDD) for

physical parameters, phytoplankton, periphyton, zooplankton, macroinvertebrates, emergent insects, macrophytes and fish. In addition the no observed effect concentrations (NOECs) were calculated by using the Williams test. Afterwards an evaluation of the NOECs was done, regarding the effect class evaluation according to EFSA (2013) and BROCK ET AL. (2014).

A total of 25 different taxa and determined parameters were classified as MDD category 1, 64 taxa and parameters were classified as MDD category 2 and 133 taxa and parameters were classified as MDD category 3 (Table 8.2.8-11). The same taxa determined with different methods (e.g. Orthocladiini unid. were found in MASS samples, sediment cores and emergent insects) were only considered once.

For macrophytes and fish parameters were determined once at the end of the study. For this single date MDD-values were classified according to EFSA–Guideline (EFSA, 2013). For three species of macrophytes the MDD was in the range to determine small to medium effects. For the three parameters determined for bluegill sunfish the MDD was low enough to indicate small effects.

Table 8.2.8-11: Overview – number of taxa of the different MDD categories.

	Category 1	Category 2	Category 3
Physical parameters	3	0	0
Phytoplankton abundance	2	16	38
Phytoplankton biomass	2	0	0
Periphyton abundance	0 (1)	1 (11)	2 (24)
Periphyton biomass	3	1	0
Zooplankton	11	3	18
Macroinvertebrates MASS	1 (4)	16 (20)	38 (52)
Macroinvertebrates sediment cores	0	4 (0)	6 (53)
Emergent insects	3	17	31
Total	25 (29)	64 (72)	133 (216)

() including all taxa determined for this parameter

Based on the calculated NOEC values, an effect class evaluation for all investigated sensitive groups and taxa was carried out. Effect classes were evaluated according to EFSA (2013) and BROCK ET AL. (2014) and are summarized in Table 2 below.

When assessed against current guidelines, there were a number of design deficiencies in the study. The sampling intervals for phytoplankton and periphyton were every four weeks. This makes the statistical assessment of effects and their potential duration very difficult to assess for these two parameters. Due to the uncertainty in the assessment of the effect interval, effects on individual sampling dates were evaluated as an effect class 2/3A i.e. it was unclear if the effect was a short term effect or not. Therefore these were assigned 2/3A* classifications, identified with an asterix, which reflect the limitations in the study design.

Taking this into account, short-term effects followed by recovery (*effect class 2*) could be observed for the sum of *Diaphanosoma* sp. in the two lowest concentrations tested.

A pronounced short term effect followed by recovery (*effect class 3A*) could be observed for the sum of *Diaphanosoma* in the treatment with 16.4 µg a.i./L.

The pronounced short-term effects followed by recovery (effect class 2/3A*), that could be observed for several periphyton parameters in the two lowest treatment levels are based on deficiencies in the sampling interval and does not necessarily reflect the real effect intervals.

Table 8.2.8-12: Summary of effect class evaluation for Fludioxonil.

	3.0 µg a.i./L	8.2 µg a.i./L	16.4 µg a.i./L	32.8 µg a.i./L
Physical parameters				
Dissolved oxygen [mg/L], 0.5 m	1	1	1	1
pH, 0.5 m	1	1	1	1
Temperature [°C] 0.5 m	1	1	1	1
Phytoplankton abundance				
Sum Individuals	1	1	1	3A
Sum Chlorophyceae	1	1	1	1
Sum Chrysophyceae	1	1	1	4B
Ankistrodesmus sp.	1	1	1	1
Kirchneriella sp.	1	1	1	2/3A*
Cyclotella sp.	1	1	1	2/3A*
Phytoplankton Chl a				
Chlorophyll a [µg/cm²]	1	1	1	5B
Phaeophytin [µg/cm²]	1	1	1	1
Periphyton Abundance				
Sum Individuals	1	1	1	3A*
Sum Chlorophyceae	1	1	1	1
Sum Bacillariophyceae	1	1	1	1
Achnanthes sp.	1	1	1	1
Sum Cyanophyceae	2/3A*	2/3A*	2/3A*	2/3A*
Ankistrodesmus sp.	2/3A**	2/3A**	4B**	4B**
Arthrodesmus sp.	1	1	1	1
Aphanizomenon sp.	2/3A**	2/3A**	2/3A**	2/3A**
Periphyton Chl a				
Chlorophyll a [µg/cm²]	2/3A*	2/3A*	2/3A*	2/3A*
Phaeophytin [µg/cm²]	2/3A*	2/3A*	2/3A*	2/3A*
Ash-free dry weight [µg/cm²]	2/3A*	2/3A*	2/3A*	2/3A*
autotrophic index [chlorophyll/ash-free dry weight]	1	1	1	2/3A*
Zooplankton				
Sum Individuals	1	1	1	2
Sum Copepoda	1	1	1	1
Sum Rotatoria	1	1	1	2
Sum *Diaphanosoma* sp.	2	2	3A	4B
Alona sp.	1	1	1	1
Chydorus sphaericus	1	1	1	1
Diaptomus siailis	1	1	1	2
Copepoda copepodites	1	1	2	2
Copepoda Nauplii	1	1	1	2
Filinia sp.	1	1	1	1
Keratella sp.	1	1	1	3A
Lecane sp.	1	1	2	2
Polyarthra sp.	1	1	1	1

	3.0 µg a.i./L	8.2 µg a.i./L	16.4 µg a.i./L	32.8 µg a.i./L
Trichocerca sp.	1	1	1	1
Diaphanosoma brachyurum	1	1	2	3A
Brachionus sp.	1	1	1	2
Macroinvertebrates Artifical Substrat Samplers				
Total Individuals	1	1	1	1
Sum Gastropoda	1	1	1	1
Sum Coleoptera	1	1	1	1
Sum Ephemeroptera	1	1	1	1
Sum Chironomidae	1	1	1	1
Physa sp.	1	1	1	1
Caenis sp.	1	1	1	1
Pentaneurini unid.	1	1	1	1
Tanytarsini unid.	1	1	1	1
Sum Trichoptera	1	1	1	2
Hydroptilidae unid.	1	1	1	2
Macroinvertebrates Sediment Cores				
Sum Individuals	1	1	1	1
Sum Gastropoda	1	1	1	1
Sum Coleoptera	1	1	1	1
Sum Chironomidae	1	1	1	1
Glyptotendipes sp.	1	1	1	1
Hydroptilidae unid.	1	1	1	1
Pentaneurini unid.	1	1	1	1
Staphylinidae unid.	1	1	1	1
Emergent insects				
Total Individuals	1	1	1	1
Sum Chironomidae	1	1	1	1
Chironomini unid.	1	1	1	1
Orthocladiini unid.	1	1	1	1
Tanytarsini unid.	1	1	1	2

* based on the deficiency in the sampling regime,
** based on both the deficiency in the sampling regime and low abundance

Legend:	
1	effect class 1: no treatment related effects
2	effect class 2: slight effects
3A	effect class 3A: clear effects with full recovery
4B	effect class 4B: clear effects, recovery could not be demonstrated due to statistical reasons
5B	effect class 5B: clear response of sensitive endpoints and full recovery cannot be demonstrated before termination of the experiment or before the start of the winter period.

(Stegger 2017)

CA 8.3 Effects on Arthropods

CA 8.3.1 Effects on bees

Table 8.3.1-1: Summary of endpoints for fludioxonil for bees

Organism	Test item	Test type	Endpoint		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
Honey bee (Acute)	Fludioxonil	Acute contact	EU	48h EC_{50} >25 µg a.s./bee	Superseded by 2001 study below.	***Hoxter (1993) CGA173506/0255***
	Fludioxonil	Acute Contact	EU	48h EC_{50} >100 µg a.s./bee	**Endpoints used for risk assessment**	***Wainright (2001) CGA173506/5376***
		Acute Oral		48h EC_{50} >100 µg a.s./bee		
	A8207M	Acute Contact	New Study	48h EC_{50} >1000 µg A8207M/bee	**Endpoints used for risk assessment** New EU data requirements	***Kling (2014) A8207M_10350***
		Acute Oral		96h EC_{50} 572 µg A8207M/bee		
	A8240B	Acute Contact	New Study	48h EC_{50} >100 µg A8240B/bee	**Endpoints used for risk assessment**	***Bew (1993) CGA173506/0366***
		Acute Oral		48h EC_{50} >101 µg A8240B/bee		
Honey bee (Chronic)	A8207M	Chronic larval	New Study	8 day NOEC = 2250 mg product/kg diet 8 day NOED = 346.5 µg product/larva/development period (8.32 µg a.s./larva)	New EU data requirements	***Eckert (2015) A8207M_10595***
		Adult chronic		10 d NOEC = 56 mg a.s./kg food 10 d LDD_{50} = 7.08 µg consumed a.s./bee/day 10 d NOED = 1.47 µg consumed a.s./bee/day		***Roig (2014) A8207M_10357***
	A8240D	Chronic larval	New study	8 day NOEC = 375 mg product/kg diet 8 day NOED = 57.8 µg product/larva (28.9 µg a.s./larva)	New EU data requirements	***Eckert (2015) A8240D_12008***
		Adult chronic		10 d LDD_{50} = >44.06 µg consumed a.s./bee/day 10 d NOED 44.06 µg consumed a.s./bee/day 10 d NOEC =3000 mg a.s./kg food		***Roig (2014) A8240D_10460***

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 ~~therefore an additional study summary is not repeated here~~. The endpoints are summarised in Table 8.3.1-1. At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Hoxter, K.A. (1993): Acute Contact Toxicity Study with the Honey Bee, Wildlife International Ltd. (Easton, MD), Easton, United States, unpublished report No. 108-348A, January 12, 1993. (Syngenta File No. CGA173506/0255).*

Guidelines: U.S. Environmental Protection Agency Pesticide Registration Guidelines, Pesticide Assessment Guidelines, FIFRA Subdivision L., Hazard Evaluation: Non-target Insects,Subsection 141-1

Deviations: None

GLP: Yes (certified laboratory)

Test Material	CGA173506 Fludioxonil technical
Lot/Batch #:	FL-891552
Purity:	95.4 %
Treatments	
Test rates:	Contact (nominal): 1.6, 3.1, 6.3, 12.5 and 25 µg fludioxonil/bee
Control:	Contact: untreated or ethanol
Administration:	Contact: cuticular absorption following the application of 2.5 µL droplet applied to abdomen/thorax of each bee
Test organisms	
Species:	*Apis mellifera* (Hymenoptera: Apidae)
Source:	Honey bees from hives maintained by Wildlife International Ltd., Easton, Maryland, USA.
Food:	50 % w/v aqueous sucrose solution, *ad libitum*
Test design	
Test cage description:	Wire mesh cages (11.5 cm height x 4.0 cm in diameter)
Replication:	2
No. of bees/arena :	25
Duration of test:	48 hours
Environmental test conditions	
Temperature:	25.0 – 27.0 °C
Humidity:	52 – 68 % RH
Photoperiod:	Darkness (except for essential procedures conducted in subdued light)

Fludioxonil (CGA 173506), ID N° FL-891552 ARS 16651. Batch No. P905004/5. Purity 95.4%.

Test species: *Apis mellifera* L. (Hymenoptera: Apidae)

One stock dosing solution was prepared for the five doses tested. A primary stock solution, representing the highest dose tested, was prepared by weighing out a calculated amount of fludioxonil and mixing it with sufficient ethanol. Lower concentration dosing solutions were prepared by serial dilution.

Newly emergent adult honeybees, 1-5 days of age, were immobilized with nitrogen gas and administered a dose of the test item in ethanol. Bees in each treatment group received a dose of the test item in a 2 µl droplet administered to the abdomen and/or thorax. Following administration of the test item, the bees in each treatment and control group were placed in test chambers and allowed to recover.

The bees then were observed over the next 48 hours for mortality and signs of toxicity. Observations for mortality and other clinical signs of toxicity were made twice on the day of test initiation (1.25 and 2hrs) and then approximately 24 and 48 hours after test initiation.

Results and Discussion

Contact mortality data for fludioxonil are summarised in the table below:

Table 8.3.1.1.1-1: Summary of acute contact toxicity of CGA173506 to the honeybee

Treatment (µg CGA173506/bee)	Mortality after 48 hours Mean %
Control	6
Solvent control	10
1.6	0
3.1	4
6.3	2
12.5	2
25.0	30
LD_{50}	> 25

Observations

Small numbers of bees were observed as immobile in both control groups during the test. All surviving bees in the control groups appeared normal throughout the test. Honey bees in the 1.6 µg a.s./bee treatment group also appeared normal throughout the test with no mortalities or overt signs of toxicity. In the 3.1 and 25 µg a.s./bee dose groups small numbers of bees were observed as immobile or lethargic during the test. All surviving bees at the 6.3 and 12.5 µg a.s./bee doses appeared normal throughout the test. The pattern of mortality in the 1.6, 3.1, 6.3 and 12.5 µg a.s./bee treatment levels was not dose responsive and did not appear to be treatment related. However, the mortality in the 25 µg a.s./bee dose group may have been treatment related.

The 48-hour no observed effect dose was determined to be 12.5 µg a.s./bee based upon possible treatment related mortality at the 25 µg a.s./bee dose.

(Hoxter 1993)

At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.6 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.2-8 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.2.4.1 Wainwright M, (2001), CGA 173506 Acute toxicity to Honey Bees (Apis mellifera), Report Number 2003721, Huntingdon Life Science Ltd, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. (Syngenta File No. CGA173506/5376).*

Guidelines

EPPO Guideline No. 170. Guideline on Test Methods for Evaluating the Side-effects of Plant Protection Products on Honeybees

GLP: Yes

Executive Summary

The acute oral and contact toxicity of CGA173506 to honeybees was assessed over 48 hours. In both the oral and contact toxicity tests, the 48-hour LD_{50} values could not be calculated but were estimated to be > 100 µg CGA173506/bee, the only concentration tested. No significant sublethal effects (affected bees) were observed throughout the oral and contact tests.

Materials

Test Material	CGA173506 Fludioxonil technical
Lot/Batch #:	P.910007
Purity:	97.9 %
Description:	Brown powder
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	June 2003

Treatments

Test rates:	Oral (nominal): 100 µg CGA173506/bee Contact (nominal): 100 µg CGA173506/bee
Control:	Oral: 50 % (w/v) aqueous sucrose solution or 50 % (w/v) sucrose solution with acetone Contact: untreated or acetone
Toxic standard:	Dimethoate technical (99.8 %) Oral (nominal): 0.01563, 0.03125, 0.0625, 0.125, 0.25, 0.5 µg dimethoate/bee Contact (nominal): 0.01563, 0.03125, 0.0625, 0.125, 0.25, 0.5 µg dimethoate/bee
Administration:	Contact: cuticular absorption following the application of droplets to the dorsal surface of the thorax of each bee Oral: ingestion in aqueous sucrose solution.

Test organisms

Species:	*Apis mellifera* (Hymenoptera: Apidae)
Source:	Worker bees, from a beekeeper (David Francis, W.J. Mead Apiarist Supplies,

	Fowlmere, Cambridgshire), collected the morning before test start
Food:	50 % w/v aqueous sucrose solution, *ad libitum*

Test design

Test cage description:	Wire mesh cages (11.5 cm height x 4.0 cm in diameter)
Replication:	6
No. of bees/arena :	10
Duration of test:	48 hours

Environmental test conditions

Temperature:	25.0 – 27.0 °C
Humidity:	52 – 68 % RH
Photoperiod:	Darkness (except for essential procedures conducted in subdued light)

Study Design and Methods

Experimental dates: 22nd to 24th June 2000

Honeybees (*Apis mellifera*) were exposed to CGA173506 via two routes of administration: contact and oral ingestion. To immobilise the bees during the course of treatment, they were anaesthetised using CO_2. The treatment of the toxic standard was conducted in parallel to the test item treatment.

Contact test procedures: The dosing solution was prepared by dissolving 0.5107 g CGA173506 in 5 mL acetone to obtain a concentration of 100 µg a.s./µL. Bees were treated with 1 µL droplets of the test solution, toxic standard or solvent control applied to the dorsal surface of the thorax using a syringe mounted in a Burkard Manufacturing Company hand micro applicator. The bees were returned to the test unit, allowed to recover and kept in a temperature-controlled room with a continuous supply of 50 % w/v aqueous sucrose solution. A group of untreated control bees was also included in the test.

Oral test procedures: The dosing solution was prepared by dispersing a 250 mL aliquot of the contact test dosing solution in 5 mL of a 50% w/v sucrose solution to achieve a concentration of 5 µg a.s./µL. Bees were starved for 1 hour before treatment, to ensure that they were equal in terms of their gut contents at the start of the test. Each group of bees was offered 200 µL (equivalent to 20 µL/bee) of the test material, or toxic standard dispersed in aqueous sucrose solution. Solvent control bees were fed a solution of 50 % sucrose containing acetone, and control bees were fed only a 50 % (w/v) aqueous sucrose solution. Once all test solutions were consumed, the feeding tubes were replaced with similar tubes containing 50 % w/v aqueous sucrose solution, *ad libitum*. Doses were fully consumed within three and five hours of introduction of the test material and of the toxic standard, respectively, and therefore feeding tubes were not weighed after the feeding period.

In both the contact and oral tests there were 6 replicates per treatment, control and solvent control, and 3 replicates per toxic standard. Mortality and sublethal effects were assessed at 4, 24 and 48 hours for the test material and toxic standard for both oral and contact tests.

The LD_{50} and 95 % confidence limits of the toxic standard were calculated using Probit analysis. The LD_{50} of the test item could not be calculated since mortality from both the oral and contact application was lower than 50 %.

Results and Discussion

Oral mortality data for CGA173506 are summarised in the table below:

Table 8.3.1.1.1-1: Summary of acute oral toxicity of CGA173506 to the honeybee

Treatment (µg CGA173506/bee)	Mortality after 4 hours Mean %	Mortality after 24 hours Mean %	Mortality after 48 hours Mean %
Control	0	1.7	1.7
Solvent control	0	0	1.7
100	1.7	1.7	1.7
LD_{50}	-	> 100	> 100

Contact mortality data for CGA173506 are summarised in the table below:

Table 8.3.1.1.1-2: Summary of acute contact toxicity of CGA173506 to the honeybee

Treatment (µg CGA173506/bee)	Mortality after 4 hours Mean %	Mortality after 24 hours Mean %	Mortality after 48 hours Mean %
Control	0	1.7	1.7
Solvent control	0	0	0
100	0	1.7	1.7
LD_{50}	-	> 100	> 100

No sublethal effects were noted in any of the controls or treatments throughout the study.

Validity criterion

The validity criterion is listed below:

- The mortality in both the contact and oral control was 1.7 % (must be < 10%)

Conclusions

In both the oral and contact toxicity tests, the 48-hour LD_{50} values could not be calculated but were estimated to be > 100 µg CGA173506/bee, the only concentration tested. No significant sublethal effects (affected bees) were observed in the oral and contact tests.

(Wainwright M, 2001)

This study complies with the current validity criteria for acute toxicity testing with honeybees. Therefore this study is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Wainwright, 2001 CGA173506/5376	EPPO No. 170	EPPO No. 170; OECD No. 213 and 214 (1998)	Compliant with OECD 213 and 214	The EPPO validity criteria were met: 1. Mortality in the negative and solvent controls was ≤ 15% (max 1.7%) The OECD validity criteria were also met: 1. Mortality in the controls was ≤ 10% (max 1.7%) 2. LD_{50} of toxic standard meets specified range of 0.10-0.30 µg a.i./bee (0.15-0.16 µg a.i./bee) The study is reliable and the endpoint can be used in the risk assessment.

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)[24].

CA 8.3.1.1.2 Acute contact toxicity

See CA 8.3.1.1.1 above.

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 fludioxonil may lead to minor potential exposure to bees, tests have been carried out with the representative formulation 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 Roig J. (2014) Fludioxonil FS (A8207M) – Honeybee (*Apis mellifera* L.) Chronic Feeding Study. Report Number S023AMC. MITOX Consultants Science Park 408, 1098XH Amsterdam, The Netherlands (Syngenta file No. A8207M_10357).

Guidelines

OECD 213. 1998.

EFSA. 2013

[24] SANCO/10329/2002 rev 2 final 17 October 2002

GLP: Yes

Executive Summary

The effects of A8207M were assessed on young adult honey bees, *Apis mellifera*, in a 10 day chronic feeding test under laboratory conditions.

The LC_{50} was calculated to be 167.15 mg a.s./kg and the NOEC was determined to be 56.00 mg a.s./kg.

The LDD_{50} (Lethal Dietary Dose) was calculated to be 7.08 µg a.s./bee/day and the NOEDD (No Observed Effect Dietary Dose) was determined to be 1.47 µg a.s./bee/day.

Materials

Test Material	A8207M Fludioxonil FS
Lot/Batch #:	SSP3D1566M
Actual content of active ingredient:	Nominal: 25 g/L; analysed: 2.41% w/w or 25.3g/L
Description:	Dark red
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	July 2016
Density:	Nominal: 1052 kg/m^3 Analysed: 1051 kg/m^3
Treatments	
Test rates:	Nominal concentrations of: 8.96, 22.40, 56.00, 140.00 and 350.00 mg a.s./kg
Control:	50 % (w/v) aqueous sucrose solution
Toxic standard:	Dimethoate BAS 152 11 I (nominal: 400 g/L)
Administration:	Oral
Test organisms	
Species:	*Apis mellifera* L. (Hymenoptera: Apidae) (young adult worker bees)
Source:	Maintained at test facility
Food:	Continuously ad libitum 50 % (w/v) aqueous sucrose solution with application solutions renewed every day and source of pollen renewed every 2 days.
Test design	
Test cage description:	Wooden cages (base: 10 x 4 cm, height: 11 cm) front and back side of the cage consisted of a loose transparent pane for observation. The glass plates were loosely fit, for air supply. The cages were lined with filter paper. On top of the cages there were 2 circular openings, one fitted with the feeder and the other closed and only used for introduction of the pollen.
Replication:	*Five*~~One~~ replicates of 10 bees
Duration of test:	10 days
Environmental test conditions	
Temperature:	32.97ºC ± 0.46
Humidity:	56.33% ± 1.16
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 8th to 19th July 2014

To obtain young adult bees a brood comb containing capped cells in an advanced stage was taken out of a honeybee colony and transferred into a climatic chamber. The brood comb also contained some honey and pollen as food for the hatched bees. Once a sufficient number of bees had emerged for the test they were introduced in the test cages and fed for 24 h with 50% sucrose solution. Prior to first application of the test treatments the test cages were checked for dead or moribund bees. Bees were moved from the comb into the units in groups of 10 by using a pooter.

Test solutions were prepared using a 50% (w/v) sucrose deionized water solution. For 10 days the bees were continuously provided with feeding solutions containing either various concentrations of the test substance, a toxic reference or untreated sucrose solution. Feeding solutions were placed in 3 mL syringes, the tips of which had been removed for access, and offered to the bees in each unit *ad libitum*. Bees in one replicate shared the feeding solution and thus received similar doses. Feeding solutions were replaced daily and the amount of feeding solution consumed was determined by weighing the syringe before and after feeding.

Mortality and sub-lethal effects were recorded every 24 h (± 1h) after each application (start of feeding). Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control were also recorded. In the adult 10d chronic studies mortality and sublethal effects were recorded every 24 h after each application (start of feeding). Sublethal effects such as symptoms of poisoning or abnormal behaviour in comparison to the control were recorded according to the following categories:

m = moribund (bees cannot walk and show only very feeble movements of legs and antennae, only weak response to stimulation; e.g light or blowing)
a = affected (bees upright and attempting to walk but with signs of reduced coordination)
c = cramps (bees contracting abdomen or entire body)
ap = apathy (bees show only low or delayed reactions to stimulation e.g. light or blowing; bees are sitting motionless in the unit or are able to walk but not correctly)

The percentage mortality in the control treatment was calculated from the number of dead individuals divided by the number of introduced test organisms. The mortality of the Test Item and Reference Item treatments was not corrected for the corresponding control mortality according to the formula of Abbott (1925) because control mortality was 0%. The number of bees showing sub-lethal effects was observed at the time of assessing the mortality.

Cumulative mortality in the different test item groups at the end of the exposure period was compared to the control group using the Mann-Whitney U test ($\alpha = 0.05$). No statistical tests were performed for the Reference item treatment. This was considered redundant due to the high mortality observed after the exposure period. SPSS Statistics 20 for Macintosh was used for statistical analyses.

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 A8207M to honey bees (*Apis mellifera* L.)

Concentration (mg a.s./kg)	Mean dose per bee per day (µg a.s./bee)	Mean uptake of feeding solution (mg/bee)	Mean Mortality (%)
Control	0.00	29.68	0.0
8.96	0.17	33.08	8
22.40	0.47	32.62	4
56.00	1.47	30.14	4
140.00	5.78	23.25	32*

350.00	14.18	11.66	98*
Reference Item	0.02	28.58	100*
LC_{50} (mg a.s./kg)	167.15 (142.33-194.16)		
NOEC (mg a.s./kg)	56.00		
LOEC (mg a.s./kg)	140.00		
LDD_{50} (µg a.s./bee/day)	7.08 (6.16-8.13)		
NOEDD (µg a.s./bee/day)	1.47		
LOEDD (µg a.s./bee/day)	5.78		

* Statistical significance between treated and control groups: Mann-Whitney U test (α = 0.05)

Sublethal effects in comparison to the control treatment were observed during the study period in the highest test item rate and the reference item treatment.

Validity criteria

According to the latest draft guidance, for a test to be considered valid the following must apply:

- Mean mortality in the control treatment should be ≤15% after 10 days (0% mortality was observed)
- Mean mortality in the toxic reference treatment should be ≥50% (100% mortality was observed)

Conclusions

In a 10-day chronic toxicity test in which adult honeybees were continuously fed solutions of a fludioxonil formulation (A8207M), the NOEC (No Observed Effect Concentration) was determined as 56.00 mg a.s./kg solution. The LC_{50} was calculated as 167.15 (142.33-194.16) mg a.s./kg. The NOEDD (No Observed Effect Dietary Dose) was determined as 1.47 µg a.s./bee/day. The LDD_{50} was calculated as 7.08 (6.16-8.13) µg a.s./bee/day.

(Roig J, 2014)

Report:	Submitted for purposes of renewal due to change in data requirements guidance: K-CA 8.3.1.2/02 Roig J (2014a). Fludioxonil WG (A8240D) – Honeybee (*Apis mellifera* L.) Chronic Feeding Multi-rate Limit Test. Report Number S024AMC. MITOX Consultants Science Park 408, 1098XH Amsterdam, The Netherlands (Syngenta file No. A8240D_11460).

Guidelines

OECD 213. 1998.

EFSA. 2013

GLP: Yes.

Executive Summary

The effects of A8240D were assessed on young adult honey bees, *Apis mellifera*, in a 10 day chronic feeding test under laboratory conditions.

The NOEC (No Observed Effect Concentration) was determined as 3000 mg a.s./kg solution.

The NOEDD (No Observed Effect Dietary Dose) was determined as 44.06 µg a.s./bee/day.

Materials

Test Material	A8240D Fludioxonil WG
Lot/Batch #:	SMO2B013
Actual content of active ingredients:	Nominal: 50% w/w or 500 g/kg analysed: 50.9% w/w or 509 g/kg
Description:	Beige granules
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	May 2015
Density:	n/a
Treatments	
Test rates:	50 % aqueous sucrose solution containing nominal concentrations of: 375, 750, 1500 and 3000 mg a.s./kg
Control:	Untreated 50 % aqueous sucrose solution
Toxic standard:	Dimethoate (batch no. 0001044987)
Administration:	Oral – in 50 % aqueous sucrose solution
Test organisms	
Species:	*Apis mellifera*. (Hymenoptera: Apidae) (young adult worker bees)
Source:	Maintained at test facility
Food:	Continuously ad libitum 50 % (w/v) aqueous sucrose solution with feeding solutions renewed every day and source of pollen renewed every 2 days
Test design	
Test cage description:	Wooden cages (base: 10 x 4 cm, height: 11 cm). Front and back side of the cage consisted of a loose transparent pane to enable observation and air supply. The cages were lined with filter paper. On top of the cages there were 2 circular openings, one fitted with the feeder and the other closed and only used for introduction of the pollen
Replication:	*Five*~~One~~ replicates of 10 bees
Duration of test:	10 days
Environmental test conditions	
Temperature:	32.58ºC ± 0.28
Humidity:	58.38% ± 1.97
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 7th to 20th July 2014

To obtain young adult bees a brood comb containing capped cells in an advanced stage was taken out of a honeybee colony and transferred into a climatic chamber. The brood comb also contained some honey

and pollen as food for the hatched bees. Once a sufficient number of bees had emerged for the test they were introduced in the test cages and fed for 24 h with 50% sucrose solution. Prior to first application of the test treatments the test cages were checked for dead or moribund bees. Bees were moved from the comb into the units in groups of 10 by using a pooter.

Test solutions were prepared using a 50% (w/v) sucrose deionized water solution. For 10 days the bees were continuously provided with feeding solutions containing either various concentrations of the test substance, a toxic reference or untreated sucrose solution. Feeding solutions were placed in 3 mL syringes, the tips of which had been removed for access, and offered to the bees in each unit *ad libitum*. Bees in one replicate shared the feeding solution and thus received similar doses. Feeding solutions were replaced daily and the amount of feeding solution consumed was determined by weighing the syringe before and after feeding.

Mortality and sub-lethal effects were recorded every 24 h (± 1h) after each application (start of feeding). Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control were also recorded. Sublethal effects such as symptoms of poisoning or abnormal behaviour in comparison to the control were recorded according to the following categories:

m = moribund (bees cannot walk and show only very feeble movements of legs and antennae, only weak response to stimulation; e.g light or blowing)
a = affected (bees upright and attempting to walk but with signs of reduced coordination)
c = cramps (bees contracting abdomen or entire body)
ap = apathy (bees show only low or delayed reactions to stimulation e.g. light or blowing; bees are sitting motionless in the unit or are able to walk but not correctly)

The percentage mortality in the control treatment was calculated from the number of dead individuals divided by the number of introduced test organisms. The mortality of the Test Item and Reference Item treatments was not corrected for the corresponding control mortality according to the formula of Abbott (1925) because control mortality was 0%. The number of bees showing sub-lethal effects was observed at the time of assessing the mortality.

