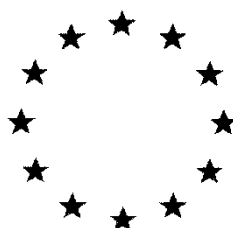


European Commission



**Draft Assessment Report prepared according to the Commission
Regulation (EU) N° 1107/2009**

XDE-729 Methyl (Halauxifen-methyl) Volume 3 – B.9

**Rapporteur Member State : United Kingdom
Co-Rapporteur Member State: France**

Version History

When	What
2013-12-19	Initial DAR

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

CONTENTS

	Page
B.9 Ecotoxicology	
B.9.1 Effects on birds	3
B.9.2 Effects on aquatic organisms	38
B.9.3 Effects on other terrestrial vertebrates	299
B.9.4 Effects on bees	308
B.9.5 Effects on other arthropod species	314
B.9.6 Effects on earthworms	321
B.9.7 Effects on other soil non-target macro-organisms	348
B.9.8 Effects on soil non-target micro-organisms	371
B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk	389
B.9.10 Effects on biological methods of sewage treatment	427
B.9.11 Overall Summary of Ecotoxicology Risk Assessment	431
B.9.12 References relied on	435

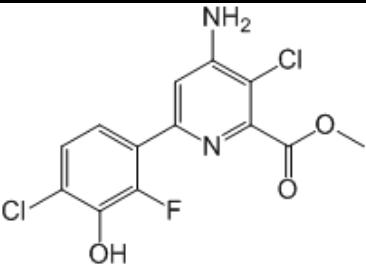
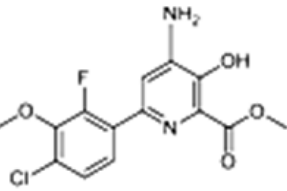
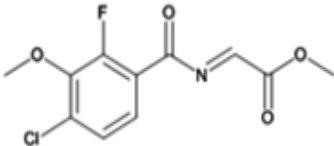
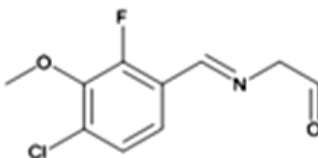
B.9 ECOTOXICOLOGY**Background information**

Table B.9.1: Summary of the proposed uses of XDE-729 as product GF-2573

Crops/ Situations	Member State/ Countries in which approval sought	Maximum individual dose (g a.s./ha)	Maximum number of treatments	Minimum interval between applications	Latest time of application/Growth stage of applications
Winter Cereals	EU	7.82 (autumn only) or 6.25 (spring only) or 7.82+6.25 (autumn + spring)	2	70 days	BBCH 10-29 (autumn) BBCH 13-45 (spring)
Spring Cereals	EU	6.25	1	-	13-45

Table B.9.2: Ecotoxicologically identified metabolites

Parent or Metabolite Name	Structure	Log K _{ow} at pH 7	Compartment of relevance
XDE-729 Methyl (X11393728)		3.76	Not Applicable (parent)
XDE-729 Acid (X11393729)		-8.3	Hydrolysis: 13% (pH 7), 99% (pH 9) at 25°C Aqueous photolysis: 10.7% Soil: 73% Water/Sediment Water Phase: 20% Water/Sediment Total System: 23.5%
X11449757		< 0.3	Aerobic Soil: 17% Water/Sediment Water Phase: 48.3% Water/Sediment Sediment Phase: 50.6% Water/Sediment Total System: 76.7%

Parent or Metabolite Name	Structure	Log K _{ow} at pH 7	Compartment of relevance
X11406790		< 0.3	Water/Sediment Water Phase: 16.5% Water/Sediment Sediment Phase: 10.6% Water/Sediment Total System: 23.6%
'Deg 10'		NA	Aqueous photolysis: 12.6 %
'Deg 11'		NA	Aqueous photolysis: 15.7%
'Deg 14'		NA	Aqueous photolysis: 11.5%

^a Metabolites identified at >10% Applied Radioactivity; only those studies in which the metabolite was detected are listed.

B.9.1 Effects on birds (IIA 8.1, IIIA 10.1)**B.9.1.1 Toxicity (IIA 8.1.1, IIIA 10.1.1)****B.9.1.1.1 Acute oral toxicity (IIA 8.1.1, IIIA 10.1.1)****Active substance**

(2011): XDE-729 Methyl: An Acute Oral Toxicity Study with the Northern Bobwhite Quail (*Colinus virginianus*).

Project Number 379-211. Dow AgroSciences unpublished report, Study Number 090026. Amended 10 May 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2% (wt/wt) XDE-729 Methyl ¹
Description:	solid
Lot No./Batch No. :	TSN031117-0004 (XR-729 methyl) E2837-51

Test system

Organism (<i>Species</i>):	Northern Bobwhite (<i>Colinus virginianus</i>)
Study Type:	Acute oral
Guidelines followed:	OPPTS Number 850.2100 and FIFRA Subdivision E, Section 71-1 Study was conducted to GLP, except for the assessment of stability, homogeneity, and verification of the test substance in the dosing solution.
Guideline deviations reported by Study Director:	None
Duration of study:	14 days
Parameters measured:	Body weight, feed consumption
Observation intervals:	Multiple observations on Day 1 and twice daily observations on remaining days.

¹ At time of receipt, the reported purity of the test substance was 99.1% ± 0.1% XDE-729 Methyl, which was used to calculate the nominal dosage level. A revised certificate of analysis reported a purity of 97.2% and a re-certification date of 30 November 2013. The dosage of 2250 mg a.s./kg was adjusted to 100% active substance based on a purity of 99.1%. When corrected for the revised purity of 97.2%, the calculated dosage is 2207 mg a.s./kg. The nominal dosage of 2250 mg a.s./kg is used for reporting purposes.

Age range of birds at test initiation:	Approximately 27 weeks
Weight range of birds at test initiation:	184-213 grams
Test concentrations:	0 and 2250 mg a.s./kg
No. of feed withholding days before dosing:	Birds were fasted for approximately 17 hours prior to dosing
Method of test item administration:	Oral gavage
Diet:	
Number of birds per dose group:	10
Number of birds per control group:	10
Housing:	GQF Manufacturing Co. (Model No. 0010)
Environmental conditions:	Temperature: $23.0 \pm 0.2^{\circ}\text{C}$ (SD) Photoperiod: 8 hrs Humidity: $32 \pm 10\%$ (SD)

Methodology

Ten northern bobwhite, five males and five females, were assigned to the treatment group and the control group by indiscriminate draw. The test consisted of a single dosage group and a control group. The birds were fasted for approximately 17 hours prior to dosing. At experimental start, a single dose of the test substance in diluent was orally intubated directly into the crop or proventriculus of each bird. Each bird was individually weighed and dosed on the basis of milligrams of active substance of test substance per kilogram of body weight. The control birds received a corresponding volume of diluent only. From test initiation until termination, all birds were observed at least twice a day. A record was maintained of all mortality, signs of toxicity, and abnormal behaviour. Body weights were measured individually at the initiation of the test and on Days 3, 7 and 14 of the test. Average feed consumption was determined by pen for each dosage group and the control group for Days 0-3, 4-7 and 8-14.

Results

There were no mortalities in the control group, and all control birds were normal in appearance and behaviour throughout the test. Additionally, there were no mortalities in the 2250 mg a.s. /kg treatment group. All birds in the 2250 mg a.s./kg dosage group were normal in appearance and behaviour for the duration of the test.

Table B 9.1.1: Effect of XDE-729 Methyl on mortality of bobwhite quail

Treatment (mg a.s./kg bw)	No. of birds	Cumulative mortality		
		At Day 7	At Day 14	Total (%)
Negative control	10	0	0	0
2250	10	0	0	0
LD50	> 2250 mg a.s./kg			
NOEL	2250 mg a.s./kg			

Table B 9.1.2: Effect of XDE-729 Methyl on body weight and feed consumption of bobwhite quail

Treatment (mg a.s./kg bw)		Observation						
		Mean body weight (g)				Feed consumption (g/bird/day)		
	Sex	Day 0	Day 3	Day 7	Day 14	Days 0-3	Days 4-7	Days 8-14
Negative control	M	202	207	209	209	28	28	22
	F	197	205	207	206	17	18	16
2250	M	201	202	207	207	15	19	17
	F	194	198	199	201	17	14	16
The acute oral LD50 value for northern bobwhite exposed to XDE-729 Methyl as a single oral dose was determined to be > 2250 mg a.s./kg.								

Conclusions

The acute oral LD50 value for northern bobwhite exposed to XDE-729 Methyl as a single oral dose was determined to be greater than 2250 mg a.s./kg. The no-mortality level was 2250 mg a.s./kg.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is an LD50 of >2250 mg a.s./kg.

██████████ (2011): XDE-729 Methyl: An Acute Oral Toxicity Study with the Zebra Finch (*Poephila guttata*). ██████████

██████████ Dow AgroSciences unpublished report, Study Number 090027. Amended 10 May 2011.

Test material

Test item:	XDE-729 methyl
Purity:	97.2% (wt/wt) XDE-729 Methyl ²
Description:	solid
Lot No./Batch No. :	TSN031117-0004 (XR-729 Methyl) E2837-51

Test system

Organism (Species)	Zebra Finch (<i>Poephila guttata</i>)
Study Type:	Acute oral
Guidelines followed:	OPPTS Number 850.2100 and FIFRA Subdivision E, Subsection 71-1. Study was conducted to GLP, except for the assessment of stability, homogeneity, and verification of the test substance in the dosing solution.
Guideline deviations reported by Study Director:	None
Duration of study:	14 days
Parameters measured:	Mortality and body weight
Observation intervals:	Multiple observations on Day 0 and twice daily observations on remaining days.
Age range of birds at test initiation:	5 to 9 months
Weight range of birds at test initiation:	13.4-17.6 grams
Test concentrations:	0 and 2250 mg a.s./kg
No. of feed withholding days before dosing:	Birds were fasted for 2-3 hours prior to dosing
Method of test item administration:	Oral capsules
Diet:	Kaytee Forti-Diet Pro Health Finch
Number of birds per dose group:	10
Number of birds per control group:	10
Housing:	Prevue Pet Products, Inc. (Model No. F040).
Environmental conditions:	Temperature: 24.2 ± 0.2 °C (SD) Photoperiod: 0.25 hr dim light/8 hrs light/0.25 hr dim light Humidity: 70 ± 6% (SD)

² At time of receipt, the reported purity of the test substance was 99.1% ± 0.1% XDE-729 Methyl, which was used to calculate the nominal dosage level. A revised certificate of analysis reported a purity of 97.2% and a re-certification date of 30 November 2013. The dosage of 2250 mg a.s./kg was adjusted to 100% active substance based on a purity of 99.1%. When corrected for the revised purity of 97.2%, the calculated dosage is 2207 mg a.s./kg. The nominal dosage of 2250 mg a.s./kg is used for reporting purposes.

Methodology

Ten zebra finch, five males and five females, were assigned to the treatment group and the control group by indiscriminate draw. The test consisted of a single dosage group and a control group. The birds were fasted for approximately 2-3 hours prior to dosing. At the experimental start, encapsulated doses (2 capsules) of the test substance were lubricated with corn oil that had been dyed blue with a food grade colourant to aid in the determination of regurgitation and orally inserted into the crop of each bird with a capsule-dosing syringe. Each bird was individually weighed and dosed on the basis of milligrams of active substance of test substance per kilogram of body weight. The control birds received two empty capsules. From test initiation until termination, all birds were observed at least twice a day. A record was maintained of all mortality, signs of toxicity, and abnormal behaviour. Body weights were measured individually at the initiation of the test and on Days 3, 7 and 14 of the test.

Results

No indications of regurgitation were noted in the control birds. Two females dosed with XDE-729 methyl were noted as regurgitating the dose approximately 30 minutes after dosing, and one female was noted as regurgitating approximately 50 minutes after dosing. The birds that regurgitated the dose were euthanized with CO₂ and not used as part of the study. Three additional female birds were dosed at the 2250 mg a.s./kg dosage, approximately 1 hour after the original ten birds were dosed, to replace those birds that had regurgitated the dose. Consequently, a total of 10 birds, 5 males and 5 females, were dosed and not noted as regurgitating the 2250 mg a.s./kg dosage. There were no mortalities in the control group and all birds in the control group were normal in appearance and behaviour for the duration of the test. There were no mortalities among the birds that did not regurgitate the 2250 mg a.s./kg dosage level. Necropsy was not conducted. All birds in the control group and 2250 mg a.s./kg dosage group were noted as feeding normally on the afternoon of the day of dosing. No abnormalities in the appearance or feeding behaviour of the birds were noted for the duration of the test.

Table B 9.1.3: Effect of XDE-729 Methyl on mortality of zebra finch

Treatment (mg a.s./kg bw)	No. of birds	Cumulative mortality		
		At Day 7	At Day 14	Total (%)
Negative control	10	0	0	0
2250	10	0	0	0
ED50	> 2250 mg a.s./kg			
95% C.I.	N/A			
NOEL	2250 mg a.s./kg			

Table B 9.1.4: Effect of XDE-729 Methyl on body weight and regurgitation of zebra finch

Treatment (mg a.s./kg bw)		Observation				
		Mean body weight (g)				Number of birds that did not regurgitate
	Sex	Day 0	Day 3	Day 7	Day 14	Day 0
Control	M	14.8	14.9	14.6	14.7	5 males
	F	15.6	15.6	15.0	15.0	5 females
2250	M	15.8	15.0	14.2	15.0	5 males
	F	16.3	15.5	14.9	15.7	5 females
The acute oral LD50 value for zebra finch exposed to XDE-729 methyl as a single oral dose was determined to be greater than 2250 mg a.s./kg.						

Conclusions

The acute oral LD50 value for zebra finch exposed to XDE-729 methyl as a single oral dose was determined to be greater than 2250 mg a.s./kg. The no-mortality level was 2250 mg a.s./kg.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is an LD50 of >2250 mg a.s./kg.

Formulation

(2011): GF-2573: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure.

Project Number 379-277. Dow AgroSciences unpublished report, Study Number 102030.

Test material

Test item:	GF-2573
Purity:	0.84% wt/wt (7.6 g/L) XR-729 methyl, 0.81w% wt/wt (7.3g/L) cloquintocet-mexyl
Description:	liquid
Lot No./Batch No. :	E2837-57

Test system

Organism (Species):	Northern Bobwhite (<i>Colinus virginianus</i>)
Study Type:	Acute oral

Guidelines followed:	OECD test guideline 223 (adopted July 2010) Study was conducted to GLP, except for the assessment of stability, homogeneity, and verification of the test substance in the dosing solution.
Guideline deviations reported by Study Director:	None
Duration of study:	14 days
Parameters measured:	Body weight, feed consumption
Observation intervals:	Multiple observations on Day 0 and twice daily observations on remaining days.
Age range of birds at test initiation:	Approximately 22 weeks
Weight range of birds at test initiation:	186-221 grams
Test concentrations:	0 and 2000 mg formulation/kg
No. of feed withholding days before dosing:	Birds were fasted for approximately 16.5 hours prior to dosing
Method of test item administration:	Oral gavage
Diet:	
Number of birds per dose group:	5
Number of birds per control group:	5
Housing:	GQE Manufacturing Co. (Model No. 0330)
Environmental conditions:	Temperature: $23.1 \pm 0.3^{\circ}\text{C}$ (SD) Photoperiod: 8 hrs light Humidity: $27 \pm 8\%$ (SD) Ventilation rate: 15 room air volumes per hour

Methodology

Five northern bobwhite were assigned to the treatment group and the control group. The test consisted of a single dosage group and a control group. The birds were fasted for approximately 16.5 hours prior to dosing. At experimental start, a single dose of the test substance in diluent was orally intubated directly into the crop or proventriculus of each bird. Each bird was individually weighed and dosed on the basis of milligrams of test substance per kilogram of body weight. The control birds received a corresponding volume of diluent only. From test initiation until termination, all birds were observed at least twice a day. A record was maintained of all mortality, signs of toxicity, and abnormal behaviour. Body weights were measured individually at the initiation of the test and on Days 3, 7 and 14 of the test. Feed consumption was determined by pen for approximately 24-hour intervals from Day 0 to Day 1, Day 1 to Day 2 and Day 2 to Day 3. Average feed consumption was then determined from Day 3 to Day 7 and From Day 7 to Day 14.

ResultsTable B 9.1.5: Effect of GF-2573 on mortality of bobwhite quail

Treatment (mg/kg bw)	No. of birds	Cumulative mortality		
		At Day 7	At Day 14	Total (%)
Negative control	5	0	0	0
2000	5	0	0	0
LD50	> 2000 mg/kg			
NOEL	2000 mg/kg			

Table B 9.1.6: Effect of GF-2573 on body weight and feed consumption of bobwhite quail

Treatment (mg/kg bw)		Observation								
		body weight (g)				Feed consumption (g/bird/day)				
		Day 0	Day 3	Day 7	Day 14	Days 0-1	Days 1-2	Days 2-3	Days 3-7	Days 7-14
Negative control	Mean SD	203 14	203 14	199 13	203 14	17 3	15 1	17 1	16 2	15 2
2000	Mean SD	197 9	197 9	195 10	198 9	16 3	15 3	18 3	17 2	16 1
The acute oral LD50 value for northern bobwhite exposed to GF-2573 as a single oral dose was determined to be > 2000 mg/kg.										

Conclusions

The acute oral LD50 value for northern bobwhite exposed to GF-2573 as a single oral dose was determined to be greater than 2000 mg/kg. The no-mortality and no-observed effect levels were 2000 mg/kg.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is an LD50 of >2000 mg form./kg.

B.9.1.1.2 Dietary toxicity (IIA 8.1.2)

(2011): XDE-729 Methyl: A Dietary LC50 Study with the Northern Bobwhite. Project Number 379-213, Dow AgroSciences unpublished report, Study Number 090028. Amended 10 May 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2% (wt/wt) XDE-729 Methyl ³
Description:	Solid
Lot No./Batch No.:	TSN 0311117-0004 E2837-51

Test system

Organism (Species):	Northern Bobwhite (<i>Colinus virginianus</i>)
Study Type:	Dietary
GLP Status:	GLP Study was conducted to GLP, except for the assessment of stability, homogeneity, and verification of the test substance in the dosing solution.
Guidelines followed:	OPPTS Number 850.2200 and FIFRA Subdivision E, Section 71-2. OECD guideline 205.
Guideline deviations reported by Study Director:	None
Duration of study:	8 days
Parameters measured:	Body weight, feed consumption
Observation intervals:	Multiple observations on Day 1 and twice daily observations on remaining days.
Age range of birds at test initiation:	10 days old
Weight range of birds at study initiation:	16-25 grams
Test concentrations:	0, 562, 1000, 1780, 3160, and 5620 mg a.s./kg diet
Analytical confirmation of test concentrations:	Yes

³ At time of receipt, the reported purity of the test substance was 99.1% ± 0.1% XDE-729 Methyl, which was used to calculate the nominal dosage level. A revised certificate of analysis reported a purity of 97.2% and a re-certification date of 30 November 2013. The dosages were adjusted to 100% active substance based on a purity of 99.1%. Dietary test concentrations of 0, 562, 1000, 1780, 3160 and 5620 mg/kg a.s. were corrected for a purity of 99.1%. When corrected for the revised purity of 97.2%, the calculated test concentrations are 0, 551, 981, 1746, 3099 and 5512 mg/kg a.s. The nominal test concentrations of 0, 562, 1000, 1780, 3160 and 5620 mg/kg a.s. are used for reporting purposes. Nominal dietary test concentrations used in this study were 0, 562, 1000, 1780, 3160 and 5620 mg/kg a.s. XDE-729 Methyl.

No. of feed withholding days before dosing:	None
Method of test item administration:	Diet
Diet:	Treated Diet from Day 0 to Day 5, Basal ration Day 6 to Day 8
Number of birds per dose group:	10
Number of birds per control group:	30
Housing:	Beacon Steel Products Co. (Model No. B735Q).
Environmental conditions:	Brooder Temperature: $38.9 \pm 1.3^{\circ}\text{C}$ (SD) Ambient Temperature: $29.2 \pm 0.8^{\circ}\text{C}$ (SD) Photoperiod: 16 hrs light/8 hrs dark Humidity: $25 \pm 6\%$ (SD)

Methodology

All birds were acclimated to the caging and facilities from the day of hatch until initiation of the test. During the test each group was fed the appropriate treated or control diet for five days. Following the five-day exposure period all groups were given untreated basal diet for three days. From test initiation until termination, all birds were observed at least twice daily. A record was maintained of all signs of toxicity and abnormal behaviour. Individual body weights were measured at the initiation of the test (Day 0), on Day 5, and at termination of the test on Day 8. Average feed consumption values were determined daily by pen for each treatment group and the control group during the exposure period (Days 0-5) and during the post-exposure observation period (Days 6-8). Mortality data were utilized to determine the LC50 value.

Results

Table B 9.1.7: Effect of XDE-729 Methyl on mortality

Treatment (mg a.s./kg diet)	No. of birds	Cumulative mortality		
		At Day 5	At Day 8	Total (%)
Negative control	30	0	1	3
562	10	0	0	0
1000	10	0	0	0
1780	10	0	0	0
3160	10	0	0	0
5620	10	0	0	0
LC50	>5620 mg a.s./kg diet			
95% C.I.	N/A			
NOEC	3160 mg a.s./kg diet			

Table B 9.1.8: Effect of XDE-729 Methyl on body weight and feed consumption

Treatment (mg a.s./kg diet)	Observation				
	Mean body weight (g)			Mean Feed consumption (g/bird/day)	
	day 0	day 5	day 8	days 0 to 5	days 6 to 8
Negative control	21	33	43	6	8
562	21	33	44	6	9
1000	19	32	42	6	8
1780	19	32	43	6	8
3160	21	33	44	6	8
5620	19	29*	38*	6	8
* Statistically different from the control group $p < 0.05$ (Dunnett's t-test)					

In all of the treatment groups, there were no overt signs of toxicity. All birds at the 562, 1000 and 3160 mg a.s./kg were normal in appearance and behaviour throughout the test. One bird in the 1780 mg a.s./ kg test concentration was noted with a lesion on the left foot on Days 5 and 6 of the test. All other birds in the 1780 mg a.s./kg test concentration were normal in appearance and behaviour for the duration of the test. One bird in the 5620 mg a.s./kg test concentration was noted as having a leg lesion and limping on the afternoon of Day 5. The bird continued to be noted with the leg lesion for the remainder of the test. All other birds at the 5620 mg a.s./kg test concentration were normal in appearance and behaviour for the duration of the test.

Samples of diet collected during the test to verify test substance concentrations for the 1000, 1780, 3160 mg a.s. /kg diet had mean measured values of 1070, 1930, and 3350 mg a.s./ kg, respectively. These values represented 107%, 109%, and 106% of nominal concentrations. Analysis of diet samples collected from feeders after being held at ambient temperature for 5 days averaged 102%, 103%, 101%, 107%, and 102% of the Day 0 values for the 562, 1000, 1780, 3160, and 5620 mg a.s./kg test concentrations, respectively.

The following data were used to determine the LC50 and NOEC in terms of daily dose:

Table B.9.1.9: Data used to determine the LC50 and NOEC in terms of daily dose

Test concentration (mg a.s./kg)	Mean body weight (g)	Mean feed consumption (g/bird/day)	Estimated daily dietary dose (mg a.s./kg/day)
0	27	6	0
562	27	6	130
1000	26	6	250
1780	26	6	434
3160	27	6	715
5620	24	6	1328

Conclusions

The dietary LC50 value for northern bobwhite exposed to XDE-729 Methyl was determined to be greater than 5620 mg a.s./kg diet (1328 mg a.s./kg/day), the highest concentration tested. The no-mortality concentration was 5620 mg a.s./kg diet. Based upon a reduction in mean body weight gain from Day 0 to Day 5 at the 5620 mg a.s./kg diet test concentration, the no-observed-effect concentration was 3160 mg a.s./kg diet (715 mg a.s./kg/day).

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is an LC50 of >5620 mg a.s./kg diet.

(2011): XDE-729 Methyl: A Dietary LC50 Study with the Mallard.

Project Number 379-214. Dow AgroSciences unpublished report, Study Number 090029. Amended 10 May 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 % wt/wt XDe-729 Methyl ⁴
Description:	Solid
Lot No./Batch No. :	TSN031117-0004 E2837-51

⁴ At time of receipt, the reported purity of the test substance was 99.1% ± 0.1% XDE-729 Methyl, which was used to calculate the nominal dosage level. A revised certificate of analysis reported a purity of 97.2% and a re-certification date of 30 November 2013. The dosages were adjusted to 100% active substance based on a purity of 99.1%. Dietary test concentrations of 0, 562, 1000, 1780, 3160 and 5620 mg a.s./kg were corrected for a purity of 99.1%. When corrected for the revised purity of 97.2%, the calculated test concentrations are 0, 551, 981, 1746, 3099 and 5512 mg a.s./kg. The nominal test concentrations of 0, 562, 1000, 1780, 3160 and 5620 mg a.s./kg are used for reporting purposes. Nominal dietary test concentrations used in this study were 0, 562, 1000, 1780, 3160 and 5620 mg a.s./kg. XDE-729 Methyl.

Test system

Organism (Species):	Mallard (<i>Anas platyrhynchos</i>)
Study Type:	Dietary
GLP Status:	GLP Study was conducted to GLP, except for the assessment of stability, homogeneity, and verification of the test substance in the dosing solution.
Guidelines followed:	OPPTS Number 850.2200 and FIFRA Subdivision E, Section 71-2. OECD guideline 205.
Guideline deviations reported by Study Director:	None
Duration of study:	8 days
Parameters measured:	Body weight, feed consumption
Observation intervals:	Multiple observations on Day 1 and twice daily observations on remaining days.
Age range of birds at test initiation:	9 days old
Weight range of birds at study initiation:	124 to 166 grams
Test concentrations:	0, 562, 1000, 1780, 3160, and 5620 mg a.s./kg diet
Analytical confirmation of test concentrations:	Yes
No. of feed withholding days before dosing:	None
Method of test item administration:	Diet
Diet:	Treated Diet from Day 0 to Day 5, Basal ration Day 6 to Day 8
Number of birds per dose group:	10
Number of birds per control group:	30
Housing:	Thermostatically controlled brooding pens manufactured by Safeguard Products, Inc.
Environmental conditions:	Brooder Temperature: $30.0 \pm 1.4^{\circ}\text{C}$ (SD) Ambient Temperature: $22.1 \pm 0.6^{\circ}\text{C}$ (SD) Photoperiod: 16 hrs light/8 hrs dark Humidity: $53 \pm 7\%$ (SD).

Methodology

All birds were acclimated to the caging and facilities from the day of hatch until initiation of the test. During the test each group was fed the appropriate treated or control diet for five days. Following the five-day exposure period all groups were given untreated basal diet for three days. From test initiation until termination, all birds were observed at least twice daily. A record was maintained of all signs of

toxicity and abnormal behaviour. Individual body weights were measured at the initiation of the test (Day 0), on Day 5, and at termination of the test on Day 8. Average feed consumption values were determined daily by pen for each treatment group and the control group during the exposure period (Days 0-5) and during the post-exposure observation period (Days 6-8). Mortality data were utilized to determine the LC50 value.

Results

Table B 9.1.10: Effect of XDE-729 Methyl on mortality

Treatment (mg a.s./kg diet)	No. of birds	Cumulative mortality		
		At Day 5	At Day 8	Total (%)
Negative control	30	0	0	0
562	10	0	0	0
1000	10	0	0	0
1780	10	0	0	0
3160	10	0	0	0
5620	10	0	0	0
LC50	>5620 mg a.s./kg diet			
95% C.I.	N/A			
NOEC	3160 mg a.s./kg diet			

Table B 9.1.11: Effect of XDE-729 Methyl on body weight and feed consumption

Treatment (mg a.s./kg diet)	Observation				
	Mean body weight (g)			Mean Feed consumption (g/bird/day)	
	day 0	day 5	day 8	days 0 to 5	days 6 to 8
Negative control	140	265	381	79	142
562	141	263	381	72	140
1000	142	265	382	90	159
1780	145	266	390	92	147
3160	142	254	374	73	130
5620	146	246	361	73	128

There were no mortalities in the control group and all birds in the control group were normal in appearance and behaviour for the duration of the test. Additionally, there were no mortalities in the 562, 1000, 1780, 3160 and 5620 mg a.s./kg treatment groups. In all of the treatment groups, there were no overt signs of toxicity and all birds at these concentrations were normal in appearance and behaviour throughout the test.

Samples of diet collected during the test to verify test substance concentrations for the 1000, 1780, 3160 mg a.s./kg diets had measured values of 1070, 1930, and

The following data were used to determine the LC50 and NOEC in terms of daily dose:

Test concentration (mg a.s./kg)	Mean body weight (g)	Mean feed consumption (g/bird/day)	Estimated daily dietary dose (mg a.s./kg/day)
0	203	79	0
562	202	72	202
1000	204	90	442
1780	206	92	796
3160	198	73	1161
5620	196	73	2088

The dietary LC50 value for mallards exposed to XDE-729 Methyl was determined to be greater than 5620 mg a.s./kg diet (2088 mg a.s./kg/day), the highest concentration tested. The no-mortality concentration was 5620 mg a.s./kg diet. Based upon a reduction in mean body weight gain From Day 0 to Day 5 at the 5620 mg a.s./kg diet test concentration, the no-observed-effect concentration was 3160 mg a.s./kg diet (1161 mg a.s./kg/day).

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is an LC50 of >5620 mg a.s./kg diet.

B.9.1.1.3 Long term/Reproductive toxicity (IIA 8.1.3)

(2011). XDE-729
Methyl: A Reproduction Study with the Northern Bobwhite.

Project No. 379-246. Dow AgroSciences
unpublished report, Study Number 101137. Study Completion Date 6 May
2011

Test material

Test item:	XDE-729 Methyl
Purity:	97.2% w/w ⁵
Description:	Solid
Lot No./Batch No. :	TXN031117-0004 E2837-51

⁵ Concentrations of the test substance in the diet were adjusted to 100% active substance.

Test system

Organism (Species):	Northern bobwhite (<i>Colinus virginianus</i>)		
Study Type:	Reproduction		
GLP Status:	GLP		
Guidelines followed:	OPPTS Number 850.2300 and FIFRA Subdivision E, Section 71-4. OECD guideline 206		
Guideline deviations reported by Study Director:	None		
Duration of study:	21 weeks		
Observation intervals:	Daily		
Age range of birds at test initiation:	17 weeks		
Weight range of birds at study initiation:	182 to 227 grams		
Test concentrations:	0, 160, 400 and 1000 mg a.s./kg diet		
Analytical confirmation of test concentrations:	Yes		
Method of test item administration:	Dietary		
Diet:	[REDACTED] basal ration with the addition of test material		
Number of birds per dose group:	32		
Number of birds per control group:	32		
Housing:	GQP Manufacturing Company (Model No. 0330)		
Environmental conditions:	Temperature °C (SD): 20.1 ± 1.6°C 14.1 ± 0.1°C 37.4 ± 0.0°C 37.3 ± 0.0°C 26.9 ± 1.0°C	Photoperiod: 8 hrs/day for 7 wks; 17 hrs/day for 14 wks 16 hrs/day	Humidity % (SD) 43 ± 18% 75 ± 8% 55 ± 0% 57 ± 1% 15 ± 5%
Adult Study Room			
Egg Cold Storage			
Egg Incubator			
Egg Hatcher			
Brooding Pens			

Methodology

Three treatment groups, each containing 16 pairs of northern bobwhite, were fed diets containing the test substance at 160, 400 or 1000 mg/kg diet for 21 weeks. A control group containing 16 pairs was maintained concurrently. The northern bobwhite were observed daily throughout the test for signs of toxicity or abnormal behaviour. Adult body weights were measured at test initiation, at the end of Weeks 2, 4, 6, 8 and at adult termination and feed consumption was measured weekly throughout the adult portion of the test. At the beginning of Week 8, the photoperiod was increased to induce egg production. Following the start of egg production, eggs were set weekly for incubation. Weekly, eggs were selected by indiscriminate draw for egg shell thickness measurement and the remaining eggs were candled and set for incubation. Hatchlings were banded for identification and weighed at hatch and again at 14 days of age.

Results

Table B 9.1.12: Summary of adult feed consumption and body weight in the northern bobwhite reproduction study

Parameter	Treatment Group			
	0	160	400	1000
Nominal concentration (mg a.s./kg diet)	0	160	400	1000
Mean measured concentration (mg a.s./kg diet)	0	162	403	1040
Daily dietary dose calculated using mean measured concentrations (mg a.s./kg b.w./day)	0	14.5	36.9	93.7
Adult body weight (g) - Males				
Test Initiation	205	204	202	202
Week 2	203	203	197	201
Week 4	205	205	202	205
Week 6	209	209	207	210
Week 8	215	216	212	216
Adult Test Termination	220	223	213	222
Adult body weight (g) - Females				
Test Initiation	201	201	201	201
Week 2	199	200	199	196
Week 4	203	203	203	201
Week 6	207	206	207	205
Week 8	211	208	213	208
Adult Test Termination	248	249	243	246

Parameter	Treatment Group			
Nominal concentration (mg a.s./kg diet)	0	160	400	1000
Adult feed consumption (g/bird/day)				
Week 1	16	16	16	16
Week 2	17	17	17	17
Week 3	16	15	16	16
Week 4	16	16	16	16
Week 5	16	15	15	16
Week 6	15	15	16	15
Week 7	14	15	15	15
Week 8	17	16	17	17
Week 9	15	16	16	16
Week 10	16	17	17	17
Week 11	18	18	18	19
Week 12	19	19	18	20
Week 13	20	20	20	21
Week 14	22	22	22	23
Week 15	22	21	22	21
Week 16	23	23	23	23
Week 17	23	23	23	25
Week 18	24	24	24	25
Week 19	24	24	23	25
Week 20	24	24	24	24
Week 21	26	25	25	25
Differences between the control and each treatment group were not significant ($p > 0.05$).				

Table B 9.1.13: Summary of adult mortalities, gross necropsy and clinical observations in the northern bobwhite reproduction study

Treat-ment (mg/kg diet)	Study Date of Mortality	Sex	Clinical Observations prior to Mortality	Necropsy Findings	Considered Treatment Related?
0	Day 2, Week 12	Female	Normal in appearance and behaviour	Small area of intra-cranial bleeding on the skull between the eyes and the heart was slightly pale.	No

Table B 9.1.14: Summary of reproductive parameters in the northern bobwhite reproduction study

Parameter	Treatment Group			
	0	160	400	1000
Nominal concentration (mg a.s./kg diet)				
Number of breeding pairs at study initiation	16	16	16	16
Number of breeding pairs at study termination	15	16	16	16
Total eggs laid	647	732	748	823
Mean egg production (eggs/hen)	43	46	47	51
Mean eggs laid/hen/reproduction day	0.44	0.47	0.48	0.52
Mean eggs laid/maximum laid (%)	62	65	67	73
Total eggs set	526	635	660	743
Total eggs cracked	23	20	15	4
Mean eggshell thickness (mm) \pm SD	0.232 \pm 0.009	0.229 \pm 0.012	0.228 \pm 0.006	0.231 \pm 0.012
Mean eggs cracked/eggs laid (%)	4	3	3	0**
Total viable embryos	490	587	573	686
Mean viable embryos/eggs set (%)	93	91	87	90
Total live 3-week embryos	485	583	572	683
Mean 3-week embryos/viable embryos (%)	99	99	100	100
Total number of hatchlings	468	556	547	602
Mean hatchlings/3-week embryos (%)	96	94	95	88**
Mean hatchlings/eggs set (%)	88	85	83	80
Mean hatchlings/maximum set (%)	48	53	53	58
Total number of 14-day surviving chicks	436	527	519	542
Mean 14-day chicks/hen	29	33	32	34
Mean 14-day chicks/hatchlings (%)	92	95	95	88
Mean 14-day chicks/eggs set (%)	82	80	79	71
Mean 14-day chicks/maximum set (%)	45	51	50	52
Mean body weight of hatchlings (g) \pm SD	6 \pm 0	6 \pm 0	6 \pm 0	6 \pm 1
Mean body weight of 14-day chicks (g) \pm SD	29 \pm 2	29 \pm 3	29 \pm 2	27 \pm 3
**Significantly different from the control at $p < 0.01$.				

Table B 9.1.15: Summary of gross pathological observations in adult birds at test termination

Parameter	Treatment Group ^a			
	0	160	400	1000
Nominal concentration (mg a.s./kg diet)				
Number of birds	30	32	32	32
External				
Feather loss	9	8	9	9
Foot lesions	0	0	1	0
Head lesions	1	1	1	1
Missing right foot	1	0	0	0
Liver				
Pale, mottled and slightly enlarged	0	0	0	1
Abdominal Cavity				
Air sacculitis, left air sac	0	0	1	0
Intra abdominal egg remnant	2	0	2	0
Slight egg yolk peritonitis	0	0	1	0
Egg yolk peritonitis	0	0	1	0
Reproductive				
Right testis small (≤ 1.5 cm)	3	4	7	2
Left testis small (≤ 1.5 cm)	0	1	2	0
Testes small (~ 1.5 cm)	1	2	0	2
Ovary regressing	0	0	0	1
Ovary regressed	0	0	1	0
Not remarkable	16	18	13	17
a Number of observations for Males and Females				
All findings observed were considered unrelated to treatment.				

The following data were used to determine the NOEC in terms of daily dose:

Test intervals (test weeks)	Test concentration (mg a.s./kg)	Mean body weight (g)	Mean feed consumption (g/bird/day)	Estimated daily dietary dose (mg a.s./kg/day)
Pre-egg production (weeks 1-11)	0	206	16	0
	160	206	16	12.5
	400	204	16	31.9
	1000	205	16	80.0
Egg production (weeks 12-21)	0	224	23	0
	160	224	23	16.1
	400	220	22	40.7
	1000	223	23	104.0
Overall (weeks 1-21)	0	211	19	0.0
	160	211	19	14.5
	400	208	19	36.9
	1000	209	20	93.7

Conclusions

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 160 or 400 mg a.s./kg diet test concentrations. At the 1000 mg a.s./kg diet test concentration, there was a statistically significant reduction in hatchability ($p < 0.01$) which was considered to be treatment-related; there were also non-significant but potentially treatment and dose related effects on mean hatchings/egg set. The no-observed-effect concentration for northern bobwhite exposed to XDE-729 Methyl in the diet during the study was therefore considered to be 400 mg a.s./kg diet (36.9 mg a.s./kg/day).

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a NOEC of 36.9 mg a.s./kg day.

(2011). XDE-729 Methyl: A Reproduction Study with the Mallard. [REDACTED]
[REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED]
[REDACTED] Project No. 379-247. Dow AgroSciences unpublished report, Study Number 101139. Study Completion Date: 6 May 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2% w/w ⁶
Description:	Solid
Lot No./Batch No. :	E2837-51

Test system

Organism (Species):	Mallard (<i>Anas platyrhynchos</i>)		
Study Type:	Reproduction		
GLP Status:	GLP		
Guidelines followed:	OPPTS Number 850.2300 and FIFRA Subdivision E, Section 71-4, OECD guideline 206		
Guideline deviations reported by Study Director:	None		
Duration of study:	20 weeks		
Observation intervals:	Daily		
Age range of birds at test initiation:	30 weeks		
Weight range of birds at study initiation:	851 to 1439 grams		
Test concentrations:	0, 160, 400 and 1000 mg a.s./kg diet		
Analytical confirmation of test concentrations:	Yes		
Method of test item administration:	Dietary		
Diet:	[REDACTED] . basal ration with the addition of test material		
Number of birds per dose group:	32		
Number of birds per control group:	32		
Housing:	Safeguard Products, Inc. (Model No. 5355)		
Environmental conditions:	Temperature °C (SD):	Photoperiod:	Humidity % (SD)
Adult Study Room	22.2 ± 1.0°C	8 hrs/day for 7 wks;	46 ± 16%
	14.2 ± 0.1°C	17 hrs/day for	78 ± 9%

⁶ Concentrations of the test substance in the diet were adjusted to 100% active substance

Egg Cold Storage	37.4 ± 0.0°C	13 wks	55 ± 0%
Egg Incubator	37.3 ± 0.0°C		60 ± 1%
Egg Hatcher	22.6 ± 0.7°C	16 hrs/day	23 ± 7%
Brooding Pens			

Methodology

Three treatment groups, each containing 16 pairs of mallard, were fed diets containing the test substance at 160, 400 or 1000 mg/kg diet for 20 weeks. A control group containing 16 pairs was maintained concurrently. The mallard were observed daily throughout the test for signs of toxicity or abnormal behaviour. Adult body weights were measured at test initiation, at the end of Weeks 2, 4, 6, 8 and at adult termination and feed consumption was measured weekly throughout the adult portion of the test. At the beginning of Week 9, the photoperiod was increased to induce egg production. Following the start of egg production, eggs were set weekly for incubation. Weekly, eggs were selected by indiscriminate draw for egg shell thickness measurement and the remaining eggs were candled and set for incubation. Hatchlings were banded for identification and weighed at hatch and again at 14 days of age.

Results

Table B 9.1.16: Summary of adult feed consumption and body weight in the mallard reproduction study

Parameter	Treatment Group			
Nominal concentration (mg a.s./kg diet)	0	160	400	1000
Mean measured concentration (mg a.s./kg diet)	0	147	380	1030
Daily dietary dose calculated using mean measured concentrations (mg a.s./kg b.w./day)	0	23.5	55.0	160.5
Adult body weight (g) - Males				
Test Initiation	1143	1147	1150	1146
Week 2	1135	1142	1149	1137
Week 4	1131	1134	1131	1133
Week 6	1124	1140	1120	1126
Week 8	1121	1135	1118	1120
Adult Test Termination	1256	1218	1243	1239
Adult body weight (g) - Females				
Test Initiation	1029	1022	1024	1032
Week 2	1028	1016	1002	1016
Week 4	1018	1001	982	1011
Week 6	1022	1020	995	1018
Week 8	1008	1028	992	1009
Adult Test Termination	1167	1150	1161	1142

Parameter	Treatment Group			
Nominal concentration (mg a.s./kg diet)	0	160	400	1000
Adult feed consumption (g/bird/day)				
Week 1	119	102	88**	101
Week 2	139	126	118	130
Week 3	123	117	102	124
Week 4	131	117	109*	124
Week 5	117	115	99	124
Week 6	141	126	115**	136
Week 7	138	130	122	141
Week 8	137	131	124	136
Week 9	135	131	128	143
Week 10	204	194	181	201
Week 11	198	181	170	195
Week 12	203	169*	150**	195
Week 13	220	184	175*	224
Week 14	213	197	192	225
Week 15	216	195	181	226
Week 16	192	178	168	200
Week 17	205	190	181	206
Week 18	218	200	193	219
Week 19	219	208	193	219
Week 20	241	226	207	242
*Significantly different from the control at $p < 0.05$.				
**Significantly different from the control at $p < 0.01$.				

Table B 9.1.17: Summary of reproductive parameters in the northern bobwhite reproduction study

Parameter	Treatment Group			
Nominal concentration (mg a.s./kg diet)	0	160	400	1000
Number of breeding pairs at study initiation	16	16	16	16
Number of breeding pairs at study termination	16	16	16	16
Total eggs laid	914	945	867	882
Mean egg production (eggs/hen)	57	59	54	55
Mean eggs laid/hen/reproduction day	0.69	0.71	0.65	0.66
Mean eggs laid/maximum laid (%)	71	73	67	68
Total eggs set	819	831	773	790
Total eggs cracked	4	3	6	4
Mean eggshell thickness (mm)	0.393 ± 0.019	0.392 ± 0.018	0.391 ± 0.019	0.389 ± 0.021
Mean eggs cracked/eggs laid (%) ± SD	1	0	1	0
Total viable embryos	791	776	669	710
Mean viable embryos/eggs set (%)	96	93	86	91
Total live 3-week embryos	787	773	659	710

Parameter	Treatment Group			
Nominal concentration (mg a.s./kg diet)	0	160	400	1000
Mean 3-week embryos/viable embryos (%)	99	100	98	100
Total number of hatchlings	683	656	578	639
Mean hatchlings/3-week embryos (%)	85	85	84	89
Mean hatchlings/eggs set (%)	82	79	74	81
Mean hatchlings/maximum set (%)	64	61	54	60
Total number of 14-day surviving chicks	683	653	571	636
Mean 14-day chicks/hen	43	41	36	40
Mean 14-day chicks/hatchlings (%)	100	100	99*	100
Mean 14-day chicks/eggs set (%)	82	79	73	81
Mean 14-day chicks/maximum set (%)	64	61	53	59
Mean body weight of hatchlings (g) \pm SD	39 \pm 3	39 \pm 3	40 \pm 2	38 \pm 3
Mean body weight of 14 day chicks (g) \pm SD	319 \pm 17	308 \pm 17	317 \pm 18	307 \pm 21
*Significantly different from the control at $p < 0.05$.				

Table B 9.1.18: Summary of gross pathological observations in adult birds at test termination

Parameter	Treatment Group ^a			
Nominal concentration (mg a.s./kg diet)	0	160	400	1000
Number of birds	32	32	32	32
External				
Breast blister	1	0	0	0
Feather loss	4	4	5	3
Moulting	1	0	0	0
Foot lesions	16	14	16	13
Head lesion(s)	0	1	1	2
Spleen				
Enlarged	1	0	1	0
Darkened colour	0	0	1	0
Abdominal Cavity				
Retained or persistent yolk sac	0	0	1	1
Slight, old egg yolk peritonitis	2	2	3	3
Old egg yolk peritonitis	1	0	1	0
Slight egg yolk peritonitis	0	1	0	1
Egg yolk peritonitis	0	2	1	1
Extensive egg yolk peritonitis	1	0	0	0

Parameter	Treatment Group ^a			
	0	160	400	1000
Reproductive				
Persistent right oviduct	0	1	0	0
4 empty follicles	0	0	0	1
Hemorrhagic follicle	1	0	1	1
Cystic follicle(s)	0	1	1	0
Ovary regressing	3	1	1	1
Ovary regressed	1	1	2	4
Right testis small, ~ 3.0 – 4.0 cm	2	0	0	2
Left testis small, ~ 3.5 – 4.5 cm	1	0	0	2
Testes small ≤ 4.5 cm	2	2	3	6
Not remarkable	9	13	11	8
^a Number of observations for Males and Females All findings observed were considered unrelated to treatment.				

Conclusions

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 160, 400 or 1000 mg a.s./kg diet test concentrations. The no-observed-effect concentration for mallard exposed to XDE-729 Methyl in the diet during the study was 1000 mg a.s. /kg diet (160.5 mg a.s./kg/day), the highest concentration tested.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a NOEC of 160.5 mg a.s./kg day.

B.9.1.2 Risk assessment

XDE-729 Methyl and GF-2573 is intended for use on winter and spring sown cereals. Thus, foliar application is expected to occur to crops during the growing season in autumn at BBCH 10 to 29 and in the spring at BBCH 13 to 45. Therefore, birds, and the foliage and/or insects that they feed upon, may be present during the application period; thus requiring a risk assessment.

Upon application, the majority of XDE-729 Methyl is expected to hydrolyse into XDE-729 acid. Therefore after application to cereals, exposure of birds will predominately be to the acid. Under current guidance for the bird risk assessment, active substance (XDE-729 Methyl) endpoints only are used. These endpoints are considered 'worse case' as exposure to the methyl automatically incorporates exposure to the metabolite X11406790 as well as to the acid (via de-esterification of the methyl). The XDE-729 acid further metabolises into X11449757 over time. Only considering exposure to the acid does not take into consideration the potential exposure from the metabolite X11406790, which is only metabolised directly from the methyl.

An acute oral formulation endpoint has also been provided for the bird risk assessment, this will also be considered.

Presented below in Table B.9.1.2-1 is a summary of the avian toxicity studies on XDE-729 Methyl. Key regulatory endpoints used in the risk assessment are highlighted in bold.

Table B.9.1.19: Summary of avian toxicity studies on XDE-729 Methyl

Test organism	Test Material	Endpoint	Reference
ACUTE ORAL TOXICITY			
Bobwhite quail (<i>Colinus virginianus</i>)	XDE-729 Methyl	LD₅₀ > 2250 mg /kg bw	(2011a) IIA 8.1.1/1
Zebra finch (<i>Poephila guttata</i>)	XDE-729 Methyl	LD ₅₀ > 2250 mg /kg bw	(2011b) IIA 8.1.1/2
Bobwhite quail (<i>Colinus virginianus</i>)	GF-2573	LD ₅₀ > 2000 mg GF-2573/kg bw	(2011) IIIA 10.1.6
SHORT TERM DIETARY TOXICITY			
Bobwhite quail (<i>Colinus virginianus</i>)	XDE-729 Methyl	LC ₅₀ > 5620 mg /kg diet LD ₅₀ > 1328 mg/kg bw/day	(2010) IIA 8.1.2/1
Mallard duck (<i>Anas platyrhynchos</i>)	XDE-729 Methyl	LC ₅₀ > 5620 mg /kg diet LD ₅₀ > 2088 mg/kg bw/day	(2011) IIA 8.1.3/1
REPRODUCTION TOXICITY			
Bobwhite quail (<i>Colinus virginianus</i>)	XDE-729 Methyl	NOEC = 400 mg /kg diet NOEL = 36.9 mg /kg /day	(2011a) IIA 8.1.4/1
Mallard duck (<i>Anas platyrhynchos</i>)	XDE-729 Methyl	NOEC = 1000 mg/kg diet NOEL = 160.5 mg/kg /day	(2011b) IIA 8.1.4/2

bold – Endpoints for use in risk assessment.