No statistical tests were performed. This was considered redundant due to the zero mortality observed after the exposure period in 3 of the doses and 8% mortality in the 375 g a.s/kg dose.

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 A8240D to honey bees (*Apis mellifera* L.)

Dose (mg a.s./kg)	Mean dose per bee per day (µg a.s./bee)	Mean uptake of feeding solution (mg/bee)	Mean Mortality (%)
Control	0.00	27.93	0
375	5.98	30.49	8
750	12.28	25.94	0
1500	23.48	25.62	0
3000	44.06	24.02	0
Reference Item	0.02	19.06	64
NOEC (mg a.s./kg)	3000		
NOEDD (µg a.s./bee/day)	44.06		

Sublethal effects in comparison to the control treatment on bees were only observed on one occasion in the same unit where mortality at the lowest rate occurred. Sublethal effects were observed several times on bees where the reference item solution was offered.

Validity criteria

According to the latest draft guidance, for a test to be considered valid the following must apply:

- Mean mortality in the control treatment should be ≤15% after 10 days (0% mortality was observed)
- Mean mortality in the toxic reference treatment should be ≥50% (64% mortality was observed)

Conclusions

In a 10-day chronic toxicity test in which adult honeybees were continuously fed solutions of a fludioxonil formulation (A8240D), The NOEC was determined as 3000 mg a.s./kg solution. The NOEDD was determined as 44.06 µg a.s./bee/day.

(Roig J. 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.

Since the use of fludioxonil may lead to potential exposure to bees, tests have been carried out with the representative formulation in lieu of the technical active substance, and are summarised below.

There is currently no validated test method in the form of an OECD guideline for the repeated exposure larval study for bees. A guidance document was issued in July 2016 (*OECD Environment, Health and Safety Publications; Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure; Series on Testing and Assessment No. 239 (July, 2016)).* It was published after the studies would have been required to be conducted ready for this submission. The larval study was conducted for the representative fludioxonil formulations (A8207M and A8240D) was for 8 days and was not intended to include assessments of development and reproduction, however sublethal assessments have been included. Both studies are considered valid for the purposes of honey bee larval risk assessment.

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.3.1.3/01 Eckert J (2015). Fludioxonil FS (A8207M) - Honey bee (*Apis mellifera* L.) Larval Toxicity Test (Repeated Exposure). Report Number S15-02449, Eurofins Agrosciences Services EcoChem GmbH, Eutinger Str. 24, 75233 Niefern-Öschelbronn, Germany (Syngenta File No. A8207M_10595).

Guidelines

OECD Draft Guidance Document on Honey Bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure (Version dated April 2015)
OECD guideline No. 237 (2013)

GLP: Yes.

Executive Summary

The objective of this study was to determine the effects of A8207M on the honey bee larvae, *Apis mellifera* L., from repeated feeding exposure in an 8 day *in vitro* test.

On day 8 the NOEC for A8207M was determined as 2250.0mg product/kg diet. On day 8 the NOED for A8207M was calculated as 346.5 µg product/larva/development period.

On day 8 the LC_{50} for A8207M was determined as 4958.5 mg product/kg diet with a lower 95 % confidence limit of 4463.7 mg product/kg diet and an upper 95 % confidence limit of 5530.3 mg product/kg diet.

On day 8 the LD_{50} for A8207M was calculated as 763.6 µg product/larva/development period with a lower confidence limit of 687.4 µg product/larva/development period and an upper confidence limit of 851.7 µg product/larva/development period.

Materials

Test Material	A8207M Fludioxonil FS
Lot/Batch #:	SSP4A1914
Content of a.i. (nominal):	2.4% w/w fludioxonil (25 g/L)
Content of a.i. (analysed):	2.43% w/w fludioxonil (25.5 g/L)
Description:	Liquid/red
Stability of test compound:	Sufficient for the test purpose (at least 1 h)
Reanalysis/Expiry date:	End of February 2018
Density:	1.049 g/mL
Treatments	
Test concentrations/doses:	86.6, 173.3, 346.5, 693.0 and 1386.0 µg product/larva/development period 562.5, 1125.0, 2250.0, 4500.0 and 9000.0 mg product/kg diet
Control:	Untreated
Toxic standard:	Dimethoate (technical) at 48.0 mg dimethoate/kg diet
Application method:	Larvae dosed directly using a micro pipette
Test organisms	
Species:	Worker honey bee larvae *Apis mellifera* carnica POLLMANN.
Age:	First instar at grafting
Source:	Maintained at test facility
Food:	Artificial diet containing glucose, fructose and royal jelly. Test item and toxic standard added to this diet
Test Design	
Test unit description:	Crystal polystyrene grafting cells (NICOTPLAST) having a diameter of 9 mm
Replication:	3
No. of larvae/replicate:	14
Environmental test conditions	

Temperature:	27.3 to 35.1 °C
Humidity:	43.3 - 100.0 %
Photoperiod:	Constant darkness except assessments
Duration of test:	8 days

Study Design and Methods

Experimental dates: 15th to 24th July 2015

The objective of this study was to determine the effects of A8207M on the honey bee larvae, *Apis mellifera* L., from repeated feeding exposure in an 8 day *in vitro* test and to determine the median lethal concentration (LC_{50}), the No Observed Effect Concentration (NOEC) and the corresponding doses (LD_{50} and NOED) for day 8, where possible.

On the first day of the dose response test synchronised honey bee larvae (*Apis mellifera carnica* POLLMANN, first instar) were transferred into 48 well-plates where they were fed a standardised amount of artificial diet. From day 3 until day 6 of the test, five different concentrations of 562.5, 1125.0, 2250.0, 4500.0 and 9000.0 mg product/kg diet were applied to larvae of the test item groups and one single concentration of 48.0 mg dimethoate/kg diet was applied to the larvae of the reference item group with diet B or C. These concentrations are equivalent to cumulative doses of 86.6, 173.3, 346.5, 693.0 and 1386.0 µg product/larva/development period and 7.4 µg dimethoate/larva/development period.

A control group was included in the test and exposed for the same period of time under identical exposure conditions to the untreated artificial diet.

Each treatment group consisted of 42 larvae from three different colonies (each colony representing a replicate). Assessments of mortality were conducted on days 4, 5, 6, 7 and day 8. The presence of uneaten food was qualitatively recorded at the end of the test on day 8. Other observations (larval appearance and size) were recorded to aid in the interpretation of mortality in comparison to the control group.

Samples of the test item stock solution, the application solutions of the test item groups and the control group were taken directly after preparation and before application. These were analysed for the content of fludioxonil in order to verify correct dosing.

Fisher's Exact Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the mortality data of the test item groups and the control group in order to determine the NOEC on day 8. The corresponding No Observed Effect Dose (NOED) was calculated by taking into account the density of the larval diet (1.1 g/cm^3) and the cumulative feeding volume per larva (140 µL diet).

On day 8 the LC_{50} with 95 % confidence limits was calculated by Probit analysis using linear max. likelihood regression. The corresponding LD_{50} was calculated by taking into account the density of the larval diet (1.1 g/cm^3) and the cumulative feeding volume per larva (140 µL diet).

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 A8207M to honeybee larvae

Item applied	Dosage [µg product/larva][1]	Concentration [mg product/kg diet]	Day 8	
			Mean cumulative mortality %	
			Absolute	Correct.
Control	-		0.0	-
Test item	86.6	562.5	0.0	0.0
	173.5	1125.0	0.0	0.0
	346.5	2250.0	0.0	0.0
	693.0	4500.0	38.1*	38.1*
	1386.0	9000.0	97.6*	97.6*
Reference item	7.4	48.0	61.9**	61.9**
Treatment	Endpoints		Day 8	
Test item doses	NOED [µg product/larva/development period]		346.5	
	LD_{50} [µg product/larva/development period]		763.6 (95% CI = 687.4 to 851.7)	
Test item concentrations	NOEC [mg product/kg diet]		2250.0	
	LC_{50} [mg product/kg diet]		4958.5	

1) Based on cumulative doses given from day 3 to day 6.
* Significantly increased compared to control (Fisher's Exact Test with Bonferroni Correction, one-sided greater, α = 0.05)
** Significantly increased compared to control (Fisher's Exact Test, one-sided greater, α= 0.05)

Uneaten food was observed in the three highest test item groups of 2250.0, 4500.0 and 9000.0 mg product/kg diet and in the reference item group. During the mortality assessments other observations such as deviating larval sizes were made in the two highest test item groups that showed affected larvae. As the test item was red colored the larvae of the three highest test item groups of 2250.0, 4500.0, 9000.0 mg product/kg diet showed a darkening of their intestines.

Analytical Verification

The actual analysed concentration was within the required range of 80-120% of the nominal concentration (actual values: 111%), thus confirming correct preparation of the test item stock solution.

Validity Criteria

All of the validity criteria were met:

- Control mortality should be ≤ 15 % for larvae across all control replicates at day 8 (actual value 0.0%)
- Reference item mortality should be ≥ 50 % for larvae across all reference replicates at day 8 (actual value 61.9%)

Conclusions

In an 8-day repeat dose test in which honeybee larvae were provided with A8207M, the NOED was determined to be 346.5 µg product/larva/development period (nominally equivalent to 8.26 µg fludioxonil/larva/development period). The LD_{50} for A8207M was calculated as 763.6 µg product/larva/development period (nominally equivalent to 18.2 µg fludioxonil/larva/development period).

(Eckert J. 2015)

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.3.1.3/02 Eckert J (2015a), Fludioxonil WG (A8240D) - Honey bee (*Apis mellifera* L.) Larval Toxicity Test (Repeated Exposure). Report Number S15-02448, Eurofins Agrosciences Services EcoChem GmbH, Eutinger Str. 24, 75233 Niefern-Öschelbronn, Germany (Syngenta File No. A8240D_12008).

Guidelines

OECD Draft Guidance Document on Honey Bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure (Version dated April 2015)
OECD guideline No. 237 (2013)

GLP: Yes.

Executive Summary

The objective of this study was to determine the effects of A8240D on the honey bee larvae, *Apis mellifera* L., from repeated feeding exposure in an 8 day *in vitro* test.

On day 8 the NOEC for A8240D was determined as 375.0 mg product/kg diet. On day 8 the NOED for A8240D was calculated as 57.8 µg product/larva/development period.

On day 8 the LC_{50} for A8240D was determined as 700.8 mg product/kg diet with a lower 95 % confidence limit of 628.1 mg product/kg diet and an upper 95 % confidence limit of 780.1 mg product/kg diet.

On day 8 the LD_{50} for A8240D was calculated as 107.9 µg product/larva/development period with a lower confidence limit of 96.7 µg product/larva/development period and an upper confidence limit of 119.9 µg product/larva/development period.

Materials

Test Material	A8204D Fludioxonil WG (50)
Lot/Batch #:	SMO3F0004
Content of a.i. (nominal):	50% w/w fludioxonil
Content of a.i. (analysed):	51.1% w/w fludioxonil
Description:	Solid/beige
Stability of test compound:	Sufficient for the test purpose (at least 1 h)
Reanalysis/Expiry date:	End of October 2016
Treatments	
Test concentrations/doses:	28.9, 57.8, 115.5, 231.0 and 297.0 µg product/larva/development period 187.5, 375.0, 750.0, 1500.0 and 3000.0 mg product/kg diet
Control:	Untreated
Toxic standard:	Dimethoate (technical) at 48.0 mg dimethoate/kg diet
Application method:	Larvae dosed directly using a micro pipette

Test organisms

Species:	Worker honey bee larvae *Apis mellifera* L.
Age:	First instar at grafting
Source:	Maintained at test facility
Food:	Artificial diet containing glucose, fructose and royal jelly. Test item and toxic standard added to this diet

Test Design

Test unit description:	Crystal polystyrene grafting cells (NICOTPLAST) having a diameter of 9 mm
Replication:	3
No. of larvae/replicate:	14

Environmental test conditions

Temperature:	27.3 to 35.1 °C
Humidity:	43.3 - 100.0 %
Photoperiod:	Constant darkness except assessments
Duration of test:	8 days

Study Design and Methods

Experimental dates: 15th to 23rd July 2015

The objective of this study was to determine the effects of A8240D on the honey bee larvae, *Apis mellifera* L., from repeated feeding exposure in an 8 day *in vitro* test and to determine the median lethal concentration (LC_{50}), the No Observed Effect Concentration (NOEC) and the corresponding doses (LD_{50} and NOED) for day 8, where possible.

On the first day of the dose response test synchronised honey bee larvae (*Apis mellifera carnica* POLLMANN, first instar) were transferred into 48 well-plates where they were fed a standardised amount of artificial diet. From day 3 until day 6 of the test, five different concentrations of 187.5, 375.0, 750.0, 1500.0 and 3000.0 mg product/kg diet were applied to larvae of the test item groups and one single concentration of 48.0 mg dimethoate/kg diet was applied to the larvae of the reference item group with diet B or C. These concentrations are equivalent to cumulative doses of 28.9, 57.8, 115.5, 231.0 and 297.0 µg product/larva/development period and 7.4 µg dimethoate/larva/development period. The analysed purity was considered for calculation of the reference item concentration. The calculation of the cumulative doses of the lowest four test item groups of 187.5, 375.0, 750.0 and 1500.00 mg product/kg diet based on the applied feeding volume of each alive larva from day 3 until day 6 of 140 µL diet and the density of the diet (1.1 g/cm^3). The calculation of the cumulative dose of the highest test item group of 3000.0 mg product/kg diet based on the applied feeding volume of each alive larva from day 3 until day 5 of 90 µL diet and the density of the diet (1.1 g/cm^3).

A control group was included in the test and exposed for the same period of time under identical exposure conditions to the untreated artificial diet.

Each treatment group consisted of 42 larvae from three different colonies (each colony representing a replicate). Assessments of mortality were conducted on day 4, day 5, day 6, day 7 and day 8. The presence of uneaten food was qualitatively recorded at the end of the test on day 8. Other observations (larval appearance and size) were recorded to aid in the interpretation of mortality in comparison to the control group.

Samples of the test item stock solution, the application solutions of the test item groups and the control group were taken directly after preparation and before application. These were analysed for the content of fludioxonil in order to verify correct dosing.

Fisher's Exact Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the mortality data of the test item groups and the control group in order to determine the No Observed Effect Concentration (NOEC) on day 8. The corresponding No Observed Effect Dose (NOED) was calculated by taking into account the density of the larval diet (1.1 g/cm^3) and the cumulative feeding volume per larva (140 µL diet).

On day 8 the LD_{50} with 95 % confidence limits was calculated by Probit analysis using linear max. likelihood regression. The calculation of the LD_{50} is based on the actual applied cumulative test item dose per larva.

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 A8240D to honeybee larvae

Item applied	Dosage [µg product/larva][1]	Concentration [mg product/kg diet]	Day 8	
			Mean cumulative mortality %	
			Absolute	Corrected
Control	-		0.0	-
Test item	28.9	187.5	0.0	0.0
	57.8	375.0	2.4	2.4
	115.5	750.0	57.1*	57.1*
	231.0	1500.0	100.0*	100.0*
	297.0	3000.0	100.0*	100.0*
Reference item	7.4	48.0	61.9**	61.9**
Treatment	Endpoints		Day 8	
Test item doses	NOED [µg product/larva/development period]		57.8	
	LD_{50} [µg product/larva/development period]		107.9 (95% CI = 96.7 to 119.9)	
Test item concentrations	NOEC [mg product/kg diet]		375.0	
	LC_{50} [mg product/kg diet]		700.8	

[1] Based on cumulative doses given from day 3 to day 6.

* Significantly increased compared to control (Fisher's Exact Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$)

** Significantly increased compared to control (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$)

Uneaten food was observed in the test item groups of 187.5 and 750.0 mg product/kg diet and in the reference item group. During the mortality assessments other observations such as deviating larval sizes were made in the three highest test item groups that showed affected larvae. As the test item was dark colored the larvae of all test item groups showed a darkening of their intestines.

Analytical Verification

The actual analysed concentration was within the required range of 80-120% of the nominal concentration (actual values: 84%), thus confirming correct preparation of the test item stock solution.

Validity Criteria

All of the validity criteria were met:

- Control mortality should be ≤ 15 % for larvae across all control replicates at day 8 (actual value 0.0%)
- Reference item mortality should be ≥ 50 % for larvae across all reference replicates at day 8 (actual value 61.9%)

Conclusions

In an 8-day repeat dose test in which honeybee larvae were provided with A8240D, the NOED was determined to be 57.8 µg product/larva/development period (nominally equivalent to 28.9 µg fludioxonil/larva/development period). The LD_{50} for was estimated to be 107.9 µg product/larva/development period (nominally equivalent to 54 µg fludioxonil/larva/development period).

(Eckert J. 2015a)

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 fludioxonil 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

Formulation studies conducted on non-target arthropods were submitted for the previous EU review to represent the active substance fludioxonil. Endpoints from these studies, and new extended Tier II studies, are presented in Tables 8.3.2-1 and 8.3.2-2. The new formulation studies are summarised in the relevant M-CP Section 10.

Table 8.3.2-1: Summary of Endpoints for Non-Target Arthropods – seed treatment formulations

Test substance	Test species	Substrate	Study type	EU agreed endpoint (Fludioxonil; EFSA Scientific Report, 2007)		Syngenta re-calculated endpoint	Endpoint to be used for the risk assessment	Reference (report number)
A8207M	*Typhlodromus pyri* (predatory mite)		Tier II, 2D exposure scenario	New study	LR_{50} > 2 000 mL formulation/ha	-	Endpoints used for risk assessment	***Fallowfield (2014) A8207M_10341***
	Aphidius rhopalosiphi (parasitic wasp)		Tier II, 2D exposure scenario		LR_{50} > 2 000 mL formulation/ha	-		***Stevens (2014) A8207M_10339***
	Aleochara bilineata (rove beetle)		Tier II, Soil incorporation		NOEC = 5.0 mg formulation/kg soil dw	-		***Röhlig (2014) A8207M_10344***
A8207I	*Poecilus cupreus* adults	Sand (14 d)	Tier I	EU	0% mortality 0% effect (consumption) at 5 g a.s./100 kg seed (307 kg seed/ha)	NOEC = 0.0205 mg a.s./kg dry soil[a]	Superseded by new variant (A8207M) data. Will not be used in the risk assessment.	***Grimm (1999) CGA173506/5115***
A8207I	*Poecilus cupreus* larvae	Sand (77d)	Tier I	EU	0% mortality at 5 g a.s./100 kg seed (943 kg seed/ha)	NOEC = 0.0629 mg a.s./kg dry soil[a]		***Reber (1999) CGA173506/5103***
A8207I	*Aleochara bilineata*	Sand (14d)	Tier I	EU	0% mortality at 5 g a.s./100 kg seed (379 kg seed/ha)	NOEC = 0.0253 mg a.s./kg dry soil[a]		***Grimm (1999) CGA173506/5116***
A8207B	*Aleochara bilineata*	Sand (77d)	Tier I	EU	0% mortality 10% effect (parasitization) at 5 g a.s./100 kg seed (200 kg seed/ha)	NOEC = 0.0133 mg a.s./kg dry soil[a]	Superseded by new variant (A8207M) data. Will not be used in the risk assessment	***Kleiner (1993) CGA173506/0493***
A8207B	*Bembidion lampros* [b]	Sand (15d)	Tier I	EU	3% mortality at 5 g a.s./100 kg seed (225 kg seed/ha)	NOEC = 0.015 mg a.s./kg dry soil[a]		***Klepa and Petto (1994) CGA173506/0423***
A8207B	*Bembidion tetracolum*	Sand (14d)	Tier I	EU	0% mortality 0% food consumption at 5 g a.s./100 kg seed (200 kg seed/ha)	NOEC = 0.0133 mg a.s./kg dry soil[a]		***Mead-Briggs (1993) CGA173506/0411***

Test substance	Test species	Substrate	Study type	EU agreed endpoint (Fludioxonil; EFSA Scientific Report, 2007)		Syngenta re-calculated endpoint	Endpoint to be used for the risk assessment	Reference (report number)
		Sand (14d)	Tier I	EU	10% mortality 18% effect (consumption) at 5 g a.s./100 kg seed (200 kg seed/ha)	EC_{18} = 0.0133 mg a.s./kg dry soil[a]		
A8207B	*Pardosa amentata*	Sand (15d)	Tier I	EU	8% mortality at 5 g a.s./100 kg seed (225 kg seed/ha)	NOEC = 0.015 mg a.s./kg dry soil[a]		***Klepa & Petto (1994) CGA173506/0415***

[a]To conduct a risk assessment according to the published ESCORT 2 document (**Candolfi et al. 2001**)[25] and the **EC Guidance Document on Terrestrial Ecotoxicology** [26] the endpoints were recalculated.
[b] Species was erroneously noted in the endpoints list as *Bembidion tetracolum*
- Not applicable

Table 8.3.2-2: Summary of Endpoints for Non-Target Arthropods – foliar treatment formulations

Test substance	Test species	Study type	EU agreed endpoint (Fludioxonil; EFSA Scientific Report, 2007)		Endpoint to be used for the risk assessment	Reference (author, date, Syngenta Ref)
A8240D	*Typhlodromus pyri*	Tier II leaf disks (2D)	New study	LR_{50} >2000 mL/ha	**Yes**	***Vaughan (2008) A8240D_10864***
	Aphidius rhopalosiphi	Tier II plant (3D)		LR_{50} >2000 mL/ha	**Yes**	***Stevens (2008) A8240D_10971***
A7850C Geoxe 50 WP (48.9%)	*Typhlodromus pyri*	Tier I glass plates [a]	EU	LR_{50} >1000 g/ha (<50% fecundity)	Superseded by new representative formulation (A8240D) data Will not be used in the risk assessment	***Kleiner (2001) CGA173506/5184***
	Aphidius rhopalosiphi	Tier 1 glass plates [a]	EU	LR_{50} >205 g/ha		***Kleiner (2001) CGA173506/5375***

[a] Erroneously stated as sand in EU DAR

Use of Extended Tier II study designs for non-target arthropod testing

Within the ESCORT 2 guidance document it is not recommended to undertake fecundity assessments at Tier 1 due to the inherent variation in the results when test organisms are exposed via glass plates. A number of member states require fecundity assessments as part of the risk assessments for non-target

[25] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

[26] EC Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329, 17 October 2002.

arthropods as this is viewed as being more conservative as they cover potential effects on reproduction that may be evident at use rates considerably lower than those that may result in mortality. However as the risk assessment is conducted with consideration for sub-lethal effects then it is inherently more conservative than the standard Tier 1 assessment. Rather than go through the tiered assessment process it is therefore deemed appropriate to start the assessment using the data generated in the extended laboratory assessments.

CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

The studies previously submitted in the EU were summarised in the previous EU review in point B.9.5 of the DAR therefore additional study summaries are not repeated here. The endpoints are summarised in Table 8.3.2-1 and Table 8.3.2-2.

New data produced on the current representative seed treatment variant is provided in **Document M-CP Section 10** (A8207M) in Section 10.3.2.2.

New data produced on the current representative solo foliar variant is provided in **Document M-CP Section 10** (A8240D) in Section 10.3.2.2.

CA 8.3.2.2 Effects on *Typhlodromus pyri*

The studies previously submitted in the EU were summarised in the previous EU review in point B.9.5 of the DAR therefore additional study summaries are not repeated here. The endpoints are summarised in Table 8.3.2-1 and Table 8.3.2-2.

New data produced on the current representative seed treatment variant is provided in **Document M-CP Section 10** (A8207M) in Section 10.3.2.2.

New data produced on the current representative solo foliar variant is provided in **Document M-CP Section 10** (A8240D) in Section 10.3.2.2.

Relevant Literature on Non-target Arthropods Other Than Bees

No relevant scientifically peer-reviewed open literature could be found on fludioxonil 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: Summary of endpoints for fludioxonil

Organism	Test substance	Test type	Endpoints (mg/kg)		Comment	Reference (author, date, Syngenta Ref)
Earthworm (*Eisenia fetida*)	Fludioxonil	Acute toxicity	EU	LC_{50} >1000	Acute endpoint no longer data requirement	***Rufli (1989) CGA173506/0008*** [b]
	Fludioxonil	Chronic toxicity	EU	NOEC = 20	**Endpoint used for risk assessment**	***Friedrich (2003) CGA173506/5665***
			EC_{10}/EC_{20} [a]	Could not be estimated	-	***Taylor & Allen (2015) CGA173506_11867***

Acute studies were included in the previous EU review in the DAR under section B.9.6.1
[a] Estimation attempted in accordance with **Commission Regulation (EU) No. 283/2013**
[b] Report number and Syngenta reference number incorrect in EU DAR

Table 8.4-2: Summary of endpoints for worm studies with metabolites of fludioxonil

Organism	Test item	Test type	Endpoints (mg/kg)		Comment	Reference (author, date, Syngenta Ref)
Earthworm (*Eisenia fetida*)	CGA192155	Acute toxicity	EU	LC_{50} = 794	Acute endpoint no longer data requirement	***Baetscher (2002) CGA192155/0015***
	CGA265378	Acute toxicity	EU	LC_{50} >1000		***Baetscher (2002) CGA265378/0011***
	CGA192155	Chronic toxicity	New study	NOEC = 26 EC_{10} = 14.3 EC_{20} = 20.7	New data requirement	***Odemer (2015) CGA192155_11296***
	CGA265378	Chronic toxicity	New study	NOEC = 200 [a]		***Kimmel (2015) CGA265378_10014***
	CGA339833	Chronic toxicity	New study	NOEC = 31.3 EC_{10} = 28.5 EC_{20} = 57.9		***Friedrich (2015) CGA339833_10009***
	SYN545245	Chronic toxicity	New study	NOEC = 55.6 EC_{10} = 61 EC_{20} = 91	New data requirement	***Friedrich (2016) SYN545245_10010***

Acute studies were included in the previous EU review in the DAR under section B.9.6.1
New study refers to studies performed since EU review of fludioxonil
[a] It was not possible to derive EC_{10}/EC_{20} values as a significant concentration response could not be plotted

A chronic worm study with the new soil metabolite SYN545245 ~~is currently being~~ has been conducted. The study ~~will be~~ has been submitted and the dossier updated as agreed with the RMS.

Table 8.4-3: Table of endpoints for soil meso- and macro-fauna (other than earthworms)

Organism	Test item	Test type	Endpoints (mg a.s./kg) [c]		Comment	Reference (author, date, Syngenta Ref)
Collembola (*Folsomia candida*)	A8207I	Chronic toxicity	EU	NOEC = 125 Reproduction: EC_{10} = 163 mg a.s./kg (original report appendix)	Superseded by new variant (A8207M) data. Will not be used in the risk assessment	***Friedrich (2002) CGA173506/5515***
			EC_{10}/EC_{20} [a]	~~Could not be determined~~ Not reliable: Reproduction: EC_{10} of 134 mg a.s/kg with 95% CI which could not be determined. Mortality: EC_{10} = 70 mg a.s/kg (95% CI n.d – 185)		***Taylor & Allen (2015) CGA173506_11870***
	A8207M	Chronic toxicity	EU	NOEC = 12.5 EC_{20} = 9.3	**Endpoint used for the risk assessment**	***Friedrich (2005) CGA173506/6294***

Organism	Test item	Test type	Endpoints (mg a.s./kg) [c]		Comment	Reference (author, date, Syngenta Ref)
	A8240D	Chronic toxicity	New study	NOEC = 1000 mg A8240D/kg $EC_{10/20}$ = >1000	New data requirement	***Friedrich ((2015) A8240D_11854***
	CGA192155	Chronic toxicity	New study	NOEC = 53 EC_{10} = 45.5 EC_{20} = 193	New data requirement	***Kimmel (2015) CGA192155_11291***
	CGA265378	Chronic toxicity	New study	NOEC = 29 EC_{10} = 2.86 EC_{20} = 42.7	New data requirement	***Kimmel (2015) CGA265378_10006***
	CGA339833	Chronic toxicity	New study	NOEC = 1000 $EC_{10/20}$ = >1000	New data requirement	***Friedrich ((2015) CGA339833_10003***
	SYN545245	Chronic toxicity	New study	NOEC = 100 $EC_{10/20}$ = >100	New data requirement	***Friedrich (2016) SYN545245_10013***
Soil mite (*Hypoaspis aculeifer*)	A8207M	Chronic toxicity	New study	NOEC = 1000 mg A8207M/kg $EC_{10/20}$ = >1000	Equivalent to 12.5 mg a.s./kg New data requirement	***Schulz (2015) A8207M_10371***
	A8240D	Chronic toxicity	New study	NOEC = 1000 mg A8240D/kg $EC_{10/20}$ >1000	Equivalent to 500 mg a.s./kg New data requirement	***Schulz (2015) A8240D_11849***
	CGA192155	Chronic toxicity	New study	NOEC = 1000 $EC_{10/20}$ = >1000	New data requirement	***Vinall (2015) CGA192155_11289***
	CGA265378	Chronic toxicity	New study	NOEC = 1000 mg a.s/kg $EC_{10/20}$ = >1000	New data requirement	***Vinall (2015) CGA265378_10000***
	CGA339833	Chronic toxicity	New study	NOEC = 500 EC_{10} = 99.4 EC_{20} = 805.2	New data requirement	***Schulz (2015) CGA339833_10004***
	SYN545245	Chronic toxicity	New study	NOEC = 1000 $EC_{10/20}$ = >1000	New data requirement	***Schulz (2016) SYN545245_10007***

New study refers to studies performed since EU review of fludioxonil
[a] This was calculated considering a nominal active substance concentration of 25 g/L and a formulation density of 1.051 g/mL
[b] Summary in CA 8.4.2.1 of the M-CA document
[c] Except where noted

RMS comment:

Minor non-transient metabolites observed but not yet identified in laboratory studies in soil should also be considered in the assessment for soil organisms. If minor non-transient metabolites are identified, ecotoxicological studies would also have to be provided for their assessment.

Syngenta response:

Syngenta believes that there are no minor non-transient metabolites observed within the aerobic metabolism or soil photolysis studies and therefore there is requirement to include in the risk assessment.

Collembolan and soil mite studies with the new soil metabolite SYN545245 ~~are currently being~~ have been conducted. The studies ~~will be~~ have been submitted and the dossier updated as agreed with the RMS.

CA 8.4.1 Earthworms – sub-lethal effects

At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The study previously submitted in the EU (Friedrich 2003, Syngenta reference CGA173506/5665) was summarised below in the previous EU review in point B.9.6.2 of the DAR~~, therefore this is not repeated here~~

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.4.3 Friedrich S (2003). Fludioxonil (CGA 173506): Sublethal toxicity of the technical material to the earthworm Eisenia fetida , Report Number 03 10 48 023, BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. (Syngenta file No. CGA173506/5665).*

Guidelines

ISO 11268-2: 1998

GLP: Yes

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to fludioxonil (CGA173506) the NOEC (based on reproduction and weight change) was determined to be 20 mg a.s./kg soil d.w, the highest concentration tested.

Materials

Test Material	Fludioxonil (CGA173506)
Lot/Batch #:	P.910007
Purity:	96.4% w/w
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	June 2003
Treatments	
Test concentrations:	1.9, 3.8, 5.2, 7.5, 9.4, 11.3 and 20 mg a.s./kg soil dry weight
Control:	Untreated
Toxic standard:	DuPont Benomyl® was tested at 1.25 kg product/ha
Test organisms	
Species:	*Eisenia fetida andrei* (BOUCHÉ)
Age and weight range at test start:	Adult worms (approximately 3 months old with clitellum); 402 – 520 mg/worm
Source:	W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany
Feeding:	3 g air-dried dry and finely ground horse manure weekly for first four weeks. 3 g manure was added for the final four weeks

Test design

Vessels:	Plastic vessel of Bellaplast (inside dimensions: 18.3 cm x 13.6 cm x 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 and 0.5% calcium carbonate. 750 g wet weight soil, corresponding to about 556 g dry weight, of artificial soil was added to each test vessel.
Replication:	4
No. of worms/arena :	10
Duration of test:	8 weeks

Environmental test conditions

Temperature:	18 – 22.0 °C	
pH of soil:	test start:	6.21
	test end:	6.42 – 6.46
Water content of soil:	test start:	35.3 (equivalent to 54.2 % of WHC)
	test end:	35.8 – 36.7 (equivalent to 55.0 – 56.4 % of WHC)
Photoperiod:	16 hours light: 8 hours dark; 600 lux	

Study Design and Methods

Experimental dates: 20th February to 20th March 2003

Sublethal effects were evaluated after incorporation of the test item at seven test concentrations (1.9, 3.8, 5.2, 7.5, 9.4, 11.3, 20 mg a.s./kg d.w.) into artificial soil. A quartz sand-treated control was also included.

The test item CGA 173506 tech. was introduced by mixing the quantity of the test item (approximating purity to 100 %), necessary to obtain the desired concentration, with quartz sand. Sufficient deionised water was added to the artificial soil to bring it to the required water content. Ten adult earthworms (approximately 6 months old with a clitellum) were placed on the substrate surface of each test vessel (4 replicates per treatment concentration).

The number of surviving adult earthworms after 4 weeks, their weight loss/increase after 4 weeks, the number of living juveniles after 8 weeks as well as behaviour (including feeding activity) and pathological symptoms were recorded. From these data, the adult mortality, the biomass increase and the NOEC value were calculated. Statistical analyses were performed using the computer program EASY ASSAY, Multiple Testing (Ratte, 1998). The multiple sequential test procedure after Dunnett-test was used to compare the control with the independent test item groups and shows a non-significant or a significant difference between both.

To demonstrate the sensitivity of the test system, the reference item DuPont Benomyl® is routinely tested at an application rate of 1.25 kg product/ha.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.1-1: Effect of fludioxonil on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg CGA173506/kg soil dry weight)							
	Control	1.9	3.8	5.2	7.5	9.4	11.3	20.0
Mean adult mortality at 28 days (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean biomass increase from 0-28 days (mg)	96.7	117.1	119.0	114.6	117.6	116.6	111.0	120.9
Mean % biomass increase of adults from 0-28 days	20.6	25.0	25.4	24.3	25.1	24.8	23.6	26.2
Mean number of juveniles after 8 weeks	307.5	371.5	356.5	312.5	292.0	289.3	250.5	267.0
Coefficient of variation for reproduction (cv %)	25.12	8.45	18.29	17.61	15.26	39.69	12.66	12.65
NOEC (biomass, reproduction)	20							

The test item caused no statistically significant decrease in worm growth (change in fresh weight after 4 weeks relative to initial fresh weight) relative to the control treatment at any concentration tested.

No statistically significant effects on number of juveniles compared to the control group were recorded for any concentration of CGA173506.

No effects on behaviour or morphology of the adult worms or on feeding activity were observed during the first four weeks of the test.

The NOEC for effects on adult earthworms was evaluated to be 20 mg CGA 173506 tech./kg dry weight of artificial soil substrate.

In the most recent test with the reference item, dated 19.11.02, the number of juveniles was reduced by 91.2 % after 8 weeks of test duration when compared to the control. Consequently, the test system was shown to be capable of demonstrating sublethal effects.

Validity criteria

The test is considered valid as:

- Adult mortality was 0.0 % in the control (< 10% required)
- Weight loss of adult worms was ≤20% (actual was an increase of 20.6)
- The number of juveniles per control replicate was 409, 242, 253 and 326 (> 30 required)
- The coefficient of variation for reproduction in the control was 25.12 % (< 30% required)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to fludioxonil (CGA173506) the NOEC (based on reproduction and weight increase) was determined to be 20 mg test item/kg soil d.w., the highest concentration tested.

(Friedrich S. 2003)

In accordance with **Commission Regulation (EU) No 283/2013**, the 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 8.4.1/01 Taylor, S., Allen M. (2015a) Fludioxonil (CGA173506 tech.) – Sublethal Toxicity of the technical material to the Earthworm *Eisenia fetida* Statistical Re-analysis. Report Number CEA.1683. Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11867).

Summary

The report from BioChem agrar, study number 03 10 48 023 (Friedrich, 2003; Syngenta file number CGA173506/5665), for the growth and reproductive toxicity test of fludioxonil with the earthworm (*Eisenia fetida*), did not provide estimates of the EC_{10} and EC_{20} for the response variables of biomass change, mortality and feeding activity. Consequently the data generated in this study have been re-analysed in an attempt to provide these values.

In line with the approach used in the original report, the nominal exposure concentrations were used in this analysis.

The following was reported from the original report:

- No effects of the test item on adult worm survival (i.e. 0 % mortality) were observed at doses of 1.9, 3.8, 5.2, 7.5, 9.4, 11.3 and 20.0 mg/kg dry weight of soil during 4 weeks.
- The test item caused no statistically significant decrease in worm growth (change in fresh weight after 4 weeks relative to initial fresh weight) relative to the control treatment at any concentration tested; i.e. a weight increase of 25.0 %, 25.4 %, 24.3 %, 25.1 %, 24.8 %, 23.6 % and 26.2 % was recorded at doses of 1.9, 3.8, 5.2, 7.5, 9.4, 11.3 and 20.0 mg/kg, respectively.
- No statistically significant effects on number of juveniles compared to the control group were recorded at doses of 1.9, 3.8, 5.2, 7.5, 9.4, 11.3 and 20.0 mg/kg. No effects on behaviour and morphology of the adult worms and on feeding activity were observed during the first four weeks of the test.

Despite these findings, the data for the number of juveniles suggested a slight treatment related effect may have occurred and this data only was selected for analysis as no treatment related effects were evident for the remaining parameters.

Statistical analysis of the available data for the number of juveniles revealed that no EC_{10} or EC_{20} value could be reliably determined due to the lack of suitable response functions.

Statistical Analysis

A 3-parameter normal cumulative distribution function (Bruce and Versteeg, 1992) was fitted to the original data and a non-linear regression without weighting was performed using the Levenberg-Marquardt algorithm to calculate the function parameters. Analysis of variance (ANOVA) and a test for the lack of fit was then performed to determine the probability that the variance explained by the regression analysis was due to chance. Where p(F:Regression) <= 0.05, a significant amount of the variance is explained by the regression and where p(F:Lack of Fit) > 0.05, there is no significant lack of fit. If both of these criteria are met, the results of the regression analysis are accepted and the point estimates from the 3-parameter normal cumulative distribution function are meaningful.

Results

Reproduction (number of juveniles)

Re-interpretation of the data using Probit analysis with linear-max likelihood regression showed that the dose response was not significant (p(F)= 0.084), supporting the conclusion that no EC_{10} or EC_{20} values could be reliably determined.

Conclusion

No statistically significant (P >0.05) treatment related effects (NOECs) were reported from the original study on any of the parameters evaluated. Despite these findings, the data for the number of juveniles suggested a slight treatment related effect may have occurred and this data only was selected for analysis. Statistical analysis of the available data for the number of juveniles revealed that no EC_{10} or EC_{20} value could be reliably determined due to the lack of suitable response functions.

(Taylor & Allen, 2015)

Metabolites of fludioxonil

New studies conducted since the original EU review are summarised below.

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.4.1/02 Odemer R. (2015), CGA192155 - Effects on Reproduction of *Eisenia fetida* (Annelida: Lumbricidae) in Artificial Soil, Report Number 20140162. Innovative Environmental Services (IES) Ltd Benkenstrasse 260 4108 Witterswil Switzerland (Syngenta file No. CGA192155_11296).

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 CGA192155, the NOEC (based on reproduction) was determined to be 26 mg product/kg soil d.w. The EC_{10}, EC_{20} and EC_{50} values for reproduction were estimated to be 14.3, 20.7 and 42.8 mg product/kg soil d.w., respectively.

Materials

Test Material	CGA192155
Lot/Batch #:	MLA-336/4
Purity:	96% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 June 2018

Treatments

Test concentrations:	1.63, 3.26, 6.5, 13, 26, 52, 104 and 208 mg product/kg dry soil.
Control:	Milli-Q water.
Solvent:	Acetone
Toxic standard:	2.5 mg carbendazim /kg dry soil.
Test organisms	
Species:	*Eisenia fetida*
Age and weight range at test start:	Adults between 2 months and a year with clitellum; Weight range was 250 to 584 mg with a mean weight per worm of 338 mg
Source:	Blades Biological Ltd, United Kingdom
Feeding:	Horse manure one day after test initiation and once a week during the first four weeks of the study. The amount of food given to the worms (between 0-6 g) depended on the consumption.
Test design	
Vessels:	Glass containers (13.5 x 10.5 x 10.5 cm) with loose-fitting glass lids to enable air exchange and minimise evaporation from artificial soil. Each test unit was filled with 650 ± 50 g wet weight of artificial soil (corresponding to 500-600 g dry weight soil).
Substrate:	Artificial soil comprising 10% sphagnum peat, 20 % kaolinite clay, 70 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and <1% calcium carbonate.
Replication:	Test item: 4 Control, Solvent control and reference item: 8
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	20.3 to 24.0 °C
pH of soil:	Test start: 5.5 to 5.9 Test end: 5.3 to 5.5
Water content of soil:	Test start: 33.8 to 38.5 % d.w. (corresponding to 56.9 and 64.8 % of the maximum WHC) Test end: 40.5 to 46.3 % (corresponding to 68.2 and 77.9 % of the maximum WHC)
Photoperiod:	16 hours light (5590 and 9740 lux)

Study Design and Methods

Experimental dates: 11th March to 29th October 2015

A stock solution was prepared by dissolving 2.607 g of test item in in 50 mL acetone. This was serially diluted further in acetone to prepare a series of application solutions. The appropriate amount of ~~stock~~ application solution was applied to 50 g of quartz sand. The sand was for 90 minutes in order for the solvent to evaporate. To obtain the test item treatment concentrations, 10 g quartz sand with the appropriate amount of application solution was added to the corresponding soil.
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 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 test initiation and 4 weeks after test initiation.

Data on the earthworm weight, adult mortality and number of juveniles was tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test and Levene's test ($p > 0.01$), respectively. The Williams Multiple Sequential t-test ($\alpha = 0.05$, one-sided smaller) was used to compare the (solvent) control with the treated groups since the data were normally distributed and homogeneous.

Reproduction NOEC was determined based on the results of the statistical procedure outlined above.

Reproduction and biomass (weight) change EC_{10}, EC_{20} and EC_{50} values and their 95 % confidence limits were calculated, when possible, by using Probit analysis with linear max. likelihood regression. Adult mortality EC_{50} could not be calculated, as the mortality in the control and all treatments was 0.0 %.

The control and solvent control were compared using a Student-t test for Homogeneous Variances ($\alpha = 0.05$). The reference treatment was compared with the solvent control for mortality and biomass (weight increase) with a Student-t test ($\alpha = 0.05$) and the number of juveniles was compared with a Fisher's Exact Binomial Test ($\alpha = 0.05$).

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.1-12 Effect of CGA192155 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg CGA192155/kg soil dry weight)									
	Control	Solvent control	1.63	3.26	6.5	13	26	52	104	208
Mean adult mortality at 28 days (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean % weight increase compared to initiation	5.7	6.4	9.6	3.6	10.9	15.6	14.7	13.3	10.6	15.5
Mean number of juveniles after 8 weeks	100.5	88.8	84.0	69.0	72.0	81.0	67.8	36.3*	11.8*	0.0*
Standard deviation (SD)	18.3	18.2	11.9	21.4	17.8	14.7	8.7	3.9	2.9	0.0
NOEC (biomass)	208									
NOEC (reproduction)	26									
EC_{10} (95% CL)	14.3 (9.7 - 18.3)									
EC_{20} (95% CL)	20.7 (15.6 - 25.2)									

* Statistically significantly lower when compared to the solvent control (Williams Multiple Sequential t-test, $\alpha = 0.05$, one-sided smaller).

Validity criteria

The test is considered valid as:

- Adult mortality was 0.0 % in the solvent control (< 10% required)
- The mean number of juveniles in the solvent control was 88.8 (> 30 required)
- The coefficient of variation for reproduction in the solvent control was 20.5 % (< 30% required)

The mean number of juveniles counted for the reference item was 10.6 which represents an 89% reduction relative to the control. This reduction was also statistically significantly different relative to the control. According to the test guideline, "significant effects should be observed between (a) 1 and 5 mg

active ingredient". The concentration of carbendazim used in this test was 2.5 mg/kg, h ~~H~~ence the worms used in this test were of suitable sensitivity.

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA192155 the NOEC based on effects on reproduction was determined to be 26 mg product/kg dry soil. The NOEC for adult adverse change in biomass was determined to be > 208 mg product/kg dry soil.

The EC_{10}, EC_{20} values for the emerged number of juveniles on Day 56 were calculated to be 14.3 and 20.7 mg CGA192155/kg dry soil, respectively.

(Odemer R. 2015)

RMS comment:

Regarding the chronic toxicity on earthworms with the metabolite CGA 192155 (Odemer, 2015, KCA 8.4.1/02):

-it is unclear if the solvent applied to the controls was in concentrations/amounts consistent with those used in the treatments. Could the notifier precise this point?

-32% effect on reproduction was observed at the statistical NOEC. The risk assessment should be based on the EC10 value.

Syngenta response:

It is apparent that the OECD summary is incorrect and following confirmation of the approach from the CRO, the OECD summary in the MCA has been updated to reflect the report. The solvent concentration was consistent across treatments.

The reported NOEC is 26 mg/kg, where there is 24% reduction in reproduction when compared to the solvent control. The test is guideline compliant adequately meeting the validity criteria for reproduction and coefficient of variation for reproduction. Probit was used for the statistics with the data satisfying the need for normal distribution and homogeneity. Therefore the NOEC is considered to be very robust.

In the risk assessment for A8240D the TERs for CGA192155 range from 440 to 870 using the NOEC which indicates a large margin of safety.

Report:	Submitted for purposes of renewal due to change in data requirements guidance K-CA 8.4.1/03 Kimmel S. (2016), CGA265378 - Effects on Reproduction of *Eisenia fetida* (Annelida: Lumbricidae) in Artificial Soil, Report Number 20140164. Innovative Environmental Services (IES) Ltd Benkenstrasse 260 4108 Witterswil Switzerland (Syngenta file No. CGA265378_10014).

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 CGA265378 the NOEC (based on reproduction) was determined to be 200 mg test item/kg soil d.w. The EC_x (confidence limits in mg test item/kg dry soil) 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*.

Materials

Test Material	CGA265378
Lot/Batch #:	MES 340/2
Purity:	98% (w/w)
Description:	Yellow solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	May 31, 2017
Treatments	
Test concentrations:	1.563, 3.125, 6.25, 12.5, 25, 50, 100 and 200 mg test item /kg soil d.w.)
Control:	Milli-Q water.
Toxic standard:	0.8 mg carbendazim/kg dry soil
Solvent:	Acetone
Test organisms	
Species:	*Eisenia fetida*
Age and weight range at test start:	Adults (< 10 months old) with clitellum; Weight range was 250 to 599 mg with a mean weight per worm of 369 mg
Source:	Blades Biological Ltd, United Kingdom
Feeding:	Between 0 – 6 g horse manure one day after test initiation and once a week for the first four weeks of the test.
Test design	
Vessels:	Plastic containers (17 x 12 x 11.5 cm) covered with perforated lids that enable air exchange Filed with 650 ± 50 g artificial soil w.w.
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 0.1% calcium carbonate.
Replication:	Control and solvent control: 8 Test item and reference item: 4
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	

Temperature:	17.0 to 28.8 °C
pH of soil:	Test start: 5.4 to 5.8 Test end: 6.2 to 6.5
Water content of soil:	Test start: 25.6 to 27.2 % (corresponding to 56.8 and 60.5 % of the maximum WHC) Test end: 28.6 to 33.7 % (corresponding to 63.6 and 74.8 % of the maximum WHC)
Photoperiod:	12 hours light (190 and 270 lux)

Study Design and Methods

Experimental dates: 21st January to 18th September 2015

A stock solution was prepared by dissolving 2.509 g of test item in in 50 mL acetone. This was serially diluted further in acetone to prepare a series of application solutions. The appropriate amount of ~~stock~~ application solution was applied to 50 g of quartz sand. The sand was stood for 90 minutes in order for the solvent to evaporate. To obtain the test item treatment concentrations, 10 g quartz sand with the appropriate amount of application solution was added to the corresponding soil.

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 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 test initiation and four weeks after application.

Data on the earthworm weights, adult mortality and number of juveniles was tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test and Levene's test ($p > 0.01$), respectively. The Williams Multiple Sequential t-test ($\alpha = 0.05$, one-sided smaller) was used to compare the control with the treated groups since the data were normally distributed and homogeneous. Reproduction NOEC was determined based on the results of the statistical procedure outlined above. Due to a lack of effect on mortality, reproduction and biomass of the earthworm *Eisenia fetida,* the EC_x were not determined. Statistical analysis was performed using ToxRat Professional, Version 3.0® ToxRat Solutions GmbH.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.1-~~2~~3: Effect of CGA265378 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg CGA265378/kg soil dry weight)									
	Control	Solvent control	1.563	3.125	6.25	12.5	25	50	100	200
Mean adult mortality at 28 days (%)	6.3	8.8	12.5	7.5	12.5	17.5	15.0	20.0	7.5	15.0
Mean % biomass change of adults from 0-28 days	11	16	9	18	14	25	6	18	13	15
Mean number of juveniles after 8 weeks	107	107	102	104	74	68	65	108	90	89
NOEC (biomass, reproduction)	200									

The EC_{10} values could not be determined for number of juveniles or biomass as there was no clear concentration-response.

Validity criteria

The test is considered valid as:

- Adult mortality was 8.8% in the solvent control (< 10% required)
- The mean number of juveniles per solvent control replicate was 107 (> 30 required)

The mean number of juveniles counted for the reference item was 54 which represents a 50% reduction relative to the control. Unfortunately the concentration of carbendazim used was 0.8 mg/kg which is lower than that recommended by the test guideline. In a study conducted with CGA192155 by the same test facility (Syngenta reference CGA192155_11296) the reference substance demonstrated suitable sensitivity of the earthworms Given that the worms were obtained from the same source for the two studies and that the timespan of the studies overlapped it can be concluded that the earthworms used in this study conducted with CGA265378 were of a suitable sensitivity.

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA265378 no effects on reproduction, mortality or biomass were observed. Therefore, the NOEC was determined to be 200 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*.

(Kimmel S. 2016)

RMS comment:

Regarding the chronic toxicity on earthworms with the metabolite CGA 265378 (Kimmel 2016, KCA 8.4.1/03), it is unclear if the solvent applied to the controls was in concentrations/amounts consistent with those used in the treatments. Could the notifier precise this point?

Regarding the chronic toxicity on earthworms with the metabolite CGA 265378 (Kimmel 2016, KCA 8.4.1/03), -the coefficient of variation in the control was 29% and thus met the validity criteria for CV

below 30% in the control. However, the coefficient of variation in solvent control was 40% with no difference with the blank control. The coefficient of variation of the pooled controls is 34%. The reliability of the study may therefore be questioned.

Syngenta response:

It is apparent that the OECD summary is incorrect and following confirmation of the approach from the CRO, the OECD summary in the MCA has been updated to reflect the report. The solvent concentration was consistent across treatments.

The study is marginally above the criteria for variability but it meets all other guideline requirements. There were no effects at any concentration tested (1.563 to 200 mg/kg soil dw.).

In the risk assessment, the TERs ranged from 4400 to 8700 indicating that there is a very low long term risk to earthworms for this metabolite. As the risk is so far above the trigger the higher variability within the study is considered acceptable.

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.1/04 Friedrich S (2015). CGA339833 - Sublethal Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil, Report Number 15 10 48 227 S, BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany . (Syngenta file No. CGA339833_10009).

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 CGA339833 the NOEC (based on reproduction) was determined to be 31.25 mg test item/kg soil d.w. The EC_{10}, EC_{20} and EC_{50} values for reproduction were estimated to be 28.5, 57.9 and 225 mg test item/kg soil d.w., respectively.

Materials

Test Material	CGA339833
Lot/Batch #:	MES 338/1
Purity:	96% w/w
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	31 May 2019

Treatments

Test concentrations:	7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000 mg test item/kg soil dry weight
Control:	Untreated
Toxic standard:	Nutdazim 50 FLOW (carbendazim, SC 500) was tested at concentrations of 5 and 10 mg product/kg soil dry weight
Test organisms	
Species:	*Eisenia fetida*
Age and weight range at test start:	Adult worms (approximately 3 months old with clitellum) 266 – 439 mg/worm
Source:	W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany
Feeding:	5 g air-dried and finely ground horse manure weekly for first four weeks. A application of 5 g manure for the final four weeks
Test design	
Vessels:	Plastic vessel of Bellaplast (inside dimensions: about 16.5 cm x 12 cm x 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. 810 g wet weight soil, corresponding to about 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	Control: 8 Treated: 4
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	20.2 – 22.0 °C
pH of soil:	test start: 6.09 – 6.24 test end: 5.72 – 6.01
Water content of soil:	test start: 34.9 – 35.1 (equivalent to 56.7 – 57.1 % of WHC) test end: 34.3 – 34.9 (equivalent to 55.8 – 56.7 % of WHC)
Photoperiod:	16 hours light: 8 hours dark; 500 lux

Study Design and Methods

Experimental dates: 20th July to 14th September 2015

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 mixing the test item with a small quantity of quartz sand , such that the required test concentrations were achieved once mixed with the artificial soil (10 g treated sand per replicate). The acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate (10 animals per test vessel).

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any

morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). 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 3.1.0 (Ratte 2015). The EC_{10}, EC_{20} and EC_{50} values (number of juveniles) were estimated by Probit analysis using linear max. likelihood regression. Confidence limits (95 %) of the EC_x values were computed by normal approximation. For identifying the NOEC values the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.1-34: Effect of CGA339833 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg CGA339833/kg soil dry weight)								
	Control	**7.81**	**15.63**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**
Mean adult mortality at 28 days (%)	0.0	2.5	0.0	2.5	0.0	0.0	2.5	0.0	0.0
Mean % biomass change of adults from 0-28 days	35.0	34.0	37.9	34.9	36.9	37.3	32.9	35.2	32.3
Mean number of juveniles after 8 weeks	149.1	152.8	150.8	137.5	116.3*	92.8*	64.8*	44.3*	36.8*
Coefficient of variation for reproduction (cv %)	18.2	24.1	17.7	22.3	25.1	29.3	29.0	57.4	36.8
% difference in reproduction relative to the control	-	-2.4	-1.1	7.8	22.0	37.8	56.6	70.3	75.4
NOEC (biomass)	1000								
NOEC (reproduction)	31.25								
EC_{10}	28.5 (95 % confidence limits 18.0 to 45.0)								
EC_{20}	57.9 (95 % confidence limits 41.5 to 80.7)								

* statistically significant compared to control (Williams-t-test, $p \leq 0.05$, one-sided smaller)
Negative values indicate an increase relative to the control

Validity criteria

The test is considered valid as:

- Adult mortality was 0.0 % in the control (< 10% required)
- The number of juveniles per control replicate was 144, 156, 131, 201, 159, 117, 122 and 163 (> 30 required)
- The coefficient of variation for reproduction was 18.2 % (< 30% required)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA339833 the NOEC (based on reproduction) was determined to be 31.25 mg test item/kg soil d.w. The EC_{10}, EC_{20} and EC_{50} values for reproduction were estimated to be 28.5, 57.9 and 225 mg test item/kg soil d.w., respectively. The NOEC for mortality and biomass were 1000 mg test item/kg soil d.w.

(Friedrich S. 2015)

A chronic worm study with the new soil metabolite SYN545245 ~~is currently being~~ has been conducted. The study ~~will be submitted~~ is summarised below and the dossier updated as agreed with the RMS.

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.1/05 Friedrich S. (2016), SYN545245 - Sublethal Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil with 5 % Peat, Report Number 16 10 48 075 S. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany . (Syngenta file No. SYN545245_10010).

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 SYN545245 the NOEC (based on reproduction) was determined to be 55.6 mg test item/kg soil d.w. The EC_{10}, EC_{20} and EC_{50} values for reproduction were estimated to be 60.6, 90.6 and >100 mg test item/kg soil d.w., respectively.

Materials

Test Material	SYN545245
Lot/Batch #:	MES 427/1
Purity:	98 % w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of October 2017
Density:	n/a
Treatments	
Test rates:	1.6, 2.9, 5.3, 9.5, 17.1, 30.9, 55.6, 100 mg test item/kg soil dry weight
Control:	Untreated
Toxic standard:	Maypon Flow (carbendazim, SC 500)
Test organisms	
Species:	*Eisenia fetida* (subspecies *Eisenia fetida* andrei)
Age and weight range at test start:	adult worms (approximately 3 months old with clitellum);

	267 – 464 mg/worm
Source:	Maintained at test facility, originally: W. Neudorff GmbH KG An der Mühle 3, 31860 Emmerthal, Germany
Feeding:	5 g air-dried and finely ground horse manure weekly for first 4 weeks
Test design	
Vessels:	plastic vessel of Bellaplast (inside dimensions: about 16.5 cm x 12 cm x 6 cm) with a lid pervious to air and light
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.3% calcium carbonate. 750 g wet weight soil, corresponding to about 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	Control: 8 Treated: 4
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	18.0 – 21.9 °C
pH of soil:	test start:5.91 – 6.05 test end: 5.71 – 5.79
Water content of soil:	test start:24.9 – 25.0 (equivalent to 57.9 – 58.1 % of WHC) test end: 24.2 – 24.9 (equivalent to 56.3 – 57.9 % of WHC)
Photoperiod:	540 lux 16 hours light

Study Design and Methods

Experimental dates: 21 January to 17 March 2016

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).

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). 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

3.2.1 (2015). The EC_{10}, EC_{20} and EC_{50} values (number of juveniles) were estimated by Logit analysis using linear max. likelihood regression. Confidence limits (95 %) of the EC_x values were computed by normal approximation. For identifying the NOEC values the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table 8.4.1-5: Effect of SYN545245 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg/kg soil dry weight)								
	Control	1.6	2.9	5.3	9.5	17.1	30.9	55.6	100
Mean adult mortality at 28 days (%)	2.5	2.5	0.0	2.5	2.5	0.0	5.0	2.5	2.5
Mean % biomass change of adults from 0-28 days	31.6	31.9	32.6	29.7	30.5	31.3	34.7	34.1	32.4
Mean number of juveniles after 8 weeks	146.5	137.5	153.8	153.0	137.0	147.8	143.0	134.5	112.0*
Coefficient of variation for reproduction (cv %)	18.9	28.9	15.0	25.2	8.7	20.5	14.2	21.7	16.0
% difference in reproduction relative to the control	-	6.1	-4.9	-4.4	6.5	-0.9	2.4	8.2	23.5
NOEC (mortality)	100								
NOEC (biomass)	100								
NOEC (reproduction)	55.6								
LC_{50}	>100								
EC_{10}	60.6								
EC_{20}	90.6								
EC_{50}	>100								

* statistically significant compared to control (Williams-t-test, $p \leq 0.05$, one-sided smaller)
Negative values indicate an increase relative to the control

Validity criteria

The test is considered valid as:

- Adult mortality was 2.5 % in the control (< 10% required)
- The mean number of juveniles per control replicate was 146.5 (> 30 required)
- The coefficient of variation for reproduction was 18.9 % (< 30% required)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to SYN545245 the NOEC (for reproduction) was determined to be 55.6 mg test item/kg soil d.w. The EC_{10}, EC_{20} and EC_{50} values for reproduction were estimated to be 60.6, 90.6 and > 100 mg test item/kg soil d.w., respectively.

(Friedrich S., 2016)

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

Collembola

The study previously submitted in the EU (*Folsomia candida* study with A8207I (old variant of new representative seed treatment formulation A8207M), Friedrich 2002, Syngenta reference CGA173506/5515) was summarised in the previous EU review in point B.9.7.1 of the DAR, therefore this is not repeated here as it was not used in the risk assessment.