On the basis of the above, the following endpoints are proposed for risk assessment purposes:

Acute, active – LD₅₀ 4248 mg a.s/kg b.w. (acute endpoint multiplied by the relevant extrapolation factor, see below for details)

An acute oral toxicity study is required for a quail species or mallard duck. For XDE-729 Methyl acute toxicity studies were conducted on bobwhite quails and zebra finch.

Although there were no major deficiencies noted from either of these studies, it is noted that Zebra finch is not a standard EU test species and does not meet the data requirement. It is considered that as there are other data available, using this study in EU regulatory risk assessment is not appropriate.

Following EFSA/2009/1438, dietary (short term) risk assessment calculations should only be done when a dietary LD₅₀ is available and this LD₅₀ value is lower than the acute LD₅₀. In this case, although the LDD₅₀ is lower than the LD₅₀, both the dietary and acute LD₅₀ are greater than values and so the acute endpoint should be used in the risk assessment. However the LD₅₀ value should first be extrapolated.

Therefore the acute study with the active on bobwhite quail is considered suitable for use in regulatory risk assessment. As no mortality was observed in this study we can extrapolate an LD₅₀ value from the limit test (EFSA Bird and mammal guidance, 2009 section 2.1.2). Ten individuals were used in this study and so an extrapolation factor of 1.888 is used. Therefore the extrapolated acute **LD₅₀ is 4248 mg a.s/kg b.w.** This is considered the appropriate endpoint for use in regulatory risk assessment.

Acute, formulation- LD₅₀ 3228 mg formⁿ./kg bw

Data have been submitted on the toxicity of the formulation to Bobwhite Quail and these indicate that the LD₅₀ is >2000 mg formⁿ./kg bw. The formulation only contains 0.84% of the active substance. The formulation study therefore tested a concentration of the a.s. that was much lower than that assessed in the standard acute oral study. The Applicant has stated that in order to test a concentration equivalent to the standard study on the active substance, the top dose would have been 268000 mg a.s./kg bw. The applicant has stated that 'the purpose of doing this study was to provide reassurance that the other formulation ingredients, which make up >99% of this formulation, will not to pose an acute risk'. As this LD₅₀ is a greater than value and as no mortality was observed in this study, it is possible to extrapolate. Five individuals were used in this study and so an extrapolation factor of 1.614 is used and a LD₅₀ value of 3228 mg formulation/kg b.w. is calculated. It is proposed to use this endpoint of **LD₅₀ 3228 mg formⁿ./kg bw** in a standard risk assessment and compare the results with the data and subsequent risk assessment for the active.

Reproduction – NOAEL = 36.9 mg a.s/kg b.w. day.

Avian reproductive toxicity studies were conducted using the active substance on the bobwhite quail and the mallard duck. The reproduction NOEC for the northern bobwhite study was 400 mg a.s./kg diet, equivalent to **36.9 mg a.s./kg bw/day**. There were no treatment related effects upon any of the reproductive parameters measured at this concentration. At 1000ppm, there was a statistically significant effect on the number of eggs cracked and the mean number of hatchlings as a percentage of live 3-week embryos (mean was ca. 8% lower than control mean). However, the applicant stated that the total number of 14-d old survivors/eggs set was greater than controls, and the mean number of hatchlings as a percentage of live 3-week embryos (and all other parameters) was within the lab's historical control range. Whilst acknowledging the applicant's point that the effect at 1000 ppm was slight, the RMS is not convinced that it is not ecologically or biologically relevant and hence has considered this to be test related and so the NOEC and therefore NOAEL is based on the lower concentration.

An acute avian risk assessment was conducted according to EFSA 2009 guidance using the formula below.

Acute risk to birds from the proposed use of XDE-729 on winter and spring cereals

*Daily dietary dose (DDD) = application rate (kg a.s./ha) * MAF * shortcut value*

TER = LD₅₀ / DDD

Within the EFSA 2009 guidance document the screening step uses a shortcut value based on 90th percentile residues and on the crop type and indicator species.

For use on cereals the indicator species given in the guidance (Table 6) is a small omnivorous bird. The shortcut value assigned to this is a value of 158.8.

XDE-729 may be applied up to twice per year, one application in the autumn at 0.00782 kg a.s./ha and then in spring at 0.00625 kg a.s./ha, with a 70 day interval between applications. Table 7 in the EFSA guideline illustrates that the MAF after 70 days would be 1, irrespective of whether 1 or 2 applications occurred. No accumulation of the active substance would be expected to occur between applications with a 70 day interval. Consequently, it is realistic to evaluate the potential risk to birds from only a single application, at the highest application rate of 0.00782 kg a.s./ha.

Table B.9.1.20: Summary of acute screening step for birds exposed to XDE-729 Methyl

Indicator species	Shortcut value	Application rate (kg a.s./ha)	MAF	DDD	Toxicity (mg a.s./kg bw)	TER*	Trigger
Small omnivorous bird	158.8	0.00782	1	1.24	4248	3421	10

*Value has been rounded up.

The TER is above the trigger value showing an acceptable acute risk to birds from the proposed use of XDE-729 Methyl on winter and spring cereals and so no further consideration is required.

Acute risk to birds from the proposed use of the formulation GF-2573 on winter and spring cereals

It is proposed to use the extrapolated formulation endpoint of **LD₅₀ 3228 mg formⁿ/kg bw** in a standard risk assessment and compare the results with the data and subsequent risk assessment for the active.

Daily dietary dose (DDD) = application rate (kg/ha) x MAF (multiple application factor) x shortcut value

$$TER = LD_{50} / DDD$$

The same indicator species and shortcut value apply, as used in the screening step given above.

However, the application rate needs to be expressed in terms of the formulation. The GAP states that the concentration of the a.s in the formulation is 7.817 g/L of XDE-729 methyl. From the application rate of 7.82 g/ha we can derive that only a litre is used for an application. Document J (IIIA 1.4.1) states that the relative density of 1 litre of the product is 0.907 g/ml. Therefore 0.907 kg formⁿ/ha is used per application.

Table B.9.1.21 Summary of acute screening step for birds exposed to GF-2573.

Indicator species	Shortcut value	Application rate (kg form ⁿ /ha)	MAF	DDD	Toxicity (mg form ⁿ /kg bw)	TER*	Trigger
Small omnivorous bird	158.8	0.907*	1	144	3228	22.41	10

*Formulation density = 907 g/L

**Value rounded to 2 decimal places.

The TER is above the trigger value showing an acceptable acute risk to birds from the proposed use of GF-2573 on winter and spring cereals and so no further consideration is required.

Reproductive risk to birds from the proposed use of XDE-729 Methyl on winter and spring cereals

Within the EFSA 2009 guidance document the screening step is used if birds may be exposed during breeding which is possible under this application.

The guidance states that for the screening step the endpoints should be presented as mg a.s./kg bw/ day. The studies submitted for this application had already presented in these units so no further conversion needs to take place. The toxicity endpoint used is the lowest of the LD₅₀/ 10 or the lowest NOAEL. Therefore, a NOAEL of 36.9 mg a.s./kg bw day is proposed (the LD₅₀ from the acute study divided by 10 is 225)

The daily dietary dose (DDD) of a compound is given by the following equation:

$$DDD = \text{application rate (kg a.s./ha)} \times \text{shortcut value} \times TWA \text{ (time weighted average)} \times MAF$$

$$TER = \frac{\text{Lowest endpoint}}{\text{Daily Dietary Dose}}$$

For use on cereals the indicator species given in the guidance (Table 10) is a small omnivorous bird. The shortcut value assigned to this is a value of 64.8. As stated above no accumulation of the active substance would be expected to occur between applications with a 70 day interval and therefore no MAF needs to be considered. A long term exposure TWA of 0.53 is to be used.

Table B.9.1.22: Summary of reproductive screening step for birds exposed to XDE-729 Methyl

Indicator species	Shortcut value	Application rate (kg a.s./ha)	TWA	MAF	DDD	Toxicity (mg/kg bw/day)	TER*	Trigger
Small omnivorous bird	64.8	0.00782	0.53	1	0.27	36.9	137	5

*Value has been rounded up.

The TER is above the trigger value showing an acceptable reproductive risk to birds from the proposed use of XDE-729 Methyl on winter and spring cereals and so no further consideration is required.

Risk assessment for substances with endocrine-disrupting properties.

A discussion of the evidence of any endocrine-disrupting properties caused by the active substance was offered by the applicant and is detailed below:

Two reproduction studies were conducted with XDE-729 Methyl; bobwhite quail and mallard duck. There were no treatment-related mortalities or effects on reproduction in the mallard duck study up to and including 1000 ppm diet. In the bobwhite quail study there was a statistically significant effect at 1000 ppm on the mean number of hatchlings as a percentage of live 3-week embryos (mean was ca. 8% lower than control mean) which were considered to be treatment-related. However, the total number of 14-d old survivors/eggs set was greater than controls, and the mean number of hatchlings as a percentage of live 3-week embryos (and all other parameters) were within the labs historical control range. The reproduction NOEC for the bobwhite study was 400 ppm diet, equivalent to 36.9 mg a.s./kg bw/day. There were no treatment related effects upon any of the reproductive parameters measured at this concentration. The metabolic pathway for XDE-729 Methyl in birds is similar to mammals (goats, rats); XDE-729 Methyl is rapidly metabolized to XDE-729 Acid, X11406790 and X11449757, followed by conjugation.

The evaluator agrees with the points made by the applicant. Furthermore, no evidence of endocrine disruption effects were seen in any of the fish or amphibian studies conducted to assess this possibility.

Based on this evidence, XDE-729 Methyl, XDE-729 Acid, X11406790 and X11449757 do not appear to exhibit endocrine-disrupting properties that would affect reproductive physiology or development of reproductive organs in wild mammals or birds. However, member states should note that there are currently no defined criteria for identifying endocrine disruptors under 2009/1107 and as such only a qualitative case can be made.

Risk assessment for metabolites formed in food items

Metabolism in plants

Following uptake of XDE-729 Methyl by plants, the molecule either undergoes de-esterification to the herbicidal moiety XDE-729 Acid and subsequent metabolism to X11449757, or XDE-729 Methyl undergoes de-methylation of the methoxy group on the phenyl ring to form X11406790, which is rapidly conjugated. The metabolites found in plants were all present at low levels (<10% AR). Thus, although it is theoretically possible that insects could ingest plant matter and thereby take up plant-derived metabolites of XDE-729 Methyl, exposure levels would be negligible. As XDE-729 acid and metabolite X1449757 were found in bird metabolism studies, the risk to birds from these metabolites has been addressed by the XDE-729 methyl assessments.

Metabolites in birds

Since XDE-729 Methyl is rapidly metabolized in birds to XDE-729 Acid, X11406790 and X11449757 (94.6 and 93.9% of the dose, cross ref section B 7.2.3: [REDACTED])

[REDACTED] *A Nature of the Residue Study in the Laying Hen with [14C]-XDE-729 Methyl Ester*), the toxicity of these metabolites is already considered within the assessments conducted with XDE-729 Methyl. Therefore, avian toxicity testing with these animal metabolites is not necessary and the risk from the metabolites can be considered to be covered by the XDE-729 methyl assessment.

Metabolites in the environment

Under aerobic soil conditions XDE-729 Methyl is rapidly converted to XDE-729 Acid and X11449757 as well as non-extractable residues and CO₂. These soil derived residues may be taken up by plants and insects living on the soil surface and therefore should be considered. As stated above, metabolites in plants were all present at low levels (<10%) so this risk is deemed acceptable and doesn't need further consideration. Furthermore, as metabolites of XDE-729 Methyl have a very low potential for bioaccumulation and biomagnification with log K_{ow} values well below the trigger value of 3, bioaccumulation of XDE-729 Methyl metabolites in soil organisms such as earthworms and aquatic organisms such as fish is negligible and not a major route of exposure to birds. As XDE-729 acid and metabolite X1449757 were found in bird metabolism studies, the risk to birds from these metabolites has been addressed by the XDE-729 methyl assessments. Exposure to metabolites in surface water is covered by the drinking water assessment below.

Drinking water

Two scenarios were identified as relevant for assessing the risk of pesticides via drinking water to birds and mammals:

- Leaf scenario. Only relevant for birds possibly drinking water from puddles in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation. As XDE-729 Methyl is applied in cereals, no pools in leaf axils are to be expected.
- Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide

to a crop or bare soil. This scenario is relevant for acute and long-term exposure.

An “escape clause” recommended in the EFSA Guidance Document for Birds and Mammals (2009) allows for screening the need for a quantitative risk assessment by a comparison between the application rate and the toxicity of the respective substance. This escape clause specifies that “due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals ..., no specific calculations of exposure and TER are necessary when the ratio of effective application rate (= application rate x MAF) (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).”.

The mean K_{oc} value for the active substance XDE-729 methyl is 987 L/kg. XDE-729 may be applied up to twice per year, one application in the autumn at 0.00782 kg a.s. /ha and then in spring at 0.00625 kg a.s. /ha, with a 70 day interval between applications. Table 7 in the EFSA guideline illustrates that the MAF after 70 days would be 1, irrespective of whether 1 or 2 applications occurred. No accumulation of the active substance would be expected to occur between applications with a 70 day interval. Consequently, it is realistic to evaluate the potential risk to birds from only a single application, at the highest application rate of 0.00782 kg a.s./ha. For more sorptive substances ($K_{oc} > 500$ L/kg) a ratio of application rate to relevant endpoint of 3000 or less indicates a low risk of poisoning via drinking water through the puddle scenario.

Table 9.1.23: Evaluation of potential concern for exposure of birds via drinking water (escape clause)

Compound	Mean K_{oc} [L/kg]	Application rate [g a.s./ha] ^{A)}	End point	Ratio (Application rate * endpoint)	“Escape clause”	Conclusion
					No concern if ratio	
XDE-729 Methyl	987	7.82	36.9 (long-term)	0.21	≤ 3000	No concern
	987	7.82	4248 (acute)	0.0018	≤ 3000	No concern

A) Worst-case application to winter cereals

An acceptable risk to birds can therefore be concluded for XDE-729 Methyl and the metabolites potentially arising from drinking water.

Bioaccumulation and food chain behaviour

According to the EFSA Guidance Document, substances with a log K_{ow} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains. XDE-729 Methyl has a **log K_{ow} value of 3.76**. Therefore, the risk from bioaccumulation to fish-eating and worm-eating birds has been carried out.

Risk to earthworm-eating birds

Risk assessment is carried out according to EFSA/2009/1438.

Dry soil approach

The bioconcentration factor for the earthworm ($BCF_{\text{earthworm}}$) is calculated by the following equation:

$$BCF_{\text{earthworm}} = \frac{0.84 + 0.012 \times K_{ow}}{f_{oc} \times K_{oc}}$$

K_{ow} -Octanol-water partition coefficient

K_{oc} -Organic carbon adsorption coefficient

f_{oc} - Organic carbon content of soil (0.02 taken as a default value)

A value for the predicted environmental concentration for dry soil (PEC_{soil}) needs to be calculated. This is then multiplied with the $BCF_{\text{earthworm}}$ to give the $PEC_{\text{earthworm}}$.

$$PEC_{\text{earthworm}} = PEC_{\text{soil}} \times BCF_{\text{earthworm}}$$

The $PEC_{\text{earthworm}}$ calculation needs to be converted into daily dietary dose (DDD) by multiplying with 1.05. This value is based on the worst case scenario of a 100g bird eating 104.6g per day.

Finally the toxicity-exposure ratio needs to be determined and compared to the respective trigger value.

$$TER = \frac{NOAEL}{DDD}$$

All input parameters and calculated values are given in the table below.

Table 9.1.24: Long-term risk from secondary poisoning to earthworm-eating birds from XDE-729

Crop	PEC_{soil} (mg a.s/kg)	K_{ow}	f_{oc}	K_{oc}	BCF	PEC_{worm} (mg/kg)	DDD (mg/kg bw/d)	NOE L (mg/kg g bw/d)	TER
Cereals	0.009	5754.4	0.02	987	3.541	0.032	0.033	36.9	1103

The TER value is greater than the trigger value of 5 indicating an acceptable risk to birds feeding on earthworms following use of XDE-729.

The log K_{OW} values for the XDE-729 Methyl metabolites are all < 3 so an acceptable risk can also be concluded for XDE-729 Acid, X11449757 and X11406790.

Risk to fish-eating birds

Risk assessment is carried out according to EFSA/2009/1438.

Values for the predicted environmental concentration for surface water (PEC_{sw}) and the whole-body BCF_{fish} have been calculated.

Estimated residues in fish can then be calculated using the following equation:

$$PEC_{fish} = PEC_{sw} \times TWA \times BCF_{fish}$$

The PEC_{fish} calculation needs to be converted into daily dietary dose (DDD) by multiplying with 0.159. This value is based on the worst case scenario of a 1000g bird eating 159g per day.

Finally the toxicity-exposure ratio needs to be determined and compared to the respective trigger value.

$$TER = \frac{NOAEL}{DDD}$$

All input parameters and calculated values are given in the table below.

Table 9.1.25: Long-term risk from secondary poisoning to fish-eating birds from XDE-729

Crop	PEC_{sw} (mg/L)	BCF_{fish}	PEC_{fish} (mg/kg)	DDD (mg/kg/bw/day)	NOEL (mg/kg bw/d)	TER
Cereals	0.001195*	217	0.260	0.041	36.9	893

* $PEC_{initial}$ (mg XDE-729 Methyl/L), as a 1st step and thus more conservative calculation, the TWA has not been used.

The TER value is greater than the trigger value of 5 indicating an acceptable risk to birds feeding on fish following the use of XDE-729.

The log K_{OW} values for the XDE-729 Methyl metabolites are all < 3 so an acceptable risk can also be concluded for XDE-729 Acid, X11449757 and X11406790.

Biomagnification

Despite the log octanol water coefficient suggesting that the active is fat soluble, the metabolism studies with livestock show that XDE-729 methyl is excreted rapidly and fat levels analysed were below the limit of quantitation (LOQ). The ADME data with rats and mice showed no evidence of accumulation in tissues / fat with either the

sodium salt of the acid or the methyl ester. Excretion is rapid and essentially complete within 72 hours. 7 days after a single dose, levels in fat were non-detectable (<0.03% of the administered dose). Reference is made to tables B.6.1.1-4 and 6.1.1-5 in the mammal toxicology DAR.

B.9.1.3 Conclusions

When applied in accordance with the proposed GAP for product GF-2573, the active substance XDE-729 methyl, XDE-729 acid and its metabolites do not pose a significant risk to birds.

B.9.2 Effects on aquatic organisms (IIA 8.2, IIIA 10.2)

B.9.2.1 Toxicity

B.9.2.1.1 Acute toxicity

B.9.2.1.1.1 Acute toxicity to fish

Active substance

██████████ (2011): XDE-729 Methyl: Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, determined Under Static-Renewal Test Conditions. ██████████. Dow AgroSciences unpublished report, Study Number 090187. 10 May 2011.

Test material

Test item	XDE-729 Methyl
Purity	97.2 wt %
Description	Off-white solid
Lot No./Batch No	E2837-51
Test system	
Organism (Species)	Rainbow Trout (<i>Oncorhynchus mykiss</i>)
Study Type	Acute
GLP Status	GLP (except the latest water characterization carried out in 2009)
Guidelines followed	OECD guideline 203
Guideline deviations reported by Study Director	None
Duration of study	96 hours
Test conditions	Static-renewal (daily)
Parameters measured	Survival
Observation intervals	0, 24, 48, 72, 96 hours
Age range of fish at test initiation	Juvenile
Weight range of fish at study initiation	1.000 to 2.113 g blotted wet weight

Range of fish length at study initiation	45 to 58 mm total length
Test concentrations	
Nominal XDE-729 Methyl concentrations	0 (control), 0 (vehicle control; 0.10 mL DMF/L), 0.25, 0.50, 1.0, 2.0, and 3.9 mg a.s./L
Mean XDE-729 Methyl measured concentrations	< MQL (control), < MQL (vehicle control), 0.215, 0.428, 0.786, 1.50, and 3.10 ⁷ mg a.s./L
Analytical confirmation of test concentrations	0, 24, 72, and 96 hours
Reference substances	XDE-729 Methyl and XDE-729 Acid
No. of holding days before dosing	>12 days
Number of fish per dose group	10
Number of fish per control group	10
Feeding	None
Environmental conditions	
Temperature	14.4 to 16.0°C
Photoperiod	16 hour light:8 hour dark, 405 lux
Dissolved Oxygen concentration (new)	9.4 to 11.5 mg/L (97 to 119% sat.)
Dissolved Oxygen (old)	6.3 to 8.9 mg/L (66 to 94% sat.)
Bioloading	0.4001 g/L
pH	6.8 to 8.7
Water alkalinity	158 mg CaCO ₃ /L
Water hardness	148 mg CaCO ₃ /L
Water conductivity	339 µS

Methodology

A 96-hour static-renewal test was performed with test concentrations of 0 (control), 0 (vehicle control; 0.10 DMF/L), 0.25, 0.50, 1.0, 2.0, and 3.9 mg a.s./L. Test fish were impartially assigned to treatments by adding one fish per chamber proceeding from controls, low to high test substance treatments, and repeating steps as necessary until five fish were present in each replicate test chamber. The treatments were replicated two times for a total of ten fish per treatment. Observations for mortality and sub-lethal responses were made at 24, 48, 72, and 96 hours. Temperature, pH, and dissolved oxygen concentration were measured in each test chamber daily. In addition, a continuous record of the temperature from the water bath was also maintained. Alkalinity, hardness, and conductivity were measured in a sample of the dilution water at test initiation.

⁷ Due to 100% mortality within 24 hours the top concentration was only assessed at 0 and 24 hours.

Results

Table B 9.2.1: Effect of XDE-729 Methyl on mortality

Treatment (mg a.s./L)		No. of fish	Cumulative Mortality				
Nominal	Mean Measured		24- h	48-h	72-h	96-h	Total (%)
Negative control	<MQL	10	0	0	0	0	0
Vehicle Control	<MQL	10	0	0	0	0	0
0.25	0.215	10	0	0	0	0	0
0.50	0.428	10	0	0	0	0	0
1.0	0.786	10	0	0	0	0	0
2.0	1.50	10	0	1	1	1	10
4.0	3.10	10	10	10	10	10	100
96 hour LC ₅₀		2.01 mg a.s./L (mean measured)					
95% C.I.		1.77 and 2.29 mg a.s./L					
96 hour NOEC		0.786 mg a.s./L (mean measured)					

Table B 9.2.2: Sub-lethal Effects of XDE-729 Methyl

Treatment (mg a.s./L)		% Affected							
Nominal	Mean Measured	Lying on Bottom				Surfacing			
		24- h	48-h	72-h	96-h	24-h	48-h	72-h	96-h
Negative control	<MQL	0	0	0	0	0	0	0	0
Vehicle Control	<MQL	0	0	0	0	0	0	0	0
0.25	0.215	0	0	0	0	0	0	0	0
0.50	0.428	0	0	0	0	0	0	0	0
1.0	0.786	0	0	0	0	0	0	0	0
2.0	1.50	10	56	33	67	0	0	0	11
4.0	3.10	---	---	---	---	---	---	---	---

In addition to determining concentrations of XDE-729 Methyl, concentrations of XDE-729 Acid were also determined. Presented in Table B 9.2.3 are the concentrations of the acid in solutions removed at 24 and 96 hours.

Table B.9.2.3: Concentration of XDE-729 Acid present in solutions removed at 24 and 96 hours

Nominal XDE-729 Methyl concentration (mg a.s./L)	Concentration of XDE-729 Acid at 24 hours	Concentration of XDE-729 Acid at 96 hours
0 (control)	<MQL	<MQL
0 (vehicle control)	<MQL	<MQL
0.25	0.0131	0.0177
0.50	0.0339	0.0399
1.0	0.0636	0.0684
2.0	0.102	0.0961
3.9	0.114	na

Conclusions

Based on mean measured concentrations, the estimated 24-hour LC₅₀ was 2.16 mg a.s./L (95% confidence limits could not be calculated, but best estimates were 1.50 and 3.10 mg a.s./L). The estimated 48-, 72-, and 96-hour LC₅₀ was 2.01 mg a.s./L with 95% confidence limits of 1.77 and 2.29 mg a.s./L. The slope of the 96-hour concentration response curve could not be calculated due to only one partial response to the test substance. The 96-hour NOEC was 0.786 mg a.s./L, based on mean measured concentrations and the lack of statistically significant mortality and sub-lethal effects at this and all lower test substance concentrations.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a mean measured 96 hour LC₅₀ of 2.01 mg a.s./L.

██████████ (2011): XDE-729 Methyl: Acute Toxicity to the Fathead Minnow, *Pimephales promelas*, Determined Under Static-Renewal Test Conditions. ██████████

██████████ Dow AgroSciences unpublished report, Study Number 090186. 16 May 2011.

Test material

Test item	XDE-729 Methyl
Purity	97.2 wt %
Description	Off-white solid
Lot No./Batch No	E2837-51
Test system	
Organism (Species)	Fathead Minnow, <i>Pimephales promelas</i>
Study Type	Acute
GLP Status	GLP (except the latest water characterization carried out in 2009)
Guidelines followed	OECD guideline 203
Guideline deviations reported by Study	None

Director	
Duration of study	96 hours
Test conditions	Static-renewal (daily)
Parameters measured	Survival
Observation intervals	0, 24, 48, 72, 96 hours
Age range of fish at test initiation	Juvenile
Weight range of fish at study initiation	0.1001 to 0.1721 g blotted wet weight
Range of fish length at study initiation	22 to 28 mm total length
Test concentrations	
Nominal XDE-729 Methyl concentrations	Nominal: 0 (control), 0 (vehicle control; 0.10 mL DMF/L), 0.25, 0.50, 1.0, 2.0, and 3.9 mg a.s./L
Mean XDE-729 Methyl measured concentrations	< MQL (control), < MQL (vehicle control), 0.225, 0.423, 0.913, 1.73, and 3.22 mg a.s./L
Analytical confirmation of test concentrations	0, 24, 72, and 96 hours
Reference substances	XDE-729 Methyl and XDE-729 Acid
No. of holding days before dosing	>12 days
Number of fish per dose group	10
Number of fish per control group	10
Feeding	None
Environmental conditions	
Temperature	21.4 to 21.9°C
Photoperiod	16 hour light:8 hour dark, 630 lux
Dissolved Oxygen concentration (new)	7.4 to 9.1 mg/L (88 to 108% sat.)
Dissolved Oxygen (old)	6.9 to 8.0 mg/L (82 to 95% sat.)
Bioloading	0.2288 g/L
pH	7.1 to 8.6
Water alkalinity	156 mg CaCO ₃ /L
Water hardness	148 mg CaCO ₃ /L
Water conductivity	336 µS

Methodology

A 96-hour static-renewal test was performed with test concentrations of 0 (control), 0 (vehicle control; 0.10 mL DMF/L), 0.25, 0.50, 1.0, 2.0, and 3.9 mg a.s./L. Test fish were impartially assigned to treatments by adding one fish per chamber proceeding from controls, low to high test substance treatments, and repeating steps as necessary until five fish were present in each replicate test chamber. The treatments were replicated two times for a total of ten fish per treatment. Observations for mortality and sub-lethal responses were made at 24, 48, 72, and 96 hours. Temperature, pH, and dissolved oxygen concentration were measured in each test chamber daily. In addition, a continuous record of the temperature from the water bath was also maintained. Alkalinity, hardness, and conductivity were measured in a sample of the dilution water at test initiation.

ResultsTable B 9.2.4: Effect of XDE-729 Methyl on mortality

Treatment (mg a.s./L)		No. of fish	Cumulative Mortality				
Nominal	Mean Measured		24- h	48-h	72-h	96-h	Total (%)
Negative control	<MQL	10	0	0	0	0	0
Vehicle Control	<MQL	10	0	0	0	0	0
0.25	0.225	10	0	0	0	0	0
0.50	0.423	10	0	0	0	0	0
1.0	0.913	10	0	0	0	0	0
2.0	1.73	10	0	0	0	0	0
3.9	3.22	10	3	3	3	3	30
96 hour LC ₅₀		>3.9 mg a.s./L (nominal); >3.22 mg a.s./L (mean measured)					
95% C.I.		Could not be calculated					
96 hour NOEC		2.0 mg a.s./L (nominal); 1.73 mg a.s./L (mean measured)					

Table B 9.2.5: Sub-lethal Effects of XDE-729 methyl

Treatment (mg a.s./L)		% Affected							
Nominal	Mean Measured	Lying on Bottom				Loss of Equilibrium			
		24-h	48-h	72-h	96-h	24-h	48-h	72-h	96-h
Negative control	<MQL	0	0	0	0	0	0	0	0
Vehicle Control	<MQL	0	0	0	0	0	0	0	0
0.25	0.225	0	0	0	0	0	0	0	0
0.50	0.423	0	0	0	0	0	0	0	0
1.0	0.913	0	0	0	0	0	0	0	0
2.0	1.73	10	20	10	0	0	0	0	0
3.9	3.22	86	100	86	43	14	0	14	57

In addition to determining concentrations of XDE-729 Methyl, concentrations of XDE-729 Acid were also determined. Presented in Table B 9.2.6 are the concentrations of the acid in solutions removed at 24 and 96 hours.

Table B 9.2.6: Concentration of XDE-729 Acid present in solutions removed at 24 and 96 hours

Nominal XDE-729 Methyl concentration (mg a.s./L)	Concentration of XDE-729 Acid at 24 hours	Concentration of XDE-729 Acid at 96 hours
0 (control)	<MQL	<MQL
0 (vehicle control)	<MQL	<MQL
0.25	0.0173	0.0242
0.50	0.0369	0.0583
1.0	0.0702	0.0938
2.0	0.116	0.155
3.9	0.210	0.296

Conclusions

Based on nominal concentrations, the estimated 24-, 48-, 72-, and 96-hour LC₅₀ was >3.9 mg a.s./L (>3.22 mg a.s./L mean measured), the highest concentration tested and maximum solubility of the test substance under the test conditions. The slope of the 96-hour concentration response curve could not be calculated due to only one partial response to the test substance. The 96-hour NOEC was 2.0 mg a.s./L, based on nominal concentrations (1.73 mg a.s./L mean measured) and the lack of statistically significant mortality and sub-lethal effects at this and all lower test substance concentrations.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a mean measured 96 hour LC₅₀ of >3.22 mg a.s./L.

(2011): XDE-729 Methyl: Acute Toxicity Test to the Sheepshead Minnow, *Cyprinodon variegatus*, Determined Under Flow-Through Conditions. [REDACTED]. Dow AgroSciences unpublished report, Study Number 090188. 25 May 2011.

Test material

Test item	XDE-729 Methyl
Purity	97.2 wt %
Description	Off-white solid
Lot No./Batch No	E2837-51
Test system	
Organism (Species)	Sheepshead Minnow (<i>Cyprinodon variegatus</i>)
Study Type	Acute
GLP Status	GLP (except the latest water characterization carried out in 2009)
Guidelines followed	OECD guideline 203
Guideline deviations reported by	Solvent concentration was 243 µL/L.

Study Director	This is not considered to have had any impact on the study since there were no observed sub-lethal effects or mortalities observed as a result of this deviation.
Duration of study	96 hours
Test conditions	Flow-through
Parameters measured	Survival
Observation intervals	0, 24, 48, 72, 96 hours
Age range of fish at test initiation	Juvenile
Weight range of fish at study initiation	0.0325 to 0.1336 g blotted weight (mean = 0.0833 ± 0.0380 g)
Range of fish length at study initiation	13 – 20 mm (mean 17 ± 2.5 mm)
Test concentrations	
Nominal XDE-729 Methyl concentrations	Nominal: 0 (control), 0 (DMF control; 243 µL DMF/L), 0.088, 0.18, 0.35, 0.70, and 1.4 mg a.s./L
Mean XDE-729 Methyl measured concentrations	< MQL (control), < MQL (DMF control), 0.0807, 0.170, 0.313, 0.676, 1.33 mg a.s./L
Analytical confirmation of test concentrations	Day -2, 0-, 48-, and 96-hours
Reference substances	XDE-729 Methyl and XDE-729 Acid
No. of holding days before dosing	
Number of fish per dose group	10
Number of fish per control group	10
Feeding	None
Environmental conditions	Temperature: 22.2 to 22.9°C
Photoperiod	16 hour light:8 hour dark
Dissolved Oxygen concentration	6.8 to 7.8 mg/L (91 to 105% sat)
Bioloading	0.0256 g/L
pH	8.1 to 9.1
Salinity	18.9 to 20.7‰
Light intensity	531 lux

Methodology

A definitive test was performed at nominal concentrations of 0 (control), 0 (DMF control), 0.088, 0.18, 0.35, 0.70, and 1.4 mg a.s./L. The definitive test was conducted for 96 hours commencing when fish were added to the test chambers. A 500-mL proportional diluter system and a Hamilton Model 420 syringe dispenser were used for the intermittent introduction of control and test substance treatment solutions to each test chamber during the definitive test. A total of 10 fish were distributed to each test chamber, with the exception of the 0.088 mg a.s./L treatment with contained 11 fish. Observations for mortality and sub-lethal responses (e.g., discolouration, loss of equilibrium, animals lying on the bottom of the test chamber, irregular respiration, etc.) were made once every 24 hours (± 1 hour) for the remainder of the test.

Table B 9.2.7: Effect of XDE-729 Methyl on Mortality

Treatment (mg a.s./L)		No. of Sheepshead Minnow	Cumulative percent mortality			
Nominal	Mean Measured		24-Hr	48-Hr	72-Hr	96-Hr
Negative control	<MQL	10	0	0	0	0
Vehicle Control	<MQL	10	0	0	0	0
0.088	0.0807	11	0	0	0	0
0.18	0.170	10	0	0	0	0
0.35	0.313	10	0	0	0	0
0.70	0.676	10	0	0	0	0
1.4	1.33	10	0	0	0	0
96 Hour LC50		>1.4 mg a.s./L (nominal) or >1.33 mg a.s./L (mean measured)				
95% C.I.		Could not be calculated				
96 Hour NOEC		1.4 mg a.s./L (nominal) or >1.33 mg a.s./L (mean measured)				

In addition to determining concentrations of XDE-729 Methyl, concentrations of XDE-729 Acid were also determined. Presented in Table B 9.2.8 are the concentrations of the acid in solutions removed at 48 and 96 hours.

Table B 9.2.8: Concentration of XDE-729 Acid present in solutions removed at 48 and 96 hours

Nominal XDE-729 Methyl concentration (mg a.s./L)	Concentration of XDE-729 Acid at 48 hours	Concentration of XDE-729 Acid at 96 hours
0 (control)	<MQL	<MQL
0 (vehicle control)	<MQL	<MQL
0.088	Not calculated	Not calculated
0.18	0.00552	Not calculated
0.35	Not calculated	Not calculated
0.70	0.0213	Not calculated
1.4	0.0412	Not calculated

Conclusions

The 24-, 48-, 72-, and 96-hour LC₅₀, was estimated to be >1.4 mg a.s./L (nominal concentration) or >1.33 mg a.s./L (mean measured concentration), the highest concentration tested and maximum solubility of the test item under the test conditions. The slope of the 96 hour concentration-response line could not be calculated due to the lack of one or more partial responses in the exposure. No sub-lethal effects were observed in the controls or test substance treatments throughout the exposure. The 96 hour NOEC was 1.4 mg a.s./L (nominal concentration) or 1.33 mg a.s./L (mean measured concentration) based on a lack of statistically significant mortality and sub-lethal effects at the maximum solubility of the test item under test conditions.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a mean measured 96 hour LC₅₀ of >1.33 mg a.s./L.

██████████. (2011): XDE-729 Acid: Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Static-Renewal Test Conditions. ██████████ Study Number 65970. Dow AgroSciences unpublished report, Study Number 101152. 17 June 2011.

Test material

Test item	XDE-729 Methyl
Purity	95.3 wt %
Description	Off-white solid
Lot No./Batch No	E2837-52
Test system	

Organism (Species)	Rainbow Trout (<i>Oncorhynchus mykiss</i>)
Study Type	Acute
GLP Status	GLP (except the latest water characterization carried out in 2011)
Guidelines followed	OECD guideline 203
Guideline deviations reported by Study Director	None
Duration of study	96 hours
Test conditions	Static-renewal (48 hours)
Parameters measured	Survival
Observation intervals	0, 24, 48, 72, 96 hours
Age range of fish at test initiation	Juvenile
Weight range of fish at study initiation	1.2013 to 2.0458 g blotted wet weight
Range of fish length at study initiation	52 to 60 mm total length
Test concentrations	
Nominal	Nominal: 0 (control) and 100 mg a.s./L
Mean measured	< MQL (control) and 107 mg a.s./L
Analytical confirmation of test concentrations	0, 24, 72, and 96 hours
Reference substances	XDE-729 Acid
No. of holding days before dosing	>12 days
Number of fish per dose group	7
Number of fish per control group	7
Feeding	None
Environmental conditions	
Temperature	15.0 to 15.8°C
Photoperiod	16 hour light:8 hour dark, 443 lux
Dissolved Oxygen concentration (new)	10.1 to 10.4 mg/L
Dissolved Oxygen (old)	5.6 to 9.5 mg/L
Bioloading	0.591 g/L
pH	7.4 to 8.4
Water alkalinity	162 mg CaCO ₃ /L
Water hardness	150 mg CaCO ₃ /L
Water conductivity	370 µS

Methodology

A 96-hour static-renewal test was performed with test concentrations of 0 (control) and 100 mg a.s./L. Test fish were impartially assigned to treatments by adding one fish per chamber proceeding from the control to the test substance treatment, and repeating steps as necessary until seven fish were present in each replicate test chamber. The treatments were not replicated. Observations for mortality and sub-lethal responses were made at 24, 48, 72, and 96 hours. Temperature, pH, and dissolved oxygen concentration were measured in each test chamber daily. In addition, a continuous record of the temperature from the water bath was also maintained. Alkalinity, hardness, and conductivity were measured in a sample of the dilution water at test initiation.

Results

Table B 9.2.9: Effect of XDE-729 Acid on mortality

Treatment (mg a.s./L)		No. of fish	Cumulative Mortality				
Nominal	Mean Measure d		24-h	48-h	72-h	96-h	Total (%)
Negative control	<MQL	7	0	0	0	0	0
100	107	7	0	0	0	0	0
96 hour LC ₅₀		>107 mg a.s./L (mean measured)					
95% C.I.		Not calculated					
96 hour NOEC		107 mg a.s./L (mean measured)					

Table B 9.2.10: Sub-lethal Effects of XDE-729 Acid

Treatment (mg a.s./L)		% Affected							
Nominal	Mean Measure d	Lying on Bottom				Surfacing			
		24-h	48-h	72-h	96-h	24-h	48-h	72-h	96-h
Negative control	<MQL	0	0	0	0	0	0	0	0
100	107	0	0	0	0	0	0	0	0

Conclusions

Based on mean measured concentrations, the estimated 24-, 48-, 72-, and 96-hour LC₅₀ was >107 mg a.s./L, the highest concentration tested. The slope of the 96-hour concentration response curve could not be calculated due to a lack of partial responses to the test substance. The 96-hour NOEC was 107 mg a.s./L, based on mean measured concentrations and the lack of statistically significant mortality and sub-lethal effects at this test substance concentration.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a mean measured 96 hour LC₅₀ of >107 mg/L.

X11449757

2011: X11449757: Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Static-Renewal Test Conditions.
66008. Dow AgroSciences unpublished report, Study Number 101166.
21 November 2011.

Test material

Test item:	X11449757
Purity:	Purity: 98.6%
Description:	Off-white solid
Lot No./Batch No. :	YB1-100780-103

Test system

Organism (Species):	Rainbow Trout (<i>Oncorhynchus mykiss</i>)
Study Type:	Acute
GLP Status:	GLP (with the exception of water characterisation performed February 2011)
Guidelines followed:	OECD guideline 203
Guideline deviations reported by Study Director:	None
Duration of study:	96 hours
Test conditions:	Static-renewal
Parameters measured:	Mortality, sub-lethal responses.
Observation intervals:	24, 48, 72 and 96 hours
Weight range of fish at study initiation:	0.6983 to 1.7330 g blotted wet weight
Range of fish length at study initiation:	45 to 58 mm total length (mean and standard deviation 51 ± 4.4 mm)
Test concentrations:	Nominal: 0 (control) and 120 mg X11449757/L Mean Measured: <MQL (control) and 124 mg X11449757/L
Analytical confirmation of test concentrations:	0 and 48 hours (new solutions); 48 and 96 hours (old solutions)
Reference substance:	X11449757
Number of fish per dose group:	14
Number of fish per control group:	14
Feeding:	None

Environmental conditions:	Temperature: 14.7 to 15.9°C Photoperiod: 16 hour light:8 hour dark (691 lux) Dissolved Oxygen (new): 8.8 to 9.9 mg/L (93 to 102% sat.) Dissolved Oxygen (old): 6.3 to 8.2 mg/L (65 to 86% sat.) Loading: 0.423 g/L pH: 7.6 to 9.7 Water alkalinity: 160 mg CaCO ₃ /L Water hardness: 150 mg CaCO ₃ /L Water conductivity: 357 µS
---------------------------	--

Methodology

A 96-hour static-renewal test was performed with nominal test concentrations of 0 (control) and 120 mg X11449757/L. Test fish were impartially assigned to treatment replicates by adding one fish per chamber until 7 fish were present in each test chamber. Fish were transferred to new solutions after 48 hours of exposure. There were two replicates per test treatment. Observations for mortality and sub-lethal responses were made at 24, 48, 72, and 96 hours. Temperature, pH, and dissolved oxygen concentration were measured in each test chamber daily. In addition, a continuous record of the temperature from the water bath was maintained. Alkalinity, hardness, and conductivity were measured in a sample of the dilution water at test initiation.

Results

Measured concentrations were within the acceptable $\pm 20\%$ of nominal range at each sampling point.

Over 96-hours no mortality occurred in either the control or treatment group. One fish exhibited curvature of the spine, observed at 96-hours only, with no other sub-lethal effects visible. All guideline-stipulated validity criteria were met (control mortality <10%, Dissolved oxygen levels maintained above 60%).

Table B 9.2.11: Effect of X11449757 on Mortality

Treatment (mg X11449757/L) Nominal	No. of fish	Cumulative mortality				
		24-h	48-h	72-h	96-h	Total (%)
Control	7	0	0	0	0	0
	7	0	0	0	0	0
120	7	0	0	0	0	0
	7	0	0	0	0	0
96 hour LC ₅₀	> 120 mg a.i./L					
95% C.I.	Not calculated					
96 hour NOEC	120 mg a.i./L					

Table B 9.2.12: Sub-Lethal Effects of X11449757

Treatment (mg X11449757/L) Nominal	Observation period (% affected)			
	24-h	48-h	72-h	96-h
Control	0	0	0	0
	0	0	0	0
120	0	0	0	14 ^c
	0	0	0	0

Key: C = one fish out of seven in this replicate had a slight curvature of the spine

Conclusion

After 96 hours of exposure, there was no mortality in the control or test substance treatment. The 24-, 48-, 72-, and 96-hour LC₅₀ values, based on nominal concentrations, were all estimated to be >120 mg X11449757/L, the highest concentration tested. The 96-hour NOEC was also estimated to be 120 mg X11449757/L, based upon nominal concentrations and a lack of mortality and treatment-related sub-lethal effects at the highest concentration tested.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a nominal 96 hour LC₅₀ of > 120 mg X11449757/L. Of note the maximum observed pH (9.7) of the test media is noticeably higher than the guideline-stated 'preferable' range of 6.5 to 8.5, although no impact to the control group organisms was observed.

(2012): X11406790 (XDE-729 Metabolite): Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Static-Renewal Test Conditions. [REDACTED] Number 68212. Dow AgroSciences unpublished report, Study Number 120020. 25 May 2012

Test material:	
Test item	X11406790
Purity	95%
Description	White solid
Lot No./Batch No	SYN-FS08644-062
Test system:	
Organism (Species)	Rainbow Trout (<i>Oncorhynchus mykiss</i>)
Study Type	Acute
GLP Status	GLP (except the latest water characterization carried out in February 2012)
Guidelines followed	OECD guideline 203
Guideline deviations reported by Study Director	None
Duration of study	96 hours
Test conditions	Static-renewal
Parameters measured	Survival
Observation intervals	Every 24 hours
Age range of fish at test initiation	Juvenile
Weight range of fish at study initiation	0.5851 to 0.9261 g blotted wet weight
Range of fish length at study initiation	42 to 48 mm total length
Test concentrations:	
Nominal XDE-729 Methyl concentrations	Nominal: 0 (control), 1.9, 3.8, 7.5, 15, and 30 mg X11406790/L
Mean XDE-729 Methyl measured concentrations	Mean Measured: <MQL (control), 1.8, 3.6, 7.1, 15, and 29 mg X11406790/L
Analytical confirmation of test concentrations	0 and 48 hours (fresh solutions); 48 and 96 hours (old solutions)
No. of holding days before dosing	14
Number of fish per dose group	10
Number of fish per control group	10
Feeding	None
Environmental conditions:	
Temperature	15.0 to 16.1°C
Photoperiod	16 hour light:8 hour dark (677 lux)
Dissolved Oxygen concentration (new)	7.6 to 9.5 mg/L (80 to 100% sat.)
Dissolved Oxygen (old)	4.8 to 8.5 mg/L (50 to 87% sat.)
Bioloading	0.433 g/L
pH	7.7 to 8.6

Water alkalinity	160 mg CaCO ₃ /L
Water hardness	158 mg CaCO ₃ /L
Water conductivity	349 µS

Methodology:

A 96-hour static-renewal test was performed with nominal test concentrations of 0 (control), 1.9, 3.8, 7.5, 15, and 30 mg X11406790/L. Test fish were impartially assigned to treatments by adding one fish per chamber proceeding from controls, low to high test substance treatments, and repeating steps as necessary until ten fish were present in each test chamber. The treatments were not replicated. Observations for mortality and sub-lethal responses were made at 24, 48, 72, and 96 hours. To maintain maximum exposure to the metabolite, both the control and treated solutions were freshly prepared and renewed every 48 hours. Temperature, pH, and dissolved oxygen concentration were measured in each test chamber daily. In addition, a continuous record of the temperature from the water bath was also maintained. Alkalinity, hardness, and conductivity were measured in a sample of the dilution water at test initiation.

Results

Table B 9.2.13: Effect of X11406790 on mortality

Treatment (mg X11406790/L)		No. of fish	Cumulative mortality				
Nominal	Mean Measured		24-h	48-h	72-h	96-h	Total (%)
Control	<MQL	10	0	0	0	0	0
1.9	1.8	10	0	0	0	0	0
3.8	3.6	10	0	0	0	0	0
7.5	7.1	10	0	0	0	0	0
15	15	10	0	0	0	0	0
30	29	10	0	0	0	0	0
96 hour LC ₅₀		>30 mg X11406790/L					
95% C.I.		Not calculated					
96 hour NOEC		30 mg X11406790/L					

Table B 9.2.14: Sub-Lethal Effects of X11406790

Treatment (mg X11406790/L)		No. of fish	Observation Period (Number affected)			
Nominal	Mean Measured		24-h	48-h	72-h	96-h
Control	<MQL	10	0	0	0	0
1.9	1.8	10	0	0	0	0
3.8	3.6	10	0	0	0	0
7.5	7.1	10	0	0	0	0
15	15	10	0	0	0	0
30	29	10	0	0	0	0

Conclusions

There was no mortality among control animals during the course of the study. Therefore, control animals satisfied test acceptability criteria for survival (i.e., $\geq 90\%$) as stated in the OECD 203 guideline. Based on nominal concentrations, the estimated 96-hour LC_{50} value was > 30 mg X11406790/L, the highest concentration tested. The 96-hour NOEC was 30 mg X11406790/L, due to a lack of mortality and observed sub lethal effects at this concentration and all lower test substance concentrations. The slope of the 96-hour concentration response curve could not be calculated due to a lack of partial responses to the test substance.

RMS comment:

The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a nominal 96 hour LC_{50} of >30 mg X11406790/L. The study has been carried out in accordance with OECD 203.

Formulation toxicity

2011: GF-2573: Acute Toxicity to the Rainbow Trout, *Oncorhynchus mykiss*, Determined Under Static-Renewal Test Conditions. 65998. Dow AgroSciences unpublished report, Study Number 101126. 30 June 2011.