The data for EC_{10} and EC_{20} were contained in the Statistical appendix at the back of the original report. The values are:

Reproduction:

EC_{10} = 163 mg a.s./kg (95% CI 27.9-270.3)
EC_{20} = 222.9 mg a.s./kg (95% CI 64.2 – 353.5).

In accordance with **Commission Regulation (EU) No 283/2013**, the 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 8.4.2.1/01 Taylor, S. & Allen M. (2015b) Fludioxonil (CGA173506) – Toxicity of a 25 g/l FS Formulation (A82071) on the reproduction of the Collembola *Folsomia candida* Statistical Re-analysis. Report Number: CEA.1598, Reference Number CCE2068_011, Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridgeshire, CB23 4NN, UK. (Syngenta File No: CGA173506_11870).

Summary

The report from BioChem Agrar study number 02 10 48 011 (Friedrich, 2002; Syngenta file number CGA173506/5515) for the reproductive toxicity test of fludioxonil on the collembola (*Folsomia candida*) did not provide estimates of the EC_{10} or 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.

The study was conducted at nominal concentrations of 10, 62.5, 125, 250, 500 and 1000 mg a.s./kg. In-line with the approach used in the original study, all reported biological results are based on these nominal concentrations.

The following parameters were analysed statistically using the methods described in the Section 3.0 of this report:

- Mortality of parental collembola
- Reproductive output as the number of juveniles

Although statistically significant differences ($P < 0.05$) were identified between the treatments and the control for mortality and reproductive output, no EC_{10} or EC_{20} values were reliably calculated, as no robust confidence limits could be determined due the lack of suitable response functions.

Statistical Analysis

Where possible, non-linear regression was used to derive the ECx values. However, in cases where this approach did not provide reliable estimates, linear max likelihood regression was used in an attempt to derive these values.

Probit analysis with linear maximum likelihood regression was used to determine the concentration response function. Chi² was used as a goodness of fit measure.

A 3-parameter normal cumulative distribution function (Bruce and Versteeg, 1992) was fitted to the original data and a non-linear regression without weighting was performed using the Levenberg-Marquardt algorithm to calculate the function parameters. Analysis of variance (ANOVA) and a test for the lack of fit was then performed to determine the probability that the variance explained by the regression analysis was due to chance. Where p(F:Regression) <= 0.05, a significant amount of the variance is explained by the regression and where p(F:Lack of Fit) > 0.05, there is no significant lack of fit. If both of these criteria are met, the results of the regression analysis are accepted and the point estimates from the 3-parameter normal cumulative distribution function are meaningful.

Results

Mortality

The relationship between dose and response was significant, p(F) = 0.036 and the slope function was 4.405. Despite this, no EC_{10} or EC_{20} values were reliably calculated, as no robust confidence limits could be determined due the lack of suitable response functions. As a result, the EC_x values and their 95% confidence limits are invalid.

Reproduction

Although statistically significant differences between treatments and the control were identified, p(F) = <0.018, no EC_{10} or EC_{20} values were reliably calculated, as no robust confidence limits could be determined due the lack of suitable response functions. As a result, the EC_x values and their 95% confidence limits are invalid.

Conclusion

Although statistically significant differences ($P < 0.05$) were identified between the treatments and the control for mortality and reproductive output, no EC_{10} or EC_{20} values were reliably calculated, as no robust confidence limits could be determined due the lack of suitable response functions.

Taylor & Allen (2015) suggested the newly recalculated values (below) were not very reliable as CEA were unable to calculate confidence intervals:

Reproduction:

EC_{10} of 134 mg a.s/kg with 95% CI which could not be determined.

Mortality:

EC_{10} = 70.0 mg a.s/kg (95% CI n.d – 185)
EC_{20} = 134 mg a.s/kg (95% CI 0.151 – 331)

(Taylor S, Allen M, 2015b)

Metabolites of fludioxonil ~ collembola

New studies conducted since the last EU review are summarised below.

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.2.1/02, Kimmel S, (2015) CGA192155 - Effects on Reproduction of *Folsomia candida* (Collembola: Isotomidae) in Artificial Soil, Report Number 20140161. Innovative Environmental Services (IES) Ltd Benkenstrasse 260, 4108 Witterswil Switzerland (Syngenta file No. CGA192155_11291).

Guidelines

OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

GLP: Yes.

Executive Summary

The effects of CGA192155 on the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC for reproduction was determined to be 53 mg a.s./kg soil dry weight. The EC_{50} for number of juvenile collembolans was calculated to be >1000 mg a.s./kg soil d.w.

Materials

Test Material	CGA192155 Fludioxonil
Lot/Batch #:	MLA-336/4
Actual content of active ingredients:	96% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30th June 2018

Treatments

Test concentrations:	16, 29, 53, 95, 172, 309, 556 and 1000 mg a.s./kg dry soil alongside a negative control and a solvent control.
Control:	Untreated + solvent control
Toxic standard:	Boric Acid
Application method:	Mixed with artificial substrate
Test organisms	
Species:	*Folsomia candida*
Age:	10 to12 day-old juveniles
Source:	Culture maintained at Test Facility
Feeding:	*ad libitum* with granulated dried baker's yeast
Test design	
Arenas:	Glass containers (8.5 cm height x 4 cm diameter) covered with lids that enabled air exchange. Filled with 30 g w.w. artificial soil (depth 2-4 cm)
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 <1% calcium carbonate. 30 g wet weight of artificial soil was added to each test vessel.
Replication:	Control: 8 Treatment: 4
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	18.1°C to 21.5°C
pH of soil:	Test start: 6.2 and 6.6. Test end: 6.9 and 7.2
Water content of soil:	Test start: 21.6 % to 25.2 % (corresponding to 44.9 % to 52.5 % of the mWHC) Test end: 19.8 % to 23.0 % (corresponding to 41.3 % to 47.9 % of the mWHC)
Photoperiod:	12 hour photo period (439 to 481 lux)

Study Design and Methods

Experimental dates: 15th December 2014 to 16th March 2015

A stock solution was prepared by diluting 667 mg of the test item in 20 mL of acetone. To obtain the required test concentrations, subsequent dilutions were prepared and 5 mL of the appropriate stock was mixed with10 g of quartz sand that was mixed into 150 g of the artificial soil, per each treatment.

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 controls. The test organisms were fed *ad libitum* with granulated dried baker's yeast.

Four weeks after introducing the test organisms, the surviving parent collembolans and offspring (juveniles) were counted. To do this the *F. candida* were extracted from the test soil and the number of juveniles were assessed by a controlled temperature gradient extractor based on principles by MacFadyen (JUMO dTRON 316 MacFayden High Gradient Extractor, ecoTech Umwelt Messsysteme GmbH,

Germany). To extract Collembola, the soil from each replicate was placed in an extraction funnel of the MacFadyen extractor and an increasing temperature regime (from 25 to 40°C) was run for 48 hours. The temperature increase forced the Collembola to fall downwards from the tunnel into a small container.

Comparison between control and solvent control was conducted using Fisher exact test (mortality) and Student t-test (reproduction). Mortality data were analyzed using Step-down Cochran-Armitage test (α: 0.05; one-sided smaller).

Reproduction data, i.e. number of juveniles, were tested for normal distribution using the Shapiro-Wilk's test and analysed using Williams Multiple Sequential t-test (α: 0.05; one-sided smaller). Probit analysis using linear maximum likelihood regression was used for determination of LCx/ECx values for mortality and reproduction. Coefficient of variation for the number of juveniles was calculated at test termination.

Statistical analysis was performed using ToxRat Professional, Version 3.0 beta.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-1: Effects of residues of CGA192155 on mortality and reproduction of *F. candida*

Endpoint	Treatment group (mg CGA192155/kg soil d.w.)									
	Control	**Solvent control**	**16**	**29**	**53**	**95**	**172**	**309**	**556**	**1000**
% Mortality of parental collembolans after 4 weeks	1.25	2.5	0	0	2.5	2.5	2.5	2.5	0	0
Mean number of juveniles after 4 weeks	1396	1471	1368	1381	1411	1266*	676*	1207*	1057*	880*
SD	164	109	88	131	80	59	140	39	119	45
CV %	11.8	7.4	6.4	9.5	5.7	4.7	20.7	3.2	11.3	5.1
NOEC (reproduction)	53									
EC_{10}	45.51 (2.54 – 109.59)									
EC_{20}	193.26 (62.46 – 373.43)									

* Statistically significantly lower when compared to the control (Williams t-test, α = 0.05; one-sided smaller)

Validity criteria

The validity criteria are as follows:

- Solvent control treatment mortality was 2.5 % (must be < 20%)
- The mean number of juvenile recorded in the solvent control treatment was 1471 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the solvent control was 7.4% (must not be > 30%)

Conclusions

In the 28-day toxicity study the NOEC and LOEC for reproduction were determined to be 53 and 95 mg a.s. /kg dry soil, respectively.

(Kimmel S., 2015)

RMS comment:

In the chronic toxicity study of CGA192155 to *Folsomia candida*, the reproduction in the toxic reference group was reduced by only 53% at 200 mg boric acid/kg dw soil (2 times the concentration recommended in the guideline). The guideline indicates that at least 50% reduction should be obtained for a concentration of 100 mg boric acid/kg dw soil. Could the notifier add a justification on the reliability of the test?

Syngenta response:

In the risk assessment for A8240D the TERs for Folsomia range from 900 to 1800. The NOEC for Folsomia would have to be below 0.295 mg/kg to lead to concerns about unacceptable long-term risk from CGA192155 following the uses of A8240D. Even assuming as a worst case, that the organisms used in the study were slightly less sensitive than the guideline suggests, it is highly unlikely this would affect the endpoint to the extent of a 180 times increase. Therefore, the study can be considered acceptable for use in the risk assessment.

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.2.1/03 Kimmel S (2015a). CGA265378 - Effects on Reproduction of *Folsomia candida* (Collembola: Isotomidae) in Artificial Soil Report Number 20140163. Innovative Environmental Services (IES) Ltd, Benkenstrasse 260, 4108 Witterswil, Switzerland (Syngenta file No. CGA265378_10006).

Guidelines

OECD Guidelines No. 232. Collembolan Reproduction test in soil (2009)

GLP: Yes.

Executive Summary

The toxicity of CGA265378 to the reproduction and the parental mortality of collembola species *Folsomia candida* was determined. The NOEC was determined to be 29 mg /kg soil dry weight. The EC_{50} for number of juvenile collembolans was calculated to be >1000 mg /kg soil d.w.

Materials

Test Material	CGA265378
Lot/Batch #:	MES 340/1
Purity:	99%
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30th June 2016

Treatments

Test concentrations:	16, 29, 53, 95, 172, 309, 556 and 1000 mg a.s. /kg dry weight.
Control:	Milli-Q water. And acetone solvent control
Toxic standard:	Boric Acid
Application method:	Mixed with artificial soil
Test organisms	
Species:	*Folsomia candida*
Age:	10 – 12 days
Source:	Culture maintained at Test Facility
Feeding:	Granulated dried baker's yeast *ad libitum*
Test design	
Arenas:	Glass containers (8.5 cm height x 4 cm diameter) covered with lids allowing gas exchange, filled with 30 g soil w.w.
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 0.5% calcium carbonate.
Replication:	Control: 8 Treatment: 4
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	17.9 and 21.9 °C
pH of soil:	Test start: 6.4 to 6.9 Test end: 6.3 and 6.7
Water content of soil:	Test start:16.8 % to 22.5 % (corresponding to 39.0 % to 52.3 % of the mWHC) Test end: 18.3 % to 22.5 % (corresponding to 42.6 % to 52.3 % of the mWHC)
Photoperiod:	12 h photoperiod (420 to 487 lux)

Study Design and Methods

Experimental dates: 16th November to 19th December 2014

A stock solution was prepared by diluting 646 mg of the test item in 20 mL of acetone. To obtain the required test concentrations, subsequent dilutions were prepared and 5 mL of the appropriate stock was mixed with 10 g of quartz sand that was mixed into 150 g of the artificial soil, per each treatment.

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 granulated dry yeast *ad libitum*. 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.

Comparison between control and solvent control was conducted using Fisher exact test (mortality) and Student t-test (reproduction). Mortality data were analysed using Step-down Cochran-Armitage test (α: 0.05; one-sided smaller). Reproduction data, i.e. number of juveniles, were tested for normal distribution using the Shapiro-Wilk's test and analysed using Williams Multiple Sequential t-test (α: 0.05; one-sided smaller).

Probit analysis using linear maximum likelihood regression was used for determination of LCx/ECx values for mortality and reproduction. Coefficient of variation for the number of juveniles was calculated at test termination. Statistical analysis was performed using ToxRat Professional, Version 3.0 beta, ToxRat Solutions GmbH

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-2: Effects of CGA265378 on mortality and reproduction of *Collembola* (*Folsomia candida*)

Endpoint	Treatment group (mg CGA265378 /kg soil d.w.)									
	Control	**Solvent control**	**16**	**29**	**53**	**95**	**172**	**309**	**556**	**1000**
% Mortality of parental collembolans after 4 weeks	1.25	2.5	0	7.5	5	5	2.5	2.5	5	2.5
Mean number of juveniles after 4 weeks	794	739	619	739	589*	600*	566*	541*	598*	458*
SD	148	63	48	51	80	60	39	83	85	104
CV %	19	8	8	7	14	10	7	15	14	23
NOEC (reproduction)	29									
EC_{10}	2.86									
EC_{20}	42.73									

* Statistically significantly lower when compared to the solvent control (Williams Multiple Sequential t-test, α = 0.05, one-sided smaller).

Validity criteria

The validity criteria are as follows:

- Solvent control treatment mortality was 2.5% (must be < 20%)
- The mean number of juvenile recorded in the solvent control treatment was 739 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the solvent control was 8% (must not be > 30%)

Conclusions

In the 28-day toxicity study the NOEC and LOEC for reproduction were determined to be 29 and 53 mg a.s. /kg dry soil, respectively.

(Kimmel S. 2015a)

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.2.1/04 Friedrich S. (2015a) CGA339833 - Effects on the Reproduction of the Collembolan (*Folsomia candida*), Report Number 14 10 48 228 S. BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6 04827 Gerichshain, Germany (Syngenta File No. CGA339833_10003).

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 CGA339833 on the reproduction and the parental mortality of the collembolan species *Folsomia candida* were determined. The NOEC was determined to be 1000 mg test item/kg soil dry weight.

Materials

Test Material	CGA339833
Lot/Batch #:	MES 338/1
Purity:	96% w/w
Description:	Not stated
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	31 May 2019
Treatments	
Test concentrations:	7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000 mg test item/kg soil dry weight
Control:	Untreated
Toxic standard:	Boric acid tested in a separate study
Application method:	Mixed with artificial soil
Test organisms	
Species:	*Folsomia candida*
Age:	9-12 days (juvenile collembolans)
Source:	Culture maintained at Test Facility (originally: Biologische Bundesanstalt (BBA) Berlin-Dahlem, Germany
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Arenas:	Glass container (approximately 150 mL) covered with a lid
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. 30 g wet weight soil of artificial soil was added to each test vessel.
Replication:	Control: 8 (+ 2 replicates not loaded with collembolans for measurement purposes) Treated: 4 (+2 replicates not loaded with collembolans for measurement purposes)
No./arena :	10
Duration of test:	4 weeks
Environmental test conditions	
Temperature:	20.2 to 22.0 ºC
pH of soil:	Test start: 5.99 to 6.06 Test end: 5.73 to 5.86
Water content of soil:	Test start: 24.9 to 25.0 (equivalent to 59.7 to 60.0 % of WHC) Test end: 24.3 – 24.8 (equivalent to 58.3 to 59.5 % of WHC)
Photoperiod:	16 hours light (480 lux)

Study Design and Methods

Experimental dates: 27th July to 24th August 2015

An exact weighed amount of the test item was mixed with finely ground quartz sand, immediately prior to application. This stock mixture was diluted with quartz sand in a way that 10 g of the mixture contained the amount of test item required for one treatment group to adjust the selected concentration. The treated quartz sand (10 g per treatment group) was added to the prepared amount of artificial soil (302.5 g wet weight) yielding 312.5 g wet artificial soil (corresponding to 250 g dry weight). The test item mixture was then mixed thoroughly with the artificial soil by intensive stirring in a laboratory mixer.

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. To do this the test substrate of each replicate was poured into an individual 150 - 200 mL container and the test organisms were floated off the substrate by the addition of water. To improve the contrast between the white collembolans and surrounding water surface, the water was stained dark with ink. After gentle stirring the numbers of parental and juvenile collembolans floating on the surface were determined. Missing parental collembolans are assumed to have died during the test period. Surviving adults and juveniles were counted using a digital image processing system (LemnaTec Scanalyzer), an automated counting technique based on a video camera connected to a digital image storage and analysis system.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

The statistical analysis was performed with the software ToxRat Professional 3.1.0 (2015). Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and Williams-t-test were used to compare the control with the independent test item groups. Mortality of adult collembolans was corrected using the formula by Abbott (1925).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-3: Effects of residues of CGA339833 on mortality and reproduction of Collembola (*Folsomia candida*)

Endpoint	Treatment group (mg CGA339833/kg soil d.w.)								
	Control	**7.81**	**15.63**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**
% Mortality of parental collembolans after 4 weeks	2.5	2.5	2.5	0.0	0.0	2.5	7.5	0.0	2.5
% corrected mortality (Abbott)	-	0	0	-3	-3	0	5	-3	0
Mean number of juveniles after 4 weeks	932	943	957	914	907	916	970	945	956
SD	123.3	56.2	214.8	26.5	130.0	99.7	83.9	41.8	74.5
CV %	13.2	6.0	22.5	2.9	14.3	10.9	8.6	4.4	7.8
% reduction compared to control	-	-1	-3	2	3	2	-4	-1	-3
NOEC (reproduction)	1000								
EC_{10}/EC_{20}	>1000								

Negative values = increase, relative to control

Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 2.5% (must be < 20%)
- The mean number of juvenile recorded in the control treatment was 932 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 13.2% (must not be > 30%)

Conclusions

In a reproduction test in which *Folsomia candida* was exposed to CGA339833, the NOEC for reproduction was considered to be 1000 mg test item/kg soil d.w., the highest concentration tested. EC_{10} and EC_{20} values were >1000 mg test item/kg soil d.w.,

(Friedrich S. 2015a)

A collembola study with the new soil metabolite SYN545245 ~~is currently being~~ has been conducted. The study ~~will be submitted~~ has been summarised below and the dossier updated as agreed with the RMS.

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.2.1/05 , Friedrich S. (2016), SYN545245 - Effects on the Reproduction of the Collembolan *Folsomia candida*, Report Number 16 10 48 073 S. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany (Syngenta file No. SYN545245_10012).

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 SYN545245 to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC was determined to be 100 mg test item/kg soil dry weight. The EC_{50} for number of juvenile collembolans was calculated to be >100 mg test item/kg soil d.w.

Materials

Test Material	SYN545245
Lot/Batch #:	MES 427/1
Purity:	98 % w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of October 2017
Density:	n/a
Treatments	
Test rates:	1.6, 2.9, 5.3, 9.5, 17.1, 30.9, 55.6, 100 mg test item/kg soil dry weight
Control:	Untreated
Toxic standard:	Boric acid
Application method:	Mixed with artificial sediment
Test organisms	
Species:	*Folsomia candida*
Age:	9-12 days (juvenile collembolans)
Source:	Culture maintained at Test Facility (originally: Biologische Bundesanstalt (BBA) Berlin-Dahlem, Germany)
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Arenas:	glass container (approximately 150 mL) covered with a lid
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. 30 g wet weight soil.
Replication:	Control: 8 (+ 2 replicates not loaded with collembolans for measurement purposes) Treated: 4 (+2 replicates not loaded with collembolans for measurement purposes)

No./arena :	10
Duration of test:	4 weeks
Environmental test conditions	
Temperature:	18.0 – 21.4 ºC
pH of soil:	test start: 5.97 – 6.03 test end: 5.71 – 5.78
Water content of soil:	test start: 24.9 – 25.0 (equivalent to 58.9 – 59.1 % of WHC) test end: 24.3 – 24.7 (equivalent to 57.4 – 58.4 % of WHC)
Photoperiod:	520 lux 16 hours light

Study Design and Methods

Experimental dates: 1 to 29 February 2016

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.

All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

The statistical analysis was performed with the software ToxRat Professional 3.2.1 (2015). Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and Williams-t-test were used to compare the control with the independent test item groups. Mortality of adult collembolans was corrected using the formula by Abbott (1925).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-4:Effects of residues of SYN545245 on mortality and reproduction of *Folsomia candida*

Endpoint	Treatment group (mg /kg soil d.w.)								
	Control	1.6	2.9	5.3	9.5	17.1	30.9	55.6	100
% Mortality of parental collembolans after 4 weeks	1.3	2.5	2.5	0.0	2.5	2.5	2.5	0.0	2.5
% corrected mortality (Abbott) [a]	-	1	1	-1	1	1	1	-1	1
Mean number of juveniles after 4 weeks	1138	1173	1119	1132	1139	1124	1111	1152	1155
SD	128.0	113.5	126.2	127.3	121.6	119.5	95.5	153.8	131.8
CV %	11.3	9.7	11.3	11.2	10.7	10.6	8.6	13.4	11.4
% reduction compared to control	-	-3	2	1	0	1	2	-1	-2
NOEC (mortality)	100								
NOEC (reproduction)	100								
LC_{50}	>100								
EC_{10}	>100								
EC_{20}	>100								
EC_{50}	>100								

Negative values indicate an increase relative to the control

Validity criteria

The validity criteria are as follows:

- Control treatment mortality was 1.3% (must be < 20%)
- The mean number of juvenile recorded in the control treatment was 1138 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 11.3% (must not be > 30%)

Conclusions

In a chronic toxicity test in which collembolans were exposed to SYN545245 the NOEC for mortality of the parental collembolans was determined to be 100 mg test item/kg soil dry weight. The LC_{50} could not be calculated, but it can be concluded that the LC_{50} is higher than 100 mg test item/kg soil d.w., the highest concentration tested.

The NOEC for reproduction was determined to be 100 mg test item/kg soil dry weight.
The EC_{10}, EC_{20} and EC_{50} values for reproduction could not be calculated, but it can be concluded that these values are higher than 100 mg test item/kg soil d.w., the highest concentration tested.

(Friedrich S., 2016)

Hypoaspis

A soil mite (*Hypoaspis aculeifer*) toxicity test was conducted with the formulation A8240D and the OECD summary is presented below.

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.2.1/05~~5~~6 Schulz L. (2015) Fludioxonil WG (A8240D) - Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer*, Report Number 15 10 48 008 S. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany(Syngenta File No. A8240D_11849).

Guidelines

OECD Guideline 226: Predatory mite (*Hypoaspis* (*Geolaelaps*) *aculeifer*) reproduction test in soil (2008)

GLP: Yes.

Executive Summary

The effects of A8240D 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.

Materials

Test Material	A8240D Fludioxonil WG (50)
Lot/Batch #:	SMO3F0004
Actual content of active ingredients:	51.1 % w/w corresponding to 511 g/kg
Description:	Beige granules
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	October 2016
Treatments	
Test concentrations:	62.5, 125, 250, 500, 1000 mg test item/kg soil dry weight
Control:	Untreated
Toxic standard:	Dimethoate
Test organisms	
Species	*Hypoaspis aculeifer* (CANESTRINI)
Source:	Cultured in test facility (originally: "Bayer CropScience AG" Monheim)
Food:	Every 2 - 3 days with cheese mites *Tyrophagus putrescentiae* (SCHRANK)
Age at test start:	
Test design	
Vessels:	100 mL SCHOTT-bottles with screw cap (4 cm diameter, 11 cm high) filled with 20 g soil d.w. (*ca*. 1.6 cm depth)
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.8 % industrial quartz sand and 0.2% calcium carbonate.

Replication:	Control group: 8 Treated group: 4
No. of mites/arena :	10
Duration of test:	14 days

Environmental test conditions

Temperature:	19.5 to 21.4 °C
pH:	Test start: 5.6 to 5.8 Test end: 5.6 to 5.7
Water content of soil:	Test start: 17.64 to 18.76 (equivalent to 48.87 to 51.99 % of WHC) Test end: 17.11 to 18.15 (equivalent to 47.40 to 50.29 % of WHC)
Photoperiod:	16 h light : 8 h dark, 529 lux

Study Design and Methods

Experimental dates: 14th to 30th January 2015

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of A8240D incorporated into the test soil.

An exactly weighed amount of the test item was mixed in a volumetric flask with deionized water to make a stock solution, without addition of solubility mediators, immediately before application. This stock solution was stepwise diluted with deionised water to prepare 5 further test solutions (serial dilution). Afterwards the test item solutions were thoroughly mixed with the artificial soil by means of a hand stirrer.

Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

Mortality (number of dead adults) in % for the treatment groups was calculated. Missing mites were counted as dead. Observations in the treatment group were expressed relative to the water control group.

The reduction of reproductive output for the treatment groups was calculated in comparison to the control. The statistical analysis was performed with the software ToxRat Professional. Fisher's Exact Binomial Test with Bonferroni Correction and the Dunnett-t-test were used to compare the control with the independent test item groups.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-45: Effects of residues of A8240D on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A8240D/kg soil d.w.)					
	Control	**62.5**	**125**	**250**	**500**	**1000**
	Mortality of adult mites after 14 days					
% mortality	0.0	5.0	0.0	0.0	0.0	0.0
	Number of juveniles after 14 days					
Mean no. progeny per replicate	265.8	272.0	289.3	261.0	269.5	251.0
Standard deviation	26.9	15.7	15.9	36.6	27.8	18.3
Coefficient of variation (CV)	10.1	5.8	5.5	14.0	10.3	7.3
% reduction compared to control	-	-2.4	-8.8	1.8	-1.4	5.6
$NOEC/EC_{10}/EC_{20}$ (reproduction)	1000					
EC_{10} (reproduction)	>1000					
EC_{20} (reproduction)	>1000					

No statistically significant differences compared to the control (Fisher's Exact Binomial with Bonferroni Correction for mortality, $p > 0.05$, one-sided greater and Dunnett-t-test for reproduction, $p > 0.05$, one-sided smaller)
Negative values indicate an increase in relation to the control

In a separate study the EC_{50} (reproduction) of the reference item dimethoate was calculated to be 6.2 mg/kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 0.0 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 265.8)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 10.1 %)

Conclusions

In a 14-day *Hypoaspis aculeifer* reproduction study with A8240D, the NOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight. EC_{10} or EC_{20} values could not be estimated as no clear concentration-response was derived from the test.

(Schulz L. 2015)

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.2.1/06~~6~~7 Schulz L (2015a) Fludioxonil FS (A8207M) - Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer*, Report Number 15 10 48 010 S. BioChem Agrar, Kupferstraβe 6, 04827 Gerichshain, Germany. (Syngenta file No. A8207M_10371).

Guidelines

OECD Guideline 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil (2008)

GLP: Yes.

Executive Summary

The effects of A8207M 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.

Materials

Test Material	Fludioxonil FS (025) A8207M
Lot/Batch #:	SSP3D1566M
Actual content of active ingredients:	Fludioxonil: 2.41 % w/w corresponding to 25.3 g/L
Description:	Red liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of July 2016
Density:	1051 kg/m^3
Treatments	
Test concentrations:	1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.8 mg A8207M/kg soil d.w.
Control:	Untreated
Toxic standard:	Dimethoate (analysed purity 99.8 %, tolerance ± 1.0 %)
Test organisms	
Species	*Hypoaspis aculeifer* (CANESTRINI)
Source:	Cultured in test facility (originally: Bayer CropScience AG, Monheim)
Food:	Cheese mites, *Tyrophagus putrescentiae* supplied two to three times per week.
Age at test start:	Adults from a synchronised culture (age difference of 2 days)
Test design	
Vessels:	Glass containers (volume: 100 mL; diameter: 4 cm; height: 11 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, 74.8 % industrial quartz sand and 0.2% calcium carbonate.
Replication:	Control group: 8 Treated group: 4
No. of mites/arena :	10
Duration of test:	14 days
Environmental test conditions	
Temperature:	19.5 to 21 °C
pH:	Test start: 5.9 – 6.0 Test end: 5.6 – 5.7
Water content of soil:	Test start: 49.69 – 51.49 % of maximum WHC Test end: 48.84 – 50.67 % of maximum WHC
Photoperiod:	16 h light : 8 h dark, 511 lux

Study Design and Methods

Experimental dates: 17th November to 12th December 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of A8207M incorporated into the test soil.

An exactly weighed amount of the test item was mixed in a volumetric flask with deionized water to make a stock solution, without addition of solubility mediators, immediately before application. This stock solution was stepwise diluted with deionised water to prepare 7 further test solutions (serial dilution). Afterwards the test item solutions were thoroughly mixed with the artificial soil by means of a hand stirrer.

Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.
Mortality data were corrected using Abbott's formula and statistically analysed using Fisher's Exact Binomial test with Bonferroni Correction ($\alpha > 0.05$, one-sided greater). Reproductive output for the treatment groups were calculated in comparison to the control and data were tested using a Dunnett-t-test ($\alpha > 0.05$, one-sided smaller). The software used to perform the statistical analysis was ToxRat Professional.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-56: Effects of residues of A8207M on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg A8207M/kg soil d.w.)								
	Control	**7.8**	**15.6**	**31.3**	**62.5**	**125**	**250**	**500**	**1000**
	Mortality of adult mites after 14 days								
% mortality	3.8	2.5	0.0	10.0	2.5	5.0	2.5	2.5	0.0
% corrected mortality	-	-1.3	-3.9	6.5	-1.3	1.3	-1.3	-1.3	-3.9
	Number of juveniles after 14 days								
Mean no. progeny per replicate	249.0	234.3	228.8	238.3	240.8	251.8	255.3	255.3	236.0
Standard deviation	19.4	6.3	11.9	15.2	19.0	19.7	15.4	16.7	19.4
CV (%)	7.8	2.7	5.2	6.4	7.9	7.8	6.0	6.5	8.2
% reduction compared to control	-	5.9	8.1	4.3	3.3	-1.1	-2.5	-2.5	5.2
NOEC (reproduction)	1000 mg A8207M/kg soil d.w.								

In a separate study the EC_{50} (reproduction) of the reference item dimethoate was calculated to be 6.2 mg/kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 3.8 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 249.0)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 7.8 %)

Conclusions

The effects of A8207M on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test.

The NOEC was considered to be 1000 mg /kg soil dry weight, the highest concentration tested. The EC_{10} and EC_{20} values were >1000 mg A8207M/kg

(Schulz L, 2015a)

Metabolites of fludioxonil ~ Hypoaspis

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.2.1/078 Vinall S (2014) CGA192155 – A laboratory test to determine the effects of fresh residues on the predatory mite *Hypoaspis aculeifer* (Acari: Laelapidae), Report Number SYN-14-97. Mambo-Tox Ltd, 2 Venture Road, University Science Park, Southampton, SO16 7NP. (Syngenta file No. CGA192155_11289).

Guidelines

OECD Guideline 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil. (2008)

GLP: Yes.

Executive Summary

The effects of CGA192155 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.

Materials

Test Material	CGA192155 CSAA794110
Lot/Batch #:	MLA-336/4
Purity:	96 ± 2% w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of June 2018

Treatments

Test concentrations:	1000, 555.6, 308.6, 171.5, 95.3 mg CGA192155 /kg
Control:	Water, acetone
Toxic standard:	BAS 152 11 I Perfekthion (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L) at a concentration of 14 mg a.s./kg

Test organisms

Species	*Hypoaspis aculeifer* (CANESTRINI)
Source:	Cultured in test facility (original source: ECT Oekotoxikologie GmbH, Germany)
Food:	At the beginning of the test juvenile springtails (*Folsomia candida*) and cheese mites, (*Tyrophagus putrescentiae*) were provided. Thereafter cheese mites were added *ad libitum* every 2-3 days
Age at test start:	30 days

Test design

Vessels:	60 mL capacity glass containers (5.5 cm tall; diameter: 5.2 cm), screw top closure with 8 mm hole and covered with 80 micron mesh, filled with 20 g soil d.w.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.8 % industrial quartz sand and 0.2% calcium carbonate.
Replication:	Control group: 8 Treated group: 4 Toxic reference group: 5
No. of mites/arena :	10 adult females
Duration of test:	14 days

Environmental test conditions

Temperature:	20.0 to 20.7 °C
pH:	Test start: 5.31 – 5.86 Test end: 5.10 – 5.84
Water content of soil:	50% of maximum WHC
Photoperiod:	16 h light : 8 h dark, 500-600 lux

Study Design and Methods

Experimental dates: 24th September to 16th December 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of CGA192155 incorporated into the test soil.

Each test item concentration was prepared separately by diluting CGA192155 in acetone. A 2.0 mL volume of the appropriate acetone solution of the test item was mixed with 31.61 g of sand in a glass dish and the acetone was allowed to evaporate. Once the treated sand was dry, it was then mixed with the artificial soil, to achieve 200 g dry weight of soil. Each aliquot of soil was then thoroughly mixed with 37.50 mL of purified water to give a final moisture content of 50% of the soil's maximum water-holding capacity (WHC).

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, juvenile springtails (*Folsomia candida*) and cheese mites (*Tyrophagus putrescentiae*) were initially added. Cheese mites were replenished every 2 to 3 days thereafter. The test was carried out under a 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 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 ($\alpha = 0.05$). Treatments were then individually compared to the acetone control either by one-way Analysis of Variance and Dunnett's t-test (one-sided, $\alpha = 0.05$) or Mann- Whitney U-test ($\alpha = 0.05$).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. SPSS (2012) software was used to perform the statistical analysis.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-67: Effects of residues of CGA192155 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint		Treatment group (mg CGA192155/kg soil d.w.)					
	Water Control	Acetone Control	1000	555.6	308.6	171.5	95.3
		Mortality of adult mites after 14 days					
% mortality	6	4	8	10	5	10	3
% reduction compared to control	-	3	4	6	1	6	0
		Number of juveniles after 14 days					
Mean no. progeny per replicate	293	301	300	272	266	271	282
Standard deviation	32.6	37.7	38.9	35.8	43.4	34.4	31.8
CV (%)	11.2	12.5	NR	NR	NR	NR	NR
% reduction relative to solvent control	3	-	0	1	12	10	6

Results compared by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). Values marked with an asterisk (*) differed significantly from the control.
NR = not reported

Validity Criteria

The validity criteria for the solvent control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 3 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 301)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 12.5 %)

In the toxic reference treatment, 94% mortality (94% corrected mortality) was recorded and this result differed significantly from the acetone control (Fisher's Exact Test, $\alpha = 0.05$).

Conclusions

The effects of CGA192155 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.

(Vinall S, 2014)

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.2.1/089 Vinall S (2014a) CGA265378 – A laboratory test to determine the effects of fresh residues on the predatory mite *Hypoaspis aculeifer* (Acari: Laelapidae), Report Number SYN-14-98. Mambo-Tox Ltd, 2 Venture Road, University Science Park, Southampton, SO16 7NP. (Syngenta file No. CGA265378_10000).

Guidelines

OECD Guideline 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil. (2008)

GLP: Yes.

Executive Summary

The effects of CGA265378 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.

Materials

Test Material	CGA265378 CSAA250411
Lot/Batch #:	MES-340/1
Description:	Yellow solid
Measured purity:	99 ± 2%
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of June 2016
Treatments	
Test rates:	1000, 555.6, 308.6, 171.5, 95.3 mg CGA265378/kg
Control:	Water, acetone
Toxic standard:	BAS 152 11 I Perfekthion (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L) at a concentration of 14 mg a.s./kg

Test organisms

Species	*Hypoaspis aculeifer* (CANESTRINI)
Source:	Cultured in test facility (original source: ECT Oekotoxikologie GmbH, Germany)
Food:	At the beginning of the test juvenile springtails (*Folsomia candida*) and cheese mites, (*Tyrophagus putrescentiae*) were provided. Thereafter cheese mites were added *ad libitum* every 2-3 days
Age at test start:	35 days

Test design

Vessels:	60 mL capacity glass containers (5.5 cm tall; diameter: 5.2 cm), screw top closure with 8 mm hole and covered with 80 micron mesh, filled with 20 g soil d.w.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.8 % industrial quartz sand and 0.2% calcium carbonate.
Replication:	Control group: 8 Treated group: 4 Toxic reference group: 5
No. of mites/arena :	10 adult females
Duration of test:	14 days

Environmental test conditions

Temperature:	19.7 to 20.6 °C
pH:	Test start: 5.98 to 6.21 Test end: 5.50 to 5.90
Water content of soil:	50% of maximum WHC
Photoperiod:	16 h light : 8 h dark, 470-630 lux

Study Design and Methods

Experimental dates: 29th September to 15th December 2014

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of CGA265378 incorporated into the test soil. Each test item concentration was prepared separately by diluting CGA192155 in acetone. A 2.0 mL volume of the appropriate acetone solution of the test item was mixed with 31.61 g of sand in a glass dish and the acetone was allowed to evaporate. Once the treated sand was dry, it was then mixed with the artificial soil, to achieve 200 g dry weight of soil. Each aliquot of soil was then thoroughly mixed with 37.50 mL of purified water to give a final moisture content of 50% of the soil's maximum water-holding capacity (WHC).

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, juvenile springtails (*Folsomia candida*) and cheese mites (*Tyrophagus putrescentiae*) were initially added. Cheese mites were replenished every 2 to 3 days thereafter. The test was carried out under a 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 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 ($\alpha = 0.05$). Treatments were then individually compared to the acetone control either by one-way Analysis of Variance and Dunnett's t-test (one-sided, $\alpha = 0.05$) or Mann- Whitney U-test ($\alpha = 0.05$).

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. SPSS (2012) software was used to perform the statistical analysis.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-78: Effects of residues of CGA265378 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint		Treatment group (mg CGA264378/kg soil d.w.)					
	Water Control	Acetone Control	1000	555.6	308.6	171.5	95.3
		Mortality of adult mites after 14 days					
% mortality	4	4	5	3	5	8	3
Corrected mortality	-	-	1	0	1	4	0
		Number of juveniles after 14 days					
Mean no. progeny per replicate	276	269	259	266	260	246	261
Standard deviation	22.6	12.3	32.5	27.9	23.1	8.2	29.6
CV (%)	8.2	4.6	NR	NR	NR	NR	NR
% reduction compared to control	-	-	4	1	4	8	3

Results compared by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). Values marked with an asterisk (*) differed significantly from the control.
NR = not reported

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 4 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 269)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 4.6 %)

In the toxic reference treatment, 100% mortality was recorded and this result differed significantly from the acetone control (Fisher's Exact Test, $\alpha = 0.05$).

Conclusions

The effects of CGA265378 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.

(Vinall S, 2014a)

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.2.1/~~09~~10 Schulz L. (2015b) CGA339833 - Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer*, Report Number 15 10 48 229 S. BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany (Syngenta file No. CGA339833_10004)

Guidelines

OECD Guideline 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil (2008)

GLP: Yes.

Executive Summary

The effects of CGA339833 on the mortality and reproductive output of the soil mite species *Hypoaspis aculeifer* were determined during a 14-day test.

The NOEC for mortality was determined to be 500 mg /kg soil dry weight.

Materials

Test Material	CGA339833
Lot/Batch #:	MES 338/1
Actual content of active ingredients:	96% w/w
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	31 May 2019
Treatments	
Test concentrations:	7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000 mg test item/kg soil dry weight
Control:	Untreated
Toxic standard:	Dimethoate tested in a separate study
Test organisms	
Species	*Hypoaspis aculeifer* (CANESTRINI)
Source:	Cultured in test facility (originally: Bayer CropScience AG)
Food:	*Tyrophagus putrescentiae* (SCHRANK), supplied twice to three times a week
Age at test start:	Adults with a difference of 2 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, 74.7 % industrial quartz sand and 0.2% calcium carbonate.
Replication:	Control group: 8 (+2 replicates for determination of water content and pH value; not loaded with predatory mites) Treated group: 4 (+2 replicates for determination of water content and pH value; not loaded with predatory mites)
No. of mites/arena :	10
Duration of test:	14 days

Environmental test conditions

Temperature:	19.7 - 21.7 °C
pH:	Test start: 5.5 - 5.8 Test end: 5.3 - 5.5
Water content of soil:	Test start: 14.36 - 16.71 (equivalent to 43.09 - 50.13 % of WHC) Test end: 14.64 - 16.27 (equivalent to 43.92 - 48.82 % of WHC)
Photoperiod:	16 h light : 8 h dark, 484 lux

Study Design and Methods

Experimental dates: 20th July to 10th August 2015

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of CGA339833 incorporated into the test soil. To prepare the various treatment concentrations the test item was mixed with finely ground quartz sand (= stock mixture). Subsequently the obtained stock mixture was stepwise diluted with quartz sand to prepare 7 further test item mixtures (serial dilution) containing the amount of test item which was required to adjust the test item concentration of the respective treatment group. Afterwards the test item mixtures were thoroughly mixed with the artificial soil by means of a hand stirrer.

Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (*Tyrophagus putrescentiae*) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

Mortality (number of dead adults) in % for the treatment groups was calculated. Missing mites were counted as dead. Observations in the treatment group were expressed relative to the water control group. The corrected mortality in the treatment groups was calculated according to ABBOTT (1925).

The reduction of reproductive output for the treatment groups was calculated in comparison to the control.

The statistical analysis was performed with the software ToxRat Professional 2.10.05. Fisher`s Exact Binomial Test with Bonferroni Correction and Williams-t-test were used to compare the control with the independent test item groups.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-89: Effects of residues of CGA339833 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg/kg soil d.w.)								
	Control	**7.81**	**15.63**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**
	Mortality of adult mites after 14 days								
% mortality	1.3	7.5	2.5	5.0	2.5	0.0	0.0	5.0	5.0
% corrected mortality	-	6.3	1.3	3.8	1.3	-1.3	-1.3	3.8	3.8
	Number of juveniles after 14 days								
Mean no. progeny per replicate	220.6	219.5	214.8	203.8	203.5	191.3	193.8	195.3	168.5*
Standard deviation	31.3	8.2	34.1	26.2	39.1	37.6	40.2	29.2	26.4
CV (%)	14.2	3.7	15.9	12.9	19.2	19.7	20.7	15.0	15.7
% reduction compared to control	-	0.5	2.7	7.6	7.8	13.3	12.2	11.5	23.6
NOEC (reproduction)	**500 mg /kg soil d.w.**								
EC_{20} (reproduction)	**805.2 mg /kg soil d.w. (95 % confidence limits 418.3 to 3361.1)**								
EC_{10} (reproduction)	**99.4 mg /kg soil d.w. (95 % confidence limits 22.3 to 193.3)**								

Calculations were done using non-rounded values
* statistically significantly different compared to the control (Williams-t-test for reproduction, $p \leq 0.05$, one-sided smaller)

In a separate study the EC_{50} (reproduction) of the reference item dimethoate was calculated to be 6.2 mg/kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

Validity Criteria

The validity criteria for the control group were met:

- Mean mortality of adult females: ≤ 20% (observed: 1.3 %)
- Mean number of juveniles per replicate: ≥ 50 (calculated: 220.6)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 14.2 %)

Conclusions

In a 14-day *Hypoaspis aculeifer* reproduction study with CGA339833, the NOEC for reproduction was determined to be 500 mg test item/kg soil dry weight. The EC_{10} for reproduction was determined to be 99.4 mg test item/kg soil dry weight and the EC_{20} was determined to be 805.2 mg test item/kg soil dry weight.

(Schulz L. 2015b)

RMS comment:

As 23% effect on reproduction were observed at 500 mg/kg dw soil (statistical NOEC) (Schulz, 2015, KCA 8.4.2.1/09), the risk assessment for the metabolite CGA 339833 should be based on the EC10 value of 94.4 mg/kg dw soil.

Syngenta response:

The 23% effect occurs at the 1000 mg/kg rate and the NOEC is 500 mg/kg. At 500 mg/kg there is only an 11.5% reduction in reproduction and very flat 7-13% reduction in repro between 31 mg/kg and 500 mg/kg. In line with that the EC_{10} is not very robust with wide confidence intervals (99.4-193.3 mg/kg). Therefore the NOEC should be used in this case.

In the risk assessment the TER's range between 7500 and 14000 so indicate a large margin of safety.

A soil mite study with the new soil metabolite SYN545245 ~~is currently being~~ has been conducted. The study ~~will be submitted~~ is summarised below and the dossier updated as agreed with the RMS.

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.4.2.1/11 Schulz L. (2016), SYN545245 - Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer*, Report Number 16 10 48 074 S. BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany (Syngenta file No. SYN545245_10007).

Guidelines

OECD Guideline 226: Predatory mite *(Hypoaspis (Geolaelaps) aculeifer)* reproduction test in soil (2008)

GLP: Yes.

Executive Summary

The effects of SYN545245 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, and the 14-day EC_{50} could not be determined but was considered to be >1000 mg /kg soil dry weight, the highest concentration tested.

Materials

Test Material	SYN545245
Lot/Batch #:	MES 427/1
Purity:	98 % w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	End of October 2016

Density:	n/a
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556, 1000 mg test item/kg soil dry weight
Control:	Untreated
Toxic standard:	Dimethoate
Test organisms	
Species	*Hypoaspis aculeifer (CANESTRINI)*
Source:	Cultured in test facility (originally: Bayer CropScience AG Monheim)
Food:	Cheese mites, Tyrophagus putrescentiae 2-3 times a week
Age at test start:	Adults from a synchronised culture with an age difference of 2 days
Test design	
Vessels:	100 mL SCHOTT-bottles with screw cap (inside dimensions: 4 cm diameter, 11 cm high)
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand and 0.2% calcium carbonate.
Replication:	Control group: 8 (+2 replicates for determination of water content ans pH value; without predatory mites) Treated group: 4 (+2 replicates for determination of water content and pH value without predatory mites)
No. of mites/arena :	10
Duration of test:	14 days
Environmental test conditions	
Temperature:	20.0 - 20.8 °C
pH:	Test start: 5.7 – 6.2 Test end: 5.3 – 5.6
Water content of soil:	test start: 17.27 - 18.67 (equivalent to 44.91 - 48.56 % of WHC) test end: 17.27 - 18.07 (equivalent to 44.93 - 47.00 % of WHC)
Photoperiod:	16 h light : 8 h dark, 444 lux

Study Design and Methods

Experimental dates: 18 April to 9 May 2016

Adult females of the soil mite Hypoaspis aculeifer were exposed to concentrations of SYN545245 incorporated into the test soil. An exactly weighed amount of the test item was mixed with purified water to make a stock solution, and appropriate volumes of this stock solution were further diluted with purified water to obtain the test concentrations such that, when added to pre-moistened artificial soil, a final moisture content value of 50% WHC was achieved. Adult females were transferred to the test vessels which contained untreated (control), reference item or test item treated artificial soil. Ten adult females were introduced to each test vessel. As a source of food, cheese mites (Tyrophagus putrescentiae) were added to the soil surface throughout the test. The test was carried out under controlled light-dark cycle. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of Hypoaspis aculeifer were extracted by heat/light extraction. From these data the mortality of the adult females and the reproductive output were calculated.

Mortality (number of dead adults) in % for the treatment groups was calculated. Missing mites were counted as dead. Observations in the treatment group were expressed relative to the control group. The corrected mortality in the treatment groups was calculated according to ABBOTT (1925).

The reduction of reproductive output for the treatment groups was calculated in comparison to the control group.

The statistical analysis was performed with the software ToxRat Professional 2.10.06 (RATTE, 2010). Fisher`s Exact Binomial Test with Bonferroni Correction and Dunnett-t-test were used to compare the control with the independent test item groups.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table 8.4.2.1-10: Effects of residues of SYN545245 on mortality and reproduction of *Hypoaspis aculeifer*

Endpoint	Treatment group (mg …/kg soil d.w.)								
	Control	16	29	53	95	171	309	556	1000
	Mortality of adult mites after 14 days								
% mortality	6.3	2.5	5.0	2.5	2.5	5.0	2.5	2.5	0.0
% corrected mortality	-	-4.0	-1.3	-4.0	-4.0	-1.3	-4.0	-4.0	-6.7
	Number of juveniles after 14 days								
Mean no. progeny per replicate	207.8	198.8	213.5	222.0	223.3	207.3	206.3	203.5	225.8
standard deviation	31.0	71.9	29.7	48.8	34.7	44.3	23.2	43.9	46.7
% reduction compared to control	-	4.3	-2.8	-6.9	-7.5	0.2	0.7	2.0	-8.7
NOEC (mortality)	1000 mg …/kg soil d.w.								
LC_{50} (mortality)	>1000 mg …/kg soil d.w.								
NOEC (reproduction)	1000 mg …/kg soil d.w.								
EC_{50} (reproduction)	>1000 mg …/kg soil d.w.								
EC_{20} (reproduction)	>1000 mg …/kg soil d.w.								
EC_{10} (reproduction)	>1000 mg …/kg soil d.w.								

Calculations were done using non-rounded values
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: 6.3 %)

- Mean number of juveniles per replicate: ≥ 50 (calculated: 207.8)
- Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (calculated: 14.9 %)

Conclusions

In a 14-day *Hypoaspis aculeifer* reproduction study with SYN545245, the LC_{50} for mortality and the EC_{10}, EC_{20} and EC_{50} values for reproduction could not be calculated, but it can be concluded that these values are greater than 1000 mg test item/kg soil dry weight, the highest concentration tested. The NOEC for mortality and the NOEC for reproduction were determined to be 1000 mg test item/kg soil dry weight.

(Schulz L., 2016)

Relevant Literature on Non-Target Soil Meso- and Macro-Fauna

No relevant scientifically peer-reviewed open literature could be found on fludioxonil 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: Summary of Endpoints for Fludioxonil

Organism	Test substance	Test type	Endpoints		Comment	Reference (author, date, Syngenta Ref)
Soil micro-organisms	Fludioxonil	C&N transformation	EU	NOEC = 1.3 mg/kg dry soil	**Endpoint used for risk assessment**	***Gallicia & Schanne (1992) CGA173506/0193***
	Fludioxonil	C&N transformation	EU	NOEC = 0.333 mg/kg	Above study (***Gallicia & Schanne (1992)*** will be used in the risk assessment as conducted at a higher rate	***Wüthrich (1993) CGA173506/0410***
Soil fungi	Fludioxonil	Growth test	EU	*Mucor circinelloides var griseocyanius* EC_{50} = >6.9 mg/kg *Marasmius orcades* EC_{50} = 8.9 mg/kg *Phytophthora nicotanae* EC_{50} = >1.68 mg/kg *Paecilomycetes maraquandii* EC_{50} = 3.7 mg/kg	Supplied for additional information. Not required for risk assessment.	***Grade (2002) CGA173506/5493***
	Fludioxonil	Growth test	New study	*Botrytis cinerea* EC_{50} = >1000 mg/kg *Mollinia fructigena* EC_{50} = 1.71 mg/kg *Mycotypha Africana* EC_{50} = 24.1 mg/kg *Pythium debaryanum* EC_{50} = >1000 mg/kg *Rhizopus stolonifer* EC_{50} = >37.5 mg/kg	Supplied for additional information. Not required for risk assessment.	***Schulz (2011) CGA173506_11470***

New study refers to studies performed since EU review of fludioxonil

Table 8.5-2: Summary of Endpoints for Metabolites of Fludioxonil

Organism	Test item	Test type	Endpoints		Comment	Reference (author, date, Syngenta Ref)
Soil micro-organisms	CGA192155	C&N transformation	EU	NOEC = 0.353 mg a.s./kg dry soil	-	***Voelkel (2001) CGA192155/0014***
	CGA265378	C&N transformation	EU	NOEC = 0.37 mg a.s./kg dry soil	-	***Voelkel (2002) CGA265378/0012***
	CGA339833	C&N transformation	New study	NOEC = 20 mg a.s./kg dry soil		***Schulz (2015) CGA339833_10007***
	SYN545245	C&N transformation	New study	NOEC = 2.07 mg a.s./kg dry soil	-	**Schulz (2016) SYN545245_10008**

New study refers to studies performed since EU review of fludioxonil

The studies previously submitted in the EU were summarised in the previous EU review in point B.9.8.1 of the DAR, therefore they are not repeated here.

At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.6 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.5-1 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.5.1 Schanné C., Galicia H., (1992). The Effects of CGA 173506 on Soil Respiration and Nitrification. Report Number 315843, RCC Umweltchemie AG, P.O. Box, CH-4452 Itingen/BL, Switzerland. (Syngenta file No. CGA173506_0193).*

Guidelines

BBA guideline VI 1-1, Auswirkungen auf die Aktivitaet der Bodenmikroflora (1990)

GLP: Yes

Executive Summary

CGA173506 was applied to samples of two soil types (sand and sandy silty loam) at concentrations of 0.133 mg a.s./kg dry soil and 1.33 mg a.s./kg dry soil.

The test item CGA173506 caused no significant adverse effects on soil respiration (measured as CO_2 evolution) in the soils tested at the end of the 28-day incubation period. Significant effects (deviation from control > 25 %) of CGA173506 on mineralization (measured as NH_4-N production), nitrification (measured as NO_2-N production), and nitrogen transformation (measured as NO_3-N-production) were

determined in the soils, after 28 days. By Day 96 the effects on mineralization, nitrification and nitrogen transformation had reduced to < 25 % difference compared to the control group.

Materials

Test Material	CGA173506
Lot/Batch #:	P.905004/5
Purity:	95.7 %
Description:	Colourless solid crystals
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	May 1993
Density:	Not applicable

Treatments

Test concentrations:	0.133 and 1.33 mg a.s./kg dry soil
Control:	Bidistilled water only
Toxic standard:	None used

Test design

Soil type:	Agricultural sand (Speyer 2.1) and sandy silty loam (Speyer F3) soils, supplied by Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA), Speyer, Germany Speyer 2.1 soil (sandy soil): 3.5 % clay, 9.1 % silt and 87.4 % sand, with an organic carbon content of 0.70 g/100 g soil, and a microbial biomass of 7.9 mg/100 g dry soil weight Speyer F3 soil (sandy silty loam soil): 15.2 % clay, 45.0 % silt and 39.8 % sand, with an organic carbon content of 1.20 g/100 g soil, and a microbial biomass of 37.7 mg/100 g dry soil weight
Test units:	250 mL Erlenmeyer flasks stoppered with a cotton wool plug
Replication:	3
Sampling intervals:	Respiration test: 0 – 3 hours, and 14 or 15, and 28 days after application (in soils Speyer 2.1 and F3, respectively) Nitrification test: 0 – 3 hours, and 14, 28, 49 or 48, 70 or 69, and 97 or 96 days after application (in soils Speyer 2.1 and F3, respectively)
Duration of test:	Respiration test: 28 days Nitrification test: 97 or 96 days (in soils Speyer 2.1 and F3, respectively)

Environmental test conditions

Temperature:	20 ± 2 °C
pH of soil:	Speyer 2.1 soil: 6.1 to 6.9 Speyer F3 soil: 7.1 to 7.7
Soil moisture content:	Speyer 2.1 soil: 12.8 g water per 100 g dry soil (40 % MWC) Speyer F3 soil: 17.8 g water per 100 g dry soil (39 % MWC)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 19th November 1991 to 5th March 1992

Soil samples of two soil types were treated with CGA173506 at two concentrations, 0.133 and 1.33 mg a.s./kg dry soil.

The test item was dissolved in 25 mL of acetone and 75 mL of distilled water was added under constant stirring with a magnet stirrer to yield a clear solution. The high concentration was prepared by adding 1 mL of the stock solution described above, to soil samples. For the lower concentration, 10 mL of the prepared test solution was made up to 100 mL with a mixture of acetone and water (ratio 25:75 mL). The test solutions were added dropwise to the soil samples and the soil samples were thoroughly mixed. After the application, 0.5 g lucerne meal per 100 g dry soil, containing 2.54 % nitrogen, was added to the soil samples for the nitrification experiment. The water content of the soil was determined prior to application and kept constant throughout the test at 40 % (Speyer 2.1) and 39 % (Speyer F3) of the respective MWC. The microbial mass was determined at the start of the test (before application).

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and the soil respiration test.

Soil respiration was determined by following the glucose induced evolution of CO_2 resulting from microbial activity. The mean CO_2 production of the soil samples was calculated as the mean quantity of CO_2 (mg) evolved during the constant respiration phase of soil samples, based on short-time experiments (approximately 24 hours) and over an incubation period of 28 days.

The determination of ammonium-, nitrite- and nitrate-nitrogen was based on a colorimetric method. Mean ammonium-, nitrite- and nitrate-nitrogen content (mg/L and mg/100 g dry soil) and standard deviation as well as percentage change relative to control were calculated for each treatment group and sampling date.

For the evaluation of the results, statistically significant variations of the test item treatment groups from the control were calculated using either the Dunnett's Test or the T-Test (2n, 5 %).

Results and Discussion

Results from the mineralization, nitrite formation, nitrogen transformation and nitrification tests and the soil respiration test are summarised in the tables below.