Test material

Test item:	GF-2573
Purity:	Purity: 0.84 % wt/wt XDE-729 methyl, 0.83% wt/wt cloquintocet-mexyl
Description:	Yellow liquid
Lot No./Batch No. :	E2837-83

Test system

Organism (Species):	Rainbow Trout (<i>Oncorhynchus mykiss</i>)
Study Type:	Acute
GLP Status:	GLP
Guidelines followed:	OECD guideline 203
Guideline deviations reported by Study Director:	None
Duration of study:	96 hours
Test conditions:	Static-renewal
Parameters measured:	Dissolved oxygen, pH, temperature, conductivity, hardness, alkalinity, and light
Observation intervals:	24 hours (DO, pH, and temperature)
Age range of fish at test initiation:	Juvenile
Weight range of fish at study initiation:	0.6603 to 1.3675 g blotted wet weight
Range of fish length at study initiation:	45 to 51 mm total length
Test concentrations:	Nominal: 0 (control), 1.9, 4.3, 9.4, 21, 45, and 100 mg GF-2573/L Mean Calculated (based on analysis of XDE-729 Methyl): <MQL (control), 1.8, 4.1, 9.2, 21, 45, and 110 mg GF-2573/L
Analytical confirmation of test concentrations:	0 and 72 hours (new solutions); 24 and 96 hours (old solutions)
Reference substance:	XDE-729 Methyl
Number of fish per dose group:	7
Number of fish per control group:	7
Feeding:	None
Environmental conditions:	Temperature: 14.3 to 15.9°C Photoperiod: 16 hour light:8 hour dark (831 lux) Dissolved Oxygen (new): 10.2 to 10.8 mg/L (105 to 111% sat.) Dissolved Oxygen (old): 7.6 to 9.5 (78 to 100% sat.) Loading: 0.391 g/L pH: 7.7 to 8.4 Water alkalinity: 154 mg CaCO ₃ /L Water hardness: 142 mg CaCO ₃ /L Water conductivity: 322 µS

Methodology

A 96-hour static-renewal test was performed with test concentrations of 0 (control), 1.9, 4.3, 9.4, 21, 45, and 100 mg GF-2573/L. Test fish were impartially assigned to treatment replicates by adding one fish per chamber until 7 fish were present in each test chamber. There was one replicate per test treatment. Fish were transferred to new solutions daily. Observations for mortality and sub-lethal

responses were made at 24, 48, 72, and 96 hours. Temperature, pH, and dissolved oxygen concentration were measured in each old and new test chamber daily. In addition, a continuous record of the temperature from the water bath was maintained. Alkalinity, hardness, and conductivity were measured in a sample of the dilution water at test initiation.

Table B 9.2.15: Effect of GF-2573 on Mortality

Treatment (mg GF-2573/L)	No. of fish	Cumulative mortality				
Nominal		24-h	48-h	72-h	96-h	Total (%)
Control	7	0	0	0	0	0
1.9	7	0	0	0	0	0
4.3	7	0	0	0	0	0
9.4	7	0	0	0	0	0
21	7	0	0	0	0	0
45	7	0	0	0	0	0
100	7	4	5	5	5	71
96 hour LC50	78.7 mg GF-2573/L					
95% C.I.	60.2 and 103 mg GF-2573/L					
96 hour NOEC	45 mg GF-2573/L					

Table B 9.2.16: Effect of GF-2573 on Sub-Lethal Effects

Treatment (mg GF-2573/L)	Observation period (# affected)			
Nominal	24-h	48-h	72-h	96-h
Control	0	0	0	0
1.9	0	0	0	0
4.3	0	0	0	0
9.4	0	0	0	0
21	0	0	0	0
45	1 S	1 S	3 S	4 S
100	3 S	2 S	2 S	1 LE, 1 S

Key: LE = Loss of equilibrium; S= Surfacing

Conclusions

Control animals satisfied test acceptability criteria for survival (i.e., $\geq 90\%$) as stated in the study protocol and the OECD 203 testing guidelines. Based on nominal concentrations, the estimated 24-hour LC50 value was 90.50 mg GF-2573/L with 95% confidence limits of 57.3 and 143 mg GF-2573/L. The estimated 48-, 72-, and 96-hour LC50 values were 78.7 mg GF-2573/L with 95% confidence limits of 60.2 and 103 mg GF-2573/L. The slope of the 96-hour concentration-

response line could not be calculated due to the lack of more than one partial response. The 96-hour NOEC was 45 mg GF-2573/L based upon nominal concentrations and a lack of statistically significant ($p < 0.05$) mortality and observed sub-lethal effects at this and all lower test substance concentrations.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a nominal 96 hour LC₅₀ of 78.7 mg formⁿ/L.

B.9.2.1.1.2 Acute toxicity to aquatic invertebrates

Active substance

Rebstock, M. (2011): XDE-729 Methyl: Acute Toxicity to the Water Flea, *Daphnia magna*, Determined Under Static Test Conditions. ABC Laboratories, Columbia, Missouri. ABC study number 64603. Dow AgroSciences unpublished report, Study Number 090185. 12 May 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt %
Description:	Off-white solid
Lot No./Lot no. :	E2837-51

Test system

Organism (Species):	Water flea (<i>Daphnia magna</i>)
Study Type:	Acute
GLP Status:	GLP (except water characterization carried out in 2009)
Guidelines followed:	OECD guideline 202
Guideline deviations reported by Study Director:	None
Duration of study:	48 hours
Test conditions:	Static
Parameters measured:	Immobility
Observation intervals:	0, 24, 48 hours
Age range of water fleas at test initiation:	<24 hours
Test concentrations: Nominal	0 (control), 0 (vehicle control), 0.25, 0.50, 1.0, 2.0, and 3.9 mg a.s./L
Test concentrations Mean measured:	<MQL (control), <MQL (vehicle control), 0.209, 0.428, 0.821, 2.09, and 3.23 mg a.s./L
Analytical confirmation of test concentrations:	0 and 48 hours
Reference substance:	XDE-729 Methyl and XDE-729 Acid
Number of water fleas per dose group:	20

Number of water fleas per control group:	20
Feeding:	None
Environmental conditions:	Temperature: 20.5 to 21.0°C Photoperiod: 16 hour light:8 hour dark, 532 lux Dissolved Oxygen concentration: 8.6 to 10.3 mg/L pH: 8.3 to 8.4 Water alkalinity: 166 mg CaCO ₃ /L Water hardness: 160 mg CaCO ₃ /L Water conductivity: 339 µS

Methodology

A definitive test was performed at nominal concentrations of 0 (control), 0 (vehicle control), 0.25, 0.50, 1.0, 2.0, and 3.9 mg a.s./L. Five neonates (<24-hours old) were added to each of four test chambers per treatment at the start of the test. The daphnids were observed for immobility and sub-lethal effects at approximately 24 and 48 hours after test initiation. Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured in all treatment replicates at initiation and 48 hours. A thermistor probe was located in a surrogate test chamber to continuously record temperature.

Results

Table B 9.2.17: Effect of XDE-729 Methyl on Immobility

Treatment (mg a.s./L)		No. of water fleas	Cumulative percent immobile	
Nominal	Mean Calculated		24-h	48-h
Negative control	Negative control	20	0	0
Vehicle control	Vehicle control	20	0	0
0.25	0.209	20	0	0
0.50	0.428	20	0	0
1.0	0.821	20	0	0
2.0	2.09	20	0	30
3.9	3.23	20	65	100
EC50		2.12 mg a.s./L		
95% C.I.		1.84 and 2.43 mg a.s./L		
NOEC		0.821 mg a.s./L		

In addition to determining concentrations of XDE-729 Methyl, concentrations of XDE-729 Acid were also determined. Presented in Table B 9.2.18 are the concentrations of the acid in solutions removed at 0 and 48 hours.

Table B 9.2.18: Concentration of XDE-729 Acid present in solutions removed at 0 and 48 hours

Nominal XDE-729 Methyl concentration (mg a.s./L)	Concentration of XDE-729 Acid at 0 hours	Concentration of XDE-729 Acid at 48 hours
0 (control)	<MQL	<MQL
0 (vehicle control)	<MQL	<MQL
0.25	Not calculated	0.0333
0.50	Not calculated	0.0633
1.0	Not calculated	0.132
2.0	Not calculated	0.288
3.9	Not calculated	0.459

Conclusions

After 48 hours of exposure, immobility was 0, 0, 0, 0, 0, 30, and 100% in the 0 (control), 0 (vehicle control), 0.209, 0.428, 0.821, 2.09, and 3.23 mg a.s./L mean measured test treatments, respectively. The 24-hour EC₅₀ value, based on mean measured concentrations, was estimated to be 2.92 mg a.s./L, with 95% confidence limits of 2.62 and 3.26 mg a.s./L. The 48-hour EC₅₀ value, based on mean measured concentrations, was estimated to be 2.12 mg a.s./L, with 95% confidence limits of 1.84 and 2.43 mg a.s./L. There were no sub-lethal effects noted in the control or treatments during the definitive test. The 48-hour NOEC was 0.821 mg a.s./L, based on mean measured concentrations and the lack of statistically significant immobility or abnormal effects at this and all lower test substance treatments.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a mean measured 48 hour EC₅₀ of 2.12 mg a.s./L.

Bergfield, A. (2011): XDE-729 Methyl: Acute Toxicity Test with the Shrimp, *Americamysis bahia*, Determined Under Flow-Through Conditions. ABC Laboratories, Columbia, Missouri, ABC Study Number 64608. Dow AgroSciences unpublished report, Study Number 090184. 27 June 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2%
Description:	Off-white solid
Lot No./Batch No. :	TSN031117-0004, E2837-51

Test system

Organism (Species):	Mysid Shrimp (<i>Americamysis bahia</i>)
Study Type:	Acute
GLP Status:	GLP (except water characterization carried out in 2011)
Guidelines followed:	OPPTS 850.1035
Guideline deviations reported by Study Director:	None
Duration of study:	96 hours
Test conditions:	Flow-Through
Parameters measured:	Dissolved oxygen, pH, temperature, salinity, light
Observation intervals:	96 hours
Age range of test organisms at test initiation:	<24 hours
Test concentrations:	Nominal: 0 (control), 0 (vehicle control), 0.088, 0.18, 0.35, 0.70, and 1.4 mg XDE-729 Methyl/L
	Mean measured: < MQL (control), < MQL (vehicle control; 0.1 mL DMF/L), 0.0893, 0.169, 0.341, 0.671, and 1.30 mg XDE-729/L
Analytical confirmation of test concentrations:	Prior to initiation (day -N) and 0, 48, and 96 hours
Reference substances:	XDE-729 Methyl and XDE-729 Acid
No. of holding days before dosing:	Not Applicable; <24 hour old mysids were used
Number of mysids per dose group:	20
Number of mysids per control group:	20
Feeding:	Mysids were fed <i>ad libitum</i> brine shrimp nauplii (<i>Artemia</i> sp.; 24-48 hours old) at least once daily.
Environmental conditions:	Temperature: 24.0 to 24.8°C Photoperiod: 16 hour light:8 hour dark, 488 to 529 lux Dissolved Oxygen: 6.7 to 7.6 mg/L (92 to 104% saturation) pH: 8.0 to 8.1 Salinity: 19.8 to 20.8‰

Methodology

A 96-hour flow-through test was performed with test concentrations of 0 (control), 0 (vehicle control), 0.088, 0.18, 0.35, 0.70, and 1.4 mg XDE-729 Methyl/L. The maximum test concentration of 1.4 mg a.s./L was selected as this represented the functional solubility of XDE-729 Methyl under the test conditions. Ten mysids were impartially added to a set of labelled containers with each container representing one treatment replicate. Each container was randomly assigned a

treatment level and replicate by a random number generation program. The individuals within each container were then released into the corresponding test chamber. The treatments were replicated two times for a total of 20 mysids per treatment. Observations for mortality and sub-lethal responses were made at 24, 48, 72, and 96 hours. Temperature, pH, dissolved oxygen, and salinity were measured in each test chamber daily. In addition, a continuous record of the temperature from the water bath was also maintained.

Results

Table B 9.2.19: Effect of XDE-729 Methyl on mortality

Treatment (mg XDE-729 Methyl/L)		No. of mysids	Cumulative Mortality				
Nominal	Mean Measured		24-h	48-h	72-h	96-h	Total (%)
Negative control	<MQL	20	0	0	0	0	0
Vehicle Control	<MQL	20	0	0	0	0	0
0.088	0.0893	20	0	1	1	1	5
0.18	0.169	20	0	0	0	0	0
0.35	0.341	20	0	0	0	0	0
0.70	0.671	20	0	0	0	0	0
1.4	1.30	20	0	8	8	8	40
96 hour LC50		>1.4 mg XDE-729 Methyl/L (nominal) or >1.30 mg XDE-729 Methyl/L (mean measured)					
95% C.I.		Could not be calculated					
96 hour NOEC		0.70 mg XDE-729 Methyl/L (nominal) or 0.671 mg XDE-729 Methyl/L (mean measured)					

Table B 9.2.20: Sub-lethal Effects of XDE-729 Methyl

Treatment (mg XDE-729 Methyl/L)		% Affected			
Nominal	Mean Measured	24-h	48-h	72-h	96-h
Negative control	<MQL	0	0	0	0
Vehicle Control	<MQL	0	0	0	0
0.088	0.0893	0	0	0	0
0.18	0.169	0	0	0	0
0.35	0.341	0	0	0	0
0.70	0.671	0	0	0	0
1.4	1.30	0	0	0	0

In addition to determining concentrations of XDE-729 Methyl, concentrations of XDE-729 Acid were also determined. Presented in Table B 9.2.21 are the concentrations of the acid in solutions removed at 0, 48 and 96 hours.

Table B 9.2.21: Concentration of XDE-729 Acid present in solutions removed at 0, 48 and 96 hours

Nominal XDE-729 Methyl concentration (mg a.s./L)	Concentration of XDE-729 Acid at 0 hours	Concentration of XDE-729 Acid at 48 hours	Concentration of XDE-729 Acid at 96 hours
0 (control)	<MQL	<MQL	<MQL
0 (vehicle control)	<MQL	<MQL	<MQL
0.088	0.00136	<MQL	<MQL
0.18	<MQL	<MQL	<MQL
0.35	<MQL	<MQL	<MQL
0.70	<MQL	<MQL	<MQL
1.4	0.0354	0.0230	<MQL

Conclusions

The 24-, 48-, 72-, and 96-hour LC50 was estimated to be >1.4 mg XDE-729 Methyl/L (based nominal concentrations) or >1.30 mg XDE-729 Methyl/L (based on mean measured concentrations), which was the highest concentration tested and the maximum functional solubility of the test item under these test conditions. There were no sub-lethal effects noted in the control or test substance treatments during the definitive test. The 96-hour NOEC was 0.70 mg XDE-729 Methyl/L (nominal) or 0.671 mg XDE-729 Methyl/L (mean measured), based on the lack of statistically significant mortality at this and lower test substance treatments.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a mean measured 96 hour LC₅₀ of >1.30 mg a.s./L.

Hicks S.L. (2011): XDE-729 Methyl: Effect on New Shell Growth of the Eastern Oyster (*Crassostrea virginica*). ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia MO 65202, ABC Study Number 64609. Dow AgroSciences unpublished report, Study Number 090120. 24 June 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt %
Description:	Off-white solid
Lot No./Batch No. :	E2837-51

Test system

Organism (Species):	Eastern Oyster (<i>Crassostrea virginica</i>)
Study Type:	Shell deposition.
GLP Status:	GLP (except water characterization carried out in 2009)
Guideline followed:	OPPTS 850.1025 FIFRA 72-3
Guideline deviations reported by Study Director:	None
Duration of study:	96-hr
Test conditions:	Flow-through
Parameters measured:	Mortality and new shell growth.
Observation intervals:	Once daily.
Age of test organisms at test initiation:	Pre-spawn condition of gonadal development.
Test concentrations (Nominal):	0 (control), 0 (vehicle control; 0.10 mL DMF/L), 0.18, 0.30, 0.50, 0.84, and 1.4 mg XDE-729 Methyl/L
Test concentrations (Mean measured):	<MQL (control), <MQL (vehicle control), 0.169, 0.260, 0.443, 0.717, 1.21 mg XDE-729 Methyl/L
Analytical confirmation of test concentrations:	On days -2 (prior to test initiation), 0, and 4.
Reference substances:	XDE-729 Methyl and XDE-729 Acid
No. of holding days before dosing:	Three
No. of oysters per dose group:	20
No. of oysters per control group:	20

Environmental conditions:	Temperature: 19.4 to 20.8°C Light intensity: 196 to 436 lux Dissolved Oxygen concentration: 6.3 to 7.9 mg/L (80 to 101% saturation) pH: 8.1 to 8.7 Salinity: 19.1 to 20.0‰
---------------------------	--

Methodology

During the definitive test, a 2000-mL proportional diluter system similar to that described by Mount and Brungs, with a Hamilton Model 420 syringe dispenser, was used for the intermittent introduction of control, vehicle control, and XDE-729 Methyl test solutions into each test chamber. The test chambers were arranged in a temperature-controlled water bath using a computer-generated random number table to assign specific treatment location. The diluter system delivered approximately 1000 mL of each solution to the appropriate test chambers with each cycle during the definitive test. A total of 140 actively growing oysters were impartially selected from the oyster culture and the shell margins were cleared of new shell growth at test initiation. Oysters were placed with the cupped valve down and the open end of the valves oriented into the flow of the recirculating water. A marine microalgal concentrate (Instant Algae Shellfish Diet 1800, Reed Mariculture, Inc.) was added manually (i.e., 3 mL added three times each day during exposure with exceptions of test initiation and termination, when 3 mL was added only once) to each test chamber during the exposure. Observations for mortality and other signs of test substance effect (e.g., slow valve closure and lack of feeding activity as evident from lack of faecal deposits) were made daily. New shell growth at test termination was measured to the nearest 0.1 mm with a vernier caliper [Manostat (15-100-109) Mecanic Type 6911]. Test solution salinity, temperature, pH, and dissolved oxygen concentration were measured daily in each test chamber.

Results

Table B 9.2.22: Effect of XDE-729 Methyl on mortality

Treatment (mg XDE-729 Methyl/L)		Days 0-4	
Nominal	Mean Measured	No. Dead	% Mortality
Negative Control	<MQL	0	0
Vehicle Control	<MQL	0	0
0.18	0.169	0	0
0.30	0.260	0	0
0.50	0.433	0	0
0.84	0.717	0	0
1.4	1.21	0	0

Table B 9.2.23: Effect of XDE-729 Methyl on new shell growth

Treatment (mg XDE-729 Methyl/L)		Observation Period: 96-hr	
Nominal	Mean Measured	Mean Length ± SD (mm)	% Change from Control
Negative Control	<MQL	3.6 ± 0.54 (range: 1.1 to 7.0)	NA
Vehicle Control	<MQL	4.3 ± 0.15 (range: 1.4 to 6.8)	NA
0.18	0.169	4.6 ± 0.85 (range: 2.4 to 8.1)	+8
0.30	0.260	4.0 ± 0.33 (range: 1.1 to 7.0)	+11
0.50	0.433	2.8 ± 0.68 (range: 0.8 to 5.6)	-22
0.84	0.717	2.7 ± 0.099 (range: 0 to 4.9)	-25
1.4	1.21	2.3 ± 0.57 (range: 0.5 to 5.7)	-36
NOEC		1.21 mg a.s./L (mean measured)	
EC50		>1.21 mg a.s./L (mean measured)	
There was no significant difference in shell growth from the control, Dunnett's test p<0.05. The new shell growth data for the negative control and vehicle control oysters were analyzed using a two-tailed planned comparison t-test and these analyses indicated there was no statistical difference between the control and vehicle control; therefore, the control was used for all further evaluations.			

In addition to determining concentrations of XDE-729 Methyl, concentrations of XDE-729 Acid were also determined. Presented in Table B 9.2.24 are the concentrations of the acid in solutions removed at 24 and 96 hours.

Table B 9.2.24: Concentration of XDE-729 Acid present in solutions removed at Day 0 and day 4

Nominal XDE-729 Methyl concentration (mg a.s./L)	Concentration of XDE-729 Acid at day 0	Concentration of XDE-729 Acid at day 4
0 (control)	<MQL	<MQL
0 (vehicle control)	<MQL	<MQL
0.18	0.00442	Not calculated
0.30	0.00560	Not calculated
0.50	Not calculated	Not calculated
0.84	Not calculated	Not calculated
1.4	0.0288	Not calculated

Conclusions

Based on mean measured concentrations of XDE-729 Methyl and new shell growth, the 96-hour EC₅₀ was >1.21 mg XDE-729 Methyl/L, the highest concentration tested and maximum solubility of the test substance under the test conditions. The 96-hour NOEC was the mean measured concentration of 1.21 mg XDE-729 Methyl/L, the highest treatment tested.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a mean measured 96 hour EC₅₀ of >1.21 mg a.s./L.

Gerke, A. 2011: XDE-729 Methyl: Whole Sediment Acute Toxicity to a Marine Amphipod (*Leptocheirus Plumulosus*), ABC Laboratories, Columbia, Missouri, ABC 66366. Dow AgroSciences unpublished report, Study Number 101132, 19 October 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 %
Description:	Off-white solid
Lot No./Batch No. :	E2837-51

Test system

Organism (<i>Species</i>):	Marine Amphipod (<i>Leptocheirus plumulosus</i>)
Study Type:	10 day acute study
GLP Status:	GLP (with the exception of the latest water characterization in August 2009)
Guideline followed:	OPPTS 850.1740
Guideline deviations reported by Study Director:	Sediment was collected eight weeks and six days prior to definitive test initiation. A total of 25 amphipods were added to replicate C of the highest treatment level. Pore water salinity was not measured at initiation of the definitive test.
Duration of study:	10 day
Test conditions:	Static
Parameters measured:	Survival
Observation intervals:	Termination
Age of test organisms at test initiation:	Juveniles (2-4 mm in length)
Test concentrations:	Nominal: 0 (Control), 0 (vehicle control), 6.3, 13, 24, 49, and 98 mg a.s./kg dry sediment Geometric Mean Measured: <MQL (Control), <MQL (Acetone Control), 4.03, 7.09, 14.5, 31.4, and 58.1 mg TRR/kg dry sediment (55-64% of nominal)
Analytical confirmation of test concentrations:	Confirmation of XDE-729 methyl and XDE-729 acid at initiation and termination using LC-MS and HPLC.
No. of holding days before dosing:	2 days
No. of amphipods per dose group:	160 (20 individuals in 8 reps)
No. of amphipods per control group:	160
Environmental conditions:	Temperature: 24.3 to 25.0°C Dissolved Oxygen: 5.8 to 7.7 mg/L (82 to 108% saturation) pH: 7.9 to 8.7 Salinity: 20.4 to 24.7 ‰ Un-ionized Ammonia: 0.112 to 0.208 mg/L Light Intensity: 649 lux

Methodology

A 10 day static test was performed with nominal test concentrations 0 (Control), 0 (Acetone Control), 6.3, 13, 24, 49, and 98 mg a.s./kg dry sediment. The dosing stock solution was prepared by combining a non-radio-labeled test solution with a radio-labeled test solution. From this stock solution, further dilutions were made in acetone. These dilutions were used to dose carrier sand, once the solvent had evaporated the sand was incorporated into the sediment by thoroughly mixing by hand.

Twenty amphipods were impartially added to a set of labeled containers with each container representing one treatment replicate. Each container was then randomly assigned to a treatment replicate by random number generator. The individuals within each container were then released from the container into the corresponding 1L glass test chamber. Approximately 217g of dosed sediment was added to each test chamber. A 700-mL volume of saltwater was carefully added to the test chambers using a deflector to minimize the disturbance to the sediments. There were eight replicates per treatment level, control and vehicle control, resulting in 160 amphipods per test treatment. 5 additional test chambers were also prepared for further analysis of the overlying water, pore water, sediment samples and pore water ammonia analysis. At test termination, the entire contents of each test chamber were removed and the live and dead amphipods were enumerated. They were carefully separated from the sediment by passing through a stainless steel sieve. Observations of general health and behaviour of the organisms were also noted. Any organisms not accounted for at test termination were considered dead.

Temperature, pH, and dissolved oxygen concentration, and salinity were measured daily in one replicate per treatment. Test chambers were placed in a temperature controlled water bath. A 24 hour light photoperiod was maintained. Aeration was provided to each test chamber through a glass pipette inserted such that its tip was 2-3 cm from the sediment surface. Aeration was provided at an initial rate of 60-100 bubbles per minute to each test chamber, this was discontinued during the addition of the animals and resumed within 50 minutes after addition was completed. Observations for sediment activity, aeration, and water level were made daily for the duration of the test. On days 0 and 10, 20 ml samples were removed from 1 to 2 cm above the sediment surface and composited by treatment for measurement of ammonia concentrations.

Results

Table B 9.2.25: Results from analysis of overlying water samples

Nominal Sediment Concentration (mg a.s./kg dry sediment)	Mean measured ¹⁴ C-labeled XDE-729 Methyl as mg TRR/L				Mean measured ¹⁴ C-labeled XDE-729 Methyl as mg TRR/kg dry sediment		
	Overlying water samples		Pore water samples		Sediment samples		
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10	Geometric Mean (Days 0-10)
0 (Control)	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
0 (Acetone Control)	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
6.3	0.0310	0.291	0.532	0.613	7.10	2.30	4.03
13	0.0415	0.628	0.855	1.21	11.5	4.44	7.09
24	0.0962	1.09	1.51	2.57	21.4	9.85	14.5
49	0.167	2.00	2.24	5.15	48.5	20.3	31.4
98	0.282	3.36	2.73	9.48	73.1	47.2	58.1
MQL(overlying water):	Day 0: 0.00239 mg TRR/L; Day 10: 0.00264 mg TRR/L						
MQL (pore water):	Day 0: 0.00372 mg TRR/L; Day 10: 0.00310 mg TRR/L						
MQL (sediment):	Day 0: 0.00365 mg TRR/kg; Day 10: 0.00369 mg TRR/kg						

*TRR- total radioactive residue

MQL- minimum quantifiable limit

Measured overlying water concentrations in two replicates of the 98 mg a.s./kg treatment level at initiation were 0.208 and 0.199 mg XDE-729 Methyl/L and 0.0958 and 0.0948 mg XDE-729 Acid/L. At termination the concentrations were 0.129 and 0.137 mg XDE-729 Methyl/L and 2.26 and 2.38 mg XDE-729 Acid/L. No residues of XDE-729 Methyl or XDE-729 Acid were detected in the control samples above the minimum quantifiable limit (MQL) values for the analytes.

Measured pore water concentrations in two replicates of the 98 mg a.s./kg treatment level at initiation were 0.696 and 0.666 mg XDE-729 Methyl/L and 2.28 and 2.24 mg XDE-729 Acid/L. At termination the concentrations were 0.101 and 0.102 mg XDE-729 Methyl/L and 5.78 and 4.86 mg XDE-729 Acid/L. No residues of XDE-729 Methyl or XDE-729 Acid were detected in the control samples above the MQL values for the analytes.

Measured sediment concentrations in two replicates of the 98 mg a.s./kg treatment level at initiation were 69.2 and 48.1 mg XDE-729 Methyl/kg and 7.76 and 6.24 mg XDE-729 Acid/kg. Measured sediment concentrations at termination were 10.6 and 16.6 mg XDE-729 Methyl/kg and 12.4 and 15.4 mg XDE-729 Acid/kg. No residues of XDE-729 Methyl or Acid were detected in the control samples above the MQL value for analytes.

Table B 9.2.26: Effect of XDE-729 Methyl on survival

Geometric Mean Measured Sediment Concentration (mg TRR/kg dry sediment)	Day 10 Survival	
	Number Surviving (total in each treatment group was 160)	Percent Survival (%)
0 (Control)	153	96
0 (Acetone Control)	152	95
4.03	149	93
7.09	154	96
14.5	151	94
31.4	153	96
58.1	157	95
NOEC	58.1 mg TRR/kg dry sediment	
LC50	>58.1 mg TRR/kg dry sediment	

There were no sub-lethal effects observed in any treatment level throughout the duration of the study.

Conclusions

The negative and vehicle control animals met the acceptability criteria for mean survival (i.e., >90%) as specified by the study protocol and the OPPTS 850.1740 testing guideline. Based on geometric mean measured sediment concentrations, the 10-day LC₅₀ was estimated to be >58.1 mg a.s./kg dry sediment, the highest concentration tested. The 10-day NOEC and LOEC values for survival were 58.1 and >58.1 mg a.s./kg dry sediment, respectively, based on geometric mean concentrations.

RMS comment: Further detail should have been recorded regarding the collection of the natural sediment i.e. the time, water depth, core depth, method of collection. The sediment in the study was used past the holding time stated in the guidelines and the pore water salinity of the sediment was not measured at initiation. Despite these minor deviations, water quality parameters and validity criteria were met so this does not affect the reliability of the study. The negative and vehicle control animals met the acceptability criteria for mean survival (i.e., >90%) as specified by the study protocol and the OPPTS 850.1740 testing guideline.

The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a mean measured, 10-day LC₅₀ of >58.1 mg a.s./kg dry sediment.

Dinehart, S. (2012). XDE-729 Methyl: Acute Toxicity to the Tadpole, *Xenopus laevis*, Determined Under Flow-Through Test Conditions. ABC Laboratories, Columbia, Missouri, ABC Study Number 64610. Dow AgroSciences unpublished report, Study Number 090121. 24 January 2012.

Test material:	
Test item	XDE-729 Methyl
Purity	97.2 wt%
Description	Off-white solid
Lot No./Batch No	TSN031117-0004, E2837-51
Test system:	
Organism (Species)	African clawed frog tadpoles (<i>Xenopus laevis</i>)
Study Type	Acute
GLP Status	GLP (except the latest water characterization carried out in February 2011)
Guidelines followed	OECD guideline 203 and ASTM 729-96
Guideline deviations reported by Study Director	Food was not withheld from tadpoles for 24 hours prior to test.
Duration of study	96 hours
Test conditions	Flow-through
Parameters measured	Survival and sub lethal effects
Observation intervals	Every 24 hours
Age range of tadpoles at test initiation	8 days post hatch
Weight range of tadpoles at study initiation	0.0182 to 0.0241 g (mean and standard deviation: 0.0207 ± 0.0021 g).
Range of fish length at study initiation	13 to 16 mm (mean and standard deviation: 14 ± 1.1 mm)
Test concentrations:	
Nominal XDE-729 Methyl concentrations	0 (control), 0 (vehicle control), 0.13, 0.25, 0.50, 1.0, and 2.0 mg XDE-729 Methyl/L
Mean XDE-729 Methyl measured concentrations	0 (control), 0 (vehicle control), 0.125, 0.239, 0.468, 0.924, and 1.75 mg XDE-729 Methyl/L
Analytical confirmation of test concentrations	Prior to initiation (day -N), 0 and 48 hours and 96 hours
Number of tadpoles per dose group	20
Number of tadpoles per control group	20
Feeding	None
Environmental conditions:	
Temperature	18.9 to 20.6°C
Photoperiod	16 hour light:8 hour dark (610 lux)
Dissolved Oxygen concentration	6.8 to 8.6 mg/L (78 to 99% saturation)
pH	7.8 to 8.5
Water alkalinity	160 mg CaCO ₃ /L
Water hardness	150 mg CaCO ₃ /L
Water conductivity	368 µS

Methodology

A 96-hour flow-through test was performed with nominal test concentrations of 0 (control), 0 (vehicle control), 0.13, 0.25, 0.50, 1.0, and 2.0 XDE-729 Methyl/L. A 2-L proportional diluter system, was used for the preparation of test solutions and intermittent introduction of the solutions to the test chambers during the definitive test. Each test chamber received approximately 1L of test solutions per cycle. The diluter cycle frequency was maintained at a cycle rate sufficient to provide approximately 6 volume turnovers per day. The test substance was diluted in dimethylformamide (DMF) and so a vehicle control was included in the definitive test.

A group of approximately 150 tadpoles were removed from the culture population and a representative sample of 40 individuals were examined under a microscope and determined to be NF (Nieuwkoop–Faber) stages 47-50. Tadpoles were impartially assigned to treatments by adding one tadpole per chamber proceeding from controls, low to high test substance treatments, and repeating steps as necessary until ten tadpoles were present in each test chamber. The treatments were replicated two times for a total of 20 tadpoles per treatment. Test chambers were immersed in a circulating water bath that maintained the temperature of the test chamber solutions at $20 \pm 2^{\circ}$ C. A computer-generated random number table was used to assign specific treatment location within the water bath. Observations for mortality and sub lethal responses were made at 24, 48, 72, and 96 hours. Tadpoles were not fed during the exposure period.

Total hardness, total alkalinity and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured daily in all replicates. A thermistor probe was positioned in a centrally located test chamber to continuously record temperature. Light intensity was determined at test initiation.

Results

Mean measured XDE-729 Methyl concentrations in the test substance treatment solutions during the 96-hour exposure were 0.125, 0.239, 0.468, 0.924, and 1.75 mg a.s./L (88 to 96% of the nominal concentrations). As the measured concentrations of XDE-729 Methyl were maintained within $\pm 20\%$ of the nominal concentrations, the biological response results were reported based upon the nominal concentrations.

Table B 9 2.27: Effect of XDE-729 Methyl on Mortality

Treatment (mg XDE-729 Methyl/L)		No. of tadpoles	Cumulative Mortality				
Nominal	Mean Measured		24-h	48-h	72-h	96-h	Total (%)
Negative control	<MQL	20	0	0	0	0	0
Vehicle Control	<MQL	20	0	0	0	0	0
0.13	0.125	20	0	0	0	0	0
0.25	0.239	20	0	0	0	0	0
0.50	0.468	20	0	0	0	0	0
1.0	0.924	20	0	0	0	0	0
2.0	1.75	20	2	2	7	9	45
96 hour LC ₅₀		>2.0 mg a.s./L (nominal)					
95% C.I.		Could not be calculated					
96 hour NOEC		1.0 mg a.s./L (nominal)					

MQL- minimum quantifiable limit

Table B 9.2.28: Sub-lethal Effects of XDE-729 Methyl

Treatment (mg XDE-729 Methyl/L)		No. of fish	Observation Period (Number affected)			
Nominal	Mean Measured		24-h	48-h	72-h	96-h
Control	<MQL	20	0	0	0	0
Vehicle Control	<MQL	20	0	0	0	0
0.13	0.125	20	0	0	0	0
0.25	0.239	20	0	0	0	0
0.50	0.468	20	0	0	0	0
1.0	0.924	20	3B	5B	0	0
2.0	1.75	20	10B, 3LE	13B, 4E, 1S	11B/DC/L E, 1DC/LE, 1DC, 2NF	11B/DC/L E, 2NF

Key to Abbreviations:

B = Organism on bottom of the test chamber; DC = Discoloration; E = Erratic swimming pattern; NF = Organism not found, counted as exposure related mortality; LE = Loss of equilibrium; S = Surfacing

Tadpoles were observed on the bottom of the test chambers in the 1.0 mg XDE-729 Methyl/L test substance treatment at 24 and 48 hours. Sub lethal effects including tadpoles on the bottom of the chamber, loss of equilibrium, discoloration, erratic swimming, and surfacing were observed in the 2.0 mg XDE-729/L test treatment throughout the test. Some effects were seen at 1.0 mg XDE-729/L for up to 48 hours however, by 96 hours these effects were not observed.

Conclusions

There was no mortality among control animals during the course of the study. Therefore, control animals satisfied test acceptability criteria for survival (i.e., $\geq 90\%$) as stated in the OECD 203 guidelines. Dissolved oxygen concentration was more than 60% of the air saturation value throughout the test.

Based on nominal concentrations, the 24-, 48-, 72-, and 96-hour LC_{50} was estimated to be >2.0 mg XDE-729 Methyl/L, the highest concentration tested. The slope of the 96-hour concentration-response line could not be calculated due to the lack of more than one partial response. The 96-hour NOEC was 1.0 mg XDE-729 Methyl/L, based on nominal concentrations and no mortality or sub lethal effects at this and all lower test substance concentrations.

RMS comment: It is worth noting that food was not withheld from the tadpoles for 24 hours prior to the test however, environmental measurements taken throughout the definitive test do not suggest that this had a negative impact on the water quality. Test substances adhesion to organic matter can take place and so not feeding prior to or during the test minimises this likelihood. It is worth considering that this guideline is not proposed for use with tadpoles, furthermore the $\log P_{ow}$ of XDE-729 Methyl is 3.76 and all its metabolites are ≤ 3 so any adhesion to organic matter is likely to be low. The study has been carried out in accordance with OECD 203. The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a 96-hour LC_{50} of >2.0 mg a.s/L based on nominal concentrations.

XDE-729 Acid

Bergfield, A. (2011): XDE-729 Acid: Acute Toxicity to the Water Flea, *Daphnia magna*, Determined Under Static-Renewal Test Conditions. ABC Laboratories, Columbia, Missouri. ABC study number 65969. Dow AgroSciences unpublished report, Study Number 101149. 27 June 2011.

Test material

Test item:	XDE-729 Acid
Purity:	95.3 wt/%
Description:	Off-white solid
Lot No./Lot no. :	E2837-52

Test system

Organism (Species):	Water flea (<i>Daphnia magna</i>)
Study Type:	Acute
GLP Status:	GLP (except water characterization carried out in 2010)
Guidelines followed:	OECD guideline 202
Guideline deviations reported by Study Director:	None
Duration of study:	48 hours
Test conditions:	Static-Renewal
Parameters measured:	Immobility
Observation intervals:	24 and 48 hours
Age range of water fleas at test initiation:	<24 hours
Test concentrations (Nominal):	0 (control), 0 (control) and 100 mg a.s./L
Test concentrations (Mean measured)	< MQL (control) and 106 mg a.s./L MQL- minimum quantifiable limit
Analytical confirmation of test concentrations:	0, 24, and 48 hours
Reference substance:	XDE-729 Acid
Number of water fleas per dose group:	20
Number of water fleas per control group:	20
Feeding:	None
Environmental conditions:	Temperature: 19.1 to 20.3°C Photoperiod: 16 hour light:8 hour dark, 414 lux Dissolved Oxygen concentration: 8.2 to 8.7 mg/L (84 to 100% sat.) (old); 8.6 to 10.0 mg/L (99 to 115% sat.) (new) pH: 7.6 to 8.6 Water alkalinity: 164 mg CaCO ₃ /L Water hardness: 156 mg CaCO ₃ /L Water conductivity: 345 µS

Methodology

A definitive test was performed at nominal concentrations of 0 (control) and 100 mg/L. Five neonates (<24-hours old) were added to each of four test chambers per treatment at the start of the test. The daphnids were observed for immobility and sub-lethal effects at approximately 24 and 48 hours after test initiation. Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured in all treatment replicates at initiation, 24, and 48 hours. A thermistor probe was located in a surrogate test chamber to continuously record temperature.

Results

Table B 9.2.29: Effect of XDE-729 Acid on Immobility

Treatment (mg a.s./L)		No. of water fleas	Cumulative percent immobile	
Nominal	Mean Measured		24-h	48-h
Negative control	Negative control	20	0	0
100	106	20	0	0
EC50		>106 mg a.s./L		
95% C.I.		Could not be calculated		
NOEC		106 mg a.s./L		

Table B 9.2.30: Effect of XDE-729 on sub-lethal Effects

Treatment (mg a.s./L)		No. of water fleas	Cumulative percent sub-lethal effects	
Nominal	Mean Measured		24-h	48-h
Negative control	Negative control	20	0	0
100	106	20	0	0

Conclusions

The test acceptability criteria were met for this study. Immobilization in the control treatments were 0%, below the acceptability limit of 10%. The dissolved oxygen concentration at the end of the test was ≥ 8.2 mg/L in control and test substance treatments, higher than the acceptability minimum of 3 mg/L. This study is classified as acceptable and satisfies the guideline requirement for an acute toxicity test with *Daphnia magna*.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a mean measured 48 hour EC50 of >106 mg a.s./L.

Metabolite X11449757

Bergfield, A. (2011): X11449757: Acute Toxicity to the Water Flea, *Daphnia magna*, Determined Under Static-Renewal Test Conditions. ABC Laboratories, Columbia, Missouri, ABC study number 66007. Dow AgroSciences unpublished report, Study Number 101163. 27 June 2011.

Test material

Test item:	X11449757
Purity:	98.6%
Description:	Off-white solid
Lot No./Lot no. :	YB1-100780-103

Test System

Organism (Species):	Water flea (<i>Daphnia magna</i>)
Study Type:	Acute
GLP Status:	GLP (except water characterization carried out in 2014)
Guidelines followed:	OECD guideline 202
Guideline deviations reported by Study Director:	None
Duration of study:	48 hours
Test conditions:	Static-Renewal
Parameters measured:	Immobility
Observation intervals:	24 hours
Age range of water fleas at test initiation:	<24 hours
Test concentrations (Nominal):	0 (control), 120 mg X11449757/L
Test concentrations (Mean Calculated):	<MQL (Control), 115 mg X11449757/L
Analytical confirmation of test concentrations:	0, 24, and 48 hours
Reference substance:	X11449757
Number of water fleas per dose group:	20
Number of water fleas per control group:	20
Feeding:	None
Environmental conditions:	Temperature: 19.6 to 20.8°C Photoperiod: 16 hour light:8 hour dark Dissolved Oxygen concentration: 8.6 to 9.2 mg/L (99-106% sat.) (new); 8.6 to 8.9 mg/L (99 to 102% sat.) (old). pH: 7.1 to 8.6 Water alkalinity: 152 mg CaCO ₃ /L Water hardness: 150 mg CaCO ₃ /L Water conductivity: 348 µS

Methodology

A definitive test was performed at nominal concentrations 0 (control), 120 mg X11449757/L. Five daphnids aged <24-hours old were impartially added to each of four test chambers per treatment at the start of the test. Daphnids were

transferred to fresh test solutions at approximately 24 hours after test initiation. The daphnids were observed for immobility and sub-lethal effects at approximately 24 and 48 hours after test initiation. Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured in all treatment replicates daily. A thermistor probe was located in a surrogate test chamber to continuously record temperature.

Results

After 48 hours of exposure there was no immobility in the control or test substance treatment and no sub-lethal effects were observed. Calculated nominal concentrations during the 48 hour exposure period were maintained within 90-103%, since the calculated analytical results were within 20% of the nominal concentrations, the biological response results were based on nominal X11449757 concentrations.

Conclusions

The estimated 24-, and 48-hour EC_{50} value was >120 mg/L, the highest concentration tested. The slope of the 48-hour concentration response curve could not be calculated due to a lack of partial responses to the test substance. The 48-hour NOEC was 120 mg/L, based on nominal concentrations and the lack of statistically significant immobility or abnormal effects at this test substance concentration.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a nominal concentration, 48 hour EC_{50} of >120 mg/L.

Metabolite X11406790

Gaertner, K. 2012: X11406790 (XDE-729 Metabolite): Acute Toxicity to the Cladoceran, *Daphnia magna*, Determined Under Static Test Conditions. ABC Laboratories, Columbia, Missouri, ABC study number 68211. Dow AgroSciences unpublished report, Study Number 120019. 29 May 2012.

Test material

Test item:	X11406790
Purity:	95%
Description:	White solid
Lot no. :	SYN-FS08644-062

Test system

Organism (Species):	Water flea (<i>Daphnia magna</i>)
Study Type:	Acute
GLP Status:	GLP (with exception of water characterisation performed February 2012)
Guidelines followed:	OECD 202
Guideline deviations reported by Study Director:	48-hour observation of organism carried out at <i>ca</i> 71-hours in the range-finding test.
Duration of study:	71 hours
Test conditions:	Static
Parameters measured:	Immobility, sub-lethal effects
Observation intervals:	At 24 and 71 hours after test initiation
Age range of water fleas at test initiation:	< 24 hours
Test concentrations: Nominal	0 (control), 1.9, 3.8, 7.5, 15, and 30 mg X11406790/L
Test concentrations Mean measured:	<MQL (Control), 1.8, 3.4, 7.0, 13, and 25 mg X11406790/L
Analytical confirmation of test concentrations:	0 and 48 hours
Reference substance:	X11406790
Number of water fleas per dose group:	20
Number of water fleas per control group:	20
Feeding:	None
Environmental conditions:	Temperature: 20.0 to 20.8°C Photoperiod: 16 hour light:8 hour dark Dissolved Oxygen concentration: 7.8 to 8.7 mg/L (91 to 101 % saturation) pH: 8.3 to 8.6 Water alkalinity: 166 mg CaCO ₃ /L Water hardness: 152 mg CaCO ₃ /L Water conductivity: 358 µS

Methodology

A definitive test was performed at nominal concentrations 0 (control), 1.9, 3.8, 7.5, 15, and 30 mg X11406790/L. Five daphnids aged <24-hours old were added to each of four test chambers per treatment at the start of the test. The daphnids were observed for immobility and sub-lethal effects at approximately 24 and 71 hours after test initiation. Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured in all treatment replicates at 0 and 48 hours. A thermistor probe was located in a surrogate test chamber to continuously record temperature.

Results

Table B 9.2.31: Measured concentrations of X11406790 in test

Nominal concentration (mg X11406790/L)	Concentration of X11406790 at 0 hours (% nominal)	Concentration of X11406790 at 48 hours (% nominal)	Mean
Control (0)	<MQL	<MQL	<MQL
1.9	1.8 (95)	1.7 (89)	1.8 (95)
3.8	3.5 (92)	3.3 (87)	3.4 (89)
7.5	7.2 (96)	6.7 (89)	7.0 (93)
15	13 (87)	13 (87)	13 (87)
30	27 (90)	22 (73)	25 (83)

MQL- minimum quantifiable limit

Table B 9.2.32: Effect of X11406790 on Immobility

Treatment (mg X11406790/L)		No. of daphnids	Cumulative percent immobile	
Nominal	Mean Calculated		24-h	48-h
Negative control	<MQL	20	0	0
1.9	1.8	20	0	0
3.8	3.4	20	5	5
7.5	7.0	20	0	5
15	13	20	5	5
30	25	20	0	10
EC50		>30 mg X11406790/L		
95% C.I.		Not calculated		
NOEC		30 mg X11406790/L		

No sub-lethal effects were observed throughout the test duration

MQL- minimum quantifiable limit

Conclusions

After 48 hours of exposure, immobility was 0, 0, 5, 5, 5 and 10% in the 0 (control), 1.9, 3.8, 7.5, 15 and 30 mg X11406790/L nominal test treatments, respectively. The 24- and 48-hour EC50 value was estimated to be >30 mg X11406790/L based on nominal concentrations and low immobility being observed at 24 and 71 hour observations points (see deviation from protocol above). The slope of the 48-hour concentration-response line could not be calculated. No sub-lethal effects were noted during the definitive test. The 48-hour NOEC was 30 mg X11406790/L, based on nominal concentrations and a lack of statistically significant immobility and sub-lethal effects at this and all lower test substance concentrations. Although measured concentration of X11406790 dropped to outside of the nominal $\pm 20\%$ at

48 hours for the top treatment group, the initial measured concentration, plus the short test duration, mean that basing results on nominal concentrations is acceptable.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a nominal 48-hour EC50 of >30 mg X11406790/L.

Formulation toxicity

Bergfield, A. 2011: GF-2573: Acute Toxicity to the Water Flea, *Daphnia magna*, Determined Under Static-Renewal Test Conditions. ABC Laboratories, Columbia, Missouri, ABC study number 65997. Dow AgroSciences unpublished report, Study Number 101123. 30 June 2011.

Test material

Test item:	GF-2573
Purity:	0.84 wt% XDE-729 methyl, 0.83 wt% cloquintocet-mexyl
Description:	Yellow liquid
Lot No./Lot no. :	E2837-83

Test system

	Water flea (<i>Daphnia magna</i>)
Study Type:	Acute
GLP Status:	GLP (except water characterization carried out in 2011)
Guidelines followed:	OECD guideline 202
Guideline deviations reported by Study Director:	None
Duration of study:	48 hours
Test conditions:	Static-Renewal
Parameters measured:	Immobility
Observation intervals:	24 hours
Age range of water fleas at test initiation:	<24 hours
Test concentrations:	Nominal: 0 (control), 0.016, 0.036, 0.079, 0.177, 0.378, and 0.84 mg a.s./L which is equivalent to 0 (control), 1.9, 4.3, 9.4, 21, 45, and 100 mg GF-2573/L
	Mean Calculated (based on analysis of XDE-729 Methyl): 0 (control), 0.016, 0.035, 0.077, 0.177, 0.378, and 0.84 mg a.s./L which is equivalent to <MQL (Control), 1.9, 4.2, 9.2, 21, 45, and 100 mg GF-2573/L

Analytical confirmation of test concentrations:	0, 24, and 48 hours
Reference substances:	XDE-729 Methyl and XDE-729 Acid
Number of water fleas per dose group:	20
Number of water fleas per control group:	20
Feeding:	None
Environmental conditions:	Temperature: 19.6 to 20.8°C Photoperiod: 16 hour light:8 hour dark Dissolved Oxygen concentration: 8.3 to 9.0 mg/L pH: 8.2 to 8.4 Water alkalinity: 152 mg CaCO ₃ /L Water hardness: 150 mg CaCO ₃ /L Water conductivity: 341 µS

Methodology

A definitive test was performed at nominal concentrations 0 (control), 1.9, 4.3, 9.4, 21, 45, and 100 mg GF-2573/L. Five neonates (<24-hours old) were added to each of four test chambers per treatment at the start of the test. The daphnids were observed for immobility and sub-lethal effects at approximately 24 and 48 hours after test initiation. Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured in all treatment replicates daily. A thermistor probe was located in a surrogate test chamber to continuously record temperature.