Table 8.5-3: Effects on mineralization in Speyer 2.1 and F3 soils after treatment with CGA173506

Days after application	Control	0.133 mg a.s./ kg soil dry weight		1.33 mg a.s./ kg soil dry weight	
	NH_4-N [mg/100 g dry soil]	NH_4-N [mg/100 g dry soil]	Variation from control [%][1)]	NH_4-N [mg/100 g dry soil]	Variation from control [%][1)]
Speyer 2.1					
0	0.09	0.11	15.4	0.11	11.1
14	0.05	0.11	104.7	0.21	303.9
28	0.03	0.03	-4.1	0.03	13.0
49	0.03	0.04	22.4	0.04	7.9
70	0.14	0.12	-17.1	0.13	-12.4
97	0.04	0.04	0.2	0.05	19.2
Speyer F3					
0	0.15	0.16	3.0	0.16	1.8
14	0.02	0.02	11.1	0.02	17.5
28	0.03	0.03	-12.1	0.03	-10.4
48	0.25	0.18	-27.9	0.17	-31.5
69	0.02	0.02	-7.9	0.02	-6.8

96	0.04	0.04	-7.9	0.05	7.3

The calculations were performed with non-rounded values
[1)] Variation of the treatment with respect to the control

Table 8.5-4: Effects on nitrite formation in Speyer 2.1 and F3 soils after treatment with CGA173506

Days after application	Control	0.133 mg a.s./ kg soil dry weight		1.33 mg a.s./ kg soil dry weight	
	NO_2-N [mg/100 g dry soil]	NO_2-N [mg/100 g dry soil]	Variation from control [%][1)]	NO_2-N [mg/100 g dry soil]	Variation from control [%][1)]
Speyer 2.1					
0	0.01	0.01	0.00	0.01	0.00
14	0.01	0.01	0.00	0.01	0.00
28	0.01	0.01	0.00	0.01	0.00
49	0.01	0.01	0.00	0.01	0.00
70	0.01	0.01	0.00	0.01	0.00
97	0.01	0.01	0.00	0.01	0.00
Speyer F3					
0	0.01	0.01	0.00	0.01	0.00
14	0.01	0.01	0.00	0.01	0.00
28	0.01	0.01	0.00	0.01	0.00
48	0.01	0.01	0.00	0.01	0.00
69	0.01	0.01	0.00	0.01	0.00
96	0.01	0.01	0.00	0.01	0.00

The calculations were performed with non-rounded values
[1)] Variation of the treatment with respect to the control

Table 8.5-5: Effects on nitrogen transformation in Speyer 2.1 and F3 soils after treatment with CGA173506

Days after application	Control	0.133 mg a.s./ kg soil dry weight		1.33 mg a.s./ kg soil dry weight	
	NO_3-N [mg/100 g dry soil]	NO_3-N [mg/100 g dry soil]	Variation from control [%][1)]	NO_3-N [mg/100 g dry soil]	Variation from control [%][1)]
Speyer 2.1					
0	0.65	0.58	-11.0	0.51	-21.5
14	0.78	0.64	-17.8	0.42	-46.5
28	2.57	2.15	-16.4	1.99	-22.6
49	4.13	4.14	0.2	3.20	-22.6
70	5.08	5.41	-13.3	3.77	-25.9
97	5.82	5.32	-8.6	5.62	-3.4
Speyer F3					
0	0.84	0.84	-0.2	0.81	-4.3

14	0.19	0.13	-31.6	0.08	-56.6
28	0.93	0.66	-29.6	0.61	-34.9
48	2.90	2.60	-10.3	2.24	-22.7
69	2.72	2.10	-22.7	1.85	-31.8
96	4.14	3.65	-11.9	3.30	-20.3

The calculations were performed with non-rounded values
[1)] Variation of the treatment with respect to the control

Table 8.5-6: Effects on nitrification in Speyer 2.1 and F3 soils after treatment with CGA173506

Days after application	Control	0.133 mg a.s./ kg soil dry weight		1.33 mg a.s./ kg soil dry weight	
	Total N [mg/100 g dry soil]	Total N [mg/100 g dry soil]	Variation from control [%][1)]	Total N [mg/100 g dry soil]	Variation from control [%][1)]
Speyer 2.1					
0	0.76	0.70	-7.5	0.63	-17.1
14	0.84	0.76	-10.0	0.64	-24.5
28	2.62	2.19	-16.1	2.04	-22.0
49	4.18	4.19	0.4	3.25	-22.3
70	5.24	4.54	-13.4	3.90	-25.5
97	5.87	5.37	-8.5	5.68	-3.3
Speyer F3					
0	1.01	1.01	0.3	0.97	-3.3
14	0.23	0.17	-26.2	0.12	-47.1
28	0.98	0.70	-28.6	0.65	-33.7
48	3.17	2.79	-11.7	2.43	-23.3
69	2.75	2.13	-22.5	1.88	-31.5
96	4.20	3.70	-11.8	3.36	-20.0

The calculations were performed with non-rounded values
[1)] Variation of the treatment with respect to the control

Table 8.5-7: Effects on respiration in Speyer 2.1 and F3 soils after treatment with CGA173506

Time after application	Control		0.133 mg a.s./ kg soil dry weight			1.33 mg a.s./ kg soil dry weight		
	Mean CO_2 evolved [mg]	±SD	Mean CO_2 evolved [mg]	± SD	Variation from control [%][1)]	Mean CO_2 evolved [mg]	± SD	Variation from control [%][1)]
Speyer 2.1								
0 – 3 hours	0.910	0.016	0.788	0.094	-13.4	0.895	0.121	-1.7
Day 14	2.679	0.210	2.937	0.048	+9.6	2.995	0.254	+11.8
Day 28	1.116	0.090	1.070	0.114	-4.1	1.131	0.044	+1.4
Speyer F3								
0 – 3 hours	2.625	0.157	2.501	0.084	-4.7	2.489	0.060	-5.2
Day 15	5.578	0.251	5.061	0.229	-9.3	5.223	0.526	-6.4
Day 28	5.844	0.399	5.493	0.421	-6.0	6.013	0.167	+2.9

SD = Standard Deviation

[1)] based on CO_2 evolution; variation of the treatment with respect to the control

Validity criteria

No validity criteria were reported.

Conclusions

The test item CGA173506 caused no significant adverse effects on soil respiration (measured as CO_2 evolution) in the soils tested at the end of the 28-day incubation period.

Significant effects (deviation from control > 25 %) of CGA173506 on mineralization (measured as NH_4-N production), nitrification (measured as NO_2-N production), and nitrogen transformation (measured as NO_3-N-production) were determined in the soils, after 28 days. By Day 96 the effects on mineralization, nitrification and nitrogen transformation had reduced to < 25 % difference compared to the control group.

(Schanné C, Galicia H, 1992)

This study above complies with the current validity criteria for toxicity testing with soil microorganisms with one exception on a single sampling occasion (as detailed in the table below). Despite some test conditions not being reported and some deviations from the recommended test design, the endpoint is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Schanné, Galicia, 1992, CGA173506/5376	BBA Guidelines Part VI I-I (1990)	OECD No. 216 and 217 (2000)	The following deviations are noted : 1. Sand content of soils used was 37.7% to 87.4% (50% to 75% is recommended). 2. No statistical analysis is included. 3. Limited information is reported regarding incubation of the soils pre-treatment and soil collection. 4. No assessments were made on Day 7 (Days 0, 7, 14 and 28 recommended). OECD 216: 1. Variation between replicate control samples was greater than recommended (16%) on Day 14 in Speyer F3 soil only. 2. Results are presented as total NO3-N formation at each assessment period (recommended: nitrate formed should be expressed in mg nitrate/kg dry weight soil/day). OECD 217: 1. Individual replicate data for the control samples were not reported, thus the variation between control samples has been calculated based on the mean and standard deviation values presented in the report. 2. The carbon dioxide released was measured for a maximum of 3.6 hours (12 consecutive hours is recommended). 3. Respiration rates are expressed in mg carbon dioxide/100g	The validity criteria were met, with one exception on a single sampling occasion: 1. OECD 216: Variation between replicate control samples was less than ± 15 (max 15% in Speyer 2.1; max 16% on Day 14 in Speyer F3 only– see explanation below). 2. OECD 217: Variation between replicate control samples was less than ± 15 (max 8%). OECD 216: The trial was carried in two soil types. On one sampling occasion in one soil only at Day 14, the variation between the controls was 16%. On all other sampling occasions, and at the end of the study the variation was within guideline limits (± 15%); therefore it is considered that this minor deviation does not affect the integrity of the results. The study has some limitations, but the endpoint can be used in the risk assessment.

			dry weight soil/per sampling period (recommended: mg carbon dioxide/kg dry weight soil/hr).	

The following study was previously submitted in the EU and was summarised in point B.9.2.6 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.5-2 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.5.1 Völkel W, (2001). The Effects of CGA 192155 (Metabolite of CGA 173506) on Soil Respiration and Nitrification. Report Number 808121, RCC Ltd, Environmental Chemistry & Pharmanalytics Division, CH-4452 Itingen, Switzerland. (Syngenta File No. CGA192155/0014).*

Guidelines

OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000)

OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)

GLP: Yes

Executive Summary

CGA192155 was applied to the soil at concentrations of 0.106 mg CGA192155/kg dry soil and 0.353 mg CGA192155/kg dry soil. The test item CGA192155 caused no significant adverse effects on soil nitrogen transformation (measured as NO_3-N- and NO_2-N- production) or on soil carbon mineralisation (measured as CO_2 evolution) at the end of the 28-day incubation period.

Materials

Test Material	CGA192155
Parent:	CGA173506
Lot/Batch #:	MLA-336/1+2
Purity:	98 ± 2 %
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	August 2003

Treatments

Test concentrations:	0.106 and 0.353 mg CGA192155/kg dry soil
Control:	Purified water only
Toxic standard:	2-sec-Butyl-4,6-dinitrophenyl acetate (Dinosebacetate PESTANAL®), 98 %, at concentration of 3.74 mg/150 g dry soil (corresponding to 25 mg/kg dry soil)

Test design

Soil type:	Agricultural sandy loam soil (Speyer 2.3) supplied by Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA), Speyer, Germany

	Speyer 2.3 soil (sandy loam): 9.0 % clay, 33.3 % silt and 57.8 % sand, with an organic carbon content of 1.32 ± 0.10 %, a microbial biomass of 143 mg microbial C/kg dry soil (equivalent to 1.1 % of organic C), and a total nitrogen content of 0.09 %. The soil was not subjected to any pesticide or organic fertiliser treatment for at least four years.
Test units:	1 L incubation flasks stoppered with cotton wool plugs
Replication:	3
Sampling intervals:	0 – 3 hours, and 7, 14 and 28 days after treatment
Duration of test:	28 days

Environmental test conditions

Temperature:	20 ± 2 °C
pH of soil:	6.5 ± 0.1 (in $CaCl_2$)
Soil moisture content:	15.2 g water per 100 g dry soil (40 % MWC)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 20th June to 25th July 2001

Soil samples were treated with CGA192155 at two concentrations, 0.106 and 0.353 mg CGA192155/kg dry soil, corresponding to three times and ten times of the maximum expected single field rate, respectively. The concentrations were calculated on the basis of the maximum use rate of the parent CGA173506 (250 g CGA173506/ha three times per year) and the maximum detected formation of CGA 192155 (13 %). Test concentrations were related to a soil depth of 5 cm and a soil density of 1.5 g soil dry weight/cm^3.

The test item was mixed with acetone to prepare the low and high concentration stock solutions and the stock solutions was subsequently added to quartz sand. After complete evaporation of the acetone under a stream of nitrogen gas, each application mixture was mixed homogeneously using a Turax mixer. The application mixtures were stored in a refrigerator until application for one day.

Thereafter, the low and high dose application mixtures were added to the soil samples. During the treatment, the soil samples were thoroughly mixed. After the application, 0.7 g lucerne meal, containing 3 % nitrogen, was added to the soil samples for the nitrification experiment.

The soil moisture content was determined about one week before initiation of the test. The soil was filled into incubation flasks and equilibrated at 20 ± 2 °C in the dark. The soil moisture content was adjusted to just below 40 % of the maximum water holding capacity (MWC) prior to application. The moisture content of the samples was monitored on a weekly basis and moisture loss was compensated by the addition of purified water.

Three replicate soil samples were prepared for each concentration and the control for the nitrogen transformation test and carbon mineralisation test.

The determination of nitrite-nitrogen was based on a colorimetric method, whilst the determination of nitrate-nitrogen was determined using a cadmium reductor, followed by a colorimetric method.

Soil respiration was determined by measuring the glucose induced evolution of CO_2 resulting from microbial activity for about 20 hours. The CO_2 production of the soil samples was measured semi-continuously using an IR gas analyser and the volume of CO_2 evolved per hour (mL/h) per kg dry soil equivalents was calculated based on the CO_2 concentration, the soil sample dry weight equivalents and

the air flow rate. The respiration rates of treated and control soil during the first 12 consecutive hours were compared.

For evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A 2-sided Dunnett's Test at 5 % significance level was performed for statistical evaluation.

The Dixon test (Sachs 1999 or Dixon 1953) was used to eliminate outliers in the respiration and nitrification experiments (one-tailed, $\alpha = 0.05$).

Results and Discussion

Results from the nitrogen transformation test and the carbon transformation test are summarised in the tables below.

Table 8.5-8: Effects on nitrite formation in soil after treatment with CGA192155

Days after application	Control	0.106 mg CGA192155/kg dry soil		0.353 mg CGA192155/kg dry soil		25 mg/kg dry soil Dinoseb acetate	
	NO_2-N [mg/kg dry soil] mean	NO_2-N [mg/kg dry soil] mean	Deviation from control [%]	NO_2-N [mg/kg dry soil] mean	Deviation from control [%]	NO_2-N [mg/kg dry soil] mean	Deviation from control [%]
0	< 0.1	< 0.1	n.a.	< 0.1	n.a.	<0.1	n.a.
7	< 0.1	< 0.1	n.a.	< 0.1	n.a.	0.2	n.a.
14	< 0.1	< 0.1	n.a.	< 0.1	n.a.	1.8	n.a.
28	< 0.1	< 0.1	n.a.	< 0.1	n.a.	<0.1	n.a.

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

Table 8.5-9: Effects on nitrate formation in soil after treatment with CGA192155

Days after application	Control	0.106 mg CGA192155/kg dry soil		0.353 mg CGA192155/kg dry soil		25 mg/kg dry soil Dinoseb acetate	
	NO_3-N [mg/kg dry soil] mean	NO_3-N [mg/kg dry soil] mean	Deviation from control [%]	NO_3-N [mg/kg dry soil] mean	Deviation from control [%]	NO_3-N [mg/kg dry soil] mean	Deviation from control [%]
0	15.0	16.5	10.0	16.1	7.3	16.5	10.0
7	3.9	3.5	-10.3	3.4	-12.8	4.4	12.8
14	11.4	11.8	3.5	14.5	27.2	20.1	76.3
28	22.1	23.6	6.8	23.7	7.2	41.9	89.6

The values represent rounded results obtained by calculation using the exact raw data

Table 8.5-10: Effects on carbon mineralisation in soil after treatment with CGA192155

Days after application	Control	0.106 mg CGA192155/kg dry soil		0.353 mg CGA192155/kg dry soil		25 mg/kg dry soil Dinoseb acetate	
	Mean Respiration rate [mL CO_2/h per kg dry soil]	Mean Respiration rate [mL CO_2/h per kg dry soil]	Deviation from control [%]	Mean Respiration rate [mL CO_2/h per kg dry soil]	Deviation from control [%]	Mean Respiration rate [mL CO_2/h per kg dry soil]	Deviation from control [%]
0	4.67	4.97	6.3	5.12	9.6	2.48	-47.0

7	4.24	4.59	8.3	4.61	8.5	2.17	-48.8
14	3.37	3.61	7.2	3.75	11.3	1.49	-55.8
28	3.93	4.10	4.2	4.46	13.3	1.33	-66.3

The values represent rounded results obtained by calculation using the exact raw data

Validity criteria

No validity criteria were reported.

Conclusions

CGA192155 was applied to the soil at concentrations of 0.106 mg CGA192155/kg dry soil and 0.353 mg CGA192155/kg dry soil. The test item CGA192155 caused no significant adverse effects on soil nitrogen transformation (measured as NO_3-N- and NO_2-N-production) and on soil carbon mineralisation (measured as CO_2 evolution) at the end of the 28-day incubation period.

(Völkel W, 2001)

This study complies with the current validity criteria for toxicity testing with soil microorganisms and despite some deviations from the recommended test design, the endpoint is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Völkel, 2001, CGA192155/0014	OECD No. 216 and 217 (2000)	OECD No. 216 and 217 (2000)	The following deviations are noted : OECD 216: 3. Results are presented as total NO_3-N formation at each assessment period (recommended: The quantities of nitrate formed should be expressed in mg nitrate/kg dry weight soil/day). OECD 217: 4. The carbon dioxide released was measured semi continuously for 24 hours (12 consecutive hours is recommended)	The validity criteria were met: 3. OECD 216: Variation between replicate control samples was less than ± 15% (max 12%). 4. OECD 217: Variation between replicate control samples was less than ± 15% (max 5%). Despite some minor deviations from the recommended test design, the endpoint is reliable and can be used in the risk assessment

The following study was previously submitted in the EU and was summarised in point B.9.2.6 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk

assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.5-2 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.5.1 Völkel W, (2002). The Effects of CGA 265378 (Metabolite of Fludioxonil) on Soil Respiration and Nitrification. Report Number 808198, RCC Ltd, Environmental Chemistry & Pharmanalytics Division, CH-4452 Itingen, Switzerland. (Syngenta File No. CGA265378/0012).*

Guidelines

OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000)

OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)

GLP: Yes

Executive Summary

CGA265378 was applied to the soil at concentrations of 0.11 mg CGA265378/kg dry soil and 0.37 mg CGA265378/kg dry soil. The test item CGA265378 caused negligible effects on soil nitrogen transformation (measured as nitrogen production) and on soil carbon mineralisation (measured as CO_2 evolution) at the end of the 28-day incubation period.

Materials

Test Material	CGA265378 Metabolite of Fludioxonil
Lot/Batch #:	MLA-256
Purity:	99 ± 2 %
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 April 2004

Treatments

Test concentrations:	0.11 mg CGA265378/kg dry soil 0.37 mg CGA265378/kg dry soil
Control:	Unamended and untreated soil
Toxic standard:	2-sec-Butyl-4,6-dinitrophenyl acetate (Dinosebacetate PESTANAL®), 98 % 3.74 mg/150 g dry soil (corresponding to 25 mg/kg dry soil)

Test design

Soil type:	Speyer 2.3 (sandy loam), consisting of 9.0 % clay, 33.3 % silt and 57.8 % sand (according to USDA classification), with an organic carbon content of 1.32 ± 0.10 %, a microbial biomass of 169 mg microbial C/kg dry soil (equivalent to 1.3 % of organic C), and a total nitrogen content of 0.06 %. The soil was not subjected to any pesticide or organic fertiliser treatment for at least four years.
Test units:	1 L incubation flasks stoppered with cotton wool plugs
Replication:	3
Sampling intervals:	At Days 0, 7, 14 (nitrification only), 18 (respiration only) and 28, respiration and

	nitrification were determined for all treatments
Duration of test:	28 days

Environmental test conditions

Temperature:	20 ± 2 °C
pH of soil:	Not reported
Soil moisture content:	15.2 g water per 100 g dry soil (40 % MWC)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 15th November to 22nd December 2001

Soil samples were treated with CGA265378 at two concentrations, 0.11 and 0.37 mg CGA265378/kg dry soil, corresponding to three times and ten times the maximum expected single field rate, respectively. The rates were calculated on the basis of the maximum use rate of the parent fludioxonil (250 g fludioxonil/ha three times per year) and the maximum detected formation of CGA265378 (10 %). Test concentrations were related to a soil depth of 5 cm and a soil density of 1.5 g soil dry weight/cm^3.

The low concentration stock solution was prepared by dissolving 0.3836 mg CGA265378 in 1000 µL acetone. For the high concentration stock solution 1.1964 mg CGA265378 was dissolved in 1000 µL acetone. Then, aliquots of 860 µL and 930 µL of the low and high concentration stock solutions were added uniformly to 30 g aliquots of quartz sand (particle size about 0.1 – 0.3 mm). After evaporation of the acetone under a stream of nitrogen gas, each application mixture was mixed homogeneously using a Vortex mixer. The application mixtures were stored in a refrigerator until application for one day.

Thereafter, 1.5 g of the low and high concentration application mixtures were added to the soil samples (150 g dry weight). During the treatment, the soil samples were thoroughly mixed. After the application, 0.7 g lucerne meal, containing 3 % nitrogen, was added to the soil samples for the nitrification experiment.

The soil moisture content was determined about one week before initiation of the test. The soil was filled into incubation flasks and equilibrated at 20 ± 2 °C in the dark. The soil moisture content was adjusted to just below 40 % of the maximum water holding capacity (MWC) prior to application.

The incubation temperature was monitored continuously. The moisture content of the samples was monitored on a weekly basis and moisture loss was compensated by the addition of purified water.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon mineralisation test.

The determination of nitrite-nitrogen was based on a colorimetric method, whilst the determination of nitrate-nitrogen was determined using a cadmium reductor, followed by a colorimetric method.

Soil respiration was determined by following the glucose induced evolution of CO_2 resulting from microbial activity for about 20 hours. The CO_2 production of the soil samples was calculated as the volume of CO_2 evolved per hour (mL/h) per kg dry soil equivalents using an IR gas analyser. The respiration rates of treated and control soil during the first 12 consecutive hours were compared.

For evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A 2-sided Dunnett's Test at 5 % significance level was performed for statistical evaluation.

The Dixon test (Sachs 1999 and Dixon 1953) was used to eliminate outliers in the respiration and nitrification experiments (one-tailed, α = 0.05).

Results and Discussion

Results from the nitrogen transformation test and the carbon transformation test are summarised in the tables below.

Table 8.5-11: Effects on nitrite formation in soil after treatment with CGA265378

Days after application	Control	0.11 mg CGA265378/kg dry soil		0.37 mg CGA265378/kg dry soil		25 mg/kg dry soil Dinoseb acetate	
	NO_2-N [mg/kg dry soil] mean	NO_2-N [mg/kg dry soil] mean	Variation from control [%]	NO_2-N [mg/kg dry soil] mean	Variation from control [%]	NO_2-N [mg/kg dry soil] mean	Variation from control [%]
0	< 0.1	< 0.1	n.a.	< 0.1	n.a.	<0.1	n.a.
7	< 0.1	< 0.1	n.a.	< 0.1	n.a.	0.2	n.a.
14	< 0.1	< 0.1	n.a.	< 0.1	n.a.	2.2	n.a.
28	< 0.1	< 0.1	n.a.	< 0.1	n.a.	0.1	n.a.

The calculations were performed with rounded values
n.a. = not applicable

Table 8.5-12: Effects on nitrate formation in soil after treatment with CGA265378

Days after application	Control	0.11 mg CGA 265378/kg dry soil		0.37 mg CGA 265378/kg dry soil		25 mg/kg dry soil Dinoseb acetate	
	NO_3-N [mg/kg dry soil] mean	NO_3-N [mg/kg dry soil] mean	Variation from control [%]	NO_3-N [mg/kg dry soil] mean	Variation from control [%]	NO_2-N [mg/kg dry soil] mean	Variation from control [%]
0	12.1	12.6	4.1	12.5	3.3	12.6	4.1
7	2.6	3.0*	15.4	2.3*	-11.5	2.2	-15.4
14	8.8	13.2*	50.0	14.4*	63.6	15.7	78.4
28	19.5	21.1*	8.2	22.3*	14.4	43.9	125.1

*Statistically significant values compared to the control (according to Dunnett's Test, two-sided, α = 0.05)
The calculations were performed with rounded values

Table 8.5-13: Effects on carbon mineralisation in soil (glucose-induced short-term respiration) after treatment with CGA265378

Days after application	Control	0.11 mg CGA 265378/kg dry soil		0.37 mg CGA 265378/kg dry soil		25 mg/kg dry soil Dinoseb acetate	
	Mean Respiration rate [mL CO_2/h per kg dry soil]	Mean Respiration rate [mL CO_2/h per kg dry soil]	Variation from control [%]	Mean Respiration rate [mL CO_2/h per kg dry soil]	Variation from control [%]	NO_2-N [mg/kg dry soil] mean	Variation from control [%]
0	4.37	3.85*	-11.8	3.90*	-10.7	2.24	-48.6
7	3.76	3.33*	-11.7	3.28*	-12.9	1.62	-56.9

18	3.67	3.29*	-10.3	3.26*	-11.1	1.29	-64.8
28	3.91	3.62*	-7.2	3.57*	-8.7	1.37	-65.0

*Statistically significant values compared to the control (according to Dunnett's Test, two-sided, $\alpha = 0.05$)
The calculations were performed with rounded values

Validity criteria

No validity criteria were reported.

Conclusions

CGA265378 was applied to the soil at concentrations of 0.11 mg CGA265378/kg dry soil and 0.37 mg CGA265378/kg dry soil. The test item CGA265378 caused negligible effects on soil nitrogen transformation (measured as nitrogen production) and on soil carbon mineralisation (measured as CO_2 evolution) at the end of the 28-day incubation period.

(Völkel W, 2002)

This study complies with the current validity criteria for toxicity testing with soil microorganisms and despite some deviations from the recommended test design, the endpoint is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Völkel, 2002, CGA265378/0012	OECD No. 216 and 217 (2000)	OECD No. 216 and 217 (2000)	The following are not reported: 1. Coefficient of Variation between replicate control samples (these have now been calculated) 2.. OECD 216: Results are presented as total NO_3-N formation at each assessment period; (recommended: The quantities of nitrate formed should be expressed in mg nitrate/kg dry weight soil/day). 3. OECD 217: Sampling was carried out at Day 0, 7, 18 and 28; (recommended: Day 0, 7, 14 and 28)	While conditions of the study were not fully reported and there were minor deviations from the current guidelines, the following validity criteria were met: 1. OECD 216: Variation between replicate control samples was less than ± 15% (max 8%). 2. OECD 217: Variation between replicate control samples was less than ± 15% (max 4%). Despite some minor deviations from the recommended test design, the endpoint is reliable and can be used in the risk assessment.

A soil micro-organism test was conducted on the fludioxonil metabolite CGA339833 and is summarised below.

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.5/01 Schulz L. (2015c) CGA339833 - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests), Report Number 15 10 48 105 C/N BioChem agrar Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6 04827 Gerichshain, Germany (Syngenta file No. CGA339833_10007)

Guidelines

OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000)

OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)

GLP: Yes

Executive Summary

CGA339833 was applied to the soil at concentrations of 2 mg test item/kg dry soil and 20 mg test item/kg dry soil. The test item caused no adverse effects on soil nitrogen transformation (measured as NO_3-N-production) and on soil carbon transformation (measured as O_2-consumption) at the end of the 28-day incubation period.

Materials

Test Material	CGA339833
Lot/Batch #:	MES 338/1
Purity:	96 % w/w
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	31 May 2019
Treatments	
Test concentrations:	2 and 20 mg test item/kg dry soil
Control:	Untreated
Toxic standard:	Dinoterb tested in a separate study
Test design	
Soil type:	Sandy loam
Test units:	Nitrogen transformation: 500 mL wide mouth glass flask containing 200 g soil d.w. Carbon transformation: 4 L steel test vessels containing 1000g soil d.w.
Replication:	3
Sampling intervals :	3 hours, 7, 14, and 28 days after application
Duration of test:	28 days

Environmental test conditions

Temperature:	19.8 - 21.2 °C
pH of soil:	6.4
Soil moisture content:	WHC: 35.59 g/100 g soil d.w
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 23rd July to 25th September 2015

Soil samples were treated with CGA339833 at two concentrations, 2 and 20 mg test item/kg dry soil. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/$cm^{3.}$

The test item was mixed with finely ground quartz sand and thoroughly mixed with the soil by means of a hand stirrer. Water was added to the soil to achieve a water content of approximately 45 % of WHC. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 - 50 % of WHC.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon transformation test.

For the nitrogen transformation test, sub-samples of 200 g soil dry weight were weighed into each test vessel. Lucerne meal (5 g/kg soil dry weight) was then added to each 200 g soil sample to provide 1.0 g lucerne meal per 200 g soil d.w. and mixed by means of a hand-stirrer.

Mean nitrogen content (mg NO_3/kg soil d.w.), standard deviation and coefficient of variation as well as the mean 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 (based on the mean nitrogen content/day) for each sampling date.

For the carbon transformation test, three replicate soil samples were prepared for each treatment rate. For each replicate a sub-sample of 1000 g soil dry weight was weighed into each test vessel and then placed into a mixing vessel of a laboratory mixer.

The cumulative O_2 consumption after 12 hours was calculated (using regression analysis; the goodness of fit (R^2) was > 0.98 in all replicates and on all days). Furthermore, standard deviation and coefficient of variation were calculated for each treatment group and sampling dates.

For evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A 2 sided Student-t-test at 5 % significance level was performed for statistical evaluation.

Results and Discussion

Results from the nitrogen transformation test and the carbon transformation test are summarised in the tables below.

Table 8.5-~~3~~14: Effects on nitrogen transformation in soil after treatment with CGA339833

Time Interval	Control	2 mg test item/kg soil dry weight	20 mg test item/kg soil dry weight

(days)	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	Deviation from control [%][1)]	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	Deviation from control [%][1)]
0 - 7	45.7	4.31	46.8	4.47	+3.5	48.2	4.72	+9.5
0 - 14	60.8	3.24	60.9	3.24	+0.1	67.8	3.76*	+16.0
0 - 28	81.2	2.35	84.2	2.45	+4.5	89.4	2.65*	+12.9

The calculations were performed with non-rounded values
1) based on NO_3-nitrogen production; - = inhibition; + = stimulation
* = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, p □ 0.05)

Table 8.5-415: Effects on carbon transformation in soil after treatment with CGA339833

Days after application	Control		2 mg test item/kg soil dry weight			20 mg test item/kg soil dry weight		
	O_2-consumption [mg/kg soil d.w./h]	CV [%]	O_2-consumption [mg/kg soil d.w./h]	CV [%]	Deviation from control [%][1)]	O_2-consumption [mg/kg soil d.w./h]	CV [%]	Deviation from control [%][1)]
0	22.61	2.4	21.93	1.4	-3.0	21.65	1.4	-4.2
7	17.06	0.5	16.70	2.9	-2.1	16.40*	0.7	-3.8
14	15.66	1.5	15.61	2.0	-0.3	14.80*	0.9	-5.5
28	14.33	1.2	14.64	3.0	+2.2	13.75	2.3	-4.0

The calculations were performed with non-rounded values.
CV [%] = Coefficient of Variation
1) based on O_2 consumption; - = inhibition; + = stimulation
* statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)

Validity criteria

The validity criteria are listed below:

- The coefficient of variation in the nitrogen and carbon transformation tests were a maximum 5.3 % and 2.4 %, respectively (must be ≤ 15 %)
- The toxic standard caused effects of +33.2 % and +46.9 % at concentrations 16.00 and 27.00 mg /kg soil d.w. in the nitrogen transformation test, demonstrating the sensitivity of the test system (must be ≥ 25 %)
- The toxic standard caused effects of -30.1 % and -39.6 % at concentrations 16.00 and 27.00 mg /kg soil d.w. in the carbon transformation test, demonstrating the sensitivity of the test system (must be ≥ 25 %)

Conclusions

The test item CGA339833 (tested at 2 mg/kg soil dry weight and 20 mg/kg soil dry weight) caused no adverse effects (deviation from control < 25 %, OECD 216/217) on soil nitrogen transformation (measured as NO_3-N-production) and on soil carbon transformation (measured as O_2-consumption) at the end of the 28-day incubation period.

(Schulz L. 2015c)

A soil micro-organism study with the new soil metabolite SYN545245 ~~is currently being~~ has been conducted. The study ~~will be submitted~~ is summarised below and the dossier updated as agreed with the RMS.

Report:	Submitted for purposes of renewal due to change in data requirements: K-CA 8.5/02 Schulz L., (2016) SYN545245 - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests), Report Number 16 10 48 020 C/N . BioChem agrar, Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany (Syngenta file No. SYN545245_10008).

Guidelines

OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000)

OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)

GLP: Yes

Executive Summary

SYN545245 was applied to the soil at concentrations of 0.207 mg test item/kg dry soil and 2.07 mg test item/kg dry soil. The test item caused no adverse effects on soil nitrogen transformation (measured as NO_3-N-production) and on soil carbon transformation (measured as O_2-consumption) at the end of the 28-day incubation period.