Table B 9.2.33: Effect of GF-2573 on immobility

Nominal	Mean Calculated	No. of water fleas	24-h	48-h
Negative control	<MQL	20	0	0
1.9	1.9	20	0	0
4.3	4.2	20	0	0
9.4	9.2	20	0	25
21	21	20	55	75
45	45	20	100	100
100	100	20	100	100
EC50	14.0 mg GF-2573/L			
95% C.I.	11.3 and 17.5 mg GF-2573/L			
NOEC	4.3 mg GF-2573/L			

MQL- minimum quantifiable limit

Table B 9.2.34: Effect of GF-2573 on sub-lethal Effects

Treatment (mg GF-2573/L)		No. of water fleas	Cumulative percent sub-lethal effects	
Nominal	Mean Calculated		24-h	48-h
Negative control	<MQL	20	0	0
1.9	1.9	20	0	0
4.3	4.2	20	0	0
9.4	9.2	20	0	0
21	21	20	0	0
45	45	20	0	0
100	100	20	0	0

MQL- minimum quantifiable limit

In addition to determining concentrations of XDE-729 Methyl, concentrations of XDE-729 Acid were also determined. Presented in Table B 9.2.35 and B 9.2.36 are the concentrations of the acid in solutions removed at 24 and 96 hours.

Table B 9.2.35: Concentration of XDE-729 Methyl present in solutions removed at 24 and 48 hours

Nominal concentration GF-2573 (mg a.s./L)	Conc ⁿ of XDE-729 Methyl at 0 hours	Conc ⁿ of XDE-729 Methyl at 24 hours (old solution)	Conc ⁿ of XDE-729 Methyl at 24 hours (new solution)	Conc ⁿ of XDE-729 Methyl at 48 hours (old solution)
0 (control)	<MQL	<MQL	<MQL	<MQL
1.9	0.0160	0.0151	0.0157	0.0153
4.3	0.0358	0.0354	0.0361	0.0348
9.4	0.0772	0.0769	0.0787	0.0761
21	0.187	0.176	0.180	0.173
45	0.380	0.368	0.384	No analysis
100	0.870	0.846	0.820	No analysis

MQL- minimum quantifiable limit

Table B.9.2.36: Concentration of XDE-729 Acid present in solutions removed at 24 and 48 hours

Nominal concentration GF-2573 (mg a.s./L)	Conc ⁿ of XDE-729 Acid at 0 hours	Conc ⁿ of XDE-729 Acid at 24 hours (old solution)	Conc ⁿ of XDE-729 Acid at 24 hours (new solution)	Conc ⁿ of XDE-729 Acid at 48 hours (old solution)
0 (control)	<MQL	<MQL	<MQL	<MQL
1.9	<MQL	0.00109	<MQL	0.00130
4.3	0.000254	0.00242	<MQL	0.00270
9.4	0.000526	0.00508	0.000390	0.00584
21	0.00110	0.0108	0.000906	0.0119
45	0.00234	0.0188	0.00173	No analysis
100	0.00420	9.0392	0.00336	No analysis

MQL- minimum quantifiable limit

Conclusions

Based on nominal concentrations, the 24-hour EC₅₀ value was estimated to be 20.0 mg GF-2573/L, with 95% confidence limits of 16.8 and 23.8 mg GF-2573/L. The 48-hour EC₅₀ value was estimated to be 14.0 mg GF-2573/L, with 95% confidence limits of 11.3 and 17.5 mg GF-2573/L. The slope of the 48-hour concentration-response line was 4.65. No sub-lethal effects were noted during the definitive test. The 48-hour NOEC was 4.3 mg GF-2573/L, based on nominal concentrations and a lack of statistically significant immobility and sub-lethal effects at this and all lower test substance concentrations.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a nominal 48 hour EC₅₀ of 14.0 mg formⁿ/L. This study is to be used in conjunction with the blank formulation acute test with *D. magna* to attempt to identify the toxic component within the formulation.

Gaertner, K. (2011): GF-2573 Blank Formulation: Acute Toxicity to the Cladoceran, *Daphnia magna*, Determined Under Static Renewal Test Conditions. ABC Laboratories, Columbia, Missouri. ABC study number 68244. Dow AgroSciences unpublished report, Study Number 120032. 30 April 2012.

Test material

Test item:	GF-2573 blank formulation (contains no active substance)
Description:	yellow liquid
Lot No./Lot no. :	E3930-03

Test system

Organism (Species):	Water flea (<i>Daphnia magna</i>)
Study Type:	Acute
GLP Status:	GLP (except water characterization carried out in February 2012 and characterisation of test substance)
Guidelines followed:	OECD guideline 202
Guideline deviations reported by Study Director:	None
Duration of study:	48 hours
Test conditions:	Static-Renewal
Parameters measured:	Immobility and sub lethal effects.
Observation intervals:	Initiation, 24 and 48 hours
Age range of water fleas at test initiation:	<24 hours
Test concentrations (Nominal):	0 (control), 1.9, 4.3, 9.4, 21, 45 and 100 mg GF-2573 /L
Analytical confirmation of test concentrations:	No analytical verification of test concentrations was performed.
Number of water fleas per dose group:	20 (five daphnia in four separate treatment containers)
Number of water fleas per control group:	20 (five daphnia in four separate treatment containers)
Feeding:	<p>During the holding period, the daphnids were fed a suspension of the algal species <i>Pseudokirchneriella subcapitata</i> at least once a day supplemented by a commercially available artificial diet consisting of a wheat grass, salmon starter, and yeast suspension.</p> <p>During the definitive test, daphnids were not fed.</p>
Environmental conditions:	<p>Temperature: 19.8 to 20.7°C</p> <p>Photoperiod: 16 hour light:8 hour dark, 749 lux</p> <p>Dissolved Oxygen concentration:</p> <p>New solutions: 8.6 to 8.8 mg/L (99 to 104% saturation)</p> <p>Old solutions: 8.3 to 8.8 mg/L (95 to 101% saturation)</p> <p>pH: 8.5 to 8.6</p> <p>Water alkalinity: 170 mg CaCO₃/L</p> <p>Water hardness: 142 mg CaCO₃/L</p> <p>Water conductivity: 303 µS</p>

Methodology

A definitive test was performed at nominal concentrations of 0 (control), 1.9, 4.3, 9.4, 21, 45 and 100 mg GF-2573 blank formulation /L. The definitive test was conducted in 250-mL glass jars containing approximately 200mL of control or test substance solution. Five neonates (<24-hours old) were impartially added to a set of labelled containers with each container representing one treatment replicate. Each container was randomly assigned to a treatment replicate using a random number generator. There were four test chambers per treatment at the start of the test. Daphnids were then transferred with a pipette from the containers to the appropriate test chamber.

Daphnids were transferred to fresh test solutions at approximately 24 hours after test initiation. The daphnids were observed for immobility and sub-lethal effects at approximately 24 and 48 hours after test initiation. Immobile daphnids, defined as those organisms not able to swim within 15 seconds after gentle agitation were discarded; therefore, immobility was synonymous with mortality.

Total hardness, total alkalinity, and conductivity were measured in the dilution water at test initiation. Temperature, dissolved oxygen concentration, and pH were measured daily in all treatment replicates. A thermistor probe was located in a surrogate test chamber to continuously record temperature. No aeration was provided to any test chamber during the test.

Results

Due to the lack of more than one partial response during the exposure, the slope of the 48-hour concentration-response line could not be calculated. No sub lethal effects were noted during the definitive test.

Table B 9.2.37: Effect of GF-2573 blank formulation on Immobility

Nominal Concentration (mg GF-2573 blank formulation /L)	No. of water fleas	Cumulative mean percent immobile	
		24 hours	48 hours
0 (control)	20	0	0
1.9	20	0	0
4.3	20	0	0
9.4	20	5	55*
21	20	70	100*
45	20	75	100*
100	20	100	100*
EC ₅₀	9.1 mg GF-2573 blank formulation/L		
95% C.I.	7.6 and 11 mg GF-2573 blank formulation/L		
NOEC	4.3 mg GF-2573 blank formulation/L		

* Statistically significant reduction in survival as compared to the control

Conclusions

The test acceptability criteria were met for this study. Immobilization in the control treatments were 0%, below the acceptability limit of 10%. The dissolved oxygen concentration at the end of the test was ≥ 8.3 mg/L in control and test substance treatments, higher than the acceptability minimum of 3 mg/L. The 24-hour EC₅₀ value was estimated to be 21 mg GF-2573 blank formulation/L. The 48-hour EC₅₀ value was estimated to be 9.1 mg GF-2573 blank formulation/L. The 48-hour NOEC was 4.3 mg GF-2573 blank formulation/L, based on nominal concentrations and a lack of statistically significant immobility and sub lethal effects at this test substance concentration. This study is classified as acceptable and satisfies the guideline requirement for an acute toxicity test with *Daphnia magna*.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes. A technical oversight in the termination pH measurement, for one replicate at the 45 mg GF-2573 blank formulation/L concentration. All other pH measurements for this treatment were 8.5. There is no indication that, a measurement carried out at this time point for this replicate would have been any different. The proposed endpoint is a nominal 48 hour EC₅₀ of 9.1 mg GF-2573 blank formulation/L.

This endpoint together with the previous study endpoint (48-hour EC₅₀ = 14.0 mg/L), indicate that the toxicity of GF-2573 to *Daphnia* is not attributed to the active substance XDE-729 methyl.

B.9.2.1.1.3 Toxicity to algae

Weber, K. (2011): Testing of Effects of XDE-729 Methyl on the Single Cell Green Alga *Pseudokirchneriella subcapitata* in a 96 h Static Test. Eurofins Agroscience Services GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany. Study code: S09-00613. Dow AgroSciences unpublished report. Study Number 090173, (29 June 2011)

Test material

Test item:	XDE-729 Methyl
Test substance no.:	TSN031117-0004
Batch no.:	E2837-51
Active substances:	XDE-729 Methyl
Content of a.s. (analysed):	97.2 % wt/wt
Appearance/colour:	solid/off-white
Certificate of analysis:	28 February 2011
Expiration date :	30 November 2013
Storage conditions:	ambient, at room temperature

Test system

Organism (Species):	Unicellular alga <i>Pseudokirchneriella subcapitata</i>
Study Type:	laboratory study assessing algal growth, static
GLP Status:	GLP
Guidelines followed:	OECD Guideline 201 (2006) OPPTS Number 850.5400 (1996)
Guideline deviations reported by Study Director:	none
Duration of study:	4 days
Parameters measured:	Test solution pH (range): 7.17 – 8.99
Environmental conditions:	Temperature (range): 22 – 23 °C Photoperiod: 24 hours (permanent) Light intensity (range): 4400 – 6000 lux
Observation intervals:	0, 1, 2, 3, 4 days
Test concentrations:	Nominal: 0, 0.0938, 0.188, 0.375, 0.75, 1.5 and 3.0 mg/L Initially measured: 89 - 117% of nominal As expected, the test item rapidly decreased. At 3 mg/L, 0.431 mg/L was found as XDE-729 Acid
Age of inoculum	Algal cells for this study were taken from a culture that had been transferred to fresh media two days prior to test initiation.
Initial cell density	0.5×10^4 per mL
Growth medium	AAP medium in accordance with OECD guideline 201
Method of test item added to the test medium	Stock solutions, dissolved in acetone, 50 µL of Acetone stock solution per L algal medium
No. of control/solvent control replicates	6 + 1 for analytical samplings
No. of test concentration replicates	3 + 1 for analytical samplings
Analytical verification:	An analytical method for the determination of XDE-729 Methyl and XDE-729 Acid was validated with regard to recovery (accuracy), linearity of detector response, repeatability (precision) and specificity. The analytical system fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000

Methodology

The test organism was *Pseudokirchneriella subcapitata* Hindák. strain SAG 61.81 (formerly *Selenastrum capricornutum*). The algae used in this study are purchased from the Collection of Algae Culture (SAG). Pflanzenphysiologisches Institut der Universität, Nikolausberger Weg 18, 37073 Göttingen, Germany

Method of cultivation: The algae are grown semi-continuously in the laboratory in aerated liquid cultures under permanent illumination. Old medium is periodically replaced by fresh mineral solution in order to keep the algae in an exponential growth state.

Test performance: Visually healthy cells from a semi-static liquid stock culture were used for the test. The cell density was adjusted to an initial concentration of 0.5×10^4 cells/mL fresh, sterile medium in each test vessel. Each test item dilution was prepared in 3 replicates. As growth control, medium without the test item was used in 6 replicates and a solvent control in 6 replicates. One additional replicate of each study group was made for analytical chemistry sampling. After 1, 2, 3 and 4 days, the cell growth was determined by fluorescence detection. The pH was measured at $t = 0$ d and 4 d. Analytical determinations were made for each concentration and control at 0 days; controls and 3 mg/L were measured every day for XDE-729 Methyl and the metabolite XDE-729 Acid. For analysing a sample of 0.5 mL was taken from each test concentration.

Culture test conditions were as follows:

Illumination: from the top, (OSRAM FQ 39 W 865 H0, Lumilux, cool daylight and OSRAM FQ 39 W 830 H0, Lumilux, warm white) approx. 4400 - 6000 lux, temperature: 22 - 23 ° C. CO₂ supply by shaking on a rotating shaker. Culture flasks: 500 mL Erlenmeyer flasks with aluminium caps and two baffles. Test volume approximately 167 mL, each

Results

The increase of cell numbers measured in the control after 3 and 4 days was 82- and 235-fold respectively. The increase in the solvent control was 68- and 238-fold respectively. The coefficient of variation (CV) of daily growth rates in the controls was 17 % after 4 days (solvent control 18 %) and did not exceed 35 %. The coefficient of variation of average growth in replicate control cultures was 2.2 % and 0.8 % after 3 and 4 days respectively (solvent control 4.9 % and 1.4 % respectively) and did not exceed 10 % during the whole test period. The test, therefore, fulfils the validity criteria of OECD Guideline 201 after 4 days of exposure.

Percentage inhibition of growth rate and yield are presented below in Tables B 9.2.38 and 39

Table B 9.2.38: Percentage inhibition of growth rate after 1, 2, 3 and 4 days

XDE-729 Methyl (mg/L)	0-1 d	0-2 d	0-3 d	0-4 d
0	0	0	0	0
Solvent control	3.3	4.5	4.4	-0.2
0.0938	22.1	5.6	3.5	-1.7
0.188	2.7	2.7	-0.4	-3.5
0.375	9.6	1.3	-4.2	-2.6
0.75	23.3	-6.6	-17.5	-2.3
1.5	10.0	-9.5	-16.4	-5.4
3	29.6	-3.5	-12.8	-0.3

- negative value mean growth promotion

Table B 9.2.39: Percentage inhibition of yield after 1, 2, 3 and 4 days

XDE-729 Methyl (mg/L)	0-1 d	0-2 d	0-3 d	0-4 d
0	0	0	0	0
Solvent control	1.9	12.8	16.5	-1.2
0.0938	33.5	15.8	14.3	-9.8
0.188	2.6	8.2	-3.1	-21.7
0.375	17.4	4.0	-21.0	-15.4
0.75	36.8	22.6	-117.3	-13.2
1.5	18.1	-34.2	-126.3	-34.7
3	45.8	-12.1	-77.0	-1.6

- negative value mean growth promotion

Table B 9.2.40: Findings (based on nominal concentrations):

EC values	XDE-729 Methyl [mg/L]
3-day and 4 day ErC50 (growth rate)	> 3.0
3-day and 4 day EyC50 (yield)	> 3.0
Lowest observed effect concentration (LOEC)	> 3.0
No observed effect concentration (NOEC)	3.0

Initial concentrations of the XDE-729 Methyl were 89 – 117 % of nominal. Thereafter, XDE-729 Methyl concentrations rapidly declined and after 1, 3 and 4 days XDE-729 Methyl concentrations declined to 54, 5 and 0.2 % nominal in the 3 mg/L treatment. After 4 days, the concentration of the hydrolysis metabolite XDE-729 Acid increased to 0.431 mg/L in the 3 mg/L test level, which is equivalent to 15 % of the nominal XDE-729 Methyl concentration. Due to rapid degradation of the test substance under these test conditions, the geometric mean measured

exposure to XDE-729 methyl over 3 days was 0.855 mg/L, and over 4 days was 0.245 mg/L at the highest treatment concentration.

Presented below in Table B 9.2.41 are the nominal and actual concentrations of XDE-729 methyl and acid present in the algal study.

Table B 9.2.41 Concentrations of XDE-729 Methyl and XDE-729 Acid

Test item Nominal mg/L	XDE-729 Methyl and XDE-729 Acid mg/L	Sampling day	XDE-729 Methyl (actual)			XDE-Acid (actual)	
			mg/L	% of nominal	% of geometric mean	mg/L	% of nominal
0	0	0 1 3 4	n.d. n.d. n.d. n.d.		-	n.d. n.d. n.d. n.d.	
0 (acetone control)	0	0 1 3 4	n.d. n.d. n.d. n.d.		-	n.d. n.d. n.d. n.d.	
0.0938	0.0912 0.0875 (acid)	0	0.107	117	-	n.d.	
0.188	0.183 0.175 (acid)	0	0.162	89	-	n.d.	
0.375	0.365 0.350 (acid)	0	0.363	99	-	n.d.	
0.750	0.729 0.700 (acid)	0	0.750	103	-	0.000817	0.1
1.50	1.46 1.40 (acid)	0	1.46	100	-	0.000637	0.05
3.00	2.92 2.80 (acid)	0 1 3 4	2.60 1.58 0.152 0.00573	89 54 5 0.2	8	0.00201 0.0245 0.235 0.431	0.07 0.9 8 15

Conclusions

The 4-day ErC50 and EyC50 for *Pseudokirchneriella subcapitata* exposed to XDE-729 Methyl was determined to be >3.0 mg/L for growth rate and for yield.

This is equivalent to a mean measured concentration of >0.245 mg/L (taking a geometric mean).

The LOEC (Lowest observed effect concentration) was >3.0 mg/L (3 and 4 days), and the NOEC (No observed effect concentration) was 3.0 mg/L (3 and 4 days); equivalent to a LOEC of greater than 0.245 mg/L and a NOEC of 0.245 mg/L.

RMS Comment: Due to the fact that concentrations were not maintained, an endpoint based on the geometric mean should be used for risk assessment. It is proposed that the endpoint from this study is a mean measured 96-hour ErC50 and EyC50 of >0.245 mg/L.

Rebstock, M. (2011): XDE-729 Methyl: Growth Inhibition Test with the Freshwater Diatom, *Navicula pelliculosa*. ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri 65202. ABC Study No. 67182. Dow AgroSciences unpublished report, Study Number 090174. 24 June 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Lot No./Batch No. :	E2837-51

Test system

Organism (Species):	Freshwater diatom, <i>Navicula pelliculosa</i>
Study Type:	Laboratory study assessing algal growth static
GLP Status:	GLP (except the latest water characterization carried out in 2011)
Guidelines followed:	U.S. EPA OPPTS 850.5400 and OECD Guideline 201
Guideline deviations reported by Study Director:	None.
Duration of study:	96 hrs
Parameters measured:	Cell Density, Growth Rate
Environmental conditions:	Test solution pH (range): 7.5 to 8.9 Test solution temperature (range): 22.7 to 24.4°C Photoperiod: Continuous light. Light intensity (range): 4096 to 4345 lux
Observation intervals:	0, 24, 48, 72, 96 hours
Test concentrations:	Nominal: 0 (control), 0 (vehicle control), 0.13, 0.25, 0.50, 1.0, 2.0, and 4.0 mg XDE-729 Methyl/L 72-hour geometric mean measured concentrations: <MQL (control), <MQL (vehicle control), 0.0408, 0.0661, 0.125, 0.346, 0.801, and 1.78 mg a.s./L 96-hour geometric mean measured concentrations:

	<MQL (control), <MQL (vehicle control), 0.0271, 0.0412, 0.0878, 0.189, 0.566, and 1.30 mg a.s./L
Age of inoculum	3 days old
Acclimation period/conditions	The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing.
Initial cell density	1.0×10^4 cells/mL
Growth medium	Name: FWAM + Si pH at test initiation: 7.5 (control solution) pH at test termination: 8.8 (control solution) Constant stirring?: Yes, all test solutions were swirled on an orbital shaker table at 100 rpm throughout the test.
Method of test item added to the test medium	A 160 mg a.s./mL primary stock solution was prepared on 09 May 2011 by diluting 1.6459 g (1.5998 g corrected for purity) of XDE-729 Methyl with dimethylformamide (DMF) in a 10-mL glass volumetric flask. Working standard solutions were prepared by serially diluting the 160 mg a.s./L primary stock solution with DMF to produce final concentrations of 5.2, 10, 20, 40, and 80 mg a.s./mL. Test solutions were prepared by diluting 0.025-mL aliquots of the primary stock and working standard solutions to a volume of 1.0 L with dilution medium for final concentrations of 0.13, 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.s./L. The vehicle control was prepared by diluting 0.025 mL of DMF to volume of 1.0 L with dilution water with a resulting solvent concentration of 0.025 mL DMF/L. The vehicle control and all treatment levels had the same solvent concentration. The control consisted of dilution medium only.
No. of control replicates	6
No. of test concentration replicates	3
Analytical verification:	Method: measuring concentrations of XDE-729 Methyl and XDE-729 Acid using LC-MS/MS Samples taken: Test initiation, 24 hours, 72, hours and termination. Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) and was 0.000246 mg XDE-729 Methyl/L or 0.000246 mg XDE-729 Acid/L.

Methodology

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and labelled with study number, treatment, replicate, and grid position. The controls were replicated six times and each test substance treatment was replicated three times. Each replicate contained 100 mL of the appropriate parent solution. Two additional replicates, each containing 100 mL of the appropriate parent solution, were prepared for the controls and each test substance treatment for analytical verification at 24- and 72-hours. Three additional replicates of the 0.13 mg/L nominal test substance treatment, containing 100 mL of the appropriate parent solution, were also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass (abiotic replicate). At test initiation, all biotic replicates were inoculated with 1.0 mL of an algal concentrate containing approximately 1.0×10^6 cells/mL, resulting in a final density of approximately 1.0×10^4 cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. At 24, 48, 72, and 96 hours (± 1 hour), cell density was measured in all replicates of the controls, as well as replicates A, B, and C of each test substance treatment by direct microscopic counting with a hemacytometer. The abiotic replicate was not inoculated with algae.

Results

Measured concentrations of XDE-729 Methyl in fresh test treatment solutions at initiation were 0.120, 0.233, 0.458, 0.852, 1.96, and 4.02 mg a.s./L, which represented 85 to 101% of the nominal concentrations. However, concentrations declined throughout the test and the 72-hour geometric mean measured XDE-729 Methyl concentrations were 0.0408, 0.0661, 0.125, 0.346, 0.801 and 1.78 mg a.s./L (25 to 45% of nominal) and the 96-hour geometric mean measured concentrations were 0.0271, 0.0412, 0.0878, 0.189, 0.566 and 1.30 mg a.s./L (16 to 33% of nominal). The biological response results were therefore reported based upon the nominal concentrations of XDE-729 Methyl, and the geometric mean of the 72- and 96-hour measured XDE-729 Methyl concentrations. Results of the chemical analysis are presented in Table B 9.2.42.

Table B 9.2.42: Measured concentrations of XDE-729 Methyl and Acid

Nominal concentration XDE-729 Methyl (mg/L)	0-hour		24-hour		72-hour		96-hour	
	Methyl	Acid	Methyl	Acid	Methyl	Acid	Methyl	Acid
0.13	0.120	<MQL	0.0656	0.00160	0.00864	0.00216	0.00796	0.00324
0.25	0.233	0.000314	0.0844	0.00254	0.0147	0.00432	0.00992	0.00618
0.50	0.458	0.000528	0.225	0.00494	0.0191	0.00536	0.0302	0.0169
1.0	0.852	0.000962	0.390	0.00892	0.125	0.0204	0.0306	0.0183
2.0	1.96	0.00208	1.16	0.0240	0.226	0.0338	0.200	0.0476
4.0	4.02	0.00412	1.96	0.0396	0.716	0.0754	0.506	0.0850

MQL- minimum quantifiable limit

Table B 9.2.43: 72 and 96-hour geometric mean methyl concentrations

Nominal concentration XDE-729 Methyl (mg/L)	72-hour	96-hour
0.13	0.0408 (31%)	0.0271 (21%)
0.25	0.0661 (26%)	0.0412 (16%)
0.50	0.125 (25%)	0.0878 (18%)
1.0	0.346 (35%)	0.189 (18%)
2.0	0.801 (40%)	0.566 (28%)
4.0	1.78 (45%)	1.30 (33%)

Table B 9.2.44: Percentage inhibition of growth rate and yield after 3 and 4 days

XDE-729 Methyl (mg/L)	0-3 d	0-4 d	0-3 d	0-4 d
	% Growth rate		% Yield	
0	-	-	-	-
Vehicle control	-	-	-	-
0.13	0	0	-1	1
0.25	0	0	-1	-2
0.50	2	1	7	2
1.0	8	4	25	16
2.0	17	10	46	36
4.0	61	52	91	92

- negative value mean growth promotion

Table B 9.2.45: Results Based on Nominal Concentrations:

Hour	EC Type	EC Value (mg a.s./L)	95% Confidence Limits (mg a.s./L)	NOEC (mg a.s./L)
72	EC10	0.95	0.81 to 1.1	0.25
	EC20	1.3	1.1 to 1.4	
	EC50	2.1	2.0 to 2.2	
	ErC10	1.6	1.5 to 1.7	0.50
	ErC20	2.1	2.0 to 2.2	
	ErC50	3.4	3.4 to 3.5	
	EyC10	1.0	0.86 to 1.2	0.25
	EyC20	1.3	1.2 to 1.4	
	EyC50	2.0	1.9 to 2.1	
96	EC10	1.4	1.3 to 1.4	0.50
	EC20	1.6	1.6 to 1.7	
	EC50	2.3	2.2 to 2.4	
	ErC10	2.0	1.8 to 2.3	0.50
	ErC20	2.6	2.4 to 2.8	
	ErC50	3.9	3.8 to 4.0	
	EyC10	1.4	1.3 to 1.4	0.50
	EyC20	1.6	1.6 to 1.7	
	EyC50	2.3	2.2 to 2.3	

EC50 for cell density, EyC50 for yield, and ErC50 for growth rate.

Table B 9.2.46: Results Based on 72-Hour Geometric Mean Measured Concentrations:

Hour	EC Type	EC Value (mg a.s./L)	95% Confidence Limits (mg a.s./L)	NOEC (mg a.s./L)
72	EC10	0.343	0.285 to 0.402	0.0661
	EC20	0.477	0.418 to 0.537	
	EC50	0.838	0.784 to 0.891	
	ErC10	0.618	0.563 to 0.673	0.125
	ErC20	0.857	0.804 to 0.910	
	ErC50	1.50	1.46 to 1.53	
	EyC10	0.369	0.308 to 0.429	0.0661
	EyC20	0.496	0.437 to 0.555	
	EyC50	0.822	0.772 to 0.872	

EC50 for cell density, EyC50 for yield, and ErC50 for growth rate.

Table B 9.2.47: Results Based on 96-Hour Geometric Mean Measured Concentrations:

Hour	EC Type	EC Value (mg a.s./L)	95% Confidence Limits (mg a.s./L)	NOEC (mg a.s./L)
96	EC10	0.356	0.340 to 0.373	0.0878
	EC20	0.450	0.434 to 0.465	
	EC50	0.669	0.654 to 0.684	
	ErC10	0.578	0.498 to 0.658	0.0878
	ErC20	0.772	0.703 to 0.840	
	ErC50	1.26	1.24 to 1.29	
	EyC10	0.360	0.344 to 0.377	0.0878
	EyC20	0.451	0.436 to 0.466	
	EyC50	0.663	0.649 to 0.677	

EC₅₀ for cell density, E_yC₅₀ for yield, and E_rC₅₀ for growth rate.

Conclusions

The test acceptability criteria were met for this study. The number of algal cells in the control at 72 hours was 33 times the number initially inoculated to verify logarithmic phase growth and greater than the 16 fold increase required. The coefficient of variation for daily growth rates in the control replicates after 72 hours was 32%, less than the 35% acceptance limit for this parameter. The coefficient of variation of average specific growth rates in control replicates after 72 hours was 1%, less than the 7% acceptance limit for this parameter. The coefficient of variation of final cell densities in the control after 96 hours was 7%, less than the 20% acceptance limit for this parameter. The pH in the control increased 1.3 units, less than the 1.5 units allowed during the study. This study satisfies the U.S. EPA and OECD guideline requirements for a growth inhibition test with *Navicula pelliculosa*.

RMS Comment: Due to the fact that concentrations were not maintained, an endpoint based on the geometric mean is proposed. It is considered that the proposed endpoint for risk assessment is a mean measured 96 hour EyC50 of 0.663 mg/L.

Weber, K. (2011): Testing of Effects of XDE-729 on the Blue Green Alga *Anabaena flos-aquae* in a 96 h Static Test. Eurofins Agrosience Services GmbH. Eutinger Str. 24. D-75223 Niefern-Öschelbronn. Germany. Study code: S09-00615. Dow AgroSciences unpublished report. Study Number 090175, (29 June 2011)

Test material

Test item:	XDE-729 Methyl
Test substance no.:	TSN031117-0004
Batch no.:	E2837-51
Active substances:	XDE-729 Methyl
Content of a.s. (analysed):	97.2 % wt/wt
Appearance/colour:	solid/off-white
Certificate of analysis:	28 February 2011
Expiration date :	30 November 2013
Storage conditions:	ambient, at room temperature

Test system

Organism (Species):	Blue green alga (<i>Cyanobacteria</i>) <i>Anabaena flos-aquae</i>
Study Type:	laboratory study assessing algal growth static
GLP Status:	GLP
Guidelines followed:	OECD Guideline 201 (2006) OPPTS Number 850.5400 (1996)
Guideline deviations reported by Study Director:	none
Duration of study:	4 days
Parameters measured	
Test solution pH (range):	7.4-8.42
Environmental conditions:	Temperature (range): 22 – 23 °C Photoperiod: 24 hours (permanent) Light intensity (range): 3000 – 4000 lux
Observation intervals:	0, 1, 2, 3, 4 days
Test concentrations:	Nominal: 0, 0.188, 0.375, 0.75, 1.5 and 3.0 mg/L. Initially measured: 72 – 90 % of nominal. As expected, the test item rapidly decreased. At 3 mg/L, 0.0400 mg/L were found as XDE-729 Acid (1% of nominal).
Inoculum	The algae used in this study were purchased as liquid culture from UMWELT-BUNDESAMT - Federal Environment Agency, FG IV 2.4, Substances Hazardous to Waters, Ecotoxicological Laboratory) Schichauweg 58, 12307 Berlin. Germany. They were originally cultured by: UTEX. The Culture Collection of Algae. The University of Texas at Austin. 1 University Station A6700, Austin TX 78712-0183 USA. Algal cells for this study were taken from a culture that had been transferred to fresh media one day prior to test initiation.
Initial cell density	0.94 x 10 ⁴ per mL
Growth medium	AAP medium in accordance with OECD guideline

	201
Method of test item added to the test medium	Stock solution: soluble in acetone, 50 µl of Acetone stock solution per L algal medium
No. of control/solvent control replicates	6 + 1 for analytical samplings
No. of test concentration replicates	3 + 1 for analytical samplings
Analytical verification:	An analytical method for the determination of XDE-729 Methyl and XDE-729 Acid was validated with regard to recovery (accuracy), linearity of detector response, repeatability (precision) and specificity. The analytical system fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000

Methodology

Method of cultivation: The algae are grown semi-continuously in the laboratory in aerated liquid cultures under permanent illumination. Old medium is periodically replaced by fresh mineral solution in order to keep the algae in an exponential growth state.

Test performance: Visually healthy cells from a semi-static liquid stock culture were used for the test. The cell density was adjusted to an initial concentration of 0.94×10^4 cells/mL fresh, sterile medium in each test vessel. Because of the filamentous growth of the algae (forms nested chains of cells), an approximate 10 mL aliquot of each test solution was pre-treated by ultra-sonication to produce enumerable single cells or short chains.

Each test item dilution was prepared in 3 replicates. As growth control, medium without the test item was used in 6 replicates and a solvent control in 6 replicates. One additional replicate of each study group was made for analytical chemistry sampling. After 1, 2, 3 and 4 days, the cell growth was determined by fluorescence detection. The pH was measured at $t = 0$ d and 4 d. An analytical sampling procedure was done for the verification of the test concentrations from the test solutions at 0, 1, 3 and 4 days. For analysing a sample of 0.5 mL was taken from each test concentration.

Culture test conditions were as follows:

Illumination: from the top, (OSRAM L 39 W 865, Lumilux, cool daylight and OSRAM L 39 W 830, Lumilux, warm white) approx. 3000 - 4000 lux

temperature: 22 - 23 °C. CO₂ supply by shaking on a rotating shaker.

Culture flasks: 500 mL Erlenmeyer flasks with aluminium caps and two baffles. Test volume approximately 167 mL, each.

Results

The increase of cell numbers measured in the control after 3 and 4 days was 36- and 150-fold respectively. The increase in the solvent control was 37- and 158-

fold respectively. The coefficient of variation (CV) of daily growth rates in the controls was 21 % after 3 days (solvent control 23 %) and 18 and 21 % respectively after 4 days and did not exceed 35 %. The coefficient of variation of average growth in replicate control cultures was 1.2 and 0.8 % after 3 and 4 days respectively (solvent control 1.4 % and 0.7 % respectively) and did not exceed 10 % during the whole test period. The test, therefore, fulfils the validity criteria of OECD Guideline 201 after 3 and 4 days of exposure.

Table B 9.2.48: Percentage inhibition of growth rate after 1, 2, 3 and 4 days

XDE-729 Methyl (mg/L)	0-1 d	0-2 d	0-3 d	0-4 d
0	0	0	0	0
Solvent control	7.4	4.4	-0.3	-1.1
0.188	-2.7	0.2	-0.4	-5.0
0.375	35.3	11.4	7.8	-1.6
0.75	33.6	8.3	5.2	2.4
1.5	61.1	12.6	4.9	-0.2
3	59.3	7.9	5.6	-2.5

- negative value mean growth promotion

Table B 9.2.49: Percentage inhibition of yield after 1, 2, 3 and 4 days

XDE-729 Methyl (mg/L)	0-1 d	0-2 d	0-3 d	0-4 d
0	0	0	0	0
Solvent control	11.1	10.7	-1.1	-5.4
0.188	-4.7	0.6	-1.9	-29.0
0.375	46.2	27.0	25.3	-8.3
0.75	45.6	20.6	17.7	11.5
1.5	72.5	29.6	15.9	-1.3
3	70.8	19.8	18.8	-13.2

- negative value mean growth promotion

Table B 9.2.50: Findings (based on nominal concentrations):

EC values	XDE-729 Methyl [mg/L]
3-day and 4 day ErC50 (growth rate)	> 3
3-day and 4 day EyC50 (yield)	> 3
Lowest observed effect concentration (LOEC)	> 3
No observed effect concentration (NOEC)	3

Initial concentrations of the XDE-729 Methyl were 72 – 90 % of nominal. Thereafter, XDE-729 Methyl concentrations rapidly declined and after 1, 3 and 4 days XDE-729 Methyl concentrations declined to 39, 17 and 9 % nominal in the 3 mg/L treatment. After 4 days, the concentration of the hydrolysis metabolite XDE-729 Acid increased to 0.04 mg/L in the 3 mg/L test level, which is equivalent to 1% of the nominal XDE-729 Methyl concentration. Due to rapid degradation of the test substance under these test conditions, the geometric mean measured exposure to XDE-729 Methyl over 3 days was 1.13 mg/L, and over 4 days was 0.775 mg/L at the highest treatment concentration.

Table B 9.2.51: Concentrations of XDE-729 Methyl and XDE-729 Acid.

Test item Nominal mg/L	XDE-729 Methyl and XDE-729 Acid mg/L	Sampling day	XDE-729 Methyl (actual)			XDE-Acid (actual)	
			mg/L	% of nominal	% of geometric mean	mg/L	% of nominal
0	0	0	n.d.	-	-	n.d.	-
		1	n.d.	-	-	n.d.	-
		3	n.d.	-	-	n.d.	-
		4	n.d.	-	-	n.d.	-
0 (acetone control)	0	0	n.d.	-	-	n.d.	-
		1	n.d.	-	-	n.d.	-
		3	n.d.	-	-	n.d.	-
		4	n.d.	-	-	n.d.	-
0.188	0.183 0.175 (acid)	0	0.131	72	-	n.d.	-
		4	n.d.	-	-	0.00532	3
0.375	0.365 0.350 (acid)	0	0.321	88	-	n.d.	-
		4	0.00599	2	-	0.00569	2
0.750	0.729 0.700 (acid)	0	0.592	81	-	n.d.	-
		4	0.0116	2	-	0.0113	2
1.50	1.46 1.40 (acid)	0	1.24	85	-	n.d.	-
		4	0.0126	1	-	0.147	11
3.00	2.92 2.80 (acid)	0	2.62	90	27	0.00964	0.3
		1	1.14	39		0.0119	0.4
		3	0.486	17		0.0287	1
		4	0.249	9		0.0400	1
3.00 (abiotic control)	2.92 2.80 (acid)	0	2.40	82	33	0.00282	0.1
		4	0.393	13		0.0686	2

Conclusions

The 4-day EC50 for *Anabaena flos-aquae* exposed to XDE-729 Methyl was determined to be > 3 mg/L for growth rate and for yield. The LOEC (Lowest observed effect concentration) was >3.0 mg/L (1-4 days) and the NOEC (No observed effect concentration) was 3.0 mg/L (1-4 days). The 72 and 96 hour EC50 are equivalent to mean measured concentrations of 1.13 mg a.s./L and 0.775 mg a.s./L.

RMS Comment: Due to the fact that concentrations were not maintained, an endpoint based on the geometric mean is proposed. It is considered that the proposed endpoint for risk assessment is a mean measured 96 hour EC50 of >0.775 mg/L. This endpoint covers both growth and yield.

Rebstock, M. (2011): XDE-729 Methyl: Static Growth Inhibition Test with the Marine Diatom, *Skeletonema costatum*. ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri 65202. ABC Study No. 64717. Dow AgroSciences unpublished report, Study Number 090176. 12 May 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Lot No./Batch No. :	E2835-51

Test system

Organism (Species):	Marine Diatom, <i>Skeletonema costatum</i>
Study Type:	laboratory study assessing diatom growth static
GLP Status:	GLP (except for the water characterization that was carried out in 2009)
Guidelines followed:	U.S. EPA OPPTS 850.5400
Guideline deviations reported by Study Director:	None.
Duration of study:	96 hrs
Parameters measured:	Test solution pH (range): 8.0 to 8.8 Test solution temperature (range): 21.2 to 21.5°C
Environmental conditions:	Temperature (range): Within the 20 ± 2°C specified in the protocol. Photoperiod: 14 hours light; 10 hours dark Light intensity (range): 4,297 to 4,488 lux
Observation intervals:	0, 24, 48, 72, 96 hours

Test concentrations:	Nominal: 0 (control), 0 (vehicle control), 0.13, 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.s./L Measured initial: <MQL (control), <MQL (vehicle control), 0.134, 0.241, 0.469, 0.939, 1.83, and 3.36 mg a.s./L (84 – 103% nominal) Geometric mean calculated concentrations: <MQL (control), <MQL (vehicle control), 0.0251, 0.0749, 0.226, 0.581, 1.33, and 1.85 mg a.s./L (19 – 67% nominal)
Age of inoculum	3 days old
Acclimation period/conditions	The prepared cultures were maintained in a temperature-controlled environmental chamber under a 14:10 day: night light cycle. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing.
Initial cell density	77×10^3 cells/mL
Growth medium	Name: SWAM pH at test initiation: 8.0 (control solution) pH at test termination: 8.7 (control solution) Constant stirring?: No, all test solutions were manually swirled twice daily throughout the test.
Method of test item added to the test medium	A 160-mg a.s./mL primary standard was prepared by dissolving 1.6142 g (1.5690 g corrected for purity) of XDE-729 Methyl in 10 mL of DMF. The primary standard was serially diluted with DMF to prepare working standards at nominal concentrations of 5.2, 10, 20, 40, and 80 mg a.s./mL. The working standards and the primary standard were used to prepare the parent test solutions by diluting 0.0125 mL of the appropriate standard solution to 0.5 L with test medium, resulting in nominal concentrations of 0.13, 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.s./L. The final vehicle concentration in each of the exposure concentrations was 25 µL DMF/L. The vehicle control was prepared by adding 0.0125 mL of DMF to 0.5 L of the dilution medium resulting in a vehicle concentration of 25 µL DMF/L. The control consisted of dilution medium without test substance or DMF.
No. of control replicates	3 control, 3 vehicle control
No. of test concentration replicates	3
Analytical verification:	Method: measuring concentrations of XDE-729 Methyl and its hydrolysis product XDE-729 Acid using LC-MS/MS.

	<p>Samples taken: Test initiation and termination.</p> <p>Limit of Detection: Not determined.</p> <p>Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) and was 0.238 ng XDE-729 Methyl/mL or 0.246 ng XDE-729 Acid/mL.</p> <p>Recoveries from QC fortifications: (range) 101 to 118% of the nominal concentrations.</p>
--	---

Methodology

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and were labelled with study number, treatment, replicate, and grid position. Prior to test initiation, the flasks were cleaned and autoclaved according to ABC standard operating procedures. The control, vehicle control, and each test substance treatment were replicated three times (replicates A, B and C). Each replicate contained 100 mL of the appropriate parent solution. An additional replicate (replicate D) of the 0.13 mg a.s./L test substance treatment, containing 100 mL of the appropriate parent solution, was also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass. At test initiation, the A, B and C replicates of each test substance treatment and the controls were inoculated with 1.0 mL of an algal concentrate containing approximately 7.7×10^6 cells/mL, resulting in a final density of approximately 77,000 cells/mL for each flask. At 24, 48, 72, and 96 (± 1 hour), cell density was measured in replicates A, B, and C of the control, vehicle control, and each test substance treatment by direct microscopic counting with a hemacytometer. Replicate D of the 0.13 mg a.s./L test substance treatment was not inoculated with algae.

Results

Table B 9.2.52: Percentage inhibition of growth rate and yield after 3 and 4 days

XDE-729 Methyl (mg/L)	0-3 d	0-4 d	0-3 d	0-4 d
	% Growth rate		% Change in cell density	
0		-	-	-
Vehicle control	-	-	-	-
0.13	2	0	5	1
0.25	-1	-1	-2	-2
0.50	-1	-2	-2	-6
1.0	-19	-9	-38	-25
2.0	-38	-30	-63	-59
4.0	-51	-44	-73	-73

- negative value mean growth promotion

Table B 9.2.53: Effects of XDE-729 Methyl on algal growth based on nominal concentrations:

Hour	EC Type ^a	EC Value ^a (mg a.s./L)	95% Confidence Limits (mg a.s./L)	NOEC (mg a.s./L)
72	EC50	1.6	1.4 to 1.8	Cell Density: 0.50
	ErC50	3.6	3.2 to 4.0	Growth Rate: 0.50
96	EC50	1.8	1.6 to 2.0	Cell Density: 0.25
	ErC50	>4.0	Not Statistically Sound	Growth Rate: 0.25

^a EC50 based on final cell density; ErC50 based on average specific growth rate.

Table B 9.2.54: Effects of XDE-729 Methyl on algal growth based on geometric mean Measured Concentrations

Hour	EC Type ^a	EC Values (mg a.s./L)	95% Confidence Limits (mg a.s./L)	NOEC (mg a.s./L)
72	EC50	0.904	0.832 to 0.975	Cell Density: 0.226
	ErC50	1.80	1.72 to 1.89	Growth Rate: 0.226
96	EC50	1.07	1.02 to 1.12	Cell Density: 0.0749
	ErC50	>1.85	Not Statistically Sound	Growth Rate: 0.0749

^a EC50 based on final cell density; ErC50 based on average specific growth rate.

Table B 9.2.55: Measured concentrations of XDE-720 Methyl and XDE-720 Acid in test solutions during the 96-hour toxicity test with the marine diatom, *Skeletonema costatum*

Nominal XDE-729 Methyl concentration (mg a.s./L)	Measured concentration as mg/L (percent nominal)				
	0 hour		96 hour		Geometric mean XDE-729 Methyl concentration
	XDE-729 Methyl	XDE-729 Acid	XDE-729 Methyl	XDE-729 Acid	
Control	<MQL	<MQL	<MQL	<MQL	-
Vehicle control	<MQL	<MQL	<MQL	<MQL	-
0.13	0.134 (103%)	Not calculated	0.00470 (4%)	0.0147	0.0251 (19%)
0.13 (abiotic)	-	-	0.0612 (47%)	0.00622	-
0.25	0.241 (96%)	Not calculated	0.0233 (9%)	0.0308	0.0749 (30%)
0.50	0.469 (94%)	Not calculated	0.109 (22%)	0.0622	0.266 (45%)
1.0	0.939 (94%)	Not calculated	0.360 (36%)	0.122	0.581 (58%)
2.0	1.83 (92%)	Not calculated	0.968 (48%)	0.146	1.33 (67%)
4.0	3.36 (84%)	Not calculated	1.02 (26%)	0.178	1.85 (46%)

MQL- minimum quantifiable limit

Conclusions

The test acceptability criteria were met for this study. The number of cells in the control at test termination was 21 times the number initially inoculated and sufficient to verify logarithmic phase growth. The coefficient of variation for cell density in the control replicates during the course of the test did not exceed 20%. This study is classified as acceptable and satisfies the guideline requirement for a growth inhibition test with *Skeletonema costatum*.

RMS Comment: Due to the fact that concentrations were not maintained, an endpoint based on the geometric mean is proposed. It is considered that the endpoint that should be used for risk assessment is a mean measured 72 hour EC50 of 0.904 mg a.s./L.

XDE-729 Acid

Rebstock, M. (2011): XDE-729 Acid: Growth Inhibition Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*. ABC 66685. Dow AgroSciences unpublished report, Study Number 102027. 01 September 2011.

Test material

Test item:	XDE-729 Acid
Purity:	95.3 wt/%
Description:	Off-white solid
Lot No./Batch No. :	E2837-52

Test system

Organism (Species):	Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>
Study Type:	Laboratory study assessing algal growth, static
GLP Status:	GLP (with the exception of the water characterization that was carried out in 2011)
Guidelines followed:	OECD Guideline 201 (2006)
Guideline deviations reported by Study Director:	None
Duration of study:	72 hrs
Parameters measured:	Cell Density, Growth Rate, Yield
Environmental conditions:	Test solution pH (range): 5.7 to 7.6 Test solution temperature (range): 23.2 to 24.4°C Photoperiod: Continuous light. Light intensity (range): 8,063 to 8,239 lux
Observation intervals:	24, 48, 72 hours
Test concentrations:	Nominal: 0 (control), 6.3, 13, 25, 50, 100 and 100 (pH adjusted to 7.5) mg XDE-729 Acid/L Geometric Mean calculated concentrations: <MQL (control), 6.60, 13.3, 24.6, 51.3, 99.7 and 110 mg XDE-729 Acid/L
Age of inoculum	4 days old
Acclimation period/conditions	The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing.
Initial cell density	5.0×10^3 cells/mL
Growth medium	Name: FWAM pH at test initiation: 7.4 (control solution) pH at test termination: 7.6 (control solution)

No. of control replicates	6
No. of test concentration replicates	3
Analytical verification:	Method: measuring concentrations of XDE-729 Acid using HPLC-UV. Samples taken: Test initiation and termination. Limit of Detection: Not determined. Limit of Quantitation: minimum quantifiable limit (MQL) = 0.204 mg/L.

Methodology

A 0.10 mg XDE-729 Acid/mL primary standard was prepared by transferring 0.2099 g of XDE-729 Acid (0.2000 g adjusted for purity) to a 2000 mL glass volumetric flask, and bringing the flask to volume with test medium. The primary standard was used as the highest test substance treatment. The five lower test substance treatments were prepared individually, each at a volume of 500 mL using appropriate volumes of the highest test substance treatment and test medium. The control consisted of test medium only.

The exposure flasks were appropriately labelled 250-mL Erlenmeyer flasks with foam stoppers. The control was replicated six times and each test substance treatment was replicated three times. Each replicate contained 100 mL of the appropriate parent solution. An additional replicate of the 6.3 mg XDE-729 Acid/L nominal test substance treatment, containing 100 mL of the appropriate parent solution, was also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass (abiotic replicate). An additional 100 mg/L test treatment was prepared by adjusting the pH of a portion of the primary standard to approximately 7.5 with 0.1N NaOH after the addition of the test substance. At test initiation, all biotic replicates were inoculated with an algal concentrate resulting in a final density of approximately 5.0×10^3 cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. The abiotic replicate was not inoculated with algae. All replicates were swirled using an orbital shaker at 100rpm throughout the test. At 24, 48, and 72 hours (± 1 hour), cell density was measured in all replicates of the control, as well as replicates A, B, and C of each test substance treatment by direct microscopic counting with a hemacytometer.

Results

Chemical analysis showed the measured test item concentrations to be within the acceptable range ($\pm 20\%$ of nominal) at all sampling points.