Materials

Test Material	SYN545245
Lot/Batch #:	MES 427/1
Purity:	98 % w/w
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	End of October 2017
Density:	n/a
Treatments	
Test rates:	0.207 and 2.07 mg test item/kg soil dry weight
Control:	Untreated
Toxic standard:	Dinoterb
Test design	
Soil type:	Sandy loam
Test units:	Nitrogen transformation: 500 mL wide mouth glass flask containing 200 g soil

	dry weight
	Carbon transformation: 4L steel test vessels containing 1000 g soil dry weight.
Replication:	3
Sampling intervals :	3 hours, 7, 14, and 28 days after application
Duration of test:	28 days
Environmental test conditions	
Temperature:	19.9 - 21.6 °C
pH of soil:	Nitrogent transformation: 5.6 to 5.9
	Carbon transformation: 5.5 to 5.8
Soil moisture content:	Nitrogen transformation: 17.97 - 18.90 g/100 g soil d.w. (equivalent to 46.32 - 48.71 % of WHC)
	Carbon transformation: 18.41 - 19.32 g/100 g soil d.w. (equivalent to 47.44 - 49.78 % of WHC)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 15 April to 18 May 2016

Soil samples were treated with the test item at two doses, 0.207 and 2.07 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.

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 approximately 45 % of WHC. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 - 50 % of WHC.

Three replicate soil samples were prepared for each treatment rate and the control for the nitrogen transformation test and carbon transformation test.

Mean nitrogen content (mg NO_3/kg soil d.w.), standard deviation and coefficient of variation as well as the mean 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 (based on the mean nitrogen content/day) for each sampling date.

The cumulative O_2 consumption after 12 hours was calculated (using regression analysis; the goodness of fit (R^2) was > 0.99 in all replicates and on all days). Furthermore, standard deviation and coefficient of variation were calculated for each treatment group and sampling dates.

For evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated for each sampling date. A 2 sided Student-t-test at 5 % significance level was performed for statistical evaluation.

Results and Discussion

Results from the Nitrogen transformation test and the Carbon transformation test are summarised in the tables below.

Table 8.5-16: Effects on Nitrogen Transformation in Soil after Treatment with the Test Item

Time Interval (days)	Control		0.207 mg test item/kg soil dry weight			2.07 mg test item/kg soil dry weight		
	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	Deviation from control [%][1)]	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	Deviation from control [%][1)]
0 - 7	56.9	4.71	55.3	4.36	-7.6	52.9	4.05	-14.0
0 - 14	71.9	3.42	69.1	3.17	-7.5	73.0	3.46	+1.1
0 - 28	86.9	2.25	85.6	2.17	-3.5	89.3	2.31	+2.8

The calculations were performed with non-rounded values
1) based on NO_3-nitrogen-production; - = inhibition; + = stimulation
No statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Table 8.5-17: Effects on Carbon Transformation in Soil after Treatment with the Test Item

Days after application	Control		0.207 mg test item/kg soil dry weight			2.07 mg test item/kg soil dry weight		
	O_2-consumption [mg/kg soil d.w./h]	CV [%]	O_2-consumption [mg/kg soil d.w./h]	CV [%]	Deviation from control [%][1)]	O_2-consumption [mg/kg soil d.w./h]	CV [%]	Deviation from control [%][1)]
0	11.99	1.4	12.46*	1.5	+3.9	12.11	1.3	+1.0
7	10.58	3.4	10.83	1.5	+2.4	10.13	2.5	-4.2
14	9.86	0.4	10.14*	1.2	+2.8	9.54	2.9	-3.2
28	10.38	2.6	10.55	2.1	+1.6	10.02	1.2	-3.5

The calculations were performed with non-rounded values.
CV [%] = Coefficient of Variation
1) based on O_2-consumption; - = inhibition; + = stimulation
* = statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Validity criteria

The validity criteria are listed below:

- The coefficient of variation in the Nitrogen and Carbon transformation tests were 5.5 % and 3.4 % respectively (must be ≤ 15 %)
- The toxic standard caused effects of +28.6 % and +40.9 % at concentrations 16.00 mg and 27.00 mg/kg soil d.w. in the Nitrogen transformation test, demonstrating the sensitivity of the test system (must be ≥ 25 %)
- The toxic standard caused effects of -34.4 % and -39.2 % at concentrations 16.00 mg and 27.00 mg/kg soil d.w. in the Carbon transformation test, demonstrating the sensitivity of the test system (must be ≥ 25 %)

Conclusions

The test item SYN545245 (tested at 0.207 mg/kg dry soil and 2.07 mg/kg dry soil) caused no adverse effects (deviation from control < 25 %, OECD 216/217) on soil nitrogen transformation (measured as

NO_3-N-production) and on soil carbon transformation (measured as O_2-consumption) at the end of the 28-day incubation period.

(Schulz L., 2016)

Fungicidal effects of fludioxonil

At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

Studies on the effects of fludioxonil on soil fungi were summarised in the previous EU review in point B.9.8.1 of the DAR, and are summarised below~~therefore they are not repeated here~~.

Report:	*KIIA 8.6 Zeun R, (2002), Fludioxonil Metabolites: Evaluation of the fungicidal activity, Report Number not reported, Research Biology Fungicides, R&T 4.33. (Syngenta file No. CGA173506/5548)*

Guidelines

Not reported

GLP: Not reported

Executive Summary

The fungicidal activity of CGA173506 and its metabolites CGA192155, CGA265378 and CGA339833 was evaluated against *Erysiphe graminis* (powdery mildew), *Botrytis cinerea* (botrytis), *Fusarium culmorum* (fusarium head blight) and *Pyricularia oryzae* (rice blast) in six pathosystems combinations: *Erysiphe graminis* on barley, *Erysiphe graminis* on wheat, *Botrytis cinerea* on apple, *Botrytis cinerea* on tomato, *Pyricularia oryzae* on rice, and *Fusarium culmorum* on wheat.

CGA173506 showed high fungicidal activity against *Erysiphe graminis* on barley, *Botrytis cinerea* on apple and *Fusarium culmorum* on wheat, and a medium activity against *Pyricularia oryzae* on rice. All metabolites were completely inactive, with the exception of a weak fungicidal activity of CGA192155 against *Erysiphe graminis* on barley.

Materials

Parent material	CGA173506 Fludioxonil
Metabolites	CGA192155 CGA265378 CGA339833
Lot/Batch #:	Not reported
Actual content of active ingredients:	Not reported
Description:	Not reported

Stability of test compound:	Not reported
Reanalysis/expiry date:	Not reported
Treatments	
Control:	Not reported
Application rate:	200 and 60 of product/foliage (no unit of measurement provided) 20 and 6 of product/pouch (no unit of measurement provided)
Application method:	Foliar/fruit application Seed application (only for 1 combination of pest species/plant species)
Test organisms	
Pest species vs. plant species:	*Erysiphe graminis* (Powdery mildew) – *Hordeum vulgare* (Barley, v, Regina) *Erysiphe graminis* (Powdery mildew) – *Triticum aestivum* (Wheat) *Botrytis cinerea* (Botrytis) – *Malus domestica* (Apple, v. Golden Delicious) *Botrytis cinerea* (Botrytis) – *Lycopersicon esculentum* (Tomato, v. Roter Gnom) *Pyricularia oryzae* (Rice blast) – *Oryza sativa* (Rice, v. Sasanishiki) *Fusarium culmorum* (Fusarium head blight) – seeds of *Triticum aestivum* (Wheat, v. Orestis)
Growth stage of plants:	1-week old barley 1-week old wheat 4-week old tomato 3-week old rice Not reported for apple – study performed on fruit
Test design	
Test systems:	Greenhouse / growth chamber
Timing of exposure to pest species, after test item treatment:	1 day after foliar application (barley and wheat) 2 hours after foliar application (apple) 2 days after foliar application (tomato and rice) At the same time with barley seed treatment
Concentration of pest species:	Not reported (powdery mildew on barley and wheat) 4 x 10^5 conidia/mL (botrytis on apple) 1 x 10^5 conidia/mL (botrytis on tomato) 1 x 10^5 conidia/mL (rice blast on rice) 7 x 10^5 conidia/mL (fusarium head blight on wheat seeds)
Incubation period:	6 days (powdery mildew on barley and wheat) 7 days (botrytis on apple) 4 days (botrytis on tomato) 6 days (rice blast on rice) 11 days (fusarium head blight on wheat seeds)
Replication:	Not reported
Duration, as sum of day of treatment, day of exposure of plants to pest species and incubation period:	7 days (powdery mildew on barley and wheat) 7 days (botrytis on apple) 6 days (botrytis on tomato) 8 days (rice blast on rice)

	11 days (fusarium head blight on wheat seeds)

Environmental conditions

Test temperature:	18 to 20 °C (powdery mildew on barley and wheat) 22 °C (botrytis on apple) 20 °C (botrytis on tomato) 25 °C (rice blast on rice) 10 to 18 °C (fusarium head blight on wheat seeds)
Humidity:	60 % R.H. (powdery mildew on barley and wheat) Not reported (botrytis on apple) 95 % R.H (botrytis on tomato) 95 % R.H (rice blast on rice) 100 % R.H (fusarium head blight on wheat seeds)
Lighting:	14-hour photoperiod (fusarium head blight on wheat seeds) Not reported for other species

Study Design and Methods

Experimental dates: Not reported

The fungicidal activity of CGA173506 and its metabolites CGA192155, CGA265378 and CGA339833 was evaluated against *Erysiphe graminis* (powdery mildew), *Botrytis cinerea* (botrytis), *Fusarium culmorum* (fusarium head blight) and *Pyricularia oryzae* (rice blast) in six pathosystems combinations: *Erysiphe graminis* on barley, *Erysiphe graminis* on wheat, *Botrytis cinerea* on apple, *Botrytis cinerea* on tomato, *Pyricularia oryzae* on rice, and *Fusarium culmorum* on wheat.

Young plants of three monocot species (*Hordeum vulgare, Triticum aestivum* and *Oryza sativa*) and two dicot species (*Malus domesticus* and *Lycopersicon esculentum*) were treated with two test concentrations of CGA173506, and of its metabolites CGA192155, CGA265378 and CGA339833, respectively. After treatment, plants were infected with a specific pest species for each, the incidence of disease was assessed after an incubation period.

In parallel, seeds of one monocot species (*Triticum aestivum*) were treated with CGA173506 and its metabolites, mixed with a conidia suspension of the pest species *F. culmorum* in a pouch equipped with filter paper. After treatment, the pouches were incubated for 11 days. The degree of disease was evaluated by assessing the occurrence of brown lesions on the roots.

Results and Discussion

Based on different ranges of observed fungicidal activity, an activity rating from 10 to 0 was given, where 10 corresponded to 100% activity, 9 corresponded to 90 – 99 % of activity, 8 corresponded to 80 – 89 % of activity, and so on, until 0 which corresponded with < 10 % of activity.

The fungicidal activity of CGA173506 and its metabolites is presented in the table below:

Table 8.5-18: Fungicidal activity of CGA173506 and its metabolites CGA192155, CGA265378 and CGA339833

Parent / Metabolite	Application rate (unit not reported)	ERY/BAR[a] ERY/WHE[b]	BOT/AP[c] BOT/TOM[d]	PYR/RIC[e]	FUS/WHE[f]

	Foliar/fruit treatment	Seed treatment	Foliar treatment	Foliar/fruit treatment	Foliar treatment	Seed treatment
CGA173506	200	20	10	10/10	6	10
	60	6	10	10/10	5	9
CGA192155	200	20	5	0	0	-*
	60	6	-*	-*	-*	-*
CGA265378	200	20	0	0	0	0
	60	6	-*	-*	0	-*
CGA339833	200	20	0	0	0	0
	60	6	0	0	0	-*

* not tested
[a] *Erysiphe graminis* / barley
[b] *Erysiphe graminis* / wheat
[c] *Botrytis cinerea* / apple
[d] *Botrytis cinerea* / tomato
[e] *Pyricularia oryzae* / rice
[f] *Fusarium culmorum* / wheat

CGA173506 showed high fungicidal activity against *Erysiphe graminis* on barley, *Botrytis cinerea* on apple and *Fusarium culmorum* on wheat, and a medium activity against *Pyricularia oryzae* on rice.

All metabolites were completely inactive, with the exception of a weak fungicidal activity of CGA192155 against *Erysiphe graminis* on barley.

Validity criteria

No validity criteria were reported.

Conclusions

CGA173506 showed high fungicidal activity against *Erysiphe graminis* on barley, *Botrytis cinerea* on apple and *Fusarium culmorum* on wheat, and a medium activity against *Pyricularia oryzae* on rice. All metabolites were completely inactive, with the exception of a weak fungicidal activity of CGA192155 against *Erysiphe graminis* on barley.

(Zeun R, 2002)

A new study on effects of fludioxonil on soil fungi was conducted since the last EU review and is summarised below.

Report:	Submitted for purposes of renewal as conducted at the request of The Netherlands to increase the number of species to enable an SSD for classification purposes: K-CA 8.5/~~02~~03 Schulz L (2009). Fludioxonil - Effects on growth of soil fungi (Soil fungi test), Report Number 09 10 48 072 S, BioChem agrar GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. (Syngenta File No. CGA173506_11470)

Guidelines

IOBC Guideline (Hokkanen *et al.* 1992), modified for soil fungi

GLP: Yes

Executive Summary

The possible effects of fludioxonil on the growth of several fungi cultures in a laboratory.

The NOEC was determined to be 100 mg test item/kg soil dry weight for *Botrytis cinerea,* 0.1 mg test item/kg soil dry weight for *Monilinia fructigena*, 1.0 mg test item/kg soil dry weight for *Mycotypha africana,* 100 mg test item/kg soil dry weight for *Pythium debaryanum,* and 3.75 mg test item/kg soil dry weight for *Rhizopus stolonifer.*

It can be concluded that the EC_{50} is greater than 1000 mg test item/kg soil dry weight for *Botrytis cinerea*, is 1.71 mg test item/kg soil dry weight for *Monilinia fructigena*, is 24.1 mg test item/kg soil dry weight for *Mycotypha africana*, is greater than 1000 mg test item/kg soil dry weight for *Pythium debaryanum*, and is greater than 37.5 mg test item/kg soil dry weight for *Rhizopus stolonifer.*

Materials

Test Material	Fludioxonil CGA173506
Lot/Batch #:	SMO5F007
Purity of active ingredient analysed:	96.6 % w/w
Description:	Grey powder
Stability of test compound:	Stable under normal conditions
Reanalysis/Expiry date:	End of September 2013
Treatments	
Test concentrations:	*Botrytis cinerea, Pythium debaryanum:* 3.75, 10, 37.5, 100, 375, 1000 mg/kg *Monilinia fructigena, Mycotypha africana, Rhizopus stolonifer :* 0.1, 0.375, 1.0, 3.75, 10, 37.5 mg/kg
Control:	Untreated
Test organism	
Soil fungi species:	*Botrytis cinerea* *Monilinia fructigena* *Mycotypha africana* *Pythium debaryanum* *Rhizopus stolonifer*
Source:	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7 B, 38124 Braunschweig, Germany
Test design	
Soil:	Agriculturally used field soil “Canitz”
Agar:	*Botrytis cinerea:* molten nutrient agar enriched with vegetable juice

	Monilinia fructigena: molten nutrient agar enriched with vegetable juice *Mycotypha africana:* molten nutrient agar *Pythium debaryanum:* molten nutrient agar enriched with vegetable juice *Rhizopus stolonifer:* molten nutrient agar enriched with vegetable juice
Test units:	Petri dishes containing 10g soil, 20 mL agar
Replication:	3
Sampling interval:	Daily
Duration:	Until the control units were fully covered by mycelium (depending on growth of the respective fungi species), i.e. 3 days for *Botrytis cinerea*, 9 days for *Monilinia fructigena*, 9 days for *Mycotypha africana*, 2 days for *Pythium debaryanum*, 1 day for *Rhizopus stolonifer*
Environmental conditions	
Test temperature:	*Botrytis cinerea, Pythium debaryanum, Rhizopus stolonifer* 20.0 - 20.9 °C *Monilinia fructigena, Mycotypha africana* 24.0 - 25.3 °C
Photoperiod:	Continuous dark
Soil moisture content at test start:	16.65 - 18.04 g/100 g soil d.w. (equivalent to 43.58 - 47.20 % of WHC)
Soil pH at test start:	5.7-5.8

Study Design and Methods

Experimental dates: 5th to 16th October 2009

Two days before test start, the test soils were prepared by mixing the test item with sterilized soil (autoclaved) and subsequently incubating for 2 days at 20 - 25 °C in an incubator. At test start (inoculation), the test soils were weighed into sterilized petri dishes (10 g soil dry weight/petri dish). Afterwards molten nutrient agar was poured on top of the soil, when the agar was at a temperature of about 50 °C. During pouring, the dishes were swirled in order to evenly distribute soil and agar. As soon as the agar was solidified and cooled off to room temperature, a piece of mycelium inoculum (5 mm diameter) was placed in the centre of each plate. After that the inoculated petri dishes were incubated at 20 - 25 °C in an incubator.

The fungi growth was monitored daily and measured twice as colony diameter (i.e. smallest colony diameter and largest colony diameter) until the control petri dishes were fully covered.

The colony diameter of each replicate (radial growth of the fungus) was measured twice (smallest and largest colony diameter) to calculate mean per replicate and mean per treatment group as well as the deviation of the test item treatment groups from the control.

Dunnett-t-test or Welch-t-test was used to compare the control with the independent test item groups. Probit analysis was performed for estimation of the EC_{50} for the last assessment day.

Results and Discussion

The results are summarised below.

Table 8.5-519: Effects of fludioxonil on radial growth of *Botrytis cinerea* and *Pythium debaryanum*

Treatment group	Soil fungi species					
	Botrytis cinerea (day 3 = last assessment day)			*Pythium debaryanum* (day 2 = last assessment day)		
Test item (mg/kg soil d.w.)	Radial growth		Deviation from control	Radial growth		Deviation from control
	Mean [Ø in cm]	CV	[%]	Mean [Ø in cm]	CV	[%]
Control	6.6	3.9	-	4.5	2.6	-
3.75	6.7	3.4	+2.0	4.4	2.4	-2.2
10	6.8	0.4	+2.8	4.3	1.8	-3.7
37.5	6.4	5.2	-2.5	4.5	2.9	+0.4
100	6.1	5.0	-7.6	4.5	1.3	±0.0
375	5.5 *	2.7	-16.5	3.7 *	4.7	-17.1
1000	5.8 *	6.5	-11.9	3.6 *	3.5	-20.1

Calculations were done using non-rounded values
Ø = diameter, CV = Coefficient of Variation
* statistically significant differences compared to control (Dunnett-t-test; $p \leq 0.05$, for homogenous variances)
- = inhibition; + = stimulation

Table 8.5-620: Effects of fludioxonil on radial growth of *Monilinia fructigena*, *Mycotypha africana* and *Rhizopus stolonifer*

Treatment group	Soil fungi species								
	Monilinia fructigena (day 9 = last assessment day)			*Mycotypha Africana* (day 9 = last assessment day)			*Rhizopus stolonifer* (day 1 = last assessment day)		
Test item (mg/kg soil d.w.)	Radial growth		Deviation from control	Radial growth		Deviation from control	Radial growth		Deviation from control
	mean [Ø in cm]	CV	[%]	mean [Ø in cm]	CV	[%]	mean [Ø in cm]	CV	[%]
Control	6.6	2.0	-	5.6	15.6	-	4.7	9.7	-
0.1	6.2	8.5	-6.3	4.9	25.3	-11.4	4.8	8.5	+3.6
0.375	5.3 *W	4.9	-20.5	4.9	13.4	-11.7	4.6	2.5	-1.8
1.0	3.9	37.8	-40.9	5.0	12.7	-10.8	4.9	9.5	+5.0
3.75	1.8 *W	2.8	-72.7	3.9 *D	13.6	-30.6	4.3	6.1	-8.6
10	1.7 *W	12.0	-74.5	3.2 *D	6.4	-42.9	3.8 *D	2.7	-17.9
37.5	0.7 *W	0.0	-89.4	2.5 *D	8.2	-55.6	2.7 *D	3.3	-43.0

Calculations were done using non-rounded values
Ø = diameter, CV = Coefficient of Variation
*D statistically significant differences compared to control (Dunnett-t-test; $p \leq 0.05$, for homogenous variances)
*W statistically significant differences compared to control (Welch-t-test; $p \leq 0.05$, for inhomogenous variances)
- = inhibition; + = stimulation

The test item fludioxonil caused a statistically significant inhibition (Dunnett-t-test ($p \leq 0.05$) compared to control of the radial growth of *Botrytis cinerea* and *Pythium debaryanum* at the two highest tested concentrations of 375 and 1000 mg test item/kg soil dry weight. It caused a statistically significant inhibition (Dunnett-t-test ($p \leq 0.05$) compared to control of the radial growth of *Monilinia fructigena*, *Mycotypha africana* and *Rhizopus stolonifer* at the two highest tested concentrations of 10 and 37.5 mg test item/kg soil dry weight and it also caused statistically significant inhibition (Dunnett-t-test ($p \leq 0.05$) compared to control of the radial growth of *Monilinia fructigena* at 0.375 and 3.75 mg test item/kg soil dry weight, and *Mycotypha africana* at 3.75 mg test item/kg soil dry weight.

Conclusions

The effects of fludioxonil on the growth of several fungi species are summarised as follows;

Table 8.5-217: Fludioxonil – Summary of endpoints

Test organism	NOEC	EC_{50}
	[mg test item/kg soil dry weight]	
Botrytis cinerea	100	> 1000
Monilinia fructigena	0.1	1.71
Mycotypha africana	1.0	24.1
Pythium debaryanum	100	> 1000
Rhizopus stolonifer	3.75	> 37.5

(Schulz L, 2009)

Fungicidal activity of metabolites

Table 8.5-228: Summary of Endpoints for fungicidal activity of metabolites of fludioxonil

Organism	Test item	Pathosystem	Endpoints	Exposure	Foliar/ Pouch	Disease pressure (%)	Activity rating (%)	Reference (author, date, Syngenta Ref)
Erysiphe graminis	Fludioxonil WP 25	Barley/wheat leaves	EU	200 mg/L	F	nr [c]	100 [b]	***Zeun (2002) CGA173506/5548*** In DAR noted as not fully acceptable as the study is not reported in sufficient detail
Botrytis cinerea		Tomato leaves			F	nr [c]	100 [b]	
Pyriculari culmorum		Rice leaves			F	nr [c]	60 [b]	
Fusarium culmoram		Wheat leaves		20 mg/L	Pouch	nr [c]	100 [b]	
Erysiphe graminis	CGA192155	Barley leaves	EU	200 mg/L	Foliar	nr [c]	50 [a b]	
Botrytis cinerea		Apple leaves			Fruit	nr [c]	0	
Pyriculari culmorum		Rice leaves		200 mg/L	Foliar	nr [c]	0	
Fusarium culmoram		Wheat leaves		-	-	-	-	
Erysiphe graminis	CGA265378	Barley leaves	EU	200 mg/L	Foliar	nr [c]	0	
Botrytis cinerea		Tomato leaves			Foliar	nr [c]	0	
Pyriculari culmorum		Rice leaves			Foliar	nr [c]	0	
Fusarium culmoram		Wheat leaves		20 mg/L	Pouch	nr [c]	0	
Erysiphe graminis	CGA339833	Wheat leaves	EU	200 mg/L	Foliar	nr [c]	0	
Botrytis cinerea		Tomato leaves			Foliar	nr [c]	0	
Pyriculari culmorum		Rice leaves			Foliar	nr [c]	0	
Fusarium culmoram		Wheat leaves		20 mg/L	Pouch	nr [c]	0	
Botrytis cinerea	Fludioxonil WP 50	Oilseed rape	New study	150 g a.s./ha	Foliar	3.8	89	***Haas (2015) CGA173506_11931***
				33 g a.s./ha		11	66	
	CGA192155			150 g a.s./ha		33	0	
	CGA265378					38	0	
	CGA339833					36	0	
Alternaria solani	Fludioxonil WP 50	Tomato	New study	60 g a.s./ha	Foliar	15	81	***Schneiter (2015) CGA173506_11933***
				20 g a.s./ha		43	46	
	CGA192155			60 g a.s./ha		78	10	
	CGA265378					80	0	
	CGA339833					80	0	

New study refers to studies performed since EU review of fludioxonil
[a] Classified as having weak activity to *Erysiphe graminis* with no activity to the other 2 species of fungi; the rate tested was very high.
[b] Originally recorded on a 0 to 10 scale, expressed as percentage (%) for consistency with other data shown in table.
[c] Disease levels in these tests conducted at that time were on average are >80% (Pers. Comm with R. Zeun).
nr: Not reported

A fungicidal activity study on fludioxonil and the metabolites CGA192155, CGA265378 and CGA339833 was summarised in the previous EU review in point B.9.9.1 of the DAR, therefore it is not repeated here.

Two new fungicidal activity tests on fludioxonil and the metabolites CGA192155, CGA265378 and CGA339833 were conducted since the last EU review and are summarised below.

Report:	Conducted at the request of the German UBA: K-CA 8.5/~~03~~04. Haas H.U. (2015), Fungicidal activity of Fludioxonil and the metabolites CSAA250411, CSAA424113, CSAA794110 against *Botrytis cinerea* on oilseed rape., Report Number PDB-2014.2928.28, Syngenta crop protection AG, Research Biological science, Disease control, Stein (Syngenta File No. CGA173506_11931).

Guidelines

None

GLP: No

Executive Summary

The fludioxonil environmental metabolites CGA265378 (CSAA250411), CGA339833 (CSAA424113) and CGA192155 (CSAA794110) showed no fungicidal activity against the fungal pathogen *Botrytis cinerea* on oil seed rape. The active substance fludioxonil showed good fungicidal activity across a range of rates as might be expected from an efficacious fungicide. Based on the results of the sensitive greenhouse test on oil seed rape all three metabolites are not considered fungicidally active against *Botrytis cinerea* on oil seed rape.

Materials

Test material	Fludioxonil CSAA250411 =CGA265378 CSAA424113 =CGA339833 CSAA794110 =CGA192155
Treatments	
Test concentrations:	Nominal concentrations of 150, 100, 75, 50 and 33.3 g a.i/ha
Solvent:	None
Test design	
Spray volume rate:	200 L spray solution/ha
Application method:	Boom sprayer
Replication:	5 plants per treatment

Duration of test: 8 days after application

Study Design and Methods

Experimental dates: 09 January 2015

17 days old oilseed rape plants var.Elektra (GS13-GS14) were sprayed with test compounds at rates of 150 g a.s./ha to 33.3. g a.s./ha. The applications were carried out with a boom sprayer and 200 L/ha spray volume on 5 plants per treatment. All compounds, including fludioxonil, were formulated using the same recipe of a WP formulation with 50 % content of test compound.

One day after application the plants were inoculated with a spore suspension of *Botrytis cinerea* containing 5 x 10^6 spores/ml in 3 % SMB+0.01 %Tween 20. After inoculation, the plants were placed randomly under closed hoods in a greenhouse at high humidity (20°C, 95 % rh).

Eight days after treatment application (7 days after inoculation) the percentage leaf area destroyed by disease was assessed visually. The percentage efficacy was then determined using the data for the individual rates and the untreated control.

The confidence interval was calculated using the student's t-distribution and a statistical significance of α=5 %.

Results and Discussion

Disease severity in untreated, infested check was 33 % in average, varying from 25 % to 55 % with one outlier of 80 %. The degree of infestation and symptoms was within the expectations.

The extremely sensitive plant showing 80 % disease severity was not used further for calculations.

Fludioxonil showed good activity on *Botrytis cinerea* on oil seed rape, with a dose response from 89 % activity at highest test rate (150 g a.s./ha) to 66 % activity at lowest test rate (33.3 g a.s./ha). Disease severity was much lower for all tested rates compared to the check. The difference was statistically significant for all treatments.

Neither CGA265378 (CSAA250411) nor CGA339833 (CSAA250411) showed any fungicidal activity against B. cinerea.

The low levels of inconsistent activity observed with CGA192155 (CSAA749110) are within the normal inherent variation observed in this pathosystem and therefore are not indicative of fungicidal activity.

The results are shown in the table below:

Table 8.5-239: Disease severity and activity of fludioxonil and metabolites at rates from 33.3 to 150 g a.s/ha to *Botrytis cinerea* on oil seed rape, 8 days after application

Compound	Rate (g a.s./ha)	Average disease severity (%)	Activity Vs Inoculated check (%)
Fludioxonil	150	3.8	89
	100	6.4	81
	75	7.4	78
	50	8.4	75
	33.3	11.4	66
CGA265378 (CSAA250411)	150	38.0	0

	100	35.0	0
	75	34.0	0
	50	37.0	0
	33.3	32.0	3
CGA339833 (CSAA424113)	150	36.0	0
	100	33.0	0
	75	39.0	0
	50	34.0	0
	33.3	35.0	0
CGA192155 (CSAA749110)	150	33.0	0
	100	27.0	18
	75	26.0	22
	50	25.0	25
	33.3	35.0	0
Inoculated check		33.1	0

Conclusions

The fludioxonil environmental metabolites CGA265378 (CSAA250411), CGA339833 (CSAA424113) and CGA192155 (CSAA794110) showed no fungicidal activity against the fungal pathogen *Botrytis cinerea* on oil seed rape, whereas the parent fludioxonil showed good fungicidal activity at high rates to mean activity at low rates. Based on the results of the sensitive greenhouse test on oil seed rape all three metabolites are without efficacy on *Botrytis cinerea* on oil seed rape.

(Haas H.U. 2015)

Report:	Conducted at the request of the German UBA: K-CA 8.5/~~0~~405 Schneiter P. (2014), Fungicidal activity of Fludioxonil and CSAA250411, CSAA424113, CSAA794110 against *Alternaria solani* on tomato., Report Number PDB-2014.2977.1B , Syngenta crop protection AG, Research Biological science, Disease control, Stein (Syngenta File No. CGA173506_11933).

Guidelines

None

GLP: Yes

Executive Summary

The environmental metabolites of fludioxonil CGA265378 (CSAA250411),CGA339833 (CSAA424113) and CGA192155 (CSAA794110) showed no activity against the fungal pathogen *Alternaria solani* on tomato at all rates tested (60, 20, 6, 2, 0.2 mg a.s./L) whereas the parent, fludioxonil, showed good fungicidal activity at higher rates (60, 20 mg a.s./L).

Materials

Test material Fludioxonil = CSAA161647
CSAA250411 =CGA265378
CSAA424113 =CGA339833
CSAA794110 =CGA192155

Treatments

Test concentrations: Nominal concentrations of 0.2, 0.6, 2, 6, 20 and 60 mg ai/L

Solvent: None

Test design

Spray volume rate: 400 L spray solution/ha

Application method: Boom sprayer

Replication: 4 plants per treatment

Duration of test: 7 days after application

Study Design and Methods

Experimental dates: 29 December 2014

Prior to application a stock solution in water was prepared by dissolving the formulated material in water to obtain the desired concentrations. To ensure the same composition of the solutions the Geoxe WP50 recipe was used for the metabolites and the parent. The spray suspensions were applied to plants in a spray cabinet (developed by the firm Caromatic AG) equivalent to a spray volume of 400 L/ha providing coverage of both upper and lower leaf surfaces (plants placed on turntable, air assisted spraying from 2 nozzles).

Four week old tomato plants cv. Roter Gnom were treated sprayed in a spray chamber with the formulated test compound diluted in water. The test plants are inoculated by spraying them with a spore suspension (strain no K5955, 9'000 spores/ml in water) two days after application. The inoculated test plants are incubated at 22/18°C (day/night) and 95% rh in a greenhouse. The percentage leaf area covered by disease is assessed when an appropriate level of disease appears on untreated check plants (5 – 7 days after application). The results are expressed as percent activity (control).

Results and Discussion

Table 8.5-2410: Disease severity and activity of fludioxonil and metabolites at rates from 0.2 to 60 g a.s/ha on *Alternaria solani* on tomato, 7 days after application

Compound	ai/ha	Average % disease damage	Efficacy
Blank formulation WP50	60	75	4
CGA265378 (CSAA250411)	60	80	0
	20	80	0
	6	80	0
	2	80	0
	0.6	80	0
	0.2	80	0
CGA339833 (CSAA424113)	60	80	0
	20	80	0
	6	80	0
	2	80	0

	0.6	80	0
	0.2	80	0
CGA192155 (CSAA749110)	60	77.5	1
	20	80	0
	6	80	0
	2	80	0
	0.6	80	0
	0.2	80	0
Fludioxonil (CSAA161647)	60	15	81
	20	42.5	46
	6	80	0
	2	80	0
	0.6	80	0
	0.2	80	0
Infected check	-	78.5	0
Uninfected check	-	0	-

Conclusions

The environmental metabolites of fludioxonil CGA265378 (CSAA250411),CGA339833 (CSAA424113) and CGA192155 (CSAA794110) showed no activity against the fungal pathogen *Alternaria solani* on tomato at all rates tested (60, 20, 6, 2, 0.2 mg a.s./L) whereas the parent, fludioxonil, showed good fungicidal activity at higher rates (60, 20 mg a.s./L).

(Schneiter P, 2014)

Relevant Literature on Nitrogen Transformation

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

CA 8.6 Effects on Terrestrial Non-Target Higher Plants

Table 8.6-1: Summary of Endpoints for Fludioxonil

Test species	Test type	Test substance	Endpoints		Comment	Reference (author, date, Syngenta Ref)
Triticum aestivum, Lactuca sativa, Raphanus sativa	Seedling emergence	A8207I	EU	ER_{50} 10 mg A8207I/kg	Equivalent to 0.261 mg a.s./kg	***Porch, & Krueger (2002) CGA173506/5496***
Allium cepa, Avena sativa, Glycine max, Beta vulgaris, Brassica napa, Cucumis sativa	Seedling emergence (Screen)	A8240D	New study	ER_{50} > 1000g A8240D/ha	-	***Bramby-Gunary 2008 A8240D_10653***
	Vegetative vigour (Screen)			ER_{50} > 1000g A8240D/ha	-	
Allium cepa, Lolium perenne, Triticum aestivum, Zea mays, Beta vulgaris, Brassica oleracea, Glycine max, Raphanus sativus, Lactuca sativa, Linum usitatissimum, Lycopersicon esculentum	Seedling emergence	A7850D Geoxe 50 WP (50.4%)	New study	ER_{50} >2.0 lbs/acre Equivalent to 2242 g a.s./ha	Superceded by new representative formulation (A8240D) study	***Porch et al. (2011) CGA173506_50433***
Glycine max, Lactuca sativa, Allium cepa, Avena sativa, Lolium perenne, Brassica oleracea, Zea mays, Cucumis sativa, Daucus carota, Lycopersicon esculentum	Seedling emergence	Fludioxonil	New study	ER_{50} 1.0 lbs/acre Equivalent to 1121 g a.s./ha	New data requirement	***Canez (1989)*** [a] ***CGA173506_11512***
Glycine max, Lactuca sativa, Allium cepa, Avena sativa, Lolium perenne, Brassica oleracea, Zea mays, Cucumis sativa, Daucus carota, Lycopersicon esculentum	Vegetative vigour	Fludioxonil	New study	ER_{50} >1.0 lbs/acre Equivalent to 1121 g a.s./ha	New data requirement	***Canez (1989)*** [a] ***CGA173506_50227***

New study refers to studies performed since EU review of fludioxonil

[a] The Canez 1989 studies were not submitted during the first EU review as data from the Switch formulation (A9219B) was used; and the Terrestrial Guidance document (2002)[27] data requirements were focused where the 'risk to terrestrial plants is considered critical' (herbicides and PGRs). The combination of the old and new data shows similar results and their use adds to the variety of plant species tested.

CA 8.6.1 Summary of screening data

The studies previously submitted in the EU were summarised in the previous EU review, therefore they are not repeated here.

New data produced on the current representative solo foliar variant is provided in **Document M-CP Section 10** (A8240D) in Section 10.6.1.

[27] Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC. Sanco/10329/2002 rev 2. Final.

CA 8.6.2 Testing on non-target plants

New data produced on a solo WP foliar formulation A7850D is provided in **Document M-CP Section 10** (A8240D) in Section 10.6.2.

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints : K-CA 8.6.2/01. Canez V.M. (1989), Nontarget phytotoxicity test seedling emergence tier 2, Report Number, Pan-agricultural labs, Inc. 32380 Avenue 10 Madera, California (Syngenta file No. CGA173506_11512).

Guidelines

U.S. EPA Guideline 122-1 (1986)

GLP: Yes

Executive Summary

The effects of the fludioxonil were evaluated on the seedling emergence, survival, height and biomass of ten species of non-target plants.

There were no adverse effects on any of the ten species after spraying fludioxonil at rates up to 1.0 lb a.s./acre. Therefore the No observed effect rate was 1.0 lb a.s./acre (1121 g a.s./ha). The ER_{25} and ER_{50} values were >1.0 lb/a.s./acre (1121 g a.s./ha), the highest application rate tested.

Materials

Test material	CGA173506 technical
Lot/Batch #:	FL-881677
Purity:	97.5%
Description:	Colourless crystals
Stability of test compound:	Stable under test conditions.
Reanalysis/expiry date:	Not stated
Treatments	
Test concentrations:	0.063, 0.125, 0.25, 0.5 and 1.0 lb a.i./a alongside an water control
Control:	Irrigation water
Spray volume:	468 L/ha
Application method:	Track sprayer

Test organisms

Species:	*Allium cepa* (onion) *Avena sativa* (oat) *Lolium perenne* (ryegrass) *Brassica oleracea* (cabbage) *Cucumis sativa* (cucumber) *Glycine max* (soybean) *Lactuca sativa* (lettuce) *Lycopersicon esculentum* (tomato) *Daucus carota* (carrot) *Zea mays* (corn)
Test soil:	Sandy loam

Test design

Test vessels:	Plastic growing pots (7.5 x 7.5 x 6.0 cm)
Sampling interval:	7, 14 and 21 days after treatment for all plants, due to slow seedling emergence, the carrot, tomato, cucumber and onion continued until 28 days after treatment.
Replication:	None
Duration:	21 to 28 days dependant on species

Study Design and Methods

Experimental dates: February 1989

Planted seeds of four monocot and six dicot species were sprayed with a series of five test concentrations of test item. Nominal test concentrations used in the definitive test for all test species ranged from 0.063 to 1.0 lb a.i. per acre. The number of emerged seedlings, number of surviving seedlings, seedling height and weight were determined at test termination.

Observations were made 7, 14 and 21 days after application to document seedling emergence and growth. Observations consisted of noting whether emergence had or had not occurred and assessing the condition of each seedling. Emergence was defined as the presence of visible plant tissue at the surface of the soil. Seedling condition was described by noting the presence or absence of possible signs of phytotoxicity such as necrosis, leaf wrinkle, chlorosis, plant lodging or plant stunting.

The growth of emerged seedlings was evaluated at the end of the test by assessing the height and biomass of seedlings. Plant biomass was estimated by measuring the total dry weight of the shoots within each replicate. Seedling height was measured with a ruler to the nearest whole centimetre from the surface of the soil to the apical meristem, or to the tip of the tallest leaf.

Results and Discussion

A one way analysis of variance was used to analyse the data from the percentage seedling emergence portions of the study. A one way ANOVA for data with unequal subsamples was used to analyse the seedling height data. Treatment mean separation was achieved using either SAS or a Lotus 1-2-3 spread sheet. The EC values were calculated using a probit analysis, if the data exhibited a dose response.

The LOER, NOER, ER_{25}, and ER_{50} for each of the ten test species are presented in tables below:

Table 8.6.2-1: Effect Rates of CGA173506 on 21-Day Emergence and Survival

Species	Emergence, biomass and survival (lbs/acre)				
	LOER	NOER	ER_{25}	ER_{50}	Mean phytotoxicity rating
Soybean	≥1.0	1.0	>1.0	>1.0	0.0
Lettuce	≥1.0	1.0	>1.0	>1.0	0.0
Carrot	≥1.0	1.0	>1.0	>1.0	0.0
Tomato	≥1.0	1.0	>1.0	>1.0	0.0
Cucumber	≥1.0	1.0	>1.0	>1.0	0.0
Cabbage	≥1.0	1.0	>1.0	>1.0	0.2
Oat	≥1.0	1.0	>1.0	>1.0	0.0
Ryegrass	≥1.0	1.0	>1.0	>1.0	0.0
Corn	≥1.0	1.0	>1.0	>1.0	0.0
Onion	≥1.0	1.0	>1.0	>1.0	0.2

Phytotoxicity ratings were based on a 0-4 scale with 0 = n effect, 1 = slight effect, 2= moderate effect, 3 = severe effect, 4 = total effect

A statistical effect was not observed following treatment with the highest test concentrations, therefore the no effect concentration is equal to or greater than 1.0 lb ai/a.

Conclusions

The effects of the fludioxonil were evaluated on the seedling emergence, survival, height and biomass of ten species of non-target plants.

There were no adverse effects on any of the ten species after spraying fludioxonil at rates up to 1.0 lb a.s./acre. Therefore the No observed effect rate was 1.0 lb a.s./acre (1121 g a.s./ha). The ER_{25} and ER_{50} values were >1.0 lb/a.s./acre (1121 g a.s./ha), the highest application rate tested.

(Canez V.M. 1989)

This study complies with the current validity criteria for toxicity testing with non-target plants (OECD 208; 2006), with the exception that the concentrations tested were not confirmed by analytical verification. However applications were made using calibrated application equipment and details of dose preparation are included in the report. Therefore the study is considered reliable and still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Canez, 1989, CGA173506_11512	U.S. EPA Guideline 122-1 (1986)	OECD No. 208 (2006)	The following were not reported: 1. The concentrations tested were not confirmed by analytical verification	Most of the validity criteria were met: 6.Seedling emergence in control was ≥70% (77 to 100%) 7.The control seedlings did not exhibit visible phytotoxic effects and plants exhibited only normal variation in growth and

				morphology. 8.The mean survival of emerged control seedlings was ≥90% for the duration of the study (no mortality reported). 9.Environmental conditions/growing media for a particular species were identical. The concentrations tested were not confirmed by analytical verification, however applications were made using calibrated application equipment and details of dose preparation are included in the report. The study is considered reliable and suitable for use in the risk assessment.

Report:	Submitted for purposes of renewal for relevance comparison with existing endpoints : K-CA 8.6.2/02. Cañez VM (1989a). Nontarget Phytotoxicity Test Vegetative Vigor Tier 2 , Report Number LR88-56A, Pan-Agricultural Labs, Inc. 32380 Avenue 10 Madera, CA 93638 USA (Syngenta file No. CGA173506_50227)

Guidelines

EPA Guideline 850.4250

GLP: Yes

Executive Summary

A vegetative vigour test was conducted in which the effects of a foliar application of fludioxonil ten species (4 monocot, 6 dicot) were exposed to a negative control and 5 application rates of the test substances, i.e. 0.063, 0.125, 0.25, 0.5 and 1.0 lb a.s./acre (71, 140, 280. 560 and 1121 g a.s./ha) were evaluated. There were no adverse effects on any of the ten species after spraying fludioxonil at rates up to 1.0 lb a.s./acre. Therefore the No observed effect rate was 1.0 lb a.s./acre (1121 g a.s./ha). The ER_{25} and ER_{50} values were >1.0 lb/a.s./acre (1121 g a.s./ha), the highest application rate tested.

Materials

Test material	Fludioxonil technical CGA173506
Lot/Batch #:	FL-881677

Purity:	97.5%
Description:	Colourless crystalline solid
Stability of test compound:	Stable under test conditions.
Reanalysis/expiry date:	n/a
Treatments	
Test concentrations:	0.063, 0.125, 0.25, 0.5 and 1.0 lb a.s./acre (71, 140, 280. 560 and 1121 g a.s./ha)
Control:	Irrigation water
Spray volume:	200 mL /ha
Application method:	Laboratory track sprayer (flat-fan nozzle)
Test organisms	
Species:	*Glycine max* (soybean) *Lactuca sativa* (lettuce) *Allium cepa* (onion) *Avena sativa* (oat) *Lolium perenne* (perennial ryegrass) *Brassica oleracea* (cabbage) *Zea mays* (corn) *Cucumis sativa* (cucumber) *Daucus carota* (carrot) *Lycopersicon esculentum* (tomato)
Test soil:	Supersoil (fir bank, redwood, Canadian peat and sand)
Test design	
Test vessels:	Plastic pots (7.5 x 7.5 x 6.0 cm)
Sampling interval:	7, 14, and 21 days after treatment
Duration:	21 days
Environmental conditions	
Test temperature:	20 to 30 °C

Study Design and Methods

Experimental dates: 14th October to 2nd November 1988

Ten plants species (*Glycine max, Lactuca sativa, Allium cepa, Avena sativa, Lolium perenne, Brassica oleracea, Zea mays, Cucumis sativa, Daucus carota & Lycopersicon esculentum*) were sprayed with a series of five test rates of fludioxonil. Test rates applied were 0.063, 0.125, 0.25, 0.5 and 1.0 lb a.s./acre (71, 140, 280. 560 and 1121 g a.s./ha).

Seeds were planted at a uniform depth of 2.5 cm for Soybean and corn, and 1.3cm for the remaining species. Ten seeds were planted per pot.

Observations were made 7, 14 and 21 days after application (DAA) to document plant condition. Observations were made 21 DAA to document plant condition and height. Plant condition was described by noting the presence or absence of possible signs of phytotoxicity such as necrosis, leaf wrinkle, chlorosis, lodging or stunting.

The growth of test plants was evaluated at the end of the test (21 DAA) by assessing height and biomass. Plant biomass was estimated by measuring the total dry weight of the shoots within each replicate. Plant height was measured with a ruler to the nearest whole centimetre from the surface of the soil to the apical meristem, or to the tip of the tallest leaf. Dead or non-emerged seedlings were assigned a height of 0 cm. Plants were then clipped at soil level, the shoots of all living plants within a replicate were placed in a labelled paper container, dried in an oven, and weighed as a group. Mean height and total replicate biomass were determined for each treatment group.

Statistical analyses were used to evaluate effects of test substance application on plant height, biomass, and survival. An analysis of variance was used to analyse the data. Due to a lack of dose response and significant differences among treatments, probit analysis could not be conducted on the data obtained.

Results and Discussion

Statistical analyses were used to evaluate effects of test substance application on plant height, biomass, and survival. An analysis of variance was used to analyse the data. Due to a lack of dose response and significant differences among treatments, probit analysis could not be conducted on the data obtained.

The LOER, NOER, ER_{25} and ER_{50} for each of the ten test species are presented in table below:

Table 8.6.2-2: Effect rates of fludioxonil on 21-day vegetative vigour

Species	Height, biomass and survival (lbs/acre)			
	LOER	NOER	ER_{25}	ER_{50}
Soybean	≥1.0	1.0	>1.0	>1.0
Lettuce	≥1.0	1.0	>1.0	>1.0
Carrot	≥1.0	1.0	>1.0	>1.0
Tomato	≥1.0	1.0	>1.0	>1.0
Cucumber	≥1.0	1.0	>1.0	>1.0
Cabbage	≥1.0	1.0	>1.0	>1.0
Oat	≥1.0	1.0	>1.0	>1.0
Ryegrass	≥1.0	1.0	>1.0	>1.0
Corn	≥1.0	1.0	>1.0	>1.0
Onion	≥1.0	1.0	>1.0	>1.0

Validity criteria

The test was considered valid based on the following criteria:

- Mean plant survival was 100% in the controls (must be > 90%)
- Control seedlings did not exhibit visible phytotoxic effects, plants exhibited only normal variation in growth and morphology for that particular species;

Conclusions

Treatment of plant seedlings with CGA173506 did not result in any significant phytotoxicity rating effects, regardless of plant species tested or treatment concentration. There were no adverse effects on any of the ten species after spraying fludioxonil at rates up to 1.0 lb a.s./acre. Therefore the No observed effect rate was 1.0 lb a.s./acre (1121 g a.s./ha). The ER_{25} and ER_{50} values were >1.0 lb/a.s./acre (1121 g a.s./ha), the highest application rate tested.

(Cañez V.M, 1989a)

Relevant Literature on Non-Target Plants

No relevant scientifically peer-reviewed open literature could be found on fludioxonil 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.

Relevant Literature

No relevant scientifically peer-reviewed open literature could be found on fludioxonil 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: Summary of Endpoints for Fludioxonil

Organism	Test type	Endpoints		Comment	Reference (author, date, Syngenta Ref)
Aquatic micro-organisms	Activated sludge respiration inhibition	EU	EC_{50} >100 mg/L	-	***Weinstock (1994) CGA173506/0503***
	Activated sludge respiration inhibition	New study	EC_{50} >1000 mg/L	Supersedes previous study as conducted to current guidance.	***McLaughlin (2007) CGA173506/7240***

New study refers to studies performed since EU review of fludioxonil

The study previously submitted in the EU was summarised in the previous EU review in point B.9.10 of the DAR, ~~therefore they are not repeated here~~.

At the request of the RMS, summaries are provided for studies that are used to derive the endpoint(s) used in the risk assessment.

The following study was previously submitted in the EU and was summarised in point B.9.2.10 of the Monograph. At the request of the RMS, this summary is provided as the endpoint is used in the risk assessment and the validity assessed against current guidance. The endpoint is summarised in Table 8.8-1 above.

	Summary for previously evaluated study submitted for purposes of renewal as requested by the RMS:
Report:	*KIIA 8.5.1 Weinstock M., (1994). Report on the test for Activated Sludge Respiration Inhibition of CGA 173506 tech., Report Number 943544, CIBA-GEIGY Ltd., Product Safety, Ecotoxicology, CH-4002 Basel, Switzerland. (Syngenta File No. CGA173506/0503).*

Guidelines

OECD Guideline 209 (April 1984)

GLP: Yes

Executive Summary

Aerobic activated sludge was incubated for three hours in the presence of CGA173506 at concentrations of 14.5, 22.0, 39.5, 62 and 102.0 mg a.s./L, together with positive and blank controls.

Up to and including the highest test concentration of 102.0 mg a.s./L, the test item had no significant inhibitory effect on the respiration rate of activated sludge at the end of the 3-hour incubation period. The 3-hour EC_{20}, EC_{50} and EC_{80} values were estimated to be > 100 mg a.s./L.

Materials

Test Material	CGA173506 Fludioxinil
Lot/Batch #:	P.805002
Purity:	97.5 %
Description:	Yellow powder
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	January 1997
Density:	Not applicable
Treatments	
Test concentrations:	Blank control; nominal concentrations of 14.5, 22.0, 39.5, 62 and 102.0 mg a.s./L
Vehicle and/or positive control:	3,5-Dichlorophenol at nominal concentrations of 3.2, 10.0 and 32.1 mg/L which gave a 3 hour EC_{50} value of 13.6 mg/L
Analysis of test concentrations:	No
Test organisms	
Organisms:	Activated sludge, concentration in test bottles: 1.72 g/L dry weight
Source:	Sewage treatment plant of CH-4153 Reinach, sludge collected on 25 May 1994
Test design	
Test vessels:	250 mL BOD flasks with gas inlet
Replication:	2 for control and 1 per test item and reference substance concentration
Exposure regime:	Static with aeration, 3-hour contact exposure period
Environmental test conditions	
Temperature:	20 ± 2 °C
pH of sludge:	Test start: 7.8 (sludge pH before use) Test end: 8.2 to 8.4
Aeration:	Yes, aeration provided for 3 hours
Oxygen:	Not reported
Lighting:	Not reported

Study Design and Methods

Experimental date: 26th May 1994

Due to the poor solubility of the test substance, a stock solution could not be prepared. The test substance was directly added to the test medium. The test vessels were prepared by adding 2.9, 4.4, 7.9, 12.4 and 20.4 mg CGA173506 substance to the test medium and the volume was adjusted to 200 mL with water. The sludge concentration in the test bottles was 1.72 g/L dry weight. Two replicate samples were prepared for the blank control a single replicate sample was prepared for each test item and reference item concentration.

The test system was aerated for 3 hours and incubated at 20 ± 2 °C. Oxygen consumption per hour was analysed with an ORION-Electrode Type 97-08. The EC values for inhibition were determined by calculating the linear regression of the results.

Results and Discussion

Results are summarised in the table below.

Table 8.8-1: Influence of CGA173506 on oxygen consumption of activated sludge in a 3-hour respiration inhibition test

Nominal concentration (mg a.s./L)	Oxygen consumption rate (mg/L/h)	Inhibition[a] (%)	pH (after 3 h aeration)
Blank 1	48.0	-5	8.2
Blank 2	43.5	5	8.3
14.5	48.2	-5	8.2
22.0	42.2	8	8.2
39.4	48.5	-6	8.2
62.0	43.7	4	8.2
102.0	45.6	0	8.2
Reference (3.2 mg/L)	42.9	6	8.2
Reference (10.0 mg/L)	27.5	40	8.2
Reference (32.1 mg/L)	10.7	77	8.4

[a] Negative inhibition results are a consequence of the comparison of oxygen consumption values obtained in the blank and the higher oxygen consumption values obtained in the test substance, which are due to biological variations between the various test samples

Validity criteria

Not reported.

Conclusions

Up to and including the highest test concentration of 102.0 mg a.s./L, the test item CGA173506 had no significant inhibitory effect on the respiration rate of activated sludge after the incubation period of 3 hours. The 3 hour EC_{20}, EC_{50}, and EC_{80} were estimated to be > 100 mg a.s./L.

(Weinstock M, 1994)

This study complies with the current validity criteria for toxicity testing with activated sludge and despite some deviations from the recommended test design, the endpoint is still valid for use in the risk assessment.

Reference	Guidance followed	Current guidance in force	Differences from guidance in force	Critical assessment
Weinstock, 1994, CGA173506/0503	OECD No. 209 (1984)	OECD No. 209 (2010)	Results are presented as mg O_2/L sludge x h; (recommended: mg O_2/g sludge x h). A settled sludge was used (centrifuged sludge is recommended) The test substance was directly applied to the test medium and not as a stock solution (due to low solubility in water)	While conditions of the study were not fully reported and there were minor deviations from the current guidelines, the following validity criteria were met: 1. Oxygen uptake rate of blank controls ≥20 mg O_2/g activated sludge (dry weight of suspended solids) in an hour. (28 and 25 mg O_2/g activated sludge/hr; control respiration rates are within 15% of each other). 2. EC_{50} of 3,5-DCP should lie in the range 2 mg/L to 25 mg/L for total respiration. (13.6 mg/L) Despite some minor deviations from the recommended test design, the endpoint is reliable and can be used in the risk assessment.

New studies conducted since the last EU review are summarised below.

Report:	Submitted for purposes of renewal as conducted at a higher concentration in line with data requirements: K-CA 8.8/01 McLaughlin S.P. (2007) Activated Sludge Respiration Inhibition Test Following OPPTS Guideline 850.6800 and OECD Guideline 209 Report number 1781.6673. Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571-1037 (Syngenta File No. CGA173506/7240)

Guidelines

OECD 209 (1984)

EPA OPPTS 850.6800

GLP: Yes

Executive Summary

Aerobic activated sludge was incubated for 3 hours in the presence of fludioxonil at 0.2, 0.6, 1.6, 10 and 1000 mg a.s./L.

The 3 hour EC_{50} value for fludioxonil was determined to be greater than 1000 mg a.s./L.

Materials

Test Material	fludioxonil CGA173506
Batch number:	AMS 273/6
Purity:	99.8%
Stability:	Reanalysis date 30th November 2007
Treatments	
Test concentrations:	Control; nominal concentration of 0.2, 0.6, 1.6, 10 and 1000 mg a.s./L.
Vehicle and/or positive control:	No vehicle; positive control was 3, 5-dichlorophenol at 3.2, 10, 30 and 100 mg/L which gave a 3 hour EC_{50} value of 10.2 mg/L.
Analysis of test concentrations:	No
Test organisms	
Organisms:	Aerobic activated sludge microorganisms, 4.0 g/L
Source:	Wareham Waste Water Treatment Plant (WWTP), Wareham, Massachusetts
Test design	
Test vessels:	1000-mL glass beaker solutions were aerated using room air, an oil-free Barnant air pump, and silicone tubing attached to a Pasteur pipet
Replication:	1 replicate in treatments 2 in the control
Exposure regime:	Static with aeration
Environmental conditions	
Temperature:	19.9 to 20.5 ºC
pH:	7.27 to 7.48.
Aeration:	Continuous aeration with compressed air
Oxygen:	23.8 to 25.7 O_2/L/hr
Lighting:	Normal laboratory lighting

Study Design and Methods

Experimental date: 3rd April 2007

At time 0, 16 mL of synthetic sewage feed was added to a 500mL graduated cylinder and then brought to a volume of 300 mL with water. Inoculum was added until the solution was at full volume (500 mL). This mixture was added to a 1-L beaker and aeration was immediately initiated at a rate of between 500 and 1000 mL/minute. This flask was identified as Control 1 (C1). An abiotic control was prepared by adding 0.5006 g of test substance to the test system along with synthetic feed and then bringing to volume with water such that the final fludioxonil concentration in the abiotic control was approximately 1000 mg/L. Lastly, the test substance test solutions were initiated following the same procedure as Control 1 with the exception that the appropriate amount of the test substance brought to volume with water was added to the sludge mixture.

Following 3 hours of aeration, a 22-mL aliquot of each sludge mixture was transferred to a 50 mL cylinder. The water bath was placed over a stir plate. A stir bar was placed in the cylinder along with a dissolved oxygen probe. The probes were attached to a Strathkelvin Instruments Model 928 oxygen system and a computer, which ran the Strathkelvin software. The Strathkelvin system was used to monitor the “SI Multisample Activated Sludge Respirometer”. Immediately after the dissolved oxygen probes

were attached to the Strathkelvin system, the computer program was initiated so that oxygen depletion rates were measured starting at time zero. The oxygen depletion rate was measured over a 10-minute period. The sludge mixtures were stirred continuously while the oxygen measurements were taken. The temperature of the environmental chamber in which the test was conducted was monitored using a minimum/maximum thermometer. Solution pH was measured with a Jenco Model 60 pH meter. The SI Multisample Activated Sludge Respirometer was used for determination of O_2 consumption. The oxygen depletion rate was measured in a single sample from each test system such that there was one replicate per test system with zero headspace.

Results and Discussion

Oxygen consumption in the test substance vessels (0.20, 0.60, 1.6, 10 and 1000 mg a.s./L) was 23.6, 22.3, 25.6, 24.1 and 23.4 mg O_2/L/hr, respectively. Compared to the controls, respiratory inhibition for the test substance vessels was 7.9, 13.1, 0.0, 5.9 and 8.8%, respectively. Based on these results, the EC_{50} value was determined to be greater than 1000 mg a.s./L.

The nominal concentrations were used for reporting of results which are presented in the table below:

Table 8.8-12: Influence of fludioxonil on oxygen consumption of activated sludge in a 3-hour respiration inhibition test

Nominal concentration (mg a.s./L)	Respiration Rate (mg O_2/L/Hr)	Inhibition (%)	pH
Abiotic	0.80	n/a	7.27
Control 1	25.7	n/a	7.43
Control 2	23.8	n/a	7.40
Fludioxonil 0.20	23.6	7.9	7.42
Fludioxonil 0.60	22.3	13.1	7.42
Fludioxonil 1.6	25.6	0.0	7.40
Fludioxonil 10	24.1	5.9	7.41
Fludioxonil 1000	23.4	8.8	7.38
3,5-dichlorophenol 3.0	19.3	22.3	7.47
3,5-dichlorophenol 10	12.6	49.2	7.33
3,5-dichlorophenol 30	2.8	88.9	7.43
3,5-dichlorophenol 100	-0.4	102	7.48

n/a – not applicable

The EC_{50} value for 3,5-dichlorophenol was 10.2 mg /L, which was within the acceptable limits (5.0 to 30 mg/L) as specified in OECD 209 Guideline and validated the test system.

Conclusion

In an aerobic activated sludge test, the EC_{50} value for fludioxonil was determined to be >1000 mg a.s./L.

(McLaughlin S.P., 2007)

CA 8.9 Monitoring Data

There are no records of reported incidents related to use of fludioxonil from monitoring data. No monitoring studies are needed for fludioxonil for ecotoxicological purposes.