After 72 hours of exposure the mean control cell density had increased to 116 times the nominal initial cell density. The coefficient of variation of average specific growth rates during the whole test period in control replicates was 1%. The mean coefficient of variation for section-by-section specific growth rates was 6% for the control replicates. The test, therefore, fulfils the validity criteria of OECD Guideline 201 after 72-hours of exposure. The mean cell density in the

XDE-729 Acid treatments at 72 hours ranged from a low of 0.852×10^4 cells/mL at the concentration of 100 mg XDE-729 Acid/L to a high of 64.1×10^4 cells/mL at the concentration of 6.3 mg XDE-729 Acid/L. Percent inhibition in algal growth at 72 hours, as compared to the control, ranged from 99% at the concentration of 100 mg XDE-729 Acid/L to -10% at the concentration of 6.3 mg XDE-729 Acid/L. Statistical comparisons of the 100 mg XDE-729 Acid/L non-pH-adjusted treatment and the 100 mg XDE-729 Acid/L pH-adjusted treatment, indicate significant reductions in growth rate and yield in the non-pH-adjusted treatment compared to the pH adjusted treatment.

Table B 9.2.56: Percentage inhibition of growth rate and cell density after 72-hours

XDE-729 Acid (mg/L)	0-72 hours Growth rate	0-72 hours Cell density	0-72 hours Yield
0	-	-	-
6.3	-2	-10	-10
13	10	37	37
25	17	55	56
50	26	71	72
100	89	99	99
100 (pH adjusted to 7.5)	31	77	77

negative value means growth promotion

Table B 9.2.57: Effects of XDE-729 Acid on algal growth based on nominal concentrations

Hour	EC Type	EC Value ^a [mg XDE-729 Acid/L]	95% Confidence Limits [mg XDE-729 Acid/L]	NOEC [mg XDE-729 Acid/L]
72	E _r C10	39	36 to 42	Growth rate: 6.3
	E _r C20	46	44 to 49	
	E _r C50	63	60 to 65	
	E _y C10	<6.3	Not statistically sound	Yield: 6.3
	E _y C20	9.7	6.0 to 13	
	E _y C50	23	18 to 27	

E_rCXX – XX% effect on growth rate

E_yCXX – XX% effect of yield

Table B 9.2.58: Concentrations of test compound

Nominal concentration (mg XDE-729 Acid/L)	Calculated concentration as mg XDE-729 Acid/L (percent Nominal)		
	0 hours	72 hours	Geometric mean
Control	<MQL	<MQL	<MQL
6.3	7.06 (112)	6.14 (97)	6.60 (105)
6.3 (abiotic)	NA	6.58 (104)	NA
13	14.6 (112)	11.9 (92)	13.3 (102)
25	26.8 (107)	22.3 (89)	24.6 (98)
50	54.8 (110)	47.7 (95)	51.3 (103)
100	104 (104)	95.4 (95)	99.7 (100)
100 (pH adjusted to 7.5)	113 (113)	106 (106)	110 (110)

MQL- minimum quantifiable limit

Conclusions

In a 72 hour growth inhibition test with *Pseudokirchneriella subcapitata* the E_rC_{50} and the E_yC_{50} were calculated to be 63 and 23 mg XDE-729 Acid/L respectively, based on nominal concentrations. The NOEC for growth rate and yield was 6.3 mg XDE-729 Acid/L. All validity criteria were met in accordance with OECD guideline 201. All concentrations of XDE-729 Acid were maintained within 80 – 120% of nominal values.

RMS Comment: This study is deemed suitable and acceptable for risk assessment purposes. It is considered that the endpoint that should be used for risk assessment is the 72 hour E_yC_{50} of 23 mg XDE-729 Acid/L, as yield was the more sensitive endpoint, based upon nominal test substance concentrations.

Rebstock, M. (2011): XDE-729 Acid: Growth Inhibition Test with the Freshwater Diatom, *Navicula pelliculosa*. ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri 65202. ABC Study No. 66687. Dow AgroSciences unpublished report, Study Number 102029. 01 September 2011.

Test material

Test Item:	XDE-729 Acid
Purity:	95.3wt/%
Description:	Off-white solid
Lot No./Batch No. :	E2837-52

Test system

Organism (Species):	Freshwater diatom, <i>Navicula pelliculosa</i>
Study Type:	Laboratory study assessing algal growth under static conditions
GLP Status:	GLP (except the latest water characterization carried out in February 2011)
Guidelines followed:	OECD Guideline 201
Guideline deviations reported by Study Director:	None
Duration of study:	72 hrs
Parameters measured:	Cell density, growth rate, yield
Environmental conditions:	Test solution pH (range): 5.7-7.6 Test solution temperature (range): 23.0 to 25.5°C Photoperiod: Continuous light. Light intensity (range): 4041 to 4097 lux
Observation intervals:	0, 24, 48, 72 hours
Test concentrations:	Nominal-0 (control), 6.3, 13, 25, 50, 100 mg/L, and 100 mg/L (pH adjusted to approximately 7.5) Mean measured concentrations: <MQL (control), 6.51, 13.4, 25.9, 51.4, 94.8, and 109 mg/L
Age of inoculum	3 days old
Acclimation period/conditions	The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing.
Initial cell density	1.0×10^4 cells/mL
Growth medium	Name: FWAM + Si pH at test initiation: 7.5 ± 0.1 (control solution) pH at test termination: 7.6 (control solution) Constant stirring?: Yes, all test solutions were swirled on an orbital shaker table at 100 rpm throughout the test.
Method of test item added to the test medium	A 0.10 mg XDE-729 Acid/mL primary standard was prepared on 27 June 2011, by transferring 0.1050 g of XDE-729 Acid (0.1001 g corrected for purity) to a 1,000 mL glass volumetric flask, and bringing the flask to volume with test medium. The primary standard was used as the highest test substance treatment. The 6.3, 13, 25, and 50 mg/L test substance treatments, each at a volume of 500 mL, were prepared individually using appropriate volumes of the highest test substance treatment and test medium. An additional 100 mg/L test treatment was prepared by adding 0.0525 g of

	XDE-729 Acid to a 500 mL glass volumetric flask, and bringing the flask to volume with test medium. The pH of this solution was adjusted to approximately 7.5 with 0.1N NaOH after the addition of the test substance. The control consisted of test medium only.
No. of control replicates	6
No. of test concentration replicates	3
Analytical verification:	Method: measuring concentrations of XDE-729 Acid using HPLC-UV Samples taken: Test initiation (0 hours) and test termination (72 hours) Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) and was 0.204 mg/L.

Methodology

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and labelled with study number, treatment, replicate, and grid position. The controls were replicated six times and each test substance treatment was replicated three times. Each replicate contained 100 mL of the appropriate parent solution. An additional replicate of the 6.3 mg/L test substance treatment, containing 100 mL of the appropriate parent solution, was also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass (abiotic replicate). At test initiation, all replicates were inoculated with 1.0 mL of an algal concentrate containing approximately 2.5×10^6 cells/mL, resulting in a final density of approximately 1.0×10^4 cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. At 24, 48 and 72 hours (± 1 hour), cell density was measured in all replicates of the control, as well as replicates of each test substance treatment by direct microscopic counting with a hemacytometer. The abiotic replicate was not inoculated with algae. Flasks were randomly positioned daily using a computer-generated random number table and incubated at $24 \pm 2^\circ\text{C}$ in a temperature controlled environmental chamber under continuous cool-white fluorescent lighting. Temperature and pH were measured in all parent solutions prior to distribution of the solutions to the test flasks. At 72 hours, temperature and pH were measured in replicate A of the controls and each test substance treatment.

Results

Chemical analysis showed the measured test item concentrations to be within 108-116% of the nominal concentrations. As these concentrations were within the acceptable range ($\pm 20\%$ of nominal), the results are based upon nominal concentrations.

Table B 9.2.59: Measured Concentrations of XDE-729 Acid in Test Solutions During the 72-Hour Toxicity Test with the Freshwater Diatom, *Navicula pelliculosa*

Nominal concentration (mg XDE-729 Acid/L)	Measured concentration as mg XDE-729 Acid/L (percent nominal)		
	0 hours	72 hours	Geometric mean
Control	MQL	MQL	MQL
6.3	6.81 (108)	6.20 (98)	6.51 (103)
6.3 (abiotic)	NA	6.55 (104)	NA
13	14.1 (108)	12.6 (97)	13.4 (103)
25	27.0 (108)	24.8 (99)	25.9 (104)
50	53.6 (107)	49.1 (98)	51.4 (103)
100	97.6 (98)	92.0 (92)	94.8 (95)
100 (pH adjusted)	111 (111)	106 (106)	109 (109)

MQL- minimum quantifiable limit

Table B 9.2.60: Percentage inhibition of cell density, growth rate and yield after 72-hours

XDE-729 Acid (mg/L)	% Inhibition (as compared to the control)		
	Cell density 72 hours	Growth rate 72 hours	Yield 72 hours
0	-	-	-
6.3	5	2	5
13	4	1	4
25	10	3	10
50	49	21	51
100	98	>100	>100
100 (pH adjusted)	33	13	35

Table B 9.2.61: Summary of EC and NOEC estimates, based on nominal concentrations, for *Navicula pelliculosa* exposed to XDE-729 acid

Hour	EC Type	EC Value ^a [mg/L]	95% Confidence Limits [mg/L]	NOEC [mg/L]
24	E _r C ₁₀	42	26 to 59	6.3
	E _r C ₂₀	46	28 to 64	
	E _r C ₅₀	53	32 to 74	
	E _y C ₁₀	41	35 to 46	6.3
	E _y C ₂₀	44	38 to 50	
	E _y C ₅₀	51	44 to 58	
48	E _r C ₁₀	42	25 to 59	13
	E _r C ₂₀	45	27 to 63	
	E _r C ₅₀	52	31 to 73	
	E _y C ₁₀	38	36 to 40	13
	E _y C ₂₀	42	39 to 44	
	E _y C ₅₀	48	45 to 50	
72	E _r C ₁₀	45	36 to 55	13
	E _r C ₂₀	49	39 to 60	
	E _r C ₅₀	56	44 to 68	
	E _y C ₁₀	40	40 to 40	13
	E _y C ₂₀	43	43 to 44	
	E _y C ₅₀	50	49 to 50	

Table B 9.2.62: Comparison of results for *Navicula pelliculosa* exposed to XDE-729 Acid and pH adjusted XDE-729 Acid treatment at 100 mg/L

Nominal Concentration mg XDE-729 Acid/L	pH		% inhibition at 72 hrs		
	0 hr	72 hr	Cell density	Growth rate	Yield
100 (non-pH-adjusted)	5.7	5.8	98	>100	>100
100 (pH adjusted)	7.4	7.4	33	13	35

Conclusion

A statistical comparison of the 100 mg/L, non-pH-adjusted treatment and the 100 mg/L, pH-adjusted treatment indicated a significant reduction in growth rate and yield in the non-pH adjusted treatment compared to the pH adjusted treatment. Based on this comparison, the initial pH shift as a result of the addition of the test substance to the dilution medium is considered a significant factor in both growth rate and yield inhibition.

The NOEC for growth rate at 72 hours was 13 mg/L, based on the lack of a statistically significant reduction in rate from time zero at this, the lowest test substance treatment. Based on growth rate, the estimated 72-hour E_rC₅₀ was 56 mg/L with 95% confidence limits of 44 and 68 mg/L.

The NOEC for yield at 72 hours was 13 mg/L, based on the lack of a statistically significant reduction in yield from time zero at this, the lowest test substance treatment. Based on yield, the estimated 72-hour E_yC_{50} value was 50 mg/L with 95% confidence limits of 49 and 50 mg/L.

RMS comment:

The study is considered to be acceptable and suitable for risk assessment purposes and the proposed endpoint is a nominal measured 72 hour E_yC_{50} value of 50 mg/L for yield. The study has been carried out in accordance with OECD 201.

Rebstock, M. (2011): XDE-729 Acid: Growth Inhibition Test with the Blue-Green Alga, *Anabaena flos-aquae*. ABC 65967. Dow AgroSciences unpublished report, Study Number 101144. 01 September 2011.

Test material

Test item:	XDE-729 Acid
Test substance no.:	TSN030751-0006
Lot no.:	E2837-52
Purity:	95.3% wt/wt
Appearance/colour:	Off-white solid
Re-certification date :	23 August 2011
Storage conditions:	Room temperature

Test system

Organism (Species):	Blue green alga (<i>Cyanobacteria, Anabaena flos-aquae</i>)
Study Type:	Laboratory study assessing algal growth
GLP Status:	GLP (with the exception of water characterisations performed February 2011)
Guidelines followed:	OECD Guideline 201 (2006)
Guideline deviations reported by Study Director:	none
Duration of study:	72 hours
Parameters measured	Cell density, Growth rate, Yield
Environmental conditions	Test solution pH (range): 5.6 – 7.8 Temperature (range): 22.9 – 25.4 °C Photoperiod: 24 hours (continuous) Light intensity (range): 2150-2200 lux
Observation intervals:	24, 48 and 72 hours
Test concentrations:	Nominal: 0, 6.3, 13, 25, 50, 100 and 100 (pH adjusted to 7.5) mg XDE-729 Acid/L Mean measured concentrations: <MQL (control), 6.53, 13.9, 26.4, 52.7, 91.9, and 108 mg XDE-729 Acid/L
Age of Inoculum	4 days

Inoculum	Prepared cultures were maintained in a temperature controlled environmental chamber under continuous light. All cultures were maintained under the same conditions as those used for testing.
Initial cell density	1×10^4 cells/mL
Growth medium	FWAM pH at test initiation: 7.5 (control solution) pH at test termination: 7.7 (control solution)
No. of control replicates	6
No. of test concentration replicates	3 (an additional abiotic replicate was prepared at treatment 6.3 mg XDE-729 Acid/mL)
Analytical verification:	Method: measuring concentrations of XDE-729 Acid using HPLC-UV. Samples taken: Test initiation and termination. Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) = 0.204 mg/L

Methodology

A 0.10 mg XDE-729 Acid/mL primary standard was prepared by transferring 0.2101 g of XDE-729 Acid (0.2002 g corrected for purity) to a 2,000 mL glass volumetric flask, and bringing the flask to volume with test medium. The primary standard was used as the highest test substance treatment. The 6.3, 13, 25, and 50 mg/L test substance treatments were prepared individually using appropriate volumes of the highest test substance treatment and test medium. An additional 100 mg/L test treatment was prepared by adjusting the pH of a portion of the primary standard to approximately 7.5 with 0.1N NaOH. The control consisted of test medium only.

The exposure flasks were appropriately labelled 250-mL Erlenmeyer flasks with foam stoppers. The control was replicated six times and each test substance treatment was replicated three times. Each replicate contained 100 mL of the appropriate parent solution. An additional replicate of the 6.3 mg XDE-729/L nominal test substance treatment, containing 100 mL of the appropriate parent solution, was also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass (abiotic replicate).

At test initiation, all biotic replicates were inoculated with 1.0 mL of an algal concentrate containing approximately 1.0×10^6 cells/mL, resulting in a final density of approximately 1.0×10^4 cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. Throughout the test all replicates were swirled by hand twice daily. At 24, 48, and 72 hours (± 1 hour), cell density was measured in all replicates of the control, as well as replicates A, B, and C of each test substance treatment by direct microscopic counting with a hemacytometer. The abiotic replicate (replicate D at 6.3 mg XDE-729 Acid/mL) was not inoculated with algae.

Results

After 72 hours of exposure the mean control cell density had increased to 24.2 times the nominal initial cell density. The coefficient of variation of average specific growth rates during the whole test period in control replicates was 2%. The mean coefficient of variation for section-by-section specific growth rates was 30% for the control replicates. The test, therefore, fulfils the validity criteria of OECD Guideline 201 after 72-hours of exposure. Percent inhibition in algal growth at 72 hours, as compared to the control group, ranged from 95% at the concentration of 100 mg XDE-729 Acid/L to 0% at the concentration of 6.3 mg XDE-729 Acid/L. A statistical comparison of the 100 mg/L, non-pH adjusted treatment and the 100 mg/L, pH-adjusted treatment indicated a significant reduction in growth rate in the non-pH-adjusted treatment compared to the pH adjusted treatment.

Measured concentrations of XDE-729 Acid in the test solutions at 0 and 72 hours were all within 80-120% of nominal, allowing results to be expressed in terms of nominal concentrations of the test substance.

Table B 9.2.63: Percentage inhibition of growth rate and cell density after 72-hours

XDE-729 Acid (mg/L)	0-72 hours Growth rate	0-72 hours Cell density	0-72 hours Yield
0	-	-	-
6.3	0	0	0
13	1	2	2
25	0	1	1
50	25	53	56
100	100	95	100
100 (pH adjusted to 7.5)	10	27	28

Table B 9.2.64: Effects of XDE-729 Acid on algal growth based on nominal concentrations

Hour	EC Type	EC Value ^a [mg XDE-729 Acid/L]	95% Confidence Limits [mg XDE-729 Acid/L]	NOEC [mg XDE-729 Acid/L]
72	E _r C10	45	30 to 59	Growth rate: 25
	E _r C20	48	33 to 64	
	E _r C50	55	38 to 73	
	E _y C10	36	7.1 to 65	Yield: 25
	E _y C20	40	19 to 62	
	E _y C50	49	44 to 53	

^a E_yC₅₀ based on final yield, E_rC₅₀ based on average specific growth rate.

Table B 9.2.65: Concentrations of test compound

Nominal concentration (mg XDE-729 Acid/L)	Calculated concentration as mg XDE-729 Acid/L (percent nominal)		
	0 hours	72 hours	Geometric mean
Control	<MQL	<MQL	<MQL
6.3	6.74 (107)	6.32 (100)	6.53 (104)
6.3 (abiotic)	NA	6.59 (105)	NA
13	14.1 (108)	13.7 (105)	13.9 (107)
25	26.3 (105)	26.5 (106)	26.4 (106)
50	53.0 (106)	52.5 (105)	52.7 (105)
100	92.2 (92)	91.7 (92)	91.9 (92)
100 (pH adjusted to 7.5)	108 (108)	108 (108)	108 (108)

MQL- minimum quantifiable limit

Conclusions

In a 72 hour growth inhibition test with *Anabaena flos-aquae* the E_rC_{50} and the E_yC_{50} were calculated to be 55 and 49 mg XDE-729 Acid/L respectively. The NOEC for growth rate and yield was 25 mg XDE-729 Acid/L. All validity criteria were met in accordance with OECD guideline 201. Results are to be based upon nominal concentrations of XDE-729 Acid.

RMS Comment: This study is deemed suitable and acceptable for risk assessment purposes. It is considered that the endpoint that should be used for risk assessment is a nominal 72 hour E_yC_{50} of 49 mg XDE-729 Acid/L, as yield was the more sensitive endpoint. While the OECD 201 guideline states a temperature range of 21- 24°C, this was not maintained (actual temperature range was 22.9 – 25.4 °C). The study protocol followed proposed an acceptable temperature range of 24 ± 2°C which was achieved. Alongside this, maximum temperatures for all treatment and control groups were similar (25.2-25.4 °C) meaning that the guideline-temperature deviation showed no bias towards certain treatment groups. All validity criteria were met, therefore this deviation is not deemed to affect the reliability of the study.

Rebstock, M. (2011): XDE-729 Acid: Static Growth Inhibition Test with the Marine Diatom, *Skeletonema costatum*. ABC 66686. Dow AgroSciences unpublished report, Study Number 102028. 19 September 2011.

Test material

Test item:	XDE-729 Acid
Purity:	95.3 wt/%
Description:	Off-white solid
Lot No./Batch No. :	E2837-52

Test system

Organism (Species):	Marine Diatom, <i>Skeletonema costatum</i>
Study Type:	Laboratory study assessing diatom growth, static
GLP Status:	GLP (with the exception of the water characterization that was carried out in February 2011)
Guidelines followed:	U.S. EPA OPPTS 850.5400
Guideline deviations reported by Study Director:	None.
Duration of study:	96 hrs
Parameters measured:	Cell Density, Growth Rate
Environmental conditions:	Test solution pH (range): 6.6 to 8.5 Test solution temperature (range): 21.2 to 21.8°C Photoperiod: 14:10 (light:dark) Light intensity (range): 4,011 to 4,176 lux
Observation intervals:	24, 48, 72 and 96 hours
Test concentrations:	Nominal: 0 (control), 6.3, 13, 25, 50 and 100 mg XDE-729 Acid/L Geometric Mean calculated concentrations: <MQL (control), 6.45, 13.4, 26.3, 52.5 and 103 mg XDE-729 Acid/L
Age of inoculum	3 days old
Acclimation period/conditions	The prepared cultures were maintained in a temperature-controlled environmental chamber under a 14:10 (light:dark) light cycle.. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing.
Initial cell density	7.7×10^4 cells/mL
Growth medium	Name: SWAM pH at test initiation: 8.0 (control solution) pH at test termination: 8.3 (control solution) Salinity: 30.2‰. All test solutions were manually swirled twice daily throughout the test.
No. of control replicates	3
No. of test concentration replicates	3
Analytical verification:	Method: measuring concentrations of XDE-729 Acid using HPLC-UV. Samples taken: Test initiation and termination. Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) = 0.204 mg/L.

Methodology

A 0.10 mg XDE-729 Acid/mL primary standard was prepared on by transferring 0.1050 g of XDE-729 Acid (0.1001 g corrected for purity) to a 1,000 mL glass volumetric flask, and bringing the flask to volume with test medium. The primary standard was used as the highest test substance treatment. The 6.3, 13, 25, and 50 mg/L test substance treatments, each at a volume of 500 mL, were prepared individually using appropriate volumes of the highest test substance treatment and test medium. The control consisted of test medium only.

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and labelled with study number, treatment, replicate, and position within the test area. The control and each test substance treatment were replicated three times. Each replicate contained 100 mL of the appropriate parent solution. An additional replicate of the 6.3 mg XDE-729 Acid/L nominal test substance treatment, containing 100 mL of the appropriate parent solution, was also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass (abiotic replicate). At test initiation, all biotic replicates were inoculated with 1.0 mL of an algal concentrate containing approximately 7.7×10^6 cells/mL, resulting in a final density of approximately 7.7×10^4 cells/mL for each flask. The abiotic replicate was not inoculated with algae. Flasks were randomly positioned in a temperature controlled environmental chamber, using a computer generated random number table.

The temperature and pH of parent solutions was measured prior to distribution of the solutions into the test flasks. At termination temperature and pH were measured in replicate A of each treatment. Light Intensity was measured daily. At 24, 48, 72 and 96 hours (± 1 hour), cell density was measured in all replicates of the control, as well as replicates A, B, and C of each test substance treatment by direct microscopic counting with a hemacytometer.

Results

After 96 hours of exposure the mean control cell density had increased to 26 times the nominal initial cell density. The coefficient of variation of average specific growth rates during the whole test period in control replicates was 8%. The cell growth in the control over the 96-hour test period and the low variability in cell densities between replicates demonstrated the acceptability of the test.

Percent inhibition in algal growth rate at 72 hours, as compared to the control, ranged from 82% at the concentration of 100 mg XDE-729 Acid/L to 0% at the concentration of 6.3 and 13 mg XDE-729 Acid/L. Percent inhibition in cell density at 72 hours, as compared to the control, ranged from 89% at the concentration of 100 mg XDE-729 Acid/L to -1% at the concentration of 6.3 mg XDE-729 Acid/L. At 96 hours percent inhibition in algal growth rate, as compared to the control, ranged from 87% at the concentration of 100 mg XDE-729 Acid/L to 1% at the concentrations of 6.3, 13 and 25 mg XDE-729 Acid/L. Percent inhibition in cell density, as compared to the control, ranged from 94% at the concentration of 100 mg XDE-729 Acid/L to 3% at the concentration of 25 mg XDE-729 Acid/L.

Table B 9.2.66: Percentage inhibition of growth rate and cell density after 72-hours

XDE-729 Acid (mg/L)	0-72 hours Growth rate	0-72 hours Cell density
0	-	-
6.3	0	-1
13	0	1
25	1	3
50	7	16
100	82	89

Negative value means growth promotion

Table B 9.2.67: Percentage inhibition of growth rate and cell density after 96-hours

XDE-729 Acid (mg/L)	0-96 hours Growth rate	0-96 hours Cell density
0	-	-
6.3	1	4
13	1	4
25	1	3
50	9	14
100	87	94

Negative value means growth promotion

Table B 9.2.68: Effects of XDE-729 Acid on algal growth based on nominal concentrations

Hour	EC Type	EC Value^a [mg XDE-729 Acid/L]	95% Confidence Limits [mg XDE-729 Acid/L]	NOEC [mg XDE-729 Acid/L]
72	E _r C50	78	72 to 83	25
	EC50	68	67 to 69	25
96	E _r C50	77	66 to 87	25
	EC50	66	64 to 67	25

^a EC50 based on final cell density, E_rC50 based on average specific growth rate.

Table B 9.2.69: Concentrations of test compound

Nominal concentration (mg XDE-729 Acid/L)	Calculated concentration as mg XDE-729 Acid/L (percent nominal)		
	0 hours	96 hours	Geometric mean
Control	<MQL	<MQL	<MQL
6.3	6.62 (105)	6.29 (100)	6.45 (102)
6.3 (abiotic)	NA	6.61 (105)	NA
13	13.3 (102)	13.6 (105)	13.4 (103)
25	26.7 (107)	26.0 (104)	26.3 (105)
50	54.0 (108)	51.1 (102)	52.5 (105)
100	109 (109)	96.6 (97)	103 (103)

MQL- minimum quantifiable limit

Conclusions

In a 96 hour growth inhibition test with *Skeletonema costatum* the 96-hour E_rC_{50} (growth rate) and the EC_{50} (cell density) were calculated to be 77 and 66 mg XDE-729 Acid/L respectively. The 96-hour NOEC for growth rate and cell density was 25 mg XDE-729 Acid/L. The 72-hour E_rC_{50} and the EC_{50} (cell density) were calculated to be 78 and 68mg XDE-729 Acid/L respectively. The 72-hour NOEC for growth rate and cell density was 25 mg XDE-729 Acid/L. All validity criteria were met in accordance with the study protocol. All concentrations of XDE-729 Acid were maintained within 80 – 120% of nominal values (97-109%).

RMS Comment: This study is deemed suitable and acceptable for risk assessment purposes. It is considered that the endpoint that should be used for risk assessment is a nominal 96 hour EC_{50} of 66 mg XDE-729 Acid/L, as cell density was the more sensitive endpoint and the 96 hour endpoint was calculated to be more sensitive, as well as the result having much tighter 95% confidence limits (64 to 67 mg XDE-729/L). An end point based on cell density is considered equivalent to biomass (i.e. the $EC_{50} = E_bC_{50}$).

Metabolite

Rebstock, M. (2011): X11449757: Growth Inhibition Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*. ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri 65202. ABC Study No. 66006. Dow AgroSciences unpublished report, Study Number 101158. 18 July 2011.

Test material

Test item:	X11449757
Purity:	98.6%
Description:	Off-white solid
Lot No./Batch No. :	YB1-100780-103

Test system

Organism (Species):	Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>
Study Type:	laboratory study assessing algal growth static
GLP Status:	GLP (with the exception of the water characterization that was carried out in 2011)
Guidelines followed:	OECD Guideline 201
Guideline deviations reported by Study Director:	None.
Duration of study:	72 hrs
Parameters measured:	Cell Density, Growth Rate
Environmental conditions:	Test solution pH (range): 7.1 to 8.0 Test solution temperature (range): 24.3 to 25.2°C Temperature (range): Within the $24 \pm 2^\circ\text{C}$ specified in the protocol. Photoperiod: Continuous light. Light intensity (range): 7979 to 8329 lux
Observation intervals:	0, 24, 48, 72 hours
Test concentrations:	Nominal: 0 (control), 0.63, 1.3, 2.5, 5.0, 10, and 20 mg a.s./L Geometric Mean calculated concentrations: <MOL (control), 0.408, 0.751, 1.72, 3.60, 7.42, and 15.8 mg a.s./L
Age of inoculum	4 days old
Acclimation period/conditions	The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing.
Initial cell density	5.0×10^3 cells/mL
Growth medium	Name: FWAM pH at test initiation: 7.5 (control solution) pH at test termination: 7.9 (control solution) Constant stirring: Yes, all test solutions were swirled on an orbital shaker table at 100 rpm throughout the test.
Method of test item added to the test medium	A 0.020 mg a.s./mL primary standard was prepared on 12 April 2011, by transferring 0.0203 g of X11449757 (0.0200 g adjusted for purity) to a 1000 mL glass volumetric flask, and bringing the flask to volume with test medium. The primary standard was used as the highest test substance treatment. The five lower test substance treatments, each at a volume of 500 mL, were

	prepared individually using appropriate volumes of the highest test substance treatment and test medium. The control consisted of test medium only.
No. of control replicates	6
No. of test concentration replicates	3
Analytical verification:	Method: measuring concentrations of XN1449757 using LC-MS/MS. Samples taken: Test initiation and termination. Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) and was 0.00202 mg a.s./L

Methodology

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and labelled with study number, treatment, replicate, and grid position. The control was replicated six times and each test substance treatment was replicated three times. Each replicate contained 100 mL of the appropriate parent solution. An additional replicate of the 0.063 mg/L nominal test substance treatment, containing 100 mL of the appropriate parent solution, was also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass (abiotic replicate). At test initiation, all biotic replicates were inoculated with 1.0 mL of an algal concentrate containing approximately 5.0×10^6 cells/mL, resulting in a final density of approximately 5.0×10^3 cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. At 24, 48, and 72 hours (± 1 hour), cell density was measured in all replicates of the control, as well as replicates A, B, and C of each test substance treatment by direct microscopic counting with a hemacytometer. The abiotic replicate was not inoculated with algae.

Results

Table B 9.2.70: Percentage inhibition of growth rate and yield after 3 days

XDE-729 Methyl (mg/L)	0-3 d Growth rate	0-3 d Cell density
0	-	-
0.408	0	-3
0.751	0	2
1.72	7	29
3.60	10	41
7.42	25	72
15.8	35	83

- negative value means growth promotion

Table B 9.2.71: Effects of X11449757 on algal growth based on geometric mean concentrations

Hour	EC Type	EC Value ^a [mg a.s./L]	95% Confidence Limits [mg a.s./L]	NOEC [mg a.s./L]
72	ErC10	2.53	1.66 and 3.41	
	ErC20	6.39	5.31 and 7.47	
	ErC50	>15.8	Not Statistically Sound	Growth rate: 0.751
	EyC10	0.795	0.503 and 1.09	
	EyC20	1.46	1.07 and 1.85	
	EyC50	4.13	3.59 and 4.67	Yield: 0.751

Table B 9.2.72: Concentrations of test compound

Nominal concentration (mg a.s./L)	Calculated concentration as mg a.s./L (percent Nominal)		
	0 hours	72 hours	Geometric mean
Control	<MQL	<MQL	<MQL
0.63	0.593 (94%)	0.281 (45%)	0.408 (65%)
0.63 (abiotic)	-	0.324 (51%)	n.a.
1.3	1.15 (88%)	0.490 (38%)	0.751 (58%)
2.5	2.36 (94%)	1.26 (50%)	1.72 (69%)
5.0	4.86 (97%)	2.66 (53%)	3.60 (72%)
10	9.48 (95%)	5.80 (58%)	7.42 (74%)
20	17.9 (90%)	14.0 (70%)	15.8 (79%)

MQL- minimum quantifiable limit

Conclusions

The test acceptability criteria were met for this study. The number of algal cells in the control at test termination was greater than 16 times the number initially inoculated to verify logarithmic phase growth. The coefficient of variation for daily growth rates in the control replicates during the course of the test did not exceed 35%. The coefficient of variation of average specific growth rates during the whole test period in control replicates did not exceed 7%. The pH in the control did not increase more than 1.5 units during the study. This study satisfies the OECD guideline requirement for a growth inhibition test with *Pseudokirchneriella subcapitata*.

RMS Comment: Due to the fact that concentrations were not maintained, an endpoint based on the geometric mean is proposed. It is considered that the endpoint that should be used for risk assessment is a mean measured 72 hour EyC50 of 4.13 mg/L.

Rebstock, M. (2012): X11406790: Growth Inhibition Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*. ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri 65202. ABC Study No. 68210. Dow AgroSciences unpublished report, Study Number 120021. 31st May 2012.

Test material

Test item:	X11406790
Purity:	95%
Description:	White solid
Lot No./Batch No. :	SYN-FS08644-062

Test system

Organism (<i>Species</i>):	Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>
Study Type:	laboratory study assessing algal growth -static
GLP Status:	GLP (with exception of the latest water characterizations performed in February 2012)
Guidelines followed:	OECD Guideline 201
Guideline deviations reported by Study Director:	Initial pH of medium 8.0 ± 0.1 rather than 7.5 ± 0.1
Duration of study:	72 hrs
Parameters measured:	Cell Density, Growth Rate, Yield.
Environmental conditions:	Test solution pH (range): 7.9 to 8.7 Test solution temperature (range): 24.0 to 24.7°C Temperature (range): Within the $24 \pm 2^\circ\text{C}$ specified in the protocol. Photoperiod: Continuous light. Light intensity (range): 8,039 to 8,238 lux
Observation intervals:	0, 24, 48, 72 hours
Test concentrations:	Nominal: 0 (control), 0.50, 1.0, 2.0, 4.0, 8.0, and 16 mg X11406790/L Mean calculated concentrations: <MQL (control), 0.26, 0.49, 0.94, 2.0, 3.4, and 5.7 mg X11406790/L
Age of inoculum	3 days old
Acclimation period/conditions	The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing.
Initial cell density	5.0×10^3 cells/mL
Growth medium	Name: FWAM pH at test initiation: 7.9 (control solution) pH at test termination: 8.4 (control solution) Constant stirring?: Yes, all test solutions were swirled on an orbital shaker table at 100 rpm

	throughout the test.
Method of test item added to the test medium	A 0.016 mg X11406790/mL primary standard was prepared on 15 April 2012 by transferring 0.0168 g of X11406790 (0.0160 g corrected for purity) to a 1,000-mL glass volumetric flask, bringing the flask to volume with test medium, and stirring the solution using a Teflon coated stir bar and magnetic stir plate for approximately 24 hour prior to use. After stirring, the primary standard was used as the high treatment and aliquots of the primary standard were diluted with dilution medium to a volume of 0.50 L to prepare the lower test substance treatments at concentrations of 0.50, 1.0, 2.0, 4.0, and 8.0 mg X11406790/L. The control consisted of test medium only.
No. of control replicates	6
No. of test concentration replicates	3 (additional abiotic replicate for test concentration at 0.50 mg X11406790/L)
Analytical verification:	Method: measuring concentrations of X11406790 using HPLC-UV. Samples taken: Test initiation and termination. Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) which was 0.029 mg X11406790/L

Methodology

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and were labelled with study number, treatment, replicate, and grid position. Prior to test initiation, the flasks were cleaned and autoclaved according to ABC standard operating procedures.

The control was replicated six times (i.e., replicates A, B, C, D, E, and F) and each test substance treatment was replicated three times (replicates A, B, and C). Each replicate contained 100 mL of the appropriate parent solution. An additional replicate (replicate D) of the 0.50 mg/L nominal test substance treatment, was also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass (abiotic replicate). At test initiation, all biotic replicates were inoculated with 1.0 mL of an algal concentrate containing approximately 5.0×10^5 cells/mL, resulting in a final density of approximately 5.0×10^3 cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. At 24, 48, and 72 hours (± 1 hour), cell density was measured in all replicates of the control, as well as replicates A, B, and C of each test substance treatment by direct microscopic counting with a hemacytometer. The abiotic replicate was not inoculated with algae.

Flasks were randomly positioned in a temperature controlled environmental chamber, using a computer generated random number table. The flasks were

swirled on an orbital shaker table at 100 rpm throughout the test. The temperature and pH of parent solutions was measured prior to distribution of the solutions into the test flasks. At termination, temperature and pH were measured in replicate A of the controls and each treatment. Light Intensity was measured daily.

Results

As the measured concentrations were not within 20 % of the nominal test concentrations, the biological response results are reported based upon the geometric mean measured concentrations.

Table B 9.2.73: Measured Concentrations of X11406790 during the 72-hour toxicity test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

Nominal concentration (mg X11406790/L)	Measured concentration as mg X11406790/L (Percent Nominal)		
	0 hours	72 hours	Geometric mean
Control	<MQL	<MQL	<MQL
0.50	0.23 (46)	0.29 (58)	0.26 (52)
0.50 (abiotic)	-	0.28 (56)	N.A
1.0	0.44 (44)	0.55 (55)	0.49 (49)
2.0	0.89 (45)	1.0 (50)	0.94 (47)
4.0	1.9 (48)	2.2 (55)	2.0 (50)
8.0	3.6 (45)	3.2 (40)	3.4 (43)
16	7.2 (45)	4.5 (28)	5.7 (36)

MQL- minimum quantifiable limit

Table B.9.2.74: Percentage inhibition of cell density, growth rate and yield after 72-hours

Geometric mean measured concentration (mg X11406790/L)	% Inhibition (as compared to the control)		
	Cell density 72 hours	Growth rate 72 hours	Yield 72 hours
Control	-	-	-
0.26	1	0	1
0.49	4	1	4
0.94	5	1	5
2.0	62	19	63
3.4	77	28	77
5.7	83	34	83

Table B 9.2.75: Summary of EC and NOEC Estimates, based on geometric mean measured concentrations, for *Pseudokirchneriella subcapitata* exposed to X11406790

Hour	EC Type	EC Value [mg X11406790/L]	95% Confidence Limits [mg X11406790/L]	NOEC [mg X11406790/L]
24	E _r C ₅₀	>5.7 ^a	Could Not Be Calculated	5.7
	E _y C ₅₀	>5.7 ^a	Could Not Be Calculated	5.7
48	E _r C ₁₀	1.3	0.76 and 1.7	0.49
	E _r C ₂₀	4.0	3.4 and 4.6	
	E _r C ₅₀	>5.7	Not Statistically Sound	
	E _y C ₁₀	0.31	0.12 and 0.50	0.49
	E _y C ₂₀	0.76	0.46 and 1.1	
	E _y C ₅₀	3.6	3.0 and 4.2	
72	E _r C ₁₀	1.1	0.65 and 1.5	0.94
	E _r C ₂₀	2.5	2.0 and 3.0	
	E _r C ₅₀	>5.7	Not Statistically Sound	
	E _y C ₁₀	0.57	0.33 and 0.82	0.94
	E _y C ₂₀	0.88	0.60 and 1.2	
	E _y C ₅₀	1.8	1.5 and 2.1	

^a EC value could not be calculated due to lack of convergence. EC50 is estimated to be greater than the highest treatment level due to less than 50% inhibition in growth rate at that treatment.

Conclusions

The test acceptability criteria were met for this study. The number of algal cells in the control at test termination was greater than 16 times the number initially inoculated to verify logarithmic phase growth. The coefficient of variation for daily growth rates in the control replicates during the course of the test did not exceed 35%. The coefficient of variation of average specific growth rates during the whole test period in control replicates did not exceed 7%. The pH in the control did not increase more than 1.5 units during the study. This study satisfies the OECD guideline requirement for a growth inhibition test with *Pseudokirchneriella subcapitata*.

RMS Comment: Despite a slight deviation in the initial pH of the test medium, validity criteria were met and so this deviation from the protocol is deemed acceptable. Due to the fact that nominal concentrations were not achieved, an endpoint based on the geometric mean is proposed. It is considered that the endpoint that should be used for risk assessment is a mean measured 72 hour E_yC₅₀ of 1.8 mg X11406790/L.

Rebstock, M. (2011): GF-2573: Growth Inhibition Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*. ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri 65202. ABC Study No. 65996. Dow AgroSciences unpublished report, Study Number 101124. 29 June 2011.

Test material

Test item:	GF-2573
Purity:	0.84 wt% XDE-729 methyl, 0.83 wt% cloquintocet-mexyl
Description:	Yellow liquid
Lot No./Batch No. :	E2837-83

Test system

Organism (Species):	Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>
Study Type:	laboratory study assessing algal growth static
GLP Status:	GLP (except the water characterization carried out in 2011)
Guidelines followed:	OECD Guideline 201
Guideline deviations reported by Study Director:	None.
Duration of study:	72 hrs
Parameters measured:	Cell Density, Growth Rate
Environmental conditions:	Test solution pH (range): 7.4 to 8.5 Test solution temperature (range): 23.7 to 24.4°C Temperature (range): Within the $24 \pm 2^\circ\text{C}$ specified in the protocol. Photoperiod: Continuous light. Light intensity (range): 8125 to 8230 lux
Observation intervals:	0, 24, 48, 72 hours
Test concentrations:	Nominal: 0 (control), 0.041, 0.12, 0.37, 1.1, 3.3, and 10 mg GF-2573/L Geometric mean calculated concentrations: <MQL (control), 0.026, 0.044, 0.078, 0.20, 0.53, and 1.3 mg GF-2573/L
Age of inoculum	3 days old
Acclimation period/conditions	The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing.
Initial cell density	5.0×10^5 cells/mL
Growth medium	Name: FWAM

	pH at test initiation: 7.5 (control solution) pH at test termination: 8.4 (control solution) Constant stirring?: Yes, all test solutions were swirled on an orbital shaker table at 100 rpm throughout the test.
Method of test item added to the test medium	A 0.010 mg GF-2573/mL primary standard was prepared on 18 April 2011, by transferring 0.0100 g of GF-2573 to a 1000 mL glass volumetric flask, and bringing the flask to volume with test medium. The primary standard was used as the highest test substance treatment. The 0.12, 0.37, 1.1, and 3.3 mg GF-2573/L test substance treatments, each at a volume of 500 mL, were prepared individually using appropriate volumes of the highest test substance treatment and test medium. The 0.041 mg GF-2573/L test treatment was prepared by diluting a 6.2-mL aliquot of the 3.3 mg GF-2573/L test solution to a volume of 500 mL with test medium. The control consisted of test medium only.
No. of control replicates	6
No. of test concentration replicates	3
Analytical verification:	Method: measuring concentrations of XDE-729 Methyl using LC-MS/MS. Samples taken: Test initiation and termination. Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) and was 0.000246 mg XDE-729 Methyl/L or 0.029 mg GF-2573/L.

Methodology

The exposure flasks were 250-mL Erlenmeyer flasks with foam stoppers and labelled with study number, treatment, replicate, and grid position. The control was replicated six times and each test substance treatment was replicated three times. Each replicate contained 100 mL of the appropriate parent solution. An additional replicate of the 0.031 mg/L nominal test substance treatment, containing 100 mL of the appropriate parent solution, was also prepared and used to evaluate the potential for incorporation of the test substance into the algal biomass (abiotic replicate). At test initiation, all biotic replicates were inoculated with 1.0 mL of an algal concentrate containing approximately 5.0×10^6 cells/mL, resulting in a final density of approximately 5.0×10^3 cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. At 24, 48, and 72 hours (± 1 hour), cell density was measured in all replicates of the control, as well as replicates A, B, and C of each test substance treatment by direct microscopic counting with a hemacytometer. The abiotic replicate was not inoculated with algae. A test with GF-2573 blank formulation at concentrations of 0.1, 1.0 and 10 mg blank GF-2573/L was also conducted.

ResultsTable B 9.2.76: Percentage inhibition of growth rate and yield after 3 days

GF-2573 (mg/L)	0-3 d Growth rate	0-3 d Cell density
0	-	-
0.0481	0	-1
0.12	-1	-6
0.37	24	66
1.1	47	88
3.3	89	99
10	92	99

Table B 9.2.77: Effects of GF-2573 on algal growth based on nominal concentrations

Hour	EC Type	EC Value [mg GF-2573/L]	95% Confidence Limits [mg GF-2573/L]	NOEC [mg GF- 2573/L]
72	ErC10	0.26	0.12 to 0.40	
	ErC20	0.44	0.27 to 0.61	
	ErC50	1.1	0.86 to 1.3	Growth rate: 0.12
	EyC10	0.11	0.024 to 0.19	
	EyC20	0.15	0.066 to 0.24	
	EyC50	0.29	0.21 to 0.36	Yield: 0.12

Table B 9.2.78: Comparison of effects of GF-2573 and blank formulation on algal growth

Nominal Concentration mg GF-2573/L	% Inhibition at 72 hrs		
	Cell density	Growth rate	Yield
0.12	-6	-1	-6
1.1	88	47	89
10	99	92	100
Nominal Concentration mg blank/L	% Inhibition at 72 hrs		
	Cell density	Growth rate	Yield
0.10	-3	-1	-3
1.0	84	36	85
10	99	85	99

Table B 9.2.79: Calculated Concentrations of GF-2573 (Based on Measured Concentrations of XDE-729 Methyl) in Test Solutions During the 72-Hour Toxicity Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*

Nominal Concentration (mg GF-2573/L)	Calculated Concentration as mg GF-2573/L (Percent Nominal)		
	0-Hour	72-Hour	Geometric mean
0.041	0.046 (112)	<MQL	0.026 (63)
0.12	0.13 (108)	<MQL	0.044 (37)
0.37	0.41 (111)	<MQL	0.078 (21)
1.1	1.1 (100)	0.036 (3)	0.20 (18)
3.3	3.5 (106)	0.081 (2)	0.53 (16)
10	11 (110)	0.15 (2)	1.3 (13)

MQL- minimum quantifiable limit

Conclusions

The test acceptability criteria were met for this study. The number of algal cells in the control at test termination was greater than 16 times the number initially inoculated to verify logarithmic phase growth. The coefficient of variation for daily growth rates in the control replicates during the course of the test did not exceed 35%. The coefficient of variation of average specific growth rates during the whole test period in control replicates did not exceed 7%. The pH in the control did not increase more than 1.5 units during the study. This study satisfies the OECD guideline requirement for a growth inhibition test with *Pseudokirchneriella subcapitata*.

The results of the additional test with the blank formulation (i.e. identical to GF-2573 but containing no XDE-729 Methyl) demonstrated that the toxicity of GF-2573 can be attributed to the other substances in the formulation and not to the XDE-729 Methyl.

RMS Comment: The study is considered to be acceptable and appropriate for risk assessment purposes. It is noted that the endpoint is presented as nominal concentrations despite the concentrations not being maintained at $\pm 20\%$. The proposed endpoint from the study is an EyC_{50} of 0.29 mg GF-2573/L. This value is based on nominal concentrations. The mean measured concentration around this concentration was approximately 21-37% of nominal. This study is to be used in conjunction with the below study on co-formulant Agnuque ME 1218 to attempt to identify the toxic component of the formulation GF-2573.

Rebstock, M. (2012): [REDACTED]: Growth Inhibition Test with the Unicellular Green Alga, *Pseudokirchneriella subcapitata*. ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri 65202. ABC Study No. 68245. Dow AgroSciences unpublished report, Study Number 120031. 27 April 2012.

Test item:	
Purity:	Not provided
Description:	Colourless liquid
Lot No. :	U20K10U023

Organism (Species):	Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i>
Study Type:	Laboratory study assessing algal growth, static
GLP Status:	GLP (with the exception of the water characterization that was carried out in 2011)
Guidelines followed:	OECD Guideline 201 (2006)
Guideline deviations reported by Study Director:	None.
Duration of study:	72 hrs
Parameters measured:	Cell Density, Growth Rate, Yield
Environmental conditions:	Test solution pH (range): 7.4 – 8.1 Test solution temperature (range): 23.5 to 24.2°C Photoperiod: Continuous light. Light intensity (range): 7,776 to 8,178 lux Stirring: constant at 100 rpm.
Observation intervals:	24, 48, 72 hours
Test concentrations:	Nominal: 0 (control), 0.12, 0.37, 1.1, 3.3, 10 mg [REDACTED] (as loading rates)
Age of inoculum	3 days old
Acclimation period/conditions	The prepared cultures were maintained in a temperature-controlled environmental chamber under continuous light. Periodically, new cultures were cloned from an existing culture derived from the parent stock. All cultures were maintained under the same conditions as those used for testing.
Initial cell density	5.0×10^3 cells/mL
Growth medium	Name: FWAM pH at test initiation: 7.5 (control solution) pH at test termination: 8.1 (control solution)
No. of control replicates	6
No. of test concentration replicates	3
Analytical verification:	Not performed.

Methodology

The control and primary test solutions were prepared as water accommodated fraction (WAF) solutions. WAF solutions were prepared by adding 0.00024, 0.00074, 0.0022, 0.0066, and 0.0200 g of [REDACTED] to 2.0 L volumes of freshwater algal medium (FWAM) in an autoclaved glass aspirator bottle containing an autoclaved Teflon stir bar for final nominal loading rates of 0.12, 0.37, 1.1, 3.3, and 10 mg [REDACTED], respectively. The WAF solutions were stirred in an environmentally controlled chamber at the approximate conditions to be used for the definitive test for approximately 24 hours with the stirring adjusted to provide a vortex <25% of the solution depth. Solutions were then allowed to settle for approximately 1 hour before being drained from the outlet of the aspirator bottle into an appropriately sized autoclaved glass container. The first approximately 100 mL of solution from each preparation was allowed to drain into a waste container.

The exposure flasks were appropriately labelled 250-mL Erlenmeyer flasks with foam stoppers. The control was replicated six times and each test substance treatment was replicated three times. Each replicate contained 100 mL of the appropriate parent solution. At test initiation replicates were inoculated with an algal concentrate resulting in a final density of approximately 5.0×10^3 cells/mL for each flask. The replicates were inoculated with algae within 30 minutes after test solution preparation. All replicates were swirled using an orbital shaker at 100rpm throughout the test. At 24, 48, and 72 hours (± 1 hour), cell density was measured in all replicates of the control, as well as replicates A, B, and C of each test substance treatment by direct microscopic counting with a hemacytometer. Temperature was monitored constantly throughout the test in a blank flask incubated alongside the test vessels. PH was measured after 0 hours in parent media and in replicate A of each treatment group after 72 hours of exposure.

Results

After 72 hours of exposure the mean control cell density had increased to 156 times the nominal initial cell density (78.1×10^4 cells/mL). The coefficient of variation of average specific growth rates during the whole test period in control replicates was 1%. The mean coefficient of variation for section-by-section specific growth rates was 12% for the control replicates. The test, therefore, fulfils the validity criteria of OECD Guideline 201 after 72-hours of exposure. Inhibition of cell growth rate and yield compared to the control group are presented in table 1. Calculated end points for effects of [REDACTED] on algal growth are presented in table 2.

Table B 9.2.80: Percentage inhibition of growth rate and cell density after 72-hours

(mg/L)	0-72 hours Growth rate	0-72 hours Yield
0	-	-
0.12	0	1
0.37	17	56
1.1	31	80
3.3	56	94
10	70	98

Table B 9.2.81: Effects of [REDACTED] on algal growth based on nominal loading rates

Hour	EC Type	EC Value ^a [mg/L]	95% Confidence Limits [mg/L]	NOEC [mg /L]
72	E _r C10	0.16	0.057 to 0.26	Growth rate: 0.12
	E _r C20	0.46	0.26 to 0.67	
	E _r C50	2.9	2.3 to 3.4	
	E _y C10	<0.12	Not statistically sound	Yield: 0.12
	E _y C20	0.14	0.078 to 0.19	
	E _y C50	0.37	0.29 to 0.45	

E_rCXX – XX% effect on growth rateE_yCXX – XX% effect of yield

Conclusions

In a 72 hour growth inhibition test with *Pseudokirchneriella subcapitata* the E_rC₅₀ and the E_yC₅₀, based on nominal concentrations, were calculated to be 2.9 and 0.37 mg [REDACTED] respectively. The NOEC for growth rate and yield was 0.12 mg [REDACTED]. All validity criteria were met in accordance with OECD guideline 201.

RMS Comment: This study resulted in an E_yC₅₀ almost the same as that obtained for GF-2573 and is provided only to help demonstrate that the toxicity of GF-2573 to *P. subcapitata* is not attributable to XDE-729 Methyl, but is most likely attributable to the methylated seed oil adjuvant [REDACTED] within the representative formulation. As such, no analytical confirmation of test substance concentration was performed on this study.

B.9.2.1.1.4 Aquatic plants**Active substance**

Rebstock, M. (2011): XDE-729 Methyl: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, *Lemna gibba*, ABC 64595, Dow AgroSciences unpublished report, Study Number 090182. 10 May 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Lot No./Batch No. :	E2837-51

Test system

Organism (Species):	Freshwater aquatic plant, <i>Lemna gibba</i>
Study Type:	Static Renewal
GLP Status:	GLP (except for the water characterisation that was performed in 2009)
Guidelines followed:	OECD guideline 221
Guideline deviations reported by Study Director:	None
Duration of study:	7 days
Test conditions:	Static Renewal, renewals daily
Parameters measured:	Frond Count, Dry weight
Observation intervals:	Days 3, 5, 7
Age of fronds at test initiation:	7 days
Number of fronds at test initiation:	12 per rep
Test concentrations:	Nominal: 0 (control), 0 (vehicle control; 0.10 mL DMF/L), 0.25, 0.50, 1.0, 2.0, and 3.9 mg a.s./L
	Geometric Mean measured: <MQL (control), <MQL (vehicle control; 0.10 mL DMF/L), 0.121, 0.237, 0.499, 1.04, and 2.27 mg a.s./L
Analytical confirmation of test concentrations:	0, 1, 3, 4, 6, and 7 days
Number of fronds per dose group:	36
Number of fronds per control group:	36
Feeding:	None

Environmental conditions:	Temperature: 23.5 to 25.1°C Photoperiod: 24 hour light Growth medium: 20X-AAP pH of fresh solutions: 7.5 to 7.6 as measured on days 0 - 6 pH of spent solutions: 8.4 to 9.1 as measured on days 1 - 7
Analytical verification:	Method: measuring concentrations of XDE-729 Methyl and XDE-729 Acid using HPLC-MS/MS. Samples taken: Test initiation and termination of each interval in fresh and spent solutions. Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) and was 0.238 ng XDE-729 Methyl/mL or 0.246 ng XDE-729 Acid/mL.

Methodology

A 7 day static renewal test with the freshwater aquatic plant, *Lemna gibba* was performed with nominal test concentrations of 0 (control), 0 (vehicle control;), 0.25, 0.50, 1.0, 2.0, and 3.9 mg a.s./L. Each test flask received three plants for a total of 12 fronds at test initiation. There were three replicates per test treatment, resulting in 36 fronds per test treatment. Frond observations and counts were made at 3, 5, and 7 days, with renewals daily.

The concentration of XDE-729 Methyl and XDE-729 Acid were measured in fresh test solution samples collected on days 0, 3 and 6, and in spent test solution samples collected on days 1, 4 and 7. Temperature and pH were measured in all fresh parent solutions, prior to distribution of the solutions to the test flasks, on days 0, 1, 2, 3, 4, 5, and 6. On days 1, 2, 3, 4, 5, 6, and 7, temperature and pH were measured in one replicate of all treatment spent solutions. Biomass (dry weight) measurements of each control and test substance treatment replicate were performed on day 7 (test termination).

Results

The measured XDE-729 Methyl concentrations in fresh test substance treatment solutions ranged from 97 to 108% of nominal concentrations. The measured XDE-729 Methyl concentrations in spent test substance treatment solutions ranged from 18 to 41% of the nominal concentrations. Since not all measured concentrations were $\pm 20\%$ of the nominal concentrations, all endpoints were calculated based on the geometric mean of the measured XDE-729 Methyl concentrations. Analytical results are presented in Table B 9.2.82.

Table B 9.2.82 Measured concentrations of XDE-729 Methyl and Acid

Nominal concentration XDE-729 Methyl (mg/L)	Day 0		Day 1 (old)		Day 3 (new)		Day 4 (old)	
	Methyl	Acid	Methyl	Acid	Methyl	Acid	Methyl	Acid
0.25	0.261	NC	0.0472	0.0102	0.253	NC	0.0607	0.0115
0.50	0.481	NC	0.0877	0.0160	0.487	NC	0.132	0.0235
1.0	1.00	NC	0.183	0.0322	1.06	NC	0.264	0.0409
2.0	1.91	NC	0.392	0.0676	2.06	NC	0.648	0.112
3.9	3.87	NC	0.992	0.155	4.02	NC	1.60	0.209

Nominal concentration XDE-729 Methyl (mg/L)	Day 6 (new)		Day 7 (old)		Geometric mean methyl concentration (mg/L) (%)
	Methyl	Acid	Methyl	Acid	
0.25	0.257	NC	0.0636	0.0258	0.121 (48%)
0.50	0.504	NC	0.130	0.0531	0.237 (47%)
1.0	1.03	NC	0.292	0.128	0.499 (50%)
2.0	0.112	NC	0.620	0.207	1.04 (52%)
3.9	2.09	NC	1.42	0.390	2.27 (58%)

Table B 9.2.83: Percentage inhibition of growth rate and yield after 7 days

Mean measured XDE-729 Methyl (mg/L)	% Growth rate inhibition		% Yield inhibition	
	Frond number	Dry weight (biomass)	Frond number	Dry weight (biomass)
Control (0)	-	-	-	-
Vehicle control	-	-	-	-
Pooled control	-	-	-	-
0.121	0	0	1	1
0.237	1	0	3	0
0.499	5	-1	14	-3
1.04	14	1	37	4
2.27	21	5	50	12

negative value mean growth promotion

Prior to the EC and NOEC calculations, the control and vehicle control groups were compared and no statistically significant differences identified for the measured

endpoints. Therefore, the control and vehicle control data were pooled for all comparisons to the treatments. Day 7 results are summarized below in Table B 9.2.84.

Table B 9.2.84: Results based on geometric mean calculated concentrations from the *Lemna* study

Endpoint	Parameter Effect Concentration as mg XDE-729 Methyl/L			
	Frond Yield (95% CL)	Frond Average Specific Growth Rate (95% CL)	Biomass Yield as Dry Weight (95% CL)	Biomass Average Specific Growth Rate as Dry Weight (95% CL)
NOEC	0.121	0.121	2.27	2.27
LOEC	0.237	0.237	>2.27	>2.27
EC10	0.269 (0.173 to 0.365)	0.813 (0.660 to 0.967)	2.02 (1.55 to 2.48)	>2.27
EC20	0.578 (0.451 to 0.704)	1.99 (1.82 to 2.16)	>2.27	>2.27
EC50	2.13 (1.88 to 2.38)	>2.27	>2.27	>2.27

The test acceptability criteria for control growth (i.e., frond doubling time < 2.5 days, greater than a seven-fold increase in the number of fronds) set by OECD 221 test guideline were met for this study. The doubling time for the control fronds was 1.5 days, corresponding to a 20-fold increase in the number of fronds, and the average specific growth rate was 0.430 day⁻¹.

Conclusions

The lowest determined 7-day EC50 for *Lemna gibba* exposed to XDE-729 Methyl under daily-renewal conditions was 2.13 mg/L based on frond yield. The 7-day EC50 values for biomass yield and specific growth rate were >2.27 mg/L, which was the highest test concentration and maximum functional solubility for XDE-729 Methyl under the test conditions.

RMS Comment: Due to the fact that concentrations were not maintained, an endpoint based on the geometric mean is proposed. It is considered that the endpoint that should be used for risk assessment is the mean measured 7-day EC50 of 2.13 mg/L based on frond yield.

Rebstock, M. (2011): XDE-729 Acid: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, *Lemna gibba*, ABC 65968. Dow AgroSciences unpublished report, Study Number 101145. 16 June 2011.

Test material

Test item:	XDE-729 Acid
Purity:	95.3 wt %
Description:	Off-white solid
Lot No./Batch No. :	E2837-52

Test system

Organism (Species):	Freshwater aquatic plant, <i>Lemna gibba</i>
Study Type:	Static Renewal
GLP Status:	GLP (except the water characterisation)
Guidelines followed:	OECD guideline 221
Guideline deviations reported by Study Director:	None
Duration of study:	7 days
Test conditions:	Static Renewal, renewals days 3 and 5
Parameters measured:	Frond Count, Dry weight
Observation intervals:	Days 3, 5, 7
Age of fronds at test initiation:	7 days
Number of fronds at test initiation:	12 per rep
Test concentrations:	Nominal: 0 (control), 0.51, 1.3, 3.2, 8.0, 20, and 50 mg a.s./L
	Mean measured: <MQL (control), 0.505, 1.30, 3.27, 8.26, 19.6, and 50 mg a.s./L
Analytical confirmation of test concentrations:	0, 3, 5, and 7 days
Number of fronds per dose group:	36
Number of fronds per control group:	36
Feeding:	None
Environmental conditions:	Temperature: 23.1 to 24.2°C Photoperiod: 24 hour light Growth medium: 20X-AAP pH of fresh solutions: 7.4 to 7.6 as measured on days 0, 3, 5 pH of spent solutions: 8.4 to 8.9 as measured on days 3, 5, 7

Analytical verification:	Method: measuring concentrations of XDE-729 Acid HPLC-UV. Samples taken: Test initiation and termination of each interval in fresh and spent solutions. Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) and was 0.204 mg XDE-729 Acid/L
--------------------------	--

Methodology

A 7 day static renewal test with the freshwater aquatic plant, *Lemna gibba* was performed with nominal test concentrations of 0 (control), 0.51, 1.3, 3.2, 8.0, 20, and 50 mg a.s./L. Each test flask received three plants for a total of 12 fronds at test initiation. There were three replicates per test treatment, resulting in 36 fronds per test treatment. Frond observations and counts were made at 3, 5, and 7 days, with renewals on day 3 and 5.

The concentration of XDE-729 Acid was measured in fresh test solution samples collected on days 0, 3 and 5, and in spent test solution samples collected on days 3, 5 and 7. Temperature and pH were measured in all fresh parent solutions, prior to distribution of the solutions to the test flasks, on days 0, 3, and 5. On days 3, 5, and 7, temperature and pH were measured in replicate A of all treatment spent solutions. Biomass (dry weight) measurements of each control and test substance treatment replicate were performed on day 7 (test termination).

Results

Measured XDE-729 Acid concentrations ranged from 96 to 114% of nominal concentrations in fresh test substance treatment solutions and from 82 to 102% of the nominal concentrations in spent test substance solutions. Since measured concentrations were $\pm 20\%$ of the nominal concentrations, all end-points were calculated based on the nominal XDE-729 Acid concentrations. Day 7 results are summarized in Table B 9.2.85.

Table B.9.2.85: Results based on nominal concentrations from the *Lemna* study

Endpoint	Parameter Effect Concentration as mg XDE-729 Acid/L			
	FronD Yield (95% CL)	FronD Average Specific Growth Rate (95% CL)	Biomass Yield as Dry Weight (95% CL)	Biomass Average Specific Growth Rate as Dry Weight (95% CL)
NOEC	1.3	1.3	50	50
LOEC	3.2	3.2	>50	>50
EC10	<0.51	Could not be calculated ^a	13 (0 to 33)	Could not be calculated ^a
EC20	1.1 (0.42 to 1.8)	Could not be calculated ^a	34 (12 to 56)	Could not be calculated ^a
EC50	15 (11 to 18)	>50 ^a	>50	>50 ^a

^a Could not be calculated due to lack of convergence. Estimated EC50 value based on percent inhibition for each parameter compared to the control.

The mean number of total fronds during a 7-day exposure of the duckweed, *Lemna gibba* to XDE-729 Acid are presented in Table B 9.2.86 below.

Table B 9.2.86: Mean number of total fronds during a 7-day exposure of the duckweed, *Lemna gibba* to XDE-729 Acid

Nominal concentration (mg acid/L)	Mean number of total fronds			% inhibition
	Day 3	Day 5	Day 7	
Control	37.0	81.0	194	-
0.51	34.7	79.0	181	7
1.3	32.3	73.3	161	17
3.2	33.0	66.3	138	29
8.0	34.0	58.0	111	43
20	32.0	47.7	91.3	53
50	28.0	42.7	79.3	50

Table B 9.2.86a: Mean frond biomass yield (as dry weight) during a 7-day exposure of the duckweed, *Lemna gibba* to XDE-729 Acid

Nominal concentration (mg acid/L)	Mean biomass yield (g)	% inhibition
	Day 7	
Control	0.0269	-
0.51	0.0259	0
1.3	0.0254	2
3.2	0.0268	-3
8.0	0.0255	2
20	0.0211	19
50	0.0196	24

Conclusions

The test acceptability criteria for control growth (i.e., frond doubling time < 2.5 days, greater than a seven-fold increase in the number of fronds) set by OECD 221 test guideline were met for this study. The doubling time for the control fronds was 1.6 days, corresponding to a 16-fold increase in the number of fronds, and the average specific growth rate was 0.397 day⁻¹. This study is classified as acceptable and satisfies the guideline requirement for a growth inhibition test with *Lemna gibba*.

RMS Comment: The study is considered acceptable for risk assessment purposes and the proposed endpoint is a mean measured EyC50 of 15 mg/L XDE-729 Acid for frond yield.

Rebstock, M. 2011: X11449757: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, *Lemna gibba*. ABC 66011. Dow AgroSciences unpublished report, Study Number 101159. 14 September 2011.

Test material

Test item:	X11449757
Purity:	98.6%
Description:	Off-white solid
Lot No./Batch No. :	YB1-100780-103

Test system

Organism (Species):	Freshwater aquatic plant, <i>Lemna gibba</i> (Duckweed)
Study Type:	Static-renewal
GLP Status:	GLP (except the water characterisation performed February 2011)
Guidelines followed:	OECD guideline 221
Guideline deviations	None

reported by Study Director:	
Duration of study:	7 days
Test conditions:	Static Renewal, renewals days 2, 4 and 6
Parameters measured:	Frond Count, dry weight, sub-lethal observations
Observation intervals:	Days 3, 5 and 7 (frond counts and sub-lethal observations), day 7 only (dry weight)
Age of fronds at test initiation:	7 days (since frond transfer to fresh nutrient medium)
Number of fronds at test initiation:	12 per replicate
Test concentrations:	Nominal: 0 (control), 6.3, 13, 25, 50 and 100 X11449757/L
	Mean measured: <MQL (control), 4.75, 11.4, 21.5, 44.8 and 92.9 mg X11449757/L
Analytical confirmation of test concentrations:	0, 2, 4, and 6 days
Number of replicates per dose and control group	3
Number of fronds per dose and control group:	36
Feeding:	None
Growth medium	20X AAP
Environmental conditions:	Temperature: 22.6 to 25.1°C Photoperiod: 24 hour (continuous) light Light intensity: 7,377 to 8,737 lux pH: 7.1 to 8.9
Analytical verification:	Method: measuring concentrations of X11449757 using HPLC-UV. Samples taken: Test initiation and termination of each interval in fresh and spent solutions. Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) and was 0.0404 mg X11449757/L.

Methodology

A 7-day static-renewal test with the freshwater aquatic plant, *Lemna gibba* was performed with nominal test concentrations of 0 (control), 6.3, 13, 25, 50 and 100 mg X11449757/L. Primary standards at a concentration of 0.10 mg X11449757/mL were prepared by transferring approximately 0.2020 g of X11449757 (0.2000 g corrected for purity) to a 2,000 mL glass volumetric flask, and bringing the flask to volume with test medium. The primary standards were used to prepare the highest test substance treatment. The four lower test substance treatments, each at a volume of 1,000 mL, were prepared individually using

appropriate volumes of the highest test concentration. Control replicates contained test medium only.

The test chambers used for the test were appropriately labelled 500-mL Erlenmeyer flasks with foam stoppers. Prior to test initiation, the flasks were cleaned and autoclaved. The control and each test substance treatment were replicated three times and each replicate contained 200 mL of the appropriate test solution. Each test flask received three plants for a total of 12 fronds, resulting in 36 fronds per test treatment. Frond observations and counts were made at 3, 5, and 7 days, with media renewals on days 2, 4 and 6.

The concentration of X11449757 was measured in fresh test solution samples collected on days 0, 2, 4 and 6, and in spent test solution samples collected on days 2, 4 and 6. Temperature and pH were measured in all fresh parent solutions, prior to distribution of the solutions to the test flasks, on days 0, 2, 4, and 6. On days 2, 4, 6 and 7, temperature and pH were measured in replicate A of all treatment spent solutions. Biomass (dry weight) measurements of each control and test substance treatment replicate were performed on day 7 (test termination).

Results

The measured X11449757 concentrations in fresh test substance treatment solutions ranged from 89 to 118% of nominal concentrations. The measured X11449757 concentrations in spent test substance treatment solutions ranged from 53 to 92% of the nominal concentrations. Since not all measured concentrations were $\pm 20\%$ of the nominal concentrations, all end-points were calculated based on the geometric mean of the measured X11449757 concentrations.

The doubling time of total frond number in the control was 1.6 days, corresponding to approximately a 17-fold increase in seven days, which met the doubling time requirement established by the OECD 221 test guideline (i.e., < 2.5 days, corresponding to approximately a seven-fold increase in seven days). The control average specific growth rate for total number of fronds on day 0 to 7 was 0.405 per day, exceeding the 0.275 per day minimum required by the test guideline. The coefficient of variation for the control average specific growth rate from day 0 to day 7 was 4%. Temperatures were maintained within $24 \pm 2^\circ\text{C}$ and control pH varied by < 1.5 units (7.5-8.8).

The percent inhibition of frond average specific growth rate as compared to the control was -3, -4, -1, 7, and 11% for the 4.75, 11.4, 21.5, 44.8, and 92.9 mg X11449757/L test substance treatments respectively (based upon mean measured concentrations). Based on the average specific growth rate, the ErC_{10} , ErC_{20} , and ErC_{50} values on day 7 were estimated to be 84.1, > 92.9 , and > 92.9 mg X11449757/L, respectively. The percent inhibition of dry weight biomass yield, as compared to the control, was -6, -6, -3, 4, and -8% for the 4.75, 11.4, 21.5, 44.8, and 92.9 mg X11449757/L test substance treatments. Based on biomass yield, the EyC_{10} , EyC_{20} and EyC_{50} values on day 7 were all estimated to be > 92.9 mg X11449757/L.

Table B 9.2.87: Results of chemical analysis

Sample day (solution age)	Measured concentration of X11449757 expressed as mg X11449757/L (% nominal)					
	Control (0)	6.3	13	25	50	100
0 (fresh)	<MQL	6.55 (104)	15.3 (118)	28.0 (112)	48.8 (98)	94.7 (95)
2 (spent)	<MQL	3.62 (57)	8.99 (69)	17.5 (70)	45.8 (92)	91.9 (92)
2 (fresh)	<MQL	5.65 (90)	14.4 (111)	29.4 (118)	51.6 (103)	116 (116)
4 (spent)	<MQL	3.62 (57)	9.42 (72)	16.4 (66)	37.2 (74)	81.7 (82)
4 (fresh)	<MQL	6.10 (97)	12.7 (98)	23.4 (94)	48.2 (96)	94.1 (94)
6 (spent)	<MQL	3.32 (53)	9.02 (69)	16.5 (66)	37.1 (74)	82.5 (83)
6 (fresh)	<MQL	5.58 (89)	11.9 (92)	23.0 (92)	47.4 (95)	93.0 (93)
Mean measured concentration	<MQL	4.75 (75)	11.4 (88)	21.5 (86)	44.8 (90)	92.9 (93)

MQL- minimum quantifiable limit

Table B 9.2.88: Mean number of total fronds during a 7-day exposure of the duckweed, *Lemna gibba* to X11449757

Mean measured concentration (mg X11449757/L)	Mean number of total fronds			% inhibition
	Day 3	Day 5	Day 7	
Control (0)	34.7	83.3	206	-
4.75	36.0	81.7	220	-7
11.4	36.7	88.3	229	-11
21.5	35.7	84.3	210	-2
44.8	34.3	72.0	169	18
92.9	36.3	69.7	151	27

Table B 9.2.88a: Mean frond biomass yield (as dry weight) during a 7-day exposure of the duckweed, *Lemna gibba* to X11449757

Mean measured concentration (mg X11449757/L)	Mean biomass yield (g)	% inhibition
	Day 7	
Control (0)	0.0415	-
4.75	0.0439	-6
11.4	0.0441	-6
21.5	0.0426	-3
44.8	0.0397	4
92.9	0.0449	-8

Table B 9.2.89: Results based on geometric mean calculated concentrations from the *Lemna gibba* study

Endpoint	Parameter Effect Concentration as mg X11449757/L			
	Frond Yield (95% CL)	Frond Average Specific Growth Rate (95% CL)	Biomass Yield as Dry Weight (95% CL)	Biomass Average Specific Growth Rate as Dry Weight (95% CL)
NOEC	44.8	44.8	92.9	92.9
LOEC	92.9	92.9	>92.9	>92.9
EC ₁₀	33.5 (8.35-58.6)	84.1 (67.0-101)	>92.9 (NC)	>92.9 (NC)
EC ₂₀	62.7 (42.6-82.8)	>92.9 (limits not sound)	>92.9 (NC)	>92.9 (NC)
EC ₅₀	>92.9 (limits not sound)	>92.9 (limits not sound)	>92.9 (NC)	>92.9 (NC)

NC – Not Calculated

Conclusions

The test acceptability criteria for control growth (i.e., frond doubling time < 2.5 days; greater than a seven-fold increase in the number of fronds) set by OECD 221 test guideline were met for this study. The doubling time for the control fronds was 1.6 days, corresponding to a 17-fold increase in the number of fronds, and the average specific growth rate was 0.405 per day. All EC₅₀ values calculated were observed to be >92.9 mg X11449757/L, based on mean measured concentrations. This study is classified as acceptable and satisfies the guideline requirement for a growth inhibition test with *Lemna gibba*.

RMS Comment: The study is considered acceptable for risk assessment purposes with the proposed endpoint of a mean measured EC₅₀ of >92.9 mg X11449757/L for all measured endpoints.

Rebstock, M. 2012: X11406790: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, *Lemna gibba*, ABC 68209. ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri 65202. ABC Study No. 68209. Dow AgroSciences unpublished report, Study Number 120022. 31st May 2012.

Test material

Test item:	X11406790
Purity:	95%
Description:	White solid
Lot No./Batch No. :	SYN-FS08644-062

Test system

Organism (<i>Species</i>):	Freshwater aquatic plant, <i>Lemna gibba</i> (G3 clone)
Study Type:	Semi- static, renewals day 3 and 5
GLP Status:	GLP (with the exception of the last water characterization performed in February 2012)
Guidelines followed:	OECD guideline 221
Guideline deviations reported by Study Director:	Initial pH of 8.0 ± 0.1 rather than 7.5 ± 0.1 Temperature of the freshly prepared 12 mg/L treatment on day 5 was 27.4°C which falls outside of the specified temperature range ($24 \pm 2^\circ\text{C}$).
Duration of study:	7 days
Parameters measured:	Frond count, growth rate, yield, biomass (dry weight)
Observation intervals:	Days 3, 5, 7

Age of fronds at test initiation:	7 days
Number of fronds at test initiation:	11 per rep
Acclimation period/conditions:	The parent stock was identified by the supplier as <i>Lemna gibba</i> G3 clone. The <i>Lemna gibba</i> cultures used to inoculate the definitive test were transferred to fresh nutrient medium seven days prior to study initiation and the number of fronds in the cultures had increased approximately 8-fold in seven days.
Test concentrations:	Nominal: 0 (control), 1.0, 2.0, 4.0, 8.0, and 16 mg X11406790/L Geometric mean measured: <MQL (control), 0.67, 1.4, 2.7, 5.5, and 12 mg X11406790/L
Analytical confirmation of test concentrations:	0, 3, 5, and 7 days
Number of fronds per dose group:	33 (3 replicates of 11 fronds)
Number of fronds per control group:	33(3 replicates of 11 fronds)
Feeding:	None
Environmental conditions:	Temperature: 23.2 to 27.4°C Photoperiod: 24 hour light Growth medium: 20X-AAP pH of fresh solutions: 7.9 to 8.0 pH of spent solutions: 8.6 to 8.9
Analytical verification:	Method: measuring concentrations of X11406790 using HPLC-UV. Samples taken: Fresh parent solutions- day 0, 3 and 5. Spent solutions- day 3, 5 and 7. Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) and was 0.020 mg a.i./L.

Methodology

A 7 day static renewal test with the freshwater aquatic plant, *Lemna gibba* was performed with nominal test concentrations of 0 (control), 1.0, 2.0, 4.0, 8.0, and 16 mg X11406790/L. The test chambers used for the test were 500-mL Erlenmeyer flasks with foam stoppers. Prior to test initiation, the flasks were cleaned and autoclaved according to ABC standard operating procedures.

Each test flask received three plants, for a total of 11 fronds, at test initiation. The control and each test substance treatment were replicated three times and each replicate contained 200 mL of the appropriate test solution. The flasks were randomly positioned on each renewal day and incubated in a temperature-controlled environmental chamber. The temperature and light intensity within the

environmental chamber were measured on test solution renewal days. Growth was measured by determining the change in the number of total fronds during the exposure period. Every frond that visibly projected beyond the edge of the parent frond was counted as a separate frond. Any change in plant development, frond size, appearance, necrosis or chlorosis was noted if observed. Frond observations and counts were performed on days 3, 5, and 7 for all replicates of the controls and each test substance treatment.

The concentrations of X11406790 were measured in fresh test solution samples collected on days 0, 3 and 5, and in spent test solution samples collected on days 3, 5, and 7. Temperature and pH were measured in all fresh parent solutions, prior to distribution of the solutions to the test flasks, on days 0, 3, and 5. On days 3, 5, and 7, temperature and pH were measured in one replicate of all treatment spent solutions. Biomass (dry weight) measurements of each control and test substance treatment replicate were performed on day 7 and also on three representative samples at test initiation. A representative sample comprised of three plants, 11 fronds per sample.

Results

The measured concentrations were not within 20% of the nominal test concentrations (fresh- 64 to 80%, spent-53 to 88%) and so all end-points were calculated based on the geometric mean measured concentrations.

Table B 9.2.90: Measured concentrations of X11406790 during a 7-day growth Inhibition toxicity test with Duckweed, *Lemna gibba*

Nominal concentration (mg X11406790/L)	Calculated Concentration as mg X11406790/L (Percent Nominal)						
	Day 0	Day 3 (old)	Day 3 (new)	Day 5 (old)	Day 5 (new)	Day 7 (old)	Geometric mean
Control	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
1.0	0.66 (66)	0.53 (53)	0.76 (76)	0.62 (62)	0.80 (80)	0.67 (67)	0.67 (67)
2.0	1.3 (65)	1.2 (60)	1.5 (75)	1.3 (65)	1.5 (75)	1.4 (70)	1.4 (70)
4.0	2.7 (68)	2.5 (63)	2.9 (73)	2.7 (68)	2.9 (73)	2.7 (68)	2.7 (68)
8.0	5.1 (64)	5.2 (65)	5.6 (70)	5.4 (68)	5.9 (74)	5.7 (71)	5.5 (69)
16	11 (69)	14 (88)	11 (69)	13 (81)	12 (75)	13 (81)	12 (75)

MQL- minimum quantifiable limit

Table B 9.2.91: Percentage inhibition of total fronds, growth rate and yield of total fronds after 72-hours

Geometric mean measured concentration (mg X11406790/L)	% Inhibition (as compared to the control)				
	Total fronds 72 hours	Growth rate 72 hours	Yield of total fronds 72 hours	Biomass yield (dry weight)	Biomass growth rate (dry weight)
Control	-	-	-	-	-
0.67	-1	-1	-1	6	2
1.4	8	3	8	-2	-1
2.7	-5	-2	-5	-16	-7
5.5	24	11	26	7	3
12	38	20	42	13	7

Table B 9.2.92: Results based on geometric mean measured concentrations of X11406790

Endpoint	Concentration (mg X11406790/L) for Day 7			
	FronD Yield (95% CL)	FronD Average Specific Growth Rate (95% CL)	Biomass Yield as Dry Weight (95% CL)	Biomass Average Specific Growth Rate as Dry Weight (95% CL)
NOEC	2.7	2.7	12	12
LOEC	5.5	5.5	>12	>12
E _y C ₁₀	2.9 (0.62 to 5.2)	-	Could Not Be Calculated ^a	-
E _r C ₁₀	-	6.2 (3.4 to 9.0)	-	Could Not Be Calculated ^a
E _y C ₂₀	5.3 (2.9 to 7.7)	-	Could Not Be Calculated ^a	-
E _r C ₂₀	-	>12	-	Could Not Be Calculated ^a
E _y C ₅₀	>12	-	>12 ^a	-
E _r C ₅₀	-	>12	-	>12 ^a

^a EC values could not be calculated due to lack of convergence. EC₅₀ is estimated to be greater than the highest treatment level due to less than 50% inhibition at that treatment

Conclusions

The test acceptability criteria were met for this study. The doubling time of total frond number in the control was 1.96 days, which met the doubling time requirement established by the OECD guideline of less than <2.5 days. The control average specific growth rate for total number of fronds was 0.353 day⁻¹, exceeding the 0.275 day⁻¹ minimum requirement. The coefficient of variation for the control average specific growth rate was 6%.

RMS Comments: Despite a slight deviation in the initial pH of the medium and also the temperature falling outside of the specified range for the 12 mg/L (mean measured) fresh solution on day 5, validity criteria were still comfortably met and so these slight deviations are deemed acceptable. The 7-day E_RC₅₀ and E_yC₅₀ values for all endpoints tested were >12 mg X11406790/L based on mean measured concentrations, which was the highest concentration tested.

Gonsior, G. (2012): XDE-729 Methyl - Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System. Eurofins Agrosience Services, EcoChem GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany. Dow AgroSciences unpublished report no: S11-02965. DAS Study No: 102023. 15 June 2012.

Test material

Name:	XDE-729 Methyl
Test substance no.:	TSN031117-0004
Batch no.:	E2837-51
Purity (analysed):	97.2 %
Appearance/colour:	solid/white

Test system

Organism (<i>Species</i>):	<i>Myriophyllum spicatum</i>
Study Type:	Growth Inhibition of <i>Myriophyllum spicatum</i> in a static Water/Sediment System
GLP Status:	GLP
Guidelines followed:	Based on the draft AMRAP Method (MALTBY et al. 2010): Growth Inhibition Test for the Rooted Aquatic Macrophyte, <i>Myriophyllum</i> sp., submitted to OECD for evaluation, 2011.
Guideline deviations reported by Study Director:	Deviations listed in the RMS comments.
Duration of study:	14 days
Parameters measured:	Biomass (fresh weight, dry weight, shoot length), growth rate and yield.
Environmental conditions:	Test vessels were maintained in a controlled environment at 20.3 ± 0.9 °C under warm and/or cool white fluorescent lighting (approx. 8000 lux at the water surface) with a 16-h day-length. The

	average pH-value was determined to be 7.18 ± 0.46 and the oxygen saturation was determined to be 95 ± 15 %. The test item had no influence on the pH-value of the test solutions.
Observation intervals:	0, 7, 14 days
Test concentrations:	Nominal: 0 (control), 0 (vehicle control), 0.0617, 0.185, 0.556, 1.67, 5.00 and 100 µg/L.
Growth medium	SMART AND BARKO medium
Method of test item added to the test medium	Stock solution: soluble in DMF Final concentration- 150 µl of DMF stock solution per 1.5 L test medium
No. of control/solvent control replicates	10
No. of test concentration replicates	5
Analytical verification:	At initiation and termination during the definitive test. Additional vessels were prepared for further chemical analysis. An analytical method for the determination of XDE-729 Methyl, XDE-729 Acid X11406790 and X11449757 was validated with regard to recovery (accuracy), linearity of detector response, repeatability (precision) and specificity. The analytical system fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000

Methodology

Seven days prior to test initiation, submerged apical shoots (without side shoots) were planted in an aquarium, in artificial sterilised sediment overlaid with SMART& BARKO medium. From this sufficient uniform material was generated for use in the following test.

For the definitive test, a 14 day growth inhibition test was conducted at nominal concentrations of 0 (control), 0 (vehicle control), 0.0617, 0.185, 0.556, 1.67, 5.00 and 100 µg/L. Moist artificial sediment was prepared and 350 g of this was added to each 2 L glass beaker. This sediment was further overlaid with a thin layer of washed quartz sand to minimise displacement of the sediment when the medium was added. Afterwards the test vessels were filled carefully with growth medium (1.5 L). One day after preparation of the test vessels and before application one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Test vessels were arranged in a completely randomised design and were re-randomised several times during the study.

Additional vessels were prepared for the chemical analysis, one set containing sediment and plants and an additional set containing no sediment or plants both in growth medium with a test substance at the highest concentration. Analytical

confirmation from these additional vessels was measured at day 0, 1, 3, 7, 14. Sediment from the first set was also sampled at termination.

The stock solution was prepared by dissolving the test item in Dimethylformamide (DMF) and thus a vehicle control was included along with a negative control. Shortly after planting, the test item was applied by stirring it gently into the growth medium. Ten replicates were used for the control and solvent control and five for each treatment group.

A representation of additional plants was selected at initiation in order to assess plant growth. These plants were blotted dry prior to assessment of plant fresh weight and shoot length. The plants were placed separately in labelled glass beakers and dried at 60 °C for > 48 hours. The weight of the dry plant samples was recorded.

Shoot height in each replicate was measured on days 7 and 14. At termination (day 14) plants were harvested from each treatment group for assessment of plant fresh weight, plant dry weight, shoot length and number and length of side shoots. In addition observations on shoot and root development (e.g. necrosis, deformation) were documented.

Water temperature, pH and dissolved oxygen content were recorded on days 0, 7 and 14. Light intensity at the water surface was measured once during the test.

Results

Measured test concentrations in the overlying water immediately after treatment were 0.0583, 0.193, 0.649, 1.90, 5.24 and 110 µg/L, which represented 94- 117 % of nominal. As the mean content of the test item was between 80 and 120 % of nominal at test start, the toxicological endpoints were evaluated using nominal concentrations of the test item. Over 14 days the concentration of XDE-729 Methyl in the overlying water declined due to adsorption to sediment and aqueous photolysis. (Hellstern, J. (2012) report was submitted by Notifier in IIA 8.6/9 and evaluated by environmental fate). At study termination approximately 26 % of the XDE-729 Methyl applied to the test systems was extracted from the sediment, while numerous degradation products were identified in the overlying water, some of which corresponded to known photolysis metabolites of XDE-729 Methyl.

The control and solvent control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots and roots. Over 14 days, the mean total shoot length increased more than 5-fold, fresh weight biomass increased more than 6-fold, and mean dry weight biomass increased more than 3-fold.

The reduction in total shoot length was due to a combination of reduced main shoot length and reduced number and length of side shoots formed during the exposure period. No side shoots were produced by plants in treatment concentrations ≥ 0.556 µg test item/L.

Table B 9.2.93: Mean total shoot length including side shoots (cm)

Nominal concentration [µg/L]	Days after application		yield [cm]	reduction in yield [%] ²⁾	growth rate [1/day]	reduction in growth rate [%] ²⁾
	0 ¹⁾	14				
control	11.4	63.5	52.1	-	0.1221	-
solvent	11.4	67.0	55.6	-	0.1258	-
Pooled controls	11.4	65.2	53.8	-	0.1239	-
0.0617	11.4	55.0*	43.6*	19.0*	0.1113*	10.2*
0.185	11.4	29.5*	18.1*	66.4*	0.0671*	45.8*
0.556	11.4	20.0*	8.6*	84.0*	0.0401*	67.6*
1.67	11.4	17.2*	5.8*	89.2*	0.0290*	76.6*
5.00	11.4	17.6*	6.2*	88.5*	0.0307*	75.2*
100	11.4	18.0*	6.6*	87.7*	0.0327*	73.6*

* significantly different to the pooled control

¹⁾ based on 10 additional plants, representative of those used in the test²⁾ compared to the pooled controls

Table B 9.2.94: Mean total plant fresh weight (g)

Nominal concentration [µg/L]	Days after application		yield [g]	reduction in yield [%] ²⁾	growth rate [1/day]	reduction in growth rate [%] ²⁾
	0 ¹⁾	14				
control	0.295	2.008	1.713*	-	0.1363*	
solvent	0.295	2.230	1.935	-	0.1440	-
Pooled controls	0.295	2.119	1.824	-	0.1401	-
0.0617	0.295	2.181	1.886	-3.4	0.1409	-0.6
0.185	0.295	1.086*	0.791*	56.6*	0.0919*	34.4*
0.556	0.295	0.899*	0.604*	66.9*	0.0792*	43.5*
1.67	0.295	0.904*	0.609*	66.6*	0.0777*	44.5*
5.00	0.295	0.958*	0.663*	63.7*	0.0838*	40.2*
100	0.295	0.995*	0.700*	61.6*	0.0861*	38.5*

* significantly different to the pooled control

1) based on 10 additional plants, representative of those used in the test

2) compared to the pooled controls

Table B 9.2.95: Mean total plant dry weight (g)

Nominal concentration [µg/L]	Days after application		yield [g]	reduction in yield [%] ²⁾	growth rate [1/day]	reduction in growth rate [%] ²⁾
	0 ¹⁾	14				
control	0.036	0.114*	0.078*	-	0.0807*	-
solvent	0.036	0.133	0.097	-	0.0927	-
0.0617	0.036	0.143	0.107	-10.3	0.0970	-4.6
0.185	0.036	0.091*	0.055*	43.3*	0.0661*	28.7*
0.556	0.036	0.093*	0.057*	41.2*	0.0672*	27.5*
1.67	0.036	0.082*	0.046*	52.6*	0.0575*	38.0*
5.00	0.036	0.091*	0.056*	43.3*	0.0662*	28.6*
100	0.036	0.097*	0.061*	37.1*	0.0700*	24.5*

* significantly different to the solvent control

¹⁾ based on 10 additional plants, representative of those used in the test²⁾ compared to the solvent control

Table B 9.2.96: Summary of biological results based on nominal concentrations

Parameter	Total Shoot Length		Mean total plant fresh weight (g)		Mean total plant dry weight (g)	
	Reduction in growth rate (µg/L)	Reduction in yield (µg/L)	Reduction in growth rate (µg/L)	Reduction in yield (µg/L)	Reduction in growth rate (µg/L)	Reduction in yield (µg/L)
14-day EC ₅₀	0.393	0.149	> 100 ¹⁾	0.543	> 100 ¹⁾	> 100 ¹⁾
95% Conf. Limits of EC ₅₀	0.288 – 0.525	0.010 – 0.198	-	0.381 – 0.765	-	-
14-day NOEC	0.0617	<0.0617	0.0617	0.0617	0.0617	0.0617
14-day LOEC	0.0617	0.0617	0.185	0.185	0.185	0.185

EC_x values calculated using Probit analysis

- = not calculable, no clear dose response.

¹⁾ no effect >50% could be observed in the highest test item concentrations of 5.00 and 100 µg/L, therefore the EC₅₀ was estimated to be >100 µg/L**Conclusion**

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to XDE-729 Methyl for 14 days, the E_yC_{50} and E_rC_{50} values based on total shoot length were 0.149 $\mu\text{g/L}$ and 0.393 $\mu\text{g/L}$ respectively. The NOEC for yield and growth rate based on total shoot length was $< 0.0617 \mu\text{g/L}$.

The E_yC_{50} and E_rC_{50} values based on biomass (fresh weight) were 0.543 $\mu\text{g/L}$ and $>100 \mu\text{g/L}$ respectively. The NOEC for yield and growth rate based on biomass (fresh weight) was 0.0617 $\mu\text{g/L}$.

The E_yC_{50} and E_rC_{50} values based on biomass (dry weight) were $>100 \mu\text{g/L}$. Due to the distribution of the data no statistical evaluation of the EC_{50} -values could be performed. The NOEC for yield and growth rate based on biomass (dry weight) was 0.0617 $\mu\text{g/L}$.

Qualitative observations indicated effects on root and shoot development compared to the control for all concentrations. At all concentrations hanging leaves, thickened nodes, smaller leaves and root reduction were observed.

RMS comment: Firstly it should be noted that the guideline used is only at draft stage and was submitted to OECD for evaluation in 2011. Despite this the following points have been raised:

The Draft AMRAP Method states some preliminary validity criteria. Growth rate for the fresh weight parameter was >0.07 per day and so was acceptable. However the coefficient variation in yield of fresh weight was not calculated and thus not stated in this study, the Draft AMRAP Method states that it should be $<35\%$. The coefficient variant was calculated to be 16.43% and so meets the criteria set out in the draft protocol.

The Draft AMRAP Method states that each vessel should contain one plant pot with 5 shoots initially, after seven days; 2 of the 5 plants should be removed to leave 3 uniformed plants. In this study only one shoot was planted in each pot. Although this could have impacted upon the statistical analysis of the study, results were statistically significant and a clear dose response pattern can be observed. The single shoots were also selected from a representative stock of over 100 uniformed plants, planted 7 days before initiation. It is however, not specified how the single shoot tips were selected from the stock. The draft protocol states that 'all clippings should be weighed individually and, for the test, only plants within a 30% weight range should be utilized'. This level of detail should have been documented however as a dose response was observed we can more confidently assume that shoot lengths selected at test initiation were representative. Therefore as both validity criteria were met and a clear dose response pattern was observed, this lack of information has been accepted.

Further environmental conditions should have been recorded; pH and oxygen concentration should have been recorded at an additional interval in the middle of the study and temperature of the medium should have been recorded continuously. However, of those measurements taken, temperature was maintained throughout the study so the limit dataset is not deemed to be an issue. Despite a slight drop in pH at study initiation, all control validity criteria were met. It is also worth noting that

culture conditions, sources of the components in the formulated sediment, methods to prevent evaporation in the test vessels and additional visual observations were not documented. Visual observations should have been recorded throughout the study and not just at termination, this may have been carried out but details of this have not been provided. This level of detail should have been documented however as validity criteria were met this lack of information has been accepted.

Analytical confirmation of the test substance concentration should have been observed for the lowest concentration, the median and the highest concentrations at initiation and termination. The highest concentration was only analysed at termination. However, this does not affect the reliability of the study as endpoints are based on the nominal concentrations.

Conductivity was not measured at any point during the test, as required in the guideline on day 0. However, no acceptable range is provided so this omission should not impact the validity of the test.

This study is acceptable. The proposed endpoint from this study is a nominal 14 day E_yC_{50} of 0.149 µg/L.

Gonsior, G. (2012): XDE-729 Acid - Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System. Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany. Unpublished report no.: S12-00215. DAS Study No.: 120533

Test material

Test item:	XDE-729 Acid
Purity:	99%
Description:	Off-white solid
Lot No./Batch No.	DC6-E2622-77

Test system

Organism (Species):	Aquatic macrophyte, <i>Myriophyllum spicatum</i> L
Study Type:	Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System
GLP Status:	GLP
Guidelines followed:	Based on the draft AMRAP Method (MALTBY et al. 2010): Growth Inhibition Test for the Rooted Aquatic Macrophyte, <i>Myriophyllum sp</i>
Guideline deviations reported by Study Director:	More replicates per treatment and control group, shoots of 5cm and not 6cm.
Duration of study:	14 days
Test conditions:	Static

Parameters measured:	Shoot length, plant fresh weight, plant dry weight, qualitative observations of shoot and root
Observation intervals:	Day 7 and 14 (shoot length), day 14 (fresh and dry weight)
Initial height of plants at day 0	5cm
Number of plants per replicate	1
Number of replicates per treatment and control group	Control (and solvent control): 10 Treatment groups: 5* *analytical vessels were prepared additionally at 100 µg/L treatment
Test concentrations:	Nominal: Control (0), solvent control (DMF @ 100 µL/L), 0.0617, 0.185, 0.556, 1.67, 5.00, 100 µg/L, Initial measured: ND*, ND*, 0.0627, 0.178, 0.523, 1.59, 4.97, 97.0 µg/L *= Not Detectable, below LOQ of 0.05 µg/L
Analytical confirmation of test concentrations:	<ul style="list-style-type: none"> Overlying media (all concentrations): day 0 Overlying media (100 µg/L): day 3, 7, 14 Sediment (100 µg/L): day 14
Feeding:	None
Sediment type	Based on artificial sediment described in OECD guideline 207: <ul style="list-style-type: none"> 4 % sphagnum peat (finely ground, air dried) 20 % kaolin clay (kaolinite content above 30 %), 75 - 76 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns) approximately 0.1 % calcium carbonate 200 mg of ammonium chloride and 200 mg of sodium phosphate per kg sediment (dry weight)
Water medium	Smart and Barko
Environmental conditions:	Temperature: 19.6 ± 0.9°C Photoperiod: 16:8 Light intensity: ~ 8000 lux pH: 8.06 ± 0.75 Dissolved Oxygen: 110 ± 18% air saturation
Analytical verification:	<ul style="list-style-type: none"> Direct injection of test medium samples into HPLC-MS/MS. Extraction of sediment samples with acetonitrile/water (1:1). Final determination by HPLC-MS/MS.

Methodology

A 14 day static test with the aquatic macrophyte, *Myriophyllum spicatum* was performed with nominal test concentrations of 0 (control), 0 (solvent control as 50 µL DMF/L media), 0.0617, 0.185, 0.556, 1.67, 5.00 and 100 µg XDE-729 Acid/L. A primary standard at a concentration of 2 mg XDE-729 Acid/mL was prepared by diluting 40 mg of the test item in 20 mL of DMF solvent. The primary standard was used to treat the 100 µg XDE-729 Acid/L group. Five subsequent standards were prepared via serial dilution starting with the primary stock and these were used to treat the other treatment groups when applied at 0.05 mL/L media. The solvent control group was treated with 0.075 mL of DMF only. The control replicates contained water media only.

The test chambers used for the test were 2 L glass beakers, (12 cm diameter, 24 cm tall). The control and solvent control treatments were replicated ten times and each treatment group was replicated five times. Each replicate contained 350 g of sediment (covered with a thin layer of quartz sand) and 1.5 L of the appropriately treated test media, at a depth of 14 cm. Each replicate had a single plant rooted in the sediment, which was planted prior to administration of the test substance. Additional replicates were prepared and treated to give a test substance concentration of 100 µg XDE-729 Acid/L. These were used as analytical vessels to determine test substance concentration throughout the test.

The concentration of XDE-729 Acid was measured in the overlying media at test initiation. On days 3, 7 and 14 analytical vessel media was sampled to determine test substance concentration. On day 14 further analytical vessels were sampled and used to determine test substance concentration in the sediment. Temperature, pH and dissolved oxygen were measured on days 0, 7 and 14. Light intensity was measured on one occasion. Shoot height in each replicate was measured on days 7 and 14, and fresh and dry weight of each replicate was measured on day 14. On day 0 ten additional plants representative of those used in the test had their mean total shoot length, fresh weight and dry weight determined.

Results

The initial measured XDE-729 Acid concentrations in test substance-treated media ranged from 95 to 103% of nominal concentrations. The measured XDE-729 Acid concentration in analytical vessels sampled on day 14 demonstrated the concentration of test substance in overlying water remained above 80% of nominal (82%). It was found also on day 14 that 19-20% of the nominal treatment concentration in each replicate was present in the sediment. On the basis of these analytical observations it is deemed appropriate to base results on nominal test concentrations.

Observations of the plants during the tests revealed that reduced numbers of roots were observed at concentrations of 0.556 µg XDE-729 Acid/L and higher. This observation was more marked at concentrations of 5.00 and 100 µg XDE-729 Acid/L. Leaves appeared to hang in concentrations of 0.185 µg XDE-729 Acid/L and higher.

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to XDE-729 Acid for 14 days, the EyC50 and ErC50 values based on total shoot length were 0.800 µg/L and 1.58 µg/L respectively. The NOEC for yield and growth rate based on total shoot length was 0.185 µg/L.

The EyC50 and ErC50 values based on biomass (fresh weight) were 0.816 µg/L and 4.52 µg/L respectively. The NOEC for yield and growth rate based on biomass (fresh weight) was 0.185 µg/L.

The ErC50 values based on biomass (dry weight) was estimated to be >100 µg/L. The EyC50 value based on biomass (dry weight) was 3.52 µg/L. The NOEC for yield and growth rate based on biomass (dry weight) was 0.556 µg/L.

Table B 9.2.97: Results of chemical analysis for initial measured concentrations

Initial measured concentration of XDE-729 Acid expressed as µg/L (% nominal)							
Control (0)	Solvent control (0)	0.0617	0.185	0.556	1.67	5.00	100
ND	ND	0.0627 (103)	0.178 (97)	0.523 (95)	1.59 (96)	4.97 (100)	97.0 (98)

ND – not detectable, below LOQ of 0.05 µg/L

Table B 9.2.98: Mean Shoot length and growth during the 14 day test with *Myriophyllum Spicatum*

Nominal Concentration (µg XDE-729 Acid/L)	Mean shoot length (cm, day 0) ¹	Mean shoot length (cm, day 14)	Yield (cm)	% reduction yield ²	Growth rate (per day)	% reduction growth rate ²
Control (0)	12.2	39.4	27.2	-	0.0832	-
Solvent control (0)	12.2	40.6	28.4	-	0.0853	-
Pooled Controls (0)	12.2	40.0	27.8	-	0.0843	-
0.0617	12.2	43.7	31.5	-13.3	0.0901	-6.9
0.185	12.2	37.6	25.4	8.6	0.0803	4.7
0.556	12.2	27.5*	15.3*	45.0*	0.0571*	32.2*
1.67	12.2	16.8*	4.6*	83.5*	0.0224*	73.4*
5.00	12.2	16.3*	4.1*	85.3*	0.0208*	75.3*
100	12.2	14.4*	2.2*	92.1*	0.0119*	85.9*

¹ Determined from 10 additional plants sampled at day 0

² Compared to the pooled controls

* Significantly different compared to the pooled controls

Table B 9.2.99: Mean plant fresh weight during the 14 day test with *Myriophyllum Spicatum*

Nominal Concentration (µg XDE-729 Acid/L)	Mean fresh weight (g, day 0) ¹	Mean fresh weight (g, day 14)	Yield (cm)	% reduction yield ²	Growth rate (per day)	% reduction growth rate ²
Control (0)	0.2696	1.2712	1.0016	-	0.1104	-
Solvent control (0)	0.2696	1.3695	1.0999	-	0.1146	-
Pooled Controls (0)	0.2696	1.3203	1.0507	-	0.1125	-
0.0617	0.2696	1.4087	1.1391	-8.4	0.1153	-2.5
0.185	0.2696	1.4459	1.1763	-12.0	0.1197	-6.4
0.556	0.2696	0.9948*	0.7252*	31.0*	0.0928*	17.5*
1.67	0.2696	0.5627*	0.2931*	72.1*	0.0510*	54.7*
5.00	0.2696	0.5423*	0.2727*	74.0*	0.0491*	56.4*
100	0.2696	0.4272*	0.1576*	85.0*	0.0320*	71.6*

¹ Determined from 10 additional plants sampled at day 0² Compared to the pooled controls

* Significantly different compared to the pooled controls

Table B 9.2.100: Mean plant dry weight during the 14 day test with *Myriophyllum Spicatum*

Nominal Concentration (µg XDE-729 Acid/L)	Mean dry weight (g, day 0) ¹	Mean dry weight (g, day 14)	Yield (cm)	% reduction yield ²	Growth rate (per day)	% reduction growth rate ²
Control (0)	0.0251	0.1211	0.0960	-	0.1120	-
Solvent control (0)	0.0251	0.1280	0.1029	-	0.1155	-
Pooled Controls (0)	0.0251	0.1246	0.0995	-	0.1138	-
0.0617	0.0251	0.1468	0.1217	-22.3	0.1245	-9.4
0.185	0.0251	0.1554	0.1303	-31.0	0.1299	-14.1
0.556	0.0251	0.1560	0.1309	-31.6	0.1300	-14.2
1.67	0.0251	0.0849*	0.0598*	39.9*	0.0856	24.8*
5.00	0.0251	0.0758*	0.0507*	49.0*	0.0779	31.5*
100	0.0251	0.0654*	0.0403*	59.5*	0.0672	40.9*

¹ Determined from 10 additional plants sampled at day 0² Compared to the pooled controls

* Significantly different compared to the pooled controls

Table B 9.2.101: Results based on nominal concentrations of XDE-729 Acid from the 14 day test with *Myriophyllum Spicatum*

Endpoint	Parameter Effect Concentration as µg XDE-729 Acid/L					
	Shoot length		Fresh weight		Dry weight	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
NOEC	0.185	0.185	0.185	0.185	0.556	0.556
LOEC	0.556	0.556	0.556	0.556	1.67	1.67
EC ₅₀ (95% confidence limits)	1.58 (1.14-2.17)	0.800 (0.586-1.06)	4.52 (2.56-8.01)	0.816 (0.363-1.43)	>100 (NC)	3.52 (0.114-12.5)

NC – Not Calculable

EC₅₀ values calculated using probit analysis

Conclusions

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to XDE-729 Acid for 14 days, the E_yC₅₀ and E_rC₅₀ values based on total shoot length were 0.800 µg XDE-729 Acid/L and 1.58 µg XDE-729 Acid/L respectively. The NOEC for yield and growth rate based on total shoot length was 0.185 µg XDE-729 Acid/L. All end points were based on nominal test concentrations.

The E_yC₅₀ and E_rC₅₀ values based on biomass (fresh weight) were 0.816 µg XDE-729 Acid/L and 4.52 µg XDE-729 Acid/L respectively. The NOEC for yield and growth rate based on biomass (fresh weight) was 0.185 µg XDE-729 Acid/L.

Due to the distribution no statistical evaluation of the E_rC₅₀ value based on biomass (dry weight) could be performed. The E_rC₅₀ values based on biomass (dry weight) was estimated to be > 100 µg XDE-729 Acid/L. The E_yC₅₀ value based on biomass (dry weight) was 3.52 µg XDE-729 Acid/L. The NOEC for yield and growth rate based on biomass (dry weight) was 0.556 µg XDE-729 Acid/L.

The pooled control results displayed a fresh weight growth rate of 0.1125 day⁻¹, which exceeds the proposed validity criteria for *Myriophyllum spicatum* in the Final report of a ring test performed to the draft AMRAP Method (MALTBY et al. 2010) of 0.07 day⁻¹. In addition, environmental parameters (light intensity, temperature and media pH) were maintained at comparable levels to those proposed in the draft method.

RMS Comment: The study is considered acceptable for risk assessment purposes with a nominal proposed EC₅₀ of 0.800 µg XDE-729 Acid/L for shoot length yield, the most sensitive endpoint. Although the guideline is only at draft stage, it should be noted that the proposed number of individual plants per treatment group is 9 (3

individuals per replicate, 3 replicates), with double that for control groups. This test used only 5 individuals per treatment group, 10 per control group. Although this could have impacted upon the statistical analysis of the study, results were statistically significant and a clear dose response pattern can be observed. The single shoots were also selected from a representative stock of over 100 uniformed plants, planted 7 days before initiation.

It is however, not specified how the single shoot tips were selected from the stock. The draft protocol states that 'all clippings should be weighed individually and, for the test, only plants within a 30% weight range should be utilized'. This level of detail should have been documented however as a dose response was observed we can more confidently assume that shoot lengths selected at test initiation were representative. A second validity criterion was proposed in the ring test final report; that the coefficient of variance for control replicate fresh weight should not exceed 35%. Although no evidence that this criterion was met is presented in the study report, it has been calculated that both the control and solvent control group replicate coefficient of variance with regard to fresh weight are < 35% (13.32% and 25.01% respectively). Conductivity was not measured at any point during the test, as required in the guideline on day 0. However, no acceptable range is provided so this omission should not impact the validity of the test as both validity criteria were met and a clear dose response pattern was observed.

Gonsior, G. (2012): X11449757 - Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System. Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany. Unpublished report no.: S12-00216. DAS Study No.: 102015

Test material

Test item:	X11449757
Purity:	97%
Description:	Off-white solid
Batch No. :	SYN-FS08644-090

Test system

Organism (Species):	Aquatic macrophyte, <i>Myriophyllum spicatum</i> L
Study Type:	Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System
GLP Status:	GLP
Guidelines followed:	Based on the draft AMRAP Method (MALTBY et al. 2010): Growth Inhibition Test for the Rooted Aquatic Macrophyte, <i>Myriophyllum sp</i>
Guideline deviations reported by Study Director:	More replicates per treatment and control group, Initial plant height was 5 cm instead of 6 cm
Duration of study:	14 days
Test conditions:	Static

Parameters measured:	Shoot length, plant fresh weight, plant dry weight, qualitative observations of shoot and root
Observation intervals:	Day 7 and 14 (shoot length), day 14 (fresh and dry weight)
Initial height of plants at day 0	5cm
Number of plants per replicate	1
Number of replicates per treatment and control group	Control (and solvent control): 10 Treatment groups: 5* *analytical vessels were prepared additionally at 100 µg/L treatment
Test concentrations:	Nominal: Control (0), 1.23, 3.70, 11.1, 33.3 and 100 µg/L Initial measured: ND*, 1.44, 3.46, 11.1, 33.6 and 104 µg/L *= Not Detectable, below LOQ of 0.05 µg/L
Analytical confirmation of test concentrations:	<ul style="list-style-type: none"> Overlying media (all concentrations): day 0 Overlying media (100 µg/L): day 3, 7, 14 Sediment (100 µg/L): day 14
Feeding:	None
Sediment type	Based on artificial sediment described in OECD guideline 207: <ul style="list-style-type: none"> 4 % sphagnum peat (finely ground, air dried) 20 % kaolin clay (kaolinite content above 30 %), 75 - 76 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns) approximately 0.1 % calcium carbonate 200 mg of ammonium chloride and 200 mg of sodium phosphate per kg sediment (dry weight)
Water medium	Smart and Barko
Environmental conditions:	Temperature: 18.7 ± 0.4°C Photoperiod: 16:8 Light intensity: ~ 8000 lux pH: 8.22 ± 0.65 Dissolved Oxygen: 113 ± 18% air saturation
Analytical verification:	<ul style="list-style-type: none"> Direct injection of test medium samples into HPLC-MS/MS. Extraction of sediment samples with acetonitrile/water (1:1). Final determination by HPLC-MS/MS.

Methodology

A 14 day static test with the aquatic macrophyte, *Myriophyllum spicatum* was performed with nominal test concentrations of 0 (control), 1.23, 3.70, 11.1, 33.3 and 100 µg X11449757/L. A primary standard at a concentration of 15 µg X11449757/mL was prepared by diluting 15 mg of the test item in 1000 mL of the test media (Smart and Barko). The primary standard was used to treat the 100 µg X11449757/L group. Four subsequent standards were prepared via serial dilution from the primary stock and these were used to treat the other treatment groups when applied at 10 mL/1.5L media (each replicate contained 1.5L untreated media). The control replicated contained water media only.

The test chambers used for the test were 2 L glass beakers, (12 cm diameter, 24 cm tall). The control treatment was replicated ten times and each treatment group was replicated five times. Each replicate contained 350 g of sediment (covered with a thin layer of quartz sand) and 1.5 L of the appropriately treated test media, at a depth of 14 cm. Each replicate had a single plant rooted in the sediment, which was planted prior to administration of the test substance. Additional replicates were prepared and treated to give a test substance concentration of 100 µg X11449757/L. These would be used as analytical vessels to determine test substance concentration throughout the test.

The concentration of X11449757 Acid was measured in the overlying media at test initiation. On days 3, 7 and 14 analytical vessel media was sampled to determine test substance concentration. On day 14 further analytical vessels were sampled and used to determine test substance concentration in the sediment. Temperature, pH and dissolved oxygen were measured on days 0, 7 and 14. Light intensity was measured on one occasion. Shoot height in each replicate was measured on days 7 and 14, and fresh and dry weight of each replicate was measured on day 14. On day 0 ten additional plants representative of those used in the test had their mean total shoot length, fresh weight and dry weight determined.

Results

The initial measured X11449757 concentrations in test substance-treated media ranged from 96 to 121% of nominal concentrations. The measured X11449757 concentration in analytical vessels sampled on day 14 demonstrated the concentration of test substance in overlying water dropped to 13% of nominal. It was found also on day 14 that 6% of the nominal treatment concentration in each replicate was present in the sediment.

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to X11449757 for 14 days, the E_yC_{50} and E_rC_{50} values based on total shoot length and biomass (fresh and dry weight) were > 100 µg/L. The NOEC for yield and growth rate based on total shoot length and biomass was 100 µg/L.

Table B 9.2.102: Results of chemical analysis for initial measured concentrations

Initial measured concentration of X11449757 expressed as µg/L (% nominal)					
Control (0)	1.23	3.70	11.1	33.3	100
ND	1.44 (121)	3.46 (96)	11.1 (103)	33.3 (104)	104 (107)

ND – not detectable, below LOQ of 0.05 µg/L

Table B 9.2.103: Mean Shoot length growth during the 14 day test with
Myriophyllum Spicatum

Nominal Concentration (µg X11449757/L)	Mean shoot length (cm, day 0) ¹	Mean shoot length (cm, day 14)	Yield (cm)	% reduction yield ²	Growth rate (per day)	% reduction growth rate ²
Control	9.4	23.0	13.6		0.0634	-
1.23	9.4	23.4	14.0	-2.9	0.0644	-1.6
3.70	9.4	24.1	14.7	-8.1	0.0672	-6.0
11.1	9.4	27.3	17.9	-31.6	0.0746	-17.7
33.3	9.4	26.5	17.1	-25.7	0.0735	-15.9
100	9.4	27.9	18.5	-36.0	0.0772	-21.8

¹ Determined from 10 additional plants sampled at day 0² Compared to the controlTable B 9.2.104: Mean plant fresh weight during the 14 day test with
Myriophyllum Spicatum

Nominal Concentration (µg X11449757/L)	Mean fresh weight (g, day 0) ¹	Mean fresh weight (g, day 14)	Yield (cm)	% reduction yield ²	Growth rate (per day)	% reduction growth rate ²
Control	0.2432	1.0803	0.8371	-	0.1046	-
1.23	0.2432	1.0351	0.7919	5.4	0.1024	2.1
3.70	0.2432	1.0337	0.7905	5.6	0.1032	1.3
11.1	0.2432	1.3277	1.0845	-29.6	0.1186	-13.4
33.3	0.2432	0.9985	0.7553	9.8	0.1007	3.7
100	0.2432	1.1334	0.8902	-6.3	0.1096	-4.8

¹ Determined from 10 additional plants sampled at day 0² Compared to the control

Table B 9.2.105: Mean plant dry weight during the 14 day test with *Myriophyllum Spicatum*

Nominal Concentration (µg X11449757/L)	Mean dry weight (g, day 0) ¹	Mean dry weight (g, day 14)	Yield (cm)	% reduction yield ²	Growth rate (per day)	% reduction growth rate ²
Control	0.02	0.1439	0.1239	-	0.1390	-
1.23	0.02	0.1383	0.1183	4.5	0.1375	1.1
3.70	0.02	0.1343	0.1143	7.7	0.1353	2.7
11.1	0.02	0.1730	0.1530	-23.5	0.1534	-10.4
33.3	0.02	0.1522	0.1322	-6.7	0.1448	-4.2
100	0.02	0.1518	0.1318	-6.4	0.1443	-3.8

¹ Determined from 10 additional plants sampled at day 0² Compared to the controlTable B 9.2.106: Results based on nominal concentrations of X11449757 from the 14 day test with *Myriophyllum Spicatum*

Endpoint	Parameter Effect Concentration as µg X11449757/L					
	Shoot length		Fresh weight		Dry weight	
	Growth rate	Yield	Growth rate	Yield	Growth rate	Yield
NOEC	100	100	100	100	100	100
LOEC	>100	>100	>100	>100	>100	>100
EC ₅₀ (95% confidence limits)	>100 (NC)	>100 (NC)	>100 (NC)	>100 (NC)	>100 (NC)	>100 (NC)

NC – Not Calculable

Conclusions

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to X11449757 for 14 days, the EyC50 and ErC50 values based on total shoot length and biomass (fresh and dry weight) were > 100 µg/L. The NOEC for yield and growth rate based on total shoot length and biomass was 100 µg/L.

Although one of the initial measured concentrations was found to be outside of the 80-120% of nominal range, it was above 80% and all other concentrations were within the desired range. This, along with the lack of significant toxic effects mean that basing results on nominal test concentrations is acceptable.

The control results displayed a fresh weight growth rate of 0.1046 day^{-1} , which exceeds the proposed validity criteria for *Myriophyllum spicatum* in the Final report of a ring test performed to the draft AMRAP Method (MALTBY et al. 2010) of 0.07 day^{-1} . In addition, environmental parameters (light intensity, temperature and media pH) were maintained at similar levels to those proposed in the draft method. Mean pH at day 0 was 7.32, outside the 7.5-8.0 recommended in the draft guideline, but this didn't appear to impact control plant performance in the test and so can be considered negligible.

RMS Comment: The study is considered acceptable for risk assessment purposes with a nominal proposed EC_{50} of $>100 \mu\text{g X11449757/L}$ for all measured endpoints. Although the guideline is only at draft stage, it should be noted that the number of individual plants per treatment group is 9 (3 individuals per replicate, 3 replicates), with double that for control groups. This test used only 5 individuals per treatment group, 10 per control group. As this study showed no evidence of any possible dose response up to and including the top tested concentration of X11449757, this is not considered to be a significant issue for this study. The single shoots were also selected from a representative stock of over 100 uniformed plants, planted 7 days before initiation. It is however, not specified how the single shoot tips were selected from the stock. The draft protocol states that 'all clippings should be weighed individually and, for the test, only plants within a 30% weight range should be utilized'.

A second validity criterion was proposed in the ring test final report; that the coefficient of variance for control replicates fresh weight should not exceed 35%. Although no evidence that this criterion was met is presented in the study report, it has been calculated and the control group replicate coefficient of variance with regard to fresh weight is $< 35\%$ (31.20%). Conductivity was not measured at any point during the test, as required in the guideline on day 0. However, no acceptable range is provided so this omission should not impact the validity of the test.

Gonsior, G. (2012): X11406790 - Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System. Eurofins Agrosience Services, EcoChem GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany. Dow AgroSciences unpublished report no: S12-00217. DAS Study No: 120534. 26 July 2012.

Test material

Name	X11406790
Test item code:	2011-008019
Batch no.:	SYN-FS08644-062
Purity (analysed):	95 %
Appearance/colour:	solid/off-white

Test system

Organism (<i>Species</i>):	<i>Myriophyllum spicatum</i>
Study Type:	Growth Inhibition of <i>Myriophyllum spicatum</i> in a static Water/Sediment System
GLP Status:	GLP
Guidelines followed:	Based on the draft AMRAP Method (MALTBY et al. 2010): Growth Inhibition Test for the Rooted Aquatic Macrophyte, <i>Myriophyllum</i> sp., submitted to OECD for evaluation, 2011.
Guideline deviations reported by Study Director:	Deviations listed in the RMS comments.
Duration of study:	14 days
Parameters measured:	Biomass (fresh weight, dry weight, shoot length), growth rate and yield.
Environmental conditions:	Test vessels were maintained in a controlled environment at 19.8 ± 0.7 °C under warm and/or cool white fluorescent lighting (approx. 8000 lux at the water surface) with a 16-h day-length. The average pH-value was determined to be 8.34 ± 0.67 and the oxygen saturation was determined to be $116 \pm 22\%$. The test item had no influence on the pH-value of the test solutions.
Observation intervals:	0, 7, 14 days
Test concentrations:	Nominal: 0 (control), 0.185, 0.556, 1.67, 5.00, 15.0 and 100 µg/L.
Growth medium	SMART AND BARKO medium
Method of test item added to the test medium	Stock solution: soluble in test medium Final concentration- 10 mL stock solution per 1.5 L test medium
No. of control solvent control replicates	10
No. of test concentration replicates	5
Analytical verification:	At initiation and termination during the definitive test. An additional set of vessels was prepared for further chemical analysis. An analytical method for the determination of XDE-729 Methyl, XDE-729 Acid X11406790 and X11449757 was validated with regard to recovery (accuracy), linearity of detector response, repeatability (precision) and specificity. The analytical system fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000

Methodology:

Seven days prior to test initiation, submerged apical shoots (without side shoots) were planted in an aquarium, in artificial sterilised sediment overlaid with SMART& BARKO medium. From this sufficient uniform material was generated for use in the following test.

For the definitive test, a 14 day growth inhibition test was conducted at nominal concentrations of 0 (control), 0.185, 0.556, 1.67, 5.00, 15.0 and 100 µg/L. Moist artificial sediment was prepared and 350 g of this was added to each 2 L glass beaker. This sediment was further overlaid with a thin layer of washed quartz sand to minimise displacement of the sediment when the medium was added. Afterwards the test vessels were filled carefully with growth medium (1.5 L). Two days after preparation of the test vessels and before application one rooted apical shoot per vessel was planted carefully, ensuring the plant was rooted into the sediment. Test vessels were arranged in a completely randomised design and were re-randomised several times during the study.

An additional set of vessels (containing sediment and plants) was prepared for further chemical analysis at the highest test concentration. Analytical confirmation from these additional vessels was measured at day 0, 3, 7, 14. Sediment was also sampled at termination.

The stock solution was prepared by dissolving the test item in medium and thus a vehicle control was not required. Shortly after planting, the test item was applied by stirring it gently into the growth medium. Ten replicates were used for the control and five for each treatment group.

A representation of additional plants was selected at initiation in order to assess plant growth. These plants were blotted dry prior to assessment of plant fresh weight and shoot length. The plants were placed separately in labelled glass beakers and dried at 60 °C for > 48 hours. The weight of the dry plant samples was recorded.

Shoot height in each replicate was measured on days 7 and 14. At termination (day 14) plants were harvested from each treatment group for assessment of plant fresh weight, plant dry weight, shoot length and number and length of side shoots. In addition observations on shoot and root development (e.g. necrosis, deformation) were documented.

Water temperature, pH and dissolved oxygen content were recorded on days 0, 7 and 14. Light intensity at the water surface was measured once during the test.

Results:

Measured test concentrations in the overlying water immediately after treatment were 0.168, 0.517, 1.50, 5.18, 14.7 and 92.6 µg/L, which represented 95, 98, 94, 109, 103 and 97 % of nominal. The mean measured content for all concentrations at test start was 99 %. As the mean content of the test item was between 80 and 120 % of nominal at test start, the toxicological endpoints were evaluated using

nominal concentrations of the test item (see SANCO/3268/2001 rev. 4 (final), chapter 2.1.4).

The analytical data showed that the X11406790 concentration in the overlying water decreased to 21 % of nominal by day 14. After 14 days 11 - 12 % of the metabolite was measured in the sediment.

The control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots and roots. Over 14 days, the mean total shoot length increased more than 3-fold, fresh weight biomass increased more than 4-fold, and mean dry weight biomass increased more than 3-fold.

Table B 9.2.107: Mean total shoot length including side shoots (cm)

Nominal concentration [µg/L]	Days after application		yield [cm]	reduction in yield [%] ²⁾	growth rate [1/day]	reduction in growth rate [%] ²⁾
	0 ¹⁾	14				
control	8.3	25.5	17.2	-	0.0787	-
0.185	8.3	20.2	11.9	30.8	0.0627	20.3
0.556	8.3	23.3	15.0	12.8	0.0735	6.6
1.67	8.3	20.7	12.4	27.9	0.0650	17.4
5.00	8.3	24.1	15.8	8.1	0.0760	3.4
15.0	8.3	23.9	15.6	9.3	0.0754	4.2
100	8.3	22.2	13.9	19.2	0.0701	10.9

¹⁾ based on 10 additional plants, representative of those used in the test

²⁾ compared to control

Table B 9.2.108: Mean total plant fresh weight (g)

Nominal concentration [µg/L]	Days after application		yield [g]	reduction in yield [%] ²⁾	growth rate [1/day]	reduction in growth rate [%] ²⁾
	0 ¹⁾	14				
Control	0.2165	1.0244	0.8079	-	0.1099	-
0.185	0.2165	0.9616	0.7451	7.8	0.1055	4.0
0.556	0.2165	1.0740	0.8575	-6.1	0.1140	-3.7
1.67	0.2165	0.9075	0.6910	14.5	0.1022	7.0
5.00	0.2165	1.0564	0.8399	-4.0	0.1128	-2.6
15.0	0.2165	1.0273	0.8108	-0.4	0.1107	-0.7
100	0.2165	0.9258	0.7093	12.2	0.1028	6.5

¹⁾ based on 10 additional plants, representative of those used in the test

²⁾ compared to the control

Table B 9.2.109: Mean total plant dry weight (g)

Nominal concentration [µg/L]	Days after application		yield [g]	reduction in yield [%] ²⁾	growth rate [1/day]	reduction in growth rate [%] ²⁾
	0 ¹⁾	14				
Control	0.0320	0.1226	0.0906	-	0.0951	-
0.185	0.0320	0.1240	0.0920	-1.5	0.0961	-1.1
0.556	0.0320	0.1340	0.1020	-12.6	0.1014	-6.6
1.67	0.0320	0.1178	0.0858	5.3	0.0929	2.3
5.00	0.0320	0.1327	0.1007	-11.1	0.1008	-6.0
15.0	0.0320	0.1268	0.0948	-4.6	0.0983	-3.4
100	0.0320	0.1268	0.0948	-4.6	0.0977	-2.7

¹⁾ based on 10 additional plants, representative of those used in the test

²⁾ compared to the control

Table B 9.2.110: Summary of Biological Results based on Nominal Concentrations

Parameter	Total Shoot Length		Mean total plant fresh weight (g)		Mean total plant dry weight (g)	
	% reduction growth rate	% reduction in yield	% reduction growth rate	% reduction in yield	% reduction growth rate	% reduction in yield
14-day EC₅₀	> 100 ¹⁾	> 100 ¹⁾	> 100 ¹⁾	> 100 ¹⁾	> 100 ¹⁾	> 100 ¹⁾
95% Conf. Limits of EC₅₀	-	-	-	-	-	-
14-day NOEC	100	100	100	100	100	100
14-day LOEC	-	-	-	-	-	-

- = not calculable, no clear dose response.

¹⁾ no effect >50% could be observed, therefore the EC₅₀ was estimated to be >100 µg/L

Conclusions:

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to X11406790 no statistically significant inhibition was determined at all test item concentrations across all parameters observed. Therefore after 14 days, the E_yC₅₀ and E_rC₅₀ values based on total shoot length and biomass (fresh and dry weight weight) were > 100 µg/L. The NOEC for yield and growth rate based on total shoot length and biomass was 100 µg/L.

Qualitative observations on shoot and root development at test termination indicated no apparent effects on root or shoot development compared to controls for concentrations < 15 µg/L. At 100 µg test item/L the leaves were hanging, and there were reduced numbers of roots.

RMS comment: Firstly it should be noted that the guideline used is only at draft stage and was submitted to OECD for evaluation in 2011. Despite this the following points have been raised:

The Draft AMRAP Method states some preliminary validity criteria. Growth rate for the fresh weight parameter was >0.07 per day and so was acceptable. However the coefficient variation in yield of fresh weight was not calculated and thus not stated in this study, the Draft AMRAP Method states that it should be <35%. The coefficient of

variance was calculated to be 27.69% and so meets the criteria set out in the draft protocol.

The Draft AMRAP Method states that each vessel should contain one plant pot with 5 shoots initially, however after seven days; 2 of the 5 plants should be removed to leave 3 uniformed plants. In this study only one shoot was planted in each pot. Results were not statistically significant; the very slight fluctuations between test concentrations for this metabolite are most likely due to natural variation rather than a dose response, not highlighted due to a lack of replicates. The single shoots were also selected from a representative stock of over 100 uniformed plants, planted 7 days before initiation. It is however, not specified how the single shoot tips were selected from the stock. The draft protocol states that 'all clippings should be weighted individually and, for the test, only plants within a 30% weight range should be utilized'. This level of detail should have been documented however as validity criteria were met together with no clear dose response across the test concentrations, this lack of information has been accepted.

Further environmental conditions should have been recorded; pH and oxygen concentration should have been recorded at an additional interval in the middle of the study and temperature of the medium should have been recorded continuously. However, from those measurements taken, temperature and pH ranges were maintained throughout the study so the limit dataset is not deemed to be an issue. It is also worth noting that culture conditions, sources of the components in the formulated sediment, methods to prevent evaporation in the test vessels and additional visual observations were not documented. Visual observations should have been recorded throughout the study and not just at termination, this may have been carried out but details of this have not been provided. This level of detail should have been documented however as validity criteria were met this lack of information has been accepted.

Analytical confirmation of the test substance concentration should have been observed for the lowest concentration, the median and the highest concentrations at initiation and termination. The highest concentration was only analysed at termination. However, this does not affect the reliability of the study as endpoints are based on the nominal concentrations.

Conductivity was not measured at any point during the test, as required in the guideline on day 0. However, no acceptable range is provided so this omission should not impact the validity of the test.

This study is acceptable. The proposed endpoint from this study is a nominal 14 day EC_{50} of $> 100 \mu\text{g X11406790/L}$.

Formulation

Rebstock, M. 2011: GF-2573: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, *Lemna gibba*, ABC 65999. Dow AgroSciences unpublished report, Study Number 101125. 25 July 2011.

Test material

Test item:	GF-2573
Purity:	0.84 wt% XDE-729 methyl, 0.83 wt% cloquintocet-mexyl
Description:	Yellow liquid
Lot No./Batch No. :	E2837-83

Test system

	Freshwater aquatic plant, <i>Lemna gibba</i>
Study Type:	Static Renewal
GLP Status:	GLP (except the water characterization)
Guidelines followed:	OECD guideline 221
Guideline deviations reported by Study Director:	None
Duration of study:	7 days
Test conditions:	Static Renewal
Parameters measured:	Frond Count, Dry weight
Observation intervals:	Days 3, 5, 7
Age of fronds at test initiation:	7 days
Number of fronds at test initiation:	12 per rep
Test concentrations:	Nominal: 0 (control), 6.3, 13, 25, 50, and 100 mg GF-2573/L
	Geometric mean calculated: <MQL (control), 3.2, 7.4, 15, 34, and 80 mg GF-2573/L
Analytical confirmation of test concentrations:	0, 1, 3, 4, 6, and 7 days
Number of fronds per dose group:	36
Number of fronds per control group:	36
Feeding:	None
Environmental conditions:	Temperature: 22.9 to 25.1°C Photoperiod: 24 hour light Growth medium: 20X-AAP pH of fresh solutions: 7.5 to 7.6 pH of spent solutions: 8.0 to 8.9
Analytical verification:	Method: measuring concentrations of XDE-729 Methyl using HPLC-MS/MS. Samples taken: Test initiation and termination of each interval in fresh and spent solutions.

	Limit of Detection: Not determined. Limit of Quantitation: Reported as the minimum quantifiable limit (MQL) and was 0.238 ng XDE 729 Methyl/mL or 0.0283 mg GF-2573/L.
--	---

Methodology

A 7 day static renewal test with the freshwater aquatic plant, *Lemna gibba* was performed with nominal test concentrations of: 0 (control), 6.3, 13, 25, 50, and 100 mg GF-2573/L. Each test flask received three plants for a total of 12 fronds at test initiation. There were three replicates per test treatment, resulting in 36 fronds per test treatment. Frond observations and counts were made at 3, 5, and 7 days, with renewals daily throughout the test. Temperature and pH were measured daily in all fresh parent solutions, on days 0 to 6, and in replicate A of all treatment spent solutions on days 1 to 7. Biomass (dry weight) measurements of each control and test substance treatment replicate were performed on day 7 (test termination).

Results

Measured concentrations of XDE-729 Methyl, the active substance of GF-2573, were used to calculate GF-2573 concentrations in test solutions. The calculated GF-2573 concentrations in fresh test substance treatment solutions ranged from 92 to 120% of nominal concentrations. The calculated GF-2573 concentrations in spent test substance treatment solutions ranged from 21 to 62% of the nominal concentrations. Since not all measured concentrations were $\pm 20\%$ of the nominal concentrations, all end-points were calculated based on the geometric mean of the calculated GF-2573 concentrations. Results of the analysis are presented below in Table B 9.2.111

Table B 9.2.111: Analytical results

Nominal concentration (mg GF-2573/L)	Calculated concentration as mg GF-2573/L (% nominal)						Geometric mean
	Day 0	Day 1 (old)	Day 3 (new)	Day 4 (old)	Day 6 (new)	Day 7 (old)	
6.3	6.5 (103)	1.5 (24)	6.2 (98)	1.3 (21)	6.5 (103)	2.2 (35)	3.2 (51)
13	14 (108)	4.1 (32)	12 (92)	3.4 (26)	14 (108)	5.0 (38)	7.4 (5.7)
25	28 (112)	7.0 (28)	26 (104)	7.4 (30)	26 (104)	11 (44)	15 (60)
50	54 (108)	19 (38)	53 (106)	20 (40)	60 (120)	22 (44)	34 (68)
100	110 (110)	52 (52)	120 (120)	53 (53)	120 (120)	62 (62)	80 (80)

Table B 9.2.112: EC50 and NOEC for *Lemna gibba* based on geometric mean calculated concentrations

Endpoint	Parameter Effect Concentration as mg GF-2573/L			
	Frond Yield (95% CL)	Frond Average Specific Growth Rate (95% CL)	Biomass Yield as Dry Weight (95% CL)	Biomass Average Specific Growth Rate as Dry Weight (95% CL)
NOEC	80	80	80	80
LOEC	>80	>80	>80	>80
EC10	77 (95%CL: 74 to 79)	>80 (95%CL: NC)	>80 (95%CL: NC)	>80 (95%CL: NC)
EC20	>80 (95%CL: NC)	>80 (95%CL: NC)	>80 (95%CL: NC)	>80 (95%CL: NC)
EC50	>80 (95%CL: NC)	>80 (95%CL: NC)	>80 (95%CL: NC)	>80 (95%CL: NC)

NC = Not calculated

The test acceptability criteria for control growth (i.e., frond doubling time < 2.5 days, greater than a seven-fold increase in the number of fronds) set by OECD 221 test guideline were met for this study. The doubling time for the control fronds was 1.5 days, corresponding to a 21-fold increase in the number of fronds, and the average specific growth rate was 0.432 day⁻¹. This study is classified as acceptable and satisfies the guideline requirement for a growth inhibition test with *Lemna gibba*.

Conclusions

The lowest determined 7-day EC50 for *Lemna gibba* exposed to GF-2573 was >80 mg GF-2573/L, based on all endpoints. The 7-day EC50 values for frond average growth rate, frond yield, biomass yield and biomass specific growth rate were all >80 mg GF-2573/L.

RMS Comment: Due to the fact that concentrations were not maintained, an endpoint based on the geometric mean is proposed. It is considered that the endpoint that should be used for risk assessment is a mean measured 7-day EC50 of >80 mg GF-2573/L.

Gonsior, G. (2012): GF-2573 – Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System. Eurofins Agroscience Services, EcoChem GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany. Dow AgroSciences unpublished report no: S12-02164. DAS Study No: 120584. 17 August 2012.

Test material

Name:	GF-2573
Test substance no.:	TSN300696
Batch no.:	ENBK-110073-002
Appearance/colour:	liquid/yellow

Test system

Organism (<i>Species</i>):	<i>Myriophyllum spicatum</i>
Study Type:	Growth Inhibition of <i>Myriophyllum spicatum</i> in a static Water/Sediment System
GLP Status:	GLP
Guidelines followed:	Based on the draft AMRAP Method (MALTBY et al. 2010): Growth Inhibition Test for the Rooted Aquatic Macrophyte, <i>Myriophyllum</i> sp., submitted to OECD for evaluation, 2011.
Guideline deviations reported by Study Director:	Deviations listed in the RMS comments.
Duration of study:	14 days
Parameters measured:	Biomass (fresh weight, dry weight, shoot length), growth rate and yield.
Environmental conditions:	Test vessels were maintained in a controlled environment at 19.7 ± 0.6 °C under warm and/or cool white fluorescent lighting (approx. 8000 lux at the water surface) with a 16-h day-length. The average pH-value was determined to be 8.13 ± 0.82 and the oxygen saturation was determined to be 123 ± 26 %. The test item had no influence on the pH-value of the test solutions.
Observation intervals:	0, 7, 14 days
Test concentrations:	Nominal: 0 (control), 2.29, 7.32, 23.4, 75.0 and 240 µg/L.
Growth medium	SMART AND BARKO medium
Method of test item added to the test medium	Stock solution: soluble in test medium Final concentration- 10 ml of test solution per 1.5 L test medium
No. of control replicates	10
No. of test concentration replicates	5

Analytical verification:	<p>At initiation and termination during the definitive test.</p> <p>At test termination on day 14, wet sediment was sampled from the test vessels for all concentration levels and control and analysis of XDE-729 methyl was performed on the sediment.</p> <p>The analytical system fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.</p>
---------------------------------	---

Methodology

Seven days prior to test initiation, submerged apical shoots (without side shoots) were planted in an aquarium, in artificial sterilised sediment overlaid with SMART& BARKO medium. From this sufficient uniform material was generated for use in the following test.

For the definitive test, a 14 day growth inhibition test was conducted at nominal concentrations of 0 (control), 2.29, 7.32, 23.4, 75.0 and 240 µg/L. Moist artificial sediment was prepared and 350 g of this was added to each 2 L glass beaker. This sediment was further overlaid with a thin layer of washed quartz sand to minimise displacement of the sediment when the medium was added. Afterwards the test vessels were filled carefully with growth medium (1.5 L). Two days after preparation of the test vessels and before application one rooted apical shoot per vessel was planted carefully. Test vessels were arranged in a completely randomised design and were re-randomised several times during the study.

The stock solution was prepared by dissolving the test item in test medium. The lower test item concentrations were made by dilution of the stock solution with test medium. Shortly after planting, the test item was applied by stirring it gently into the growth medium. Ten replicates were used for the control and five for each treatment group.

A representation of additional plants was selected at initiation in order to assess plant growth. These plants were blotted dry prior to assessment of plant fresh weight and shoot length. The plants were placed separately in labelled glass beakers and dried at 60 °C for > 48 hours. The weight of the dry plant samples was recorded.

Shoot height in each replicate was measured on days 7 and 14. At termination (day 14) plants were harvested from each treatment group for assessment of plant fresh weight, plant dry weight, shoot length and number and length of side shoots. In addition observations on shoot and root development (e.g. necrosis, deformation) were documented.

Water temperature, pH and dissolved oxygen content were recorded on days 0, 7 and 14. Light intensity at the water surface was measured once during the test.

Results

Measured test concentrations in the overlying water immediately after treatment were 0.0153, 0.0497, 0.158, 0.560 and 1.91 µg/L, which represented 78- 93 % of nominal. As the initial GF-2573 test concentrations were between 80 and 120 % of nominal at test start, the toxicological endpoints were evaluated using nominal concentrations of the test item. Over 14 days the concentration of XDE-729 Methyl in the overlying water declined. A maximum concentration of 1 % of nominal by day 14 was measured at the highest concentration level of 240 µg/L test item. After 14 days measured XDE-729 methyl concentrations in the sediment were below the LOQ.

The control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots and roots. Over 14 days, the mean total shoot length increased more than 2.5-fold, fresh weight biomass increased more than 3.5-fold, and mean dry weight biomass increased more than 3-fold.

The reduction in total shoot length was mainly due a reduction in the number and length of side shoots formed during the exposure period.

Table B 9.2.113: Mean total shoot length including side shoots (cm)

Nominal concentration [µg/L]	Days after application		yield [cm]	reduction in yield [%]	growth rate [1/day]	reduction in growth rate [%]
	0 ¹⁾	14				
control	8.3	22.9	44.6	-	0.0717	-
2.29	8.3	21.6	13.3	8.9	0.0673	6.1
7.32	8.3	24.2	15.9	-8.9	0.0761	-6.1
23.4	8.3	20.2	11.9	18.5	0.0625	12.8
75.0	8.3	13.7*	5.4*	63.0*	0.0357*	50.2*
240	8.3	11.6*	3.3*	77.4*	0.0234*	67.4*

* significantly different reduction compared to the control

¹⁾ based on 10 additional plants, representative of those used in the test

Table B 9.2.114: Mean total plant fresh weight (g)

Nominal concentration [µg/L]	Days after application		yield [cm]	reduction in yield [%]	growth rate [1/day]	reduction in growth rate [%]
	0 ¹⁾	14				
control	0.2276	0.8805	0.6529	-	0.0955	-
2.29	0.2276	0.8230	0.5954	8.8	0.0882	7.6
7.32	0.2276	0.9379	0.7103	-8.8	0.1000	-4.7
23.4	0.2276	0.6004*	0.3728*	42.9*	0.0687*	28.1*
75.0	0.2276	0.5149*	0.2873*	56.0*	0.0567*	40.6*
240	0.2276	0.3501*	0.1225*	81.2*	0.0245*	74.3*

* significantly different reduction compared to the control

¹⁾ based on 10 additional plants, representative of those used in the test

Table B 9.2.115: Mean total plant dry weight (g)

Nominal concentration [µg/L]	Days after application		yield [cm]	reduction in yield [%]	growth rate [1/day]	reduction in growth rate [%]
	0 ¹⁾	14				
control	0.0368	0.1145	0.0777	-	0.0795	-
2.29	0.0368	0.1235	0.0867	-11.6	0.0807	-1.5
7.32	0.0368	0.1249	0.0881	-13.4	0.0839	-5.5
23.4	0.0368	0.0955	0.0587	24.5	0.0661	16.9
75.0	0.0368	0.0815*	0.0447*	42.5*	0.0560	29.6
240	0.0368	0.0651*	0.0283*	63.6*	0.0349*	56.1*

* significantly different reduction compared to the control

¹⁾ based on 10 additional plants, representative of those used in the test

Table B 9.2.116: Summary of Biological Results based on Nominal Concentrations

Parameter	Total Shoot Length		Mean total plant fresh weight (g)		Mean total plant dry weight (g)	
	Reduction in growth rate (µg/L)	Reduction in yield (µg/L)	Reduction in growth rate (µg/L)	Reduction in yield (µg/L)	Reduction in growth rate (µg/L)	Reduction in yield (µg/L)
14-day EC₅₀	102	66.6	84.4	40.2	189	112
95% Conf. Limits of EC₅₀	81.0-132	52.8-82.9	63.5-115	24.6-56.4	133-328	80.2-171
14-day NOEC	23.4	23.4	7.32	7.32	75.0	23.4
14-day LOEC	75.0	75.0	23.4	23.4	240	75.0

EC_x values calculated using Probit analysis

Conclusion

Following exposure of the aquatic macrophyte *Myriophyllum spicatum* to GF-2573 for 14 days, the E_rC₅₀ and E_yC₅₀ values based on total shoot length were 102 µg/L and 66.6 µg/L respectively. The NOEC for yield and growth rate based on total shoot length was 23.4 µg/L.

The E_rC₅₀ and E_yC₅₀ values based on biomass (fresh weight) were 84.4 µg/L and 40.2 µg/L respectively. The NOEC for yield and growth rate based on biomass (fresh weight) was 7.32 µg/L.

The E_rC₅₀ and E_yC₅₀ values based on biomass (dry weight) were 189 µg/L and 112 µg/L respectively. The NOEC for yield and growth rate based on biomass (dry weight) were 75.0 µg/L and 23.4 µg/L respectively.

Qualitative observations on shoot and root development at test termination indicated no apparent effects on root or shoot development compared to controls for concentrations < 23.4 µg/L. At all concentrations > 23.4 µg/L the leaves were hanging, and there were reduced numbers of roots.

RMS comment: Firstly it should be noted that the guideline used is only at draft stage and was submitted to OECD for evaluation in 2011. Despite this the following points have been raised:

The Draft AMRAP Method states some preliminary validity criteria. Growth rate for the control fresh weight parameter was >0.07 per day and so was acceptable. However the coefficient variation in yield of fresh weight in the controls was not calculated and thus not stated in this study, the Draft AMRAP Method states that it

should be <35%. Having worked out the coefficient of variance it is 26.49 % and so meets the criteria set out in the draft protocol.

The Draft AMRAP Method states that each vessel should contained one plant pot with 5 shoots initially, after seven days; 2 of the 5 plants should be removed to leave 3 uniformed plants. In this study only one shoot was planted in each pot. Although this could have impacted upon the statistical analysis of the study, results were statistically significant and a dose response pattern can be observed. The single shoots were also selected from a representative stock of over 100 uniformed plants. It is however, not specified how the single shoot tips were selected from the stock, planted 7 days before initiation. The draft protocol states that 'all clippings should be weighted individually and, for the test, only plants within a 30% weight range should be utilized'. This level of detail should have been documented however as validity criteria were met this lack of information has been accepted.

Further environmental conditions should have been recorded; pH and oxygen concentration should have been recorded at an additional interval in the middle of the study and temperature of the medium should have been recorded continuously. However, the temperature and pH ranges were maintained throughout the study so this is not deemed to be an issue. It is also worth noting that culture conditions, sources of the components in the formulated sediment, methods to prevent evaporation in the test vessels and additional visual observations were not documented. Visual observations should have been recorded throughout the study and not just at termination, this may have been carried out but details of this have not been provided. This level of detail should have been documented however as validity criteria were met this lack of information has been accepted.

This study is classified as acceptable. The proposed endpoint from this study is a nominal 14 day EyC_{50} of 40.2 $\mu\text{g GF-2573/L}$.

B.9.2.1.2 Chronic toxicity**B.9.2.1.2.1 Chronic toxicity to fish**

██████████ (2011): XDE-729 Methyl: Early Life-Stage Toxicity Test with the Fathead Minnow, *Pimephales promelas*, Under Flow-Through Test Conditions. ██████████ 65896. Dow AgroSciences unpublished report, Study Number 101134. 25 May 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Test Substance No./Lot No.	E2837-51

Test system

Organism (Species):	Fathead minnow (<i>Pimephales promelas</i>)
Study Type:	Early-Life Stage
GLP Status:	GLP
Guidelines followed:	OECD Guideline 210 (except the water characterization)
Guideline deviations reported by Study Director:	Dissolved oxygen concentrations on day 0 could not be confirmed. Dissolved concentrations through the remainder of the study ranged from 76 to 117% saturation.
Duration of study:	35 days (29 days post-hatch)
Test conditions:	Flow through
Parameters measured:	Egg hatchability, fry survival, and growth (i.e., standard length and blotted wet weight)
Observation intervals:	Egg hatchability and fry survival were observed daily; growth was measured at test termination.
Stage of embryonic development at test initiation:	<24 hours post-fertilization
Test concentrations:	Nominal: 0 (control), 0 (vehicle control), 0.072, 0.14, 0.29, 0.58, 1.2, and 2.3 mg a.s./L Mean measured: <MQL (control), <MQL (vehicle control), 0.0710, 0.129, 0.259, 0.536, 1.08, and 2.14 mg a.s./L
Analytical confirmation of test concentrations:	On days: -N, 0, 7, 14, 21, 28, and 35
Reference substance:	XDE-729 Methyl and XDE-729 Acid
Brood stock holding conditions:	Approximately 25°C
No. of holding days before dosing:	Not applicable; fertilized embryos were <24 hours post-fertilization.
Number of eggs per dose	80

group:	
Number of eggs per control group:	80
Feeding regime:	The fish were fed <i>ad libitum</i> at least three times daily during the week and at least twice a day on weekends. Fish were fed a commercial fish food and/or brine shrimp nauplii (<i>Artemia</i> sp.)
Environmental conditions:	Loading rate: maximum of 0.0315 g/L/day Temperature: 24.0 to 25.1 °C as measured weekly in all replicates. Photoperiod: 16-hour light and 8-hour dark with 30-minute simulated dawn and dusk transition periods. Dissolved oxygen concentration: 6.0 to 9.5 mg/L as measured weekly in all replicates (76 to 117% saturation) pH: 8.1 to 8.7 as measured weekly in all replicates. Total hardness: 140 to 152 mg CaCO ₃ /L.

Methodology

A 2-L proportional equal solvent diluter system similar to that described by Mount and Brungs, with a Hamilton 420 syringe dispenser, was used for the intermittent introduction of control and XDE-729 Methyl test solutions into each test chamber during the definitive test. The diluter cycle rate during the test was maintained at approximately 4.6 cycles/hour, which was sufficient to provide approximately 5.5 volume additions to each test chamber over a 24-hour period. Test chambers consisted of glass aquaria measuring approximately 15 cm wide by 31 cm long by 29 cm high with a test solution depth of 22 cm. These dimensions yielded a test solution volume of approximately 10 L. During the definitive testing, each treatment was replicated four times. Aquaria were arranged in a temperature-controlled water bath using a computer-generated random number table. The test was initiated when a target number of 20 embryos were distributed to an egg cup (glass cups constructed from 9-cm diameter glass jars with Nitex® screen replacing the bottom and suspended within each replicate chamber) in each of four test chambers for the control and each test substance treatment, yielding a target number of 80 embryos per treatment group. To facilitate test solution circulation, the cups were oscillated vertically in each chamber by means of a rocker arm apparatus driven by a low rpm electric motor. On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded. Day 0 post-hatch was based on ≥95% hatch in the control group. On study day 13 (i.e., day 7 post-hatch), all live fry were counted and released into their respective replicate growth chamber. Survival was monitored daily by visually inspecting each test chamber, and any behavioural or physical changes were recorded, including abnormalities. The test chambers were cleaned periodically (at least two times each week following the initial feeding) during the test to remove waste material and uneaten food and to minimize biological growth on the sides and bottom of the test chamber. After 29 days of post-hatch growth (Study Day 35), surviving fish were carefully netted from each replicate chamber and euthanized.

with tricaine methanesulfonate (MS-222; Argent Chemical Laboratories). All individuals were measured for standard length (i.e., tip of the snout to the caudal peduncle) using a millimeter scale and blotted wet weight using an electronic balance.

Results

Results of the chemical analysis are presented in Table B.9.2.117:

Table B.9.2.117: Results of chemical analysis.

Measured concentrations as mg a.s./L (% of nominal)												
Study day	0.072		0.14		0.29		0.58		1.2		2.3	
-N	0.0624	0.00254	0.122	0.00450	0.248	0.0100	0.470	0.0160	0.982	0.0418	1.68	0.22
0	0.0644	0.00215	0.110	0.00511	0.232	0.00894	0.460	0.0197	0.942	0.0347	1.88	0.0801
7	0.0750	NC	0.131	0.00290	0.271	0.00756	0.555	0.0159	1.09	0.0292	2.22	0.0504
14	0.0740	NC	0.129	0.00352	0.257	0.00932	0.578	0.0161	1.09	0.0276	2.21	0.0468
21	0.0708	0.00262	0.135	0.00336	0.266	0.00604	0.539	NC	1.16	0.0246	2.25	0.0832
28	0.0666	0.00292	0.124	0.00480	0.240	0.00824	0.468	0.0173	1.03	0.0272	1.94	0.0616
35	0.0724	0.00788	0.145	0.00326	0.285	0.00836	0.613	0.0171	1.18	NC	2.34	NC
Mean	0.0710	n.a.	0.129	n.a.	0.259	n.a.	0.536	n.a.	1.08	a.s.	2.14	na

Table B.9.2.118: Effects of XDE-729 Methyl on hatchability, survival and growth.

Mean Measured XDE-729 Methyl Concentration (mg a.s./L)	No. of eggs at study initiation	% egg hatchability	No. of surviving fry	% fry survival	Mean length of surviving fish (mm)	Mean wet weight of surviving fish (g)
Negative control	80	93	72	97	16.6	0.0840
Vehicle control	80	94	69	92	17.0	0.0922
0.0710	80	93	70	95	16.7	0.0879
0.129	80	94	73	97	16.4	0.0872
0.259	80	91	70	96	16.3	0.0879
0.536	80	95	56	74*	15.7*	0.0746
1.08	80	94	31	41*	14.7*	0.0633*
2.14	80	66*	0	0*	---	---
	Hatchability		Survival		Growth (based on length)	Growth (blotted wet weight)
NOEC (mg a.s./L)	1.08		0.259		0.259	0.536
LOEC (mg a.s./L)	2.14		0.536		0.536	>0.536
MATC (mg a.s./L)	1.52		0.373		0.373	nc

* Statistically significant difference in mean percent hatch as compared to the control mean for this parameter (Dunnett's test; $p < 0.05$).

Conclusions

Based on mean measured concentrations of XDE-729 Methyl, the NOEC, LOEC, and MATC values for fathead minnow egg hatchability were 1.08, 2.14, and 1.52 mg a.s./L, respectively. Based on mean measured concentrations of XDE-729 Methyl, the NOEC, LOEC, and MATC values for fry survival and standard length were 0.259, 0.536, and 0.373 mg a.s./L, respectively. The NOEC and LOEC values for blotted wet weight were 0.536 and >0.536 mg a.s./L, respectively.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes. It is proposed that for risk assessment purposes a mean measured NOEC of 0.259 mg a.s./L based on fry survival should be used.

2011: XDE-729 Acid: Early Life-Stage Toxicity Test with the Fathead Minnow, *Pimephales promelas*, Under Flow-Through Conditions. 65971. Dow AgroSciences unpublished report, Study Number 101151. 17 June 2011.

Test material

Test item:	XDE-729 Acid
Purity:	95.3 wt/%
Description:	Off-white solid
Test Substance No./Lot No. :	E2837-52

Test system

Organism (Species):	Fathead minnow (<i>Pimephales promelas</i>)
Study Type:	Early-Life Stage
GLP Status:	GLP (except the water characterization)
Guidelines followed:	OECD Guideline 210
Guideline deviations reported by Study Director:	Dissolved oxygen concentrations ranged between 79 and 108% saturation.
Duration of study:	33 days (28 days post-hatch)
Test conditions:	Flow through
Parameters measured:	Egg hatchability, fry survival, and growth (i.e., standard length and blotted wet weight)
Observation intervals:	Egg hatchability and fry survival were observed daily; growth was measured at test termination.
Stage of embryonic development at test initiation:	<24 hours post-fertilization
Test concentrations:	<p>Normal: 0 (control), 0 (vehicle control), 0.63, 1.3, 2.5, 5.0, and 10 mg a.s./L</p> <p>Mean measured: <MQL (control), <MQL (vehicle control), 0.756, 1.00, 2.93, 5.87, and 11.8 mg a.s./L</p>
Analytical confirmation of test concentrations:	On days: -N, 0, 7, 11, 20, 26, and 33
Reference substance:	XDE-729 Acid
Brood stock holding conditions:	Approximately 25°C
No. of holding days before dosing:	Not applicable; fertilized embryos were <24 hours post-fertilization.
Number of eggs per dose group:	80
Number of eggs per control group:	80
Feeding regime:	The fish were fed <i>ad libitum</i> at least three times daily during the week and at least twice a day on weekends. Fish were fed a commercial fish food and/or brine shrimp nauplii (<i>Artemia</i> sp.)
Environmental conditions:	<p>Loading rate: maximum of 0.0354 g/L/day</p> <p>Temperature: 23.5 to 24.6 °C as measured weekly in all replicates.</p> <p>Photoperiod: 16-hour light and 8-hour dark with 30-minute simulated dawn and dusk transition</p>

	<p>periods.</p> <p>Dissolved oxygen concentration: 6.4 to 8.4 mg/L as measured weekly in all replicates (79 to 108% saturation).</p> <p>pH: 8.0 to 8.4 as measured weekly in all replicates.</p> <p>Total hardness: 140 to 152 mg CaCO₃/L</p>
--	--

Methodology

A 2-L proportional equal solvent diluter system similar to that described by Mount and Brungs, with a Hamilton 420 syringe dispenser, was used for the intermittent introduction of control and XDE-729 Acid test solutions into each test chamber during the definitive test. The diluter cycle rate during the test was maintained at approximately 4.1 cycles/hour, which was sufficient to provide approximately 5.5 volume additions to each test chamber over a 24-hour period. Test chambers consisted of glass aquaria measuring approximately 18 cm wide by 20 cm long by 33 cm high with a test solution depth of 25 cm. These dimensions yielded a test solution volume of approximately 10 L. During the definitive testing, each treatment was replicated four times. Aquaria were arranged in a temperature-controlled water bath using a computer-generated random number table. The test was initiated when a target number of 20 embryos were distributed to an egg cup (glass cups constructed from 9-cm diameter glass jars with Nitex® screen replacing the bottom and suspended within each replicate chamber) in each of four test chambers for the control and each test substance treatment, yielding a target number of 80 embryos per treatment group. To facilitate test solution circulation, the cups were oscillated vertically in each chamber by means of a rocker arm apparatus driven by a low rpm electric motor. On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded. Day 0 post-hatch was based on ≥95% hatch in the control group. On study day 13 (i.e., day 7 post-hatch), all live fry were counted and released into their respective replicate growth chamber. Survival was monitored daily by visually inspecting each test chamber, and any behavioural or physical changes were recorded, including abnormalities. The test chambers were cleaned periodically during the test to remove waste material and uneaten food and to minimize biological growth on the sides and bottom of the test chamber. After 28 days of post-hatch growth (Study Day 33), surviving fish were carefully netted from each replicate chamber and euthanized with tricaine methanesulfonate (MS-222; Argent Chemical Laboratories). All individuals were measured for standard length (i.e., tip of the snout to the caudal peduncle) using a millimeter scale and blotted wet weight using an electronic balance.

Results

Results of the chemical analysis are presented in Table B.9.2.119

Table B.9.2.119 Results of chemical analysis

Nominal concentration (mg/L)	Day-N	Day 0	Day 7	Day 11	Day 20	Day 26	Day 33	Mean measured concentration
0.63	0.780	0.774	0.711	0.760	0.809	0.742	0.740	0.756 (120)
1.3	1.40	1.27	0.0802	0.862	0.208	1.42	1.45	1.00 (77)
2.5	2.97	2.72	2.80	3.10	3.16	2.82	2.96	2.93 (117)
5.0	5.57	5.34	5.57	6.49	6.38	5.46	5.98	5.87 (117)
10	12.0	11.8	11.2	12.4	12.8	11.1	11.7	11.8 (118)

Biological results are presented in Table B.9.2.120.

Table B.9.2.120 Effects of XDE-729 Acid on hatchability, survival and growth.

Mean Measured XDE-729 Acid Concentration (mg a.s./L)	No. of eggs at study initiation	% egg hatchability	No. of surviving fry	% fry survival	Mean length of surviving fish (mm)	Mean wet weight of surviving fish (g)
Negative control	80	80	57	89	18.6	0.1070
Vehicle control	80	84	56	84	18.7	0.1113
0.756	80	84	58	87	18.2	0.1015
1.00	80	75	57	95	18.5	0.1039
2.93	80	86	61	88	17.6*	0.0852*
5.87	80	90	65	90	17.8*	0.0966
11.8	80	80	56	88	18.1	0.1051
	Hatchability		Survival		Growth (based on length)	
NOEC (mg a.s./L)	11.8		11.8		11.8	
LOEC (mg a.s./L)	NA		NA		NA	
MATC (mg a.s./L)	NA		NA		NA	

* Statistically significant difference in mean growth (i.e., standard length or blotted wet weight) as compared to the control mean for this parameter (Dunnett's test; $p < 0.05$).

Conclusions

Hatching success in the control and vehicle control was 80 and 84%, respectively, and ranged from 75 to 90% in the test substance treatments. Post-hatch survival of fry in the control and vehicle control was 89 and 84%, respectively, and ranged from 87 to 95% in the test substance treatments. The negative and vehicle control animals met the acceptability criteria for mean percent hatch (i.e., >66%) and percent post-hatch survival (i.e., >70%) and there were no statistically significant differences between the control groups for any of the test parameters as specified by the study protocol and the OECD 210 testing guideline. Mean standard length was 18.6 and 18.7 mm in the control and vehicle control, respectively, and ranged

from 17.6 to 18.5 mm in the test substance treatments. Mean blotted wet weight was 0.1070 and 0.1113 g in the control and vehicle control, respectively, and ranged from 0.0852 to 0.1051 g in the test substance treatments. Water quality parameters were within acceptable limits throughout the exposure.

Based on mean measured concentrations of XDE-729 Acid, the NOEC for fathead minnow egg hatchability, fry survival, standard length, and blotted wet weight was 11.8 mg a.s./L.

RMS Comment: The study is considered to be acceptable for risk assessment purposes. It is proposed that the NOEC based on mean measured concentrations of 11.8 mg/L should be used for risk assessment purposes.

2012: XDE-729 Methyl: Early Life-Stage Toxicity Test with the Sheepshead Minnow, *Cyprinodon variegatus*, Under Flow-Through Conditions. 68313. Dow AgroSciences unpublished report, Study Number 120017. 30 July 2012.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Lot No.	E2837-51

Test system

Organism (Species):	Sheepshead Minnow (<i>Cyprinodon variegatus</i>)
Study Type:	Fish early-life stage toxicity test
GLP Status:	GLP (with the exception of water characterisation performed Feb 2012)
Guidelines followed:	OPPTS Number 850.1400
Guideline deviations reported by Study Director:	None
Duration of study:	36 days (29 days post-hatch)
Test conditions:	Flow through
Parameters measured:	Egg hatchability, fry survival, and growth (i.e. standard length and blotted wet weight)
Observation intervals:	Egg hatchability and fry survival were observed daily; growth was measured at test termination.
Stage of embryonic development at test initiation:	<24 hours post-fertilization
Test concentrations:	Nominal: 0 (control), 0 (vehicle control; 50 µL dimethylformamide/L), 3.0, 6.0, 12, 23, 45, and 90 µg XDE-729 Methyl/L Mean measured: <MQL (control), <MQL (vehicle control; 50 µL dimethylformamide/L), 2.72, 5.69,

	11.5, 22.9, 46.1, and 93.8 µg XDE-729 Methyl/L
Analytical confirmation of test concentrations:	On days: -N, 0, 3, 6, 13, 20, 27, 34 and 36
Reference substance:	XDE-729 Methyl
Brood stock holding conditions:	Salinity: 20±3‰
No. of holding days before dosing:	Not applicable; fertilized embryos were < 24 hours post-fertilization.
Number of eggs per dose group:	80
Number of eggs per control group:	80
Feeding regime:	The fish were fed <i>ad libitum</i> at least three times daily, except on study day 35 when they were fed once. Fish were fed brine shrimp nauplii (<i>Artemia</i> sp.)
Environmental conditions:	Loading rate: maximum of 0.1263 g/L/day Temperature: 24.1 to 25.6°C as measured weekly in all replicates. Photoperiod: 16-hour light and 8-hour dark with 30-minute simulated dawn and dusk transition periods. Light intensity: 511 to 694 lux Dissolved oxygen concentration: 4.42 to 7.26 mg/L as measured at least weekly in all replicates. pH: 7.82-8.37 as measured at least weekly in all replicates. Salinity: 19.3-20.3‰ as measured at least weekly in all replicates. Total hardness: Not measured

Methodology

Diluter stock solutions were prepared at a target nominal concentration of 1.80 g XDE-729 Methyl/L by diluting 0.1852 g (0.1800 g as active ingredient) to a volume of 0.10 L with dilution water. At each cycle of the diluter system, the Hamilton syringe dispenser introduced approximately 0.10 mL volumes of the diluter stock solution to the diluter system where the solution was diluted with approximately 2,000 mL of dilution water.

A 2L proportional equal solvent diluter system with a Hamilton 420 syringe dispenser, was used for the intermittent introduction of control, vehicle control (50 µL dimethylformamide/L), and XDE-729 Methyl test solutions into each test chamber during the definitive test. The diluter system mixing/flow-splitting cells delivered the dilution water control, vehicle control, and each of the six test solutions to the test chambers during the definitive test. Each mixing/flow-splitting cell divided each 1L volume four ways resulting in a volume of approximately 250 mL being delivered to each test chamber with each cycle during the definitive test. The diluter cycle rate during the definitive test was maintained

at approximately 4.0 cycles/hour, which was sufficient to provide approximately six volume additions to each test chamber over a 24-hour period.

Test chambers consisted of glass aquaria measuring approximately 14 cm wide by 22.6 cm long by 16.9 cm high with a test solution depth of 13 cm. These dimensions yielded a test solution volume of approximately 4 L. Each treatment was replicated four times and the replicate test chambers were held in a temperature-controlled water bath maintained at 25 ± 2 °C during the test. During the pre-hatch portion of the test, developing embryos were incubated in glass cups constructed from 9-cm diameter glass jars with nylon screen replacing the bottom and were oscillated vertically in each aquarium by means of an electric rocker arm apparatus. On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded. Day 0 post-hatch was based on $\geq 95\%$ hatch in the control treatment. On study day 13 (i.e., day 6 post-hatch), all live fry were counted and released into their respective replicate growth chamber. Embryos that had not yet hatched by the date of release were maintained in the egg cup until they had hatched, at which time they were released into their respective replicate growth chamber. Survival was monitored daily by visually inspecting each test chamber, and any behavioural or physical changes were recorded, including abnormalities. After 29 days of post-hatch growth (Study Day 36), surviving fish were carefully netted from each replicate chamber and euthanized with tricaine methanesulfonate (MS-222; Argent Chemical, Inc.). All individuals were measured for standard length (i.e., tip of the snout to the caudal peduncle) using a millimeter scale and blotted wet weight using an electronic balance.

Results

All guideline-prescribed validity criteria were met for this fish early life stage toxicity test. Temperature and dissolved oxygen levels within the test media were maintained within the stated range. There was no adverse effect to any of the recorded parameters in the vehicle control group. The guideline-given minimum control hatching success and survival were also met. Control and vehicle control hatching success were 84% and 93 % respectively (criteria of $>75\%$ required). Control and vehicle control fry survival were 96% and 95% respectively (criteria of $>80\%$ required).

Measured concentrations throughout the test were in the range 15-113%, meaning that endpoints should be based upon mean measured concentrations. The day 0 analytical sample at 3.0 µg/L resulted in only 15% of the nominal test substance concentration being present. This was confirmed by re-dilution and duplicate re-analysis. Although all other samples at this test concentration were found to be within 80-120% of the nominal value, it is considered suitably conservative to base results on mean measured concentrations. Based upon these, the NOEC and LOEC values for sheepshead minnow egg hatchability were 93.8 and >93.8 µg XDE-729 Methyl/L, respectively. The NOEC, LOEC, and MATC values for fry survival were 11.5, 22.9, and 16.2 µg XDE-729 Methyl/L, respectively. The NOEC and LOEC values for blotted wet weight were 11.5 and >11.5 µg XDE-729 Methyl/L, respectively. The NOEC, LOEC, and MATC values for fry standard length were 2.72, 5.69, and 3.93 µg XDE-729 Methyl/L, respectively.

Table B.9.2.121: Results of chemical analysis.

Measured concentrations as µg XDE-729 Methyl/L (% of nominal)								
Study day	Control (0)	Vehicle control	3.0	6.0	12	23	45	90
-N	<MQL	<MQL	2.64 (88)	4.90 (82)	9.26 (77)	19.8 (86)	38.2 (85)	75.0 (83)
0	<MQL	<MQL	0.442 (15)	6.32 (105)	12.8 (107)	24.4 (106)	48.8 (108)	93.1 (103)
3	<MQL	<MQL	3.16 (105)	6.08 (101)	12.9 (108)	23.1 (100)	47.2 (103)	90.1 (100)
6	<MQL	<MQL	3.34 (111)	5.94 (99)	11.1 (93)	23.9 (104)	46.0 (102)	94.6 (104)
13	<MQL	<MQL	3.14 (105)	5.70 (95)	12.5 (104)	23.7 (103)	47.2 (105)	102 (113)
20	<MQL	<MQL	3.06 (102)	5.68 (95)	10.4 (87)	21.5 (93)	45.2 (100)	92.4 (103)
27	<MQL	<MQL	3.00 (100)	5.28 (88)	11.6 (97)	22.7 (99)	46.0 (102)	92.5 (103)
34	<MQL	<MQL	2.68 (89)	5.06 (84)	9.70 (81)	20.4 (89)	41.6 (92)	84.0 (93)
36	<MQL	<MQL	2.90 (97)	5.48 (91)	11.2 (93)	23.2 (101)	46.8 (104)	102 (113)
Mean	<MQL	<MQL	2.72 (91)	5.69 (95)	11.5 (96)	22.9 (100)	46.1 (102)	93.8 (104)

Table B.9.2.122: Effects of XDE-729 Methyl on hatchability, survival and growth.

Treatment Expressed as Mean Measured Concentration (µg XDE-729 Methyl/L)	No. of eggs at study initiation	% egg hatchability	No. of surviving fry	% fry survival	Mean length of surviving fish (mm)	Mean wet weight of surviving fish (g)
Negative control	80	84	64	96	16.8	0.157
Vehicle control	80	93	70	95	16.4	0.142
2.72	80	89	68	96	16.1	0.149
5.69	80	83	66	100	15.7 [*]	0.142
11.5	80	91	70	96	15.6 [*]	0.140
22.9	80	83	50	76 [*]	15.7 ^a	0.150 ^a
46.1	80	88	16	23 [*]	16.2 ^a	0.174 ^a
93.8	80	89	5	7 [*]	16.8 ^a	0.168 ^a
	Hatchability		Survival		Standard length	
NOEC (µg XDE-729 Methyl/L)	93.8 µg XDE-729 Methyl/L		11.5 µg XDE-729 Methyl/L		2.72 µg XDE-729 Methyl/L	
LOEC (µg XDE-729 Methyl/L)	>93.8 µg XDE-729 Methyl/L		22.9 µg XDE-729 Methyl/L		5.69 µg XDE-729 Methyl/L	
MATC (µg XDE-729 Methyl/L)	Could not be calculated		16.2 µg XDE-729 Methyl/L		3.93 µg XDE-729 Methyl/L	
* Statistically significant reduction (Dunnett's test; <i>p</i> < 0.05) as compared to the control mean for this endpoint.						
^a Not included in statistical analysis for this endpoint due to significant reduction in fry survival at this treatment level compared to the control.						

Conclusions

The lowest reported NOEC (2.72 μg XDE-729 Methyl/L) was based on length; no similar effect was seen for weight. Since test substance treatment levels for which a significant reduction in fry survival was detected were excluded from statistical analysis for growth endpoints, this obscures that the mean length of the surviving fish in the higher treatment levels was similar to the controls and indicates no dose-related trend. Therefore the NOEC for length is likely to be highly conservative compared to the NOEC obtained for survival (11.5 μg XDE-729 Methyl/L). All water quality parameters were maintained within guideline-acceptable limits throughout the test.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes. It is proposed that for risk assessment purposes a mean

measured NOEC of 11.5 µg XDE-729 Methyl/L based on fry survival should be used. This follows discussion with the applicant in which it was agreed that survival was the most appropriate endpoint, not standard length (see conclusions).

2012: X11449757: Early Life-Stage Toxicity Test with the Fathead Minnow, *Pimephales promelas*, Under Flow-Through Conditions. 66009. Dow AgroSciences unpublished report, Study Number 101165. 22 August 2012.

Test material

Test item:	X11449757
Purity:	97%
Description:	White solid
Test Substance No./Lot No.	SYN-FS08644-090

Test system

Organism (Species):	Fathead minnow (<i>Pimephales promelas</i>)
Study Type:	Early-life stage
GLP Status:	GLP (exception was latest water characterization performed in February 2012)
Guidelines followed:	OECD 210
Guideline deviations reported by Study Director:	None
Duration of study:	34 days (29 days post-hatch)
Test conditions:	Flow-through
Parameters measured:	Hatch start, hatch completion, Egg hatchability, fry survival, and growth (i.e., standard length and blotted wet weight)
Observation intervals:	Egg hatchability and fry survival were observed daily; growth was measured at test termination.
Stage of embryonic development at test initiation:	<24 hours post-fertilization
Test concentrations:	Nominal: 0 (control), 0.33, 0.65, 1.3, 2.5, 5.0, and 10 mg/L Mean: <MQL (control), 0.25, 0.54, 1.0, 2.2, 4.4, and 8.9 mg/L
Analytical confirmation of test concentrations:	On days: -N, 0, 7, 14, 21, 28, and 34
Brood stock holding conditions:	Approximately 25°C
No. of holding days before dosing:	Not applicable; fertilized embryos were <24 hours post-fertilization.
Number of eggs per dose group:	80 (exception for level 1 replicate A and level 5 replicate D. 21 eggs were added at initiation so

	there were more than 80 eggs in these two dose replicates)
Number of eggs per control group:	80
Feeding regime:	Initially fry were fed brine shrimp nauplii (<i>Artemia</i> sp.) Fish were fed live brine shrimp <i>ad libitum</i> twice on study day 3, and three times daily during the remainder of the definitive test. A standard commercial fish food was added to the diet on study day 17.
Environmental conditions:	Loading rate: maximum of 0.0842 g/L/day Temperature: 24.4 to 25.1°C as measured weekly in all replicates. Photoperiod: 16-hour light and 8-hour dark with 30-minute simulated dawn and dusk transition periods. Dissolved oxygen concentration: 5.6 to 8.2 mg/L as measured weekly in all replicates. pH: 8.1 to 8.4 as measured weekly in all replicates. Total hardness: 142 to 150 mg CaCO ₃ /L

Methodology

A 34 day study (of which 29 days were post-hatch), flow through test was performed with nominal test concentrations of 0 (control), 0.33, 0.65, 1.3, 2.5, 5.0 and 10 mg/L. A 2-L proportional equal solvent diluter system similar to that described by Mount and Brungs, with a Luft Systematic pump, was used for the intermittent introduction of control and X11449757 test solutions into each test chamber during the definitive test. The diluter cycle rate during the test was maintained at approximately 4.25 cycles/hour, which was sufficient to provide approximately 5.1 volume additions to each test chamber over a 24-hour period. Test chambers consisted of glass aquaria measuring approximately 15 cm wide by 31 cm long by 29 cm high with a test solution depth of 22 cm. These dimensions yielded a test solution volume of approximately 10 L. During the definitive testing, each treatment was replicated four times. Aquaria were arranged in a temperature-controlled water bath using a computer-generated random number table. The test was initiated when a target number of 20 embryos were distributed to an egg cup (glass cups constructed from 9-cm diameter glass jars with Nitex® screen replacing the bottom) suspended in each of four test chambers for the control and each test substance treatment, yielding a target number of 80 embryos per treatment group. The embryos were impartially selected and distributed into each replicate incubation cup until the total number of embryos was achieved within each replicate. To facilitate test solution circulation, the cups were oscillated vertically in each chamber by means of a rocker arm apparatus driven by a low rpm electric motor. On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded. Day 0 post-hatch was based on ≥95% hatch in the control group. On study day 7 (i.e., day 2 post-hatch), all live fry were counted and released into their respective replicate growth chamber.

Survival was monitored daily by visually inspecting each test chamber, and any behavioural or physical changes were recorded, including abnormalities. The test chambers were cleaned periodically during the test to remove waste material and uneaten food and to minimize biological growth on the sides and bottom of the test chamber. After 29 days of post-hatch growth (study day 34), surviving fish were carefully netted from each replicate chamber and euthanized with tricaine methanesulfonate. All individuals were measured for standard length (i.e., tip of the snout to the caudal peduncle) using a millimeter scale and blotted wet weight using an electronic balance.

Results

Table B.9.2.123: Measured Concentrations of X11449757 During the Flow-Through Early Life-Stage Toxicity Test with Fathead Minnow (*Pimephales promelas*)

Measured concentrations as mg/L (% of nominal)								
Study day	Control	Level 1 (0.33) ^a	Level 2 (0.65) ^a	Level 3 (1.3) ^a	Level 4 (2.5) ^a	Level 5 (5.0) ^a	Level 6 (10) ^a	Diluter Stock (98) ^a
-N	<MQL ^b	0.25 (76)	0.51 (78)	0.95 (73)	2.1 (84)	4.1 (82)	8.4 (84)	NA
0	<MQL ^b	0.26 (79)	0.53 (82)	1.0 (77)	2.2 (88)	4.1 (82)	8.9 (89)	81 (83)
7	<MQL ^b	0.26 (79)	0.52 (80)	0.97 (75)	2.1 (84)	4.3 (86)	8.7 (87)	85 (87)
14	<MQL ^b	0.23 (70)	0.51 (78)	0.95 (73)	2.1 (84)	4.3 (86)	8.3 (83)	85 (87)
21	<MQL ^b	0.22 (67)	0.51 (78)	0.93 (72)	2.1 (84)	4.2 (84)	8.2 (82)	84 (86)
28	<MQL ^b	0.26 (79)	0.59 (91)	1.1 (85)	2.4 (96)	4.8 (96)	9.9 (99)	87 (89)
34	<MQL ^b	0.28 (85)	0.57 (88)	1.1 (85)	2.2 (88)	4.7 (94)	9.3 (93)	85 (87)
Mean	<MQL ^b	0.25 (76)	0.54 (83)	1.0 (77)	2.2 (88)	4.4 (88)	8.9 (89)	85 (87)

^a Nominal Concentration as mg/L.

^b Minimum Quantifiable Limit (MQL) = 0.050 mg/L.

NA = Not applicable.

Table B.9.2.124: Effects of X11449757 on hatchability, survival and growth

Mean Measured X11449757 Concentration (mg/L)	No. of eggs at study initiation	% egg hatchability	No. of surviving fry	% fry survival	Mean length of surviving fish (mm)	Mean wet weight of surviving fish (g)
Negative control	80	100	76	95	21.9	0.199
0.25	81	99	69	86	22.0	0.217
0.54	80	98	70	90	22.0	0.212
1.0	80	98	69	88	22.3	0.224
2.2	80	100	71	89	22.8	0.227
4.4	81	98	70	89	22.4	0.220
8.9	80	99	73	92	22.4	0.230
	Hatchability		Survival		Growth (based on length)	
NOEC (mg/L)	8.9		8.9		8.9	
LOEC (mg/L)	>8.9		>8.9		>8.9	
MATC (mg/L)	NC		NC		NC	

NC = Not calculated

Conclusions

Based on mean measured concentrations of X11449757, the NOEC and LOEC values for fathead minnow for all endpoints observed were 8.9 and >8.9 mg/L, respectively. There was no statistically significant difference in days to hatch start, hatch completion, hatch success, mean growth (i.e., standard length or blotted wet weight) or mean survival as compared to the control mean. The MATC values could not be calculated.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes. The study has been carried out in accordance with OECD 210. It is proposed that for risk assessment purposes a mean measured NOEC of 8.9 mg X11449757/L should be used.

B.9.2.1.2.2 Chronic toxicity to aquatic invertebrates

Bergfield, W.A. (2011): XDE-729 Methyl: Chronic Toxicity with the Water Flea, *Daphnia magna*, Exposed Under Static-Renewal Test Conditions. ABC Laboratories, Columbia, Missouri, ABC study number 65897. Dow AgroSciences unpublished report, Study Number 101133. 27 June 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Lot No./Lot no. :	E2837-51

Test system

Organism (Species):	Water flea (<i>Daphnia magna</i>)
Study Type:	Chronic
GLP Status:	GLP (except the water characterization)
Guidelines followed:	U.S. EPA OPPTS 850.1300 and OECD guideline 211
Guideline deviations reported by Study Director:	None
Duration of study:	21 days
Test conditions:	Static-Renewal (daily renewal)
Parameters measured:	Immobility, reproduction, and length
Observation intervals:	24 hours
Age range of water fleas at test initiation:	<24 hours
Test concentrations:	Nominal: 0 (control), 0 (vehicle control), 0.062, 0.13, 0.25, 0.49, 0.98, and 2.0 mg a.s./L
	Mean measured: <MQL (control), <MQL (vehicle control), 0.0620, 0.144, 0.279, 0.484, 0.920, and 1.63 mg a.s./L
Analytical confirmation of test concentrations:	Day 0, 5, 14, 20 (fresh solutions) Day 1, 6, 15, 21 (24h old solutions)
Reference substance:	XDE-729 Methyl and XDE-729 Acid
Number of Daphnids per dose group:	10
Number of Daphnids per control group:	10
Feeding regime:	Daily
Environmental conditions:	Temperature: 19.4 to 21.7°C Photoperiod: 16 hour light:8 hour dark Light intensity: 528 lux Dissolved Oxygen concentration: 8.2 to 9.2 mg/L (94 to 106% of saturation) in fresh solutions; 7.0 to 8.6 mg/L (80 to 99% of saturation) in spent solutions pH: 8.0 to 8.7 Water alkalinity: 156 to 168 mg CaCO ₃ /L

	Water hardness: 144 to 152 mg CaCO ₃ /L Water conductivity: 316 to 359 µS
--	--

Methodology

A definitive test was performed at nominal concentrations of 0 (control), 0 (vehicle control), 0.062, 0.13, 0.25, 0.49, 0.98, and 2.0 mg a.s./L. The definitive test was conducted in 150-mL glass jars containing approximately 80 mL of control or test substance solution. A single daphnid was added to labelled containers until 80 containers contained one daphnid. The jars were covered with Teflon-lined lids. The test chambers were maintained at $20 \pm 2^{\circ}\text{C}$ in a temperature-controlled water bath. Fluorescent lighting was maintained on a 16-hour daylight photoperiod with two 30-minute simulated dawn and dusk periods. The light intensity during the definitive test was 528 lux as measured on Day 0 with a LI-COR Model LI-189 light meter equipped with a photometric sensor.

Results

Initial concentrations of XDE-729 methyl were generally between 85 – 118 % nominal concentrations, with deviations from this range considered to be most probably due to post-sampling contamination rather than incorrect treatment preparation. Daily renewal of test solutions resulted in XDE-729 Methyl concentrations in the 24h old solutions generally remaining above 80% nominal. As a result, mean measured test concentrations of XDE-729 Methyl were 0.0620, 0.144, 0.279, 0.484, 0.920 and 1.63 mg a.s./L (i.e. between 85 and 142% nominal). Analytical results are presented in Table B.9.2.125-126.

Table B.9.2.125: Analytical results – XDE-729 methyl

Sample day	Measured concentration of XDE-729 Methyl expressed as mg a.s./L (% nominal)					
	0.062	0.13	0.25	0.49	0.98	2.0
0 (fresh)	0.0878 (142)	0.139 (107)	0.240 (96)	0.515 (105)	0.942 (96)	1.66 (85)
1 (spent)	0.0474 (76)	0.0992 (76)	0.212 (85)	0.429 (88)	0.866 (88)	1.60 (82)
5 (fresh)	0.0616 (99)	0.298 (229)	0.404 (162)	0.504 (103)	0.952 (97)	na
6 (spent)	0.0556 (90)	0.108 (83)	0.200 (80)	0.445 (91)	0.886 (90)	na
14 (fresh)	0.0624 (101)	0.138 (106)	0.343 (137)	0.510 (104)	0.928 (95)	na
15 (spent)	0.0530 (85)	0.106 (82)	0.212 (85)	0.434 (89)	0.882 (90)	na
20 (fresh)	0.0684 (110)	0.142 (109)	0.387 (155)	0.577 (118)	0.992 (101)	na
21 (spent)	0.0596 (96)	0.123 (95)	0.234 (94)	0.457 (93)	0.910 (93)	na
Average measured concentration	0.0620 (111)	0.144 (111)	0.279 (112)	0.484 (99)	0.920 (94)	1.63 (83)

Table B.9.2.126: Analytical results – XDE-729 acid

Sample day	Measured concentration of XDE-729 Acid expressed as mg a.s./L					
	0.062	0.13	0.25	0.49	0.98	2.0
Treatment (mg XDE-729 methyl/L)						
0 (fresh)	0.00139	0.00218	0.00398	0.00868	0.0157	0.0792
1 (spent)	0.00350	0.00672	0.0134	0.0293	0.0622	0.123
5 (fresh)	0.000794	0.00344	0.00468	0.00620	0.0116	na
6 (spent)	0.00320	0.00566	0.0115	0.0238	0.0530	na
14 (fresh)	0.000420	0.000778	0.00190	0.00320	0.00566	na
15 (spent)	0.00304	0.00578	0.0116	0.0232	0.0662	na
20 (fresh)	0.000598	0.00113	0.00264	0.00486	0.00906	na
21 (spent)	0.00334	0.00608	0.0110	0.0234	0.0405	na

Table B.9.2.127: Effects of XDE-729 Methyl on survival, growth, and reproduction of daphnids

Treatment (mg a.s./L)		Day 0 to 21	At test termination			
Nominal	Mean Measured	Survival of adult daphnids (%)	Days to First Brood	Total No. of live offspring produced	Mean number of live offspring produced per surviving daphnid	Mean length of surviving adults (mm)
Negative control	<MQL	80	10	701	88	4.0
Vehicle control	<MQL	90	10	789	83	4.1
0.062	0.0620	90	11	742	79	3.9
0.13	0.144	80	10	634	76	4.2
0.25	0.279	100	10	509	51*	4.1
0.49	0.484	80	10	570	71	4.1
0.98	0.920	90	12	208	23*	3.4
2.0	1.63	0*	---	---	---	---
		Survival	Reproduction			Growth
EC50 (mg/L) a		1.18	NC	NC	0.706	NC
NOEC (mg/L) a		0.920	0.484	NC	0.144	0.484
LOEC (mg/L) a		1.63	0.920	NC	0.279	0.920
MATC (mg/L) a, b		1.22	0.667	NC	NC	0.667

a Based on mean measured concentrations. Values expressed as mg a.s./L.

b MATC is defined by US EPA as the geometric mean of the NOEC and LOEC for the most sensitive endpoint.

NC = not calculated

*Statistically significant ($p = 0.05$)

Table B.9.2.128: Sub-lethal effects of XDE-729 Methyl on appearance or behaviour.

Treatment (mg a.s./L)		(% affected)		
Nominal	Mean Measured	Day 7	Day 14	Day 21
Negative control	<MQL	0	0	0
Vehicle control	<MQL	0	0	0
0.062	0.0620	0	0	0
0.13	0.144	0	0	0
0.25	0.279	0	0	0
0.49	0.484	0	0	0
0.98	0.920	0	0	0
2.0	1.63	0	0	0

Conclusions

The test acceptability criteria were met for this study. The mortality of the parent animal in the control did not exceed 20% at the end of the test. The mean number of live young produced per parent animal surviving at the end of the test was ≥ 60 . This study is classified as acceptable and satisfies the guideline requirement for a reproduction test with *Daphnia magna*. Total young per surviving adult and adult length were the most sensitive biological parameters.

After 21 days of exposure, the 21-day NOEC and LOEC values for survival were 0.920 and 1.63 mg a.s./L, respectively. The 21-day EC50 based on immobilization of the first generation daphnids was 1.18 mg a.s./L with 95% confidence limits of 1.07 and 1.30 mg a.s./L. The MATC for adult survival was 1.22 mg a.s./L. Based on mean number of live young per surviving the 21-day NOEC and LOEC values were 0.144 and 0.920 mg a.s./L, respectively. The 21-day EC50 for total young per surviving adult was 0.706 mg a.s./L with 95% confidence limits of 0.643 and 0.769 mg a.s./L. The MATC for days to first brood, number of young per live adult, and adult length was 0.667 mg a.s./L.

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes. It is proposed that the mean measured NOEC of 0.144 mg a.s./L based on mean offspring production be used for risk assessment purposes.

Bergfield, W.A. 2011: XDE-729 Acid: Chronic Toxicity Test with the Water Flea, *Daphnia magna*, Exposed Under Static-Renewal Conditions. ABC Laboratories, Columbia, Missouri, ABC study no 65972. Dow AgroSciences unpublished report, Study Number 101150. 14 December 2011.

Test material

Test item:	XDE-729 Acid
Purity:	95.3%
Description:	Off white solid
Lot No./Lot no. :	E2837-52

Test system

Organism (Species):	<i>Daphnia magna</i>
Study Type:	Chronic
GLP Status:	GLP (with exception of water characterization performed in February 2011)
Guidelines followed:	OECD 211
Guideline deviations reported by Study Director:	None
Duration of study:	21 day
Test conditions:	Static renewal (2-3 days)
Parameters measured:	Immobility, growth (length), reproductive output and abnormalities.
Observation intervals:	Daily, length on day 21 only.
Age range of water fleas at test initiation:	<24 hours old
Test concentrations: Nominal	0 (control), 0.95, 3.1, 9.8, 31, and 100 mg a.i./L
Test concentrations Mean measured:	<MQL (control), 0.946, 3.10, 10.0, 30.8, and 98.3 mg XDE-729 acid/L
Analytical confirmation of test concentrations:	Fresh test solutions- initiation, days 9, 14, and 19. Spent solutions- days 2, 12, 16, and 21
Number of water fleas per dose group:	10
Number of water fleas per control group:	10
Feeding:	During the holding period, the daphnids were fed a suspension of the algal species <i>Pseudokirchneriella subcapitata</i> (formerly <i>Selenastrum capricornutum</i>) at least once a day supplemented by a prepared artificial diet consisting of a wheat grass, salmon starter, and yeast suspension. The daphnids were fed daily a diet consisting of 1.0 mL of a 3.0×10^7 cells/mL concentrated algal suspension (<i>Pseudokirchneriella subcapitata</i> , formerly <i>Selenastrum capricornutum</i>) and 0.5 mL of a 2.4 g/L YTC daphnid feed mixture.
Environmental conditions:	Temperature: 19.2 to 20.3°C

	<p>Photoperiod: 16 hour light: 8 hour dark, 30 minutes simulated dawn and dusk period.</p> <p>Light intensity: 447 lux</p> <p>Dissolved Oxygen concentration: 8.2 to 9.8 mg/L (94 to 107% of saturation) in fresh solutions; 7.0 to 8.2 mg/L (82 to 94% of saturation) in spent solutions.</p> <p>pH: 7.6 to 8.6</p> <p>Water alkalinity: 158 to 178 mg CaCO₃/L</p> <p>Water hardness: 140 to 158 mg CaCO₃/L</p> <p>Water conductivity: 297 to 328 µS</p>
--	---

Methodology

An initial definitive test was performed with 0 (control), 6.5, 13, 25, 50 and 100 mg XDE-729 acid/L. There was no significant effect on time to first brood, or on final body length of the adult daphnids in this first test however, statistical analysis indicated that reproduction in the controls was higher than all treatment concentrations. Although a statistically significant difference was identified, this was not a dose-related response, indicating that this difference was not treatment related. Nevertheless, to confirm this, a second definitive test was performed at nominal concentrations of 0 (control), 0.95, 3.1, 9.8, 31, and 100 mg XDE-729 acid/L for 21 days, therefore covering a range of concentrations used in the first definitive test.

This second definitive test (from which the endpoints are derived) was conducted in 150-mL glass jars containing approximately 80 mL of control or test substance solution. Ten daphnids were impartially added to a set of labelled containers with each container representing one treatment replicate. Each container was randomly assigned to a treatment replicate using a random number generator. Daphnids were then transferred with a pipette from the containers to the appropriate test chamber. Observations were made daily on the number of surviving adult daphnids, occurrence of abnormalities, and production of neonates. Immobile daphnids, defined as those organisms not able to swim within 15 seconds after gentle agitation were discarded; therefore, immobility was synonymous with mortality.

Test solutions were renewed every Monday, Wednesday, and Friday to ensure concentrations of the test item were maintained. Fresh test solutions were analysed for test item concentration at test initiation and on days 9, 14 and 19. Spent test solutions were analysed for test item concentration at on days 2, 12, 16 and 21. The daphnids were fed daily. At test termination, the length (head to base of spine) of each surviving adult was measured with an ocular micrometer on a dissecting microscope. Total hardness, total alkalinity, and conductivity were measured in a sample of the dilution water control from fresh and spent solutions once per week. Temperature, dissolved oxygen concentration, and pH were measured in the fresh and spent solutions on renewal days. No aeration was provided to any control or test substance treatment during the test. A continuous recording of the water bath temperature was made during the test using a datalogger and thermistor probe in

addition to daily temperature measurements of the water bath using a verified thermometer

Results

Chemical analysis showed the measured test item concentrations to be within the acceptable range ($\pm 20\%$ of nominal) at all sampling points.

Table B.9.2.129: Effects of XDE-729 Acid on survival, growth, and reproduction of daphnids.

Nominal (mg /L)	Mean Measured (mg/L)	Survival of adult daphnids (%)	Days to First Brood	Total No. of live offspring produced	Mean number of live offspring produced per surviving daphnid	Mean length of surviving adults (mm)
Negative control	<MQL	100	8	1,281	128	4.8
0.95	0.946	100	8-10	1,343	134	4.8
3.1	3.10	100	8-9	1,432	143	4.8
9.8	10.0	80	8	1,017	127	4.9
31	30.8	90	8	1,311	146	4.9
100	98.3	90	8	1,344	149	5.0
		Survival	Reproduction			Growth
EC ₅₀ (mg/L) ^a		>100	>100	>100	NC	NC
NOEC (mg/L) ^a		100	100	100	100	100
LOEC (mg/L) ^a		>100	>100	>100	>100	>100

^a Based on nominal concentrations. Values expressed as mg XDE-729 acid/L.
NC = not calculated

No sub-lethal effects were observed.

Conclusions

The test acceptability criteria were met for this study. The parental daphnid mortality in the control group did not exceed 20% at the end of the test. The mean number of live young produced per surviving parental daphnid at the end of the test was ≥ 60 , with a coefficient of variance of $<30\%$ (9%). This study is classified as acceptable and satisfies the guideline requirement for a reproduction test with *Daphnia magna*.

The measured XDE-729 Acid concentrations ranged from 92-107% of the nominal concentrations. Since all analytical results were within 20% of the nominal XDE-729 Acid concentrations, the biological response results were reported based upon the nominal concentrations. After 21 days of exposure, there was no significant reduction in survival in the test substance treatments as compared to the control survival. Based

on survival, the 21 day NOEC and LOEC were 100 and >100 mg XDE-729 acid/L respectively. There was no significant difference in total number of young per surviving adult or number of days to first brood release in the test substance treatments as compared to the control. Based on nominal concentrations, the NOEC and LOEC for all reproductive endpoints were 100 and >100 mg XDE-729 acid/L, respectively. The 21-day EC₅₀ based on immobilization of the first generation daphnids, total young per surviving adult, and number of days to first brood was >100 mg XDE-729 acid/L, the highest concentration tested. Based on length, the 21-day NOEC and LOEC were 100 and >100 mg XDE-729 acid/L, respectively.

RMS comment: The study is considered to be acceptable and suitable for risk assessment purposes. The study satisfies the OECD 211 guideline. The proposed endpoint is a nominal 21 day NOEC of 100 mg XDE-729 acid/L. Ideally the report would confirm the neonates used to initiate the definitive test were not from the first brood produced by the parent stock.

Hicks, Stephen L. (2011): XDE-729 Methyl: Life-Cycle Toxicity Test of the Saltwater Mysid, *Americamysis bahia*, Conducted under Flow-Through Conditions. ABC Laboratories, 7200 E. ABC Lane, Columbia, Missouri 65202, ABC Laboratories Project Number 65895. Dow AgroSciences unpublished report, Study Number 101131. 08 July 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Test Substance No./Lot No.:	E2837-51

Test system

Organism (Species):	Mysid (<i>Americamysis bahia</i>)
Study Type:	Life-cycle
GLP Status:	GLP (except the water characterization)
Guidelines followed:	U.S. EPA OPPTS 850.1350 and OPPTS 850.1000, and FIFRA Subdivision E, Section 72-4
Guideline deviations reported by Study Director:	None
Duration of study:	28 days
Test conditions:	Flow through
Parameters measured:	Dissolved oxygen, pH, temperature, salinity, light
Observation intervals:	Daily
Age of test organisms at test initiation:	<24 hours
Test concentrations:	0 (control), 0 (vehicle control), 0.090, 0.18, 0.35, 0.70, and 1.4 mg a.s./L Mean measured Methyl: <MQL (control), <MQL (vehicle control), 0.0815, 0.152, 0.325, 0.646, and

	1.27 mg a.s./L
Analytical confirmation of test concentrations:	On days: -N, 0, 7, 14, 16, 21, and 28
Reference substance:	XDE-729 Methyl and XDE-729 Acid
No. of holding days before dosing:	Not Applicable; <24 hour old mysids were used
Number of mysids per dose group:	90
Number of mysids per control group:	90
Feeding regime:	Mysids were fed <i>ad libitum</i> brine shrimp nauplii (<i>Artemia</i> sp.; 24-48 hours old) at least two times daily.
Environmental conditions:	Temperature: 24.1 to 25.0°C Photoperiod: 14 hours light, 10 hours dark, 604 – 800 lux Dissolved oxygen concentration: 5.9 to 8.6 mg/L (80 to 108% saturation) pH: 7.9 to 8.6 Salinity: 18.8 to 21.2‰

Methodology

The in-life phase of the flow-through definitive test was performed from 28 February to 28 March 2011. The testing was performed at nominal concentrations of 0 (control), 0 (vehicle control), 0.090, 0.18, 0.35, 0.70, and 1.4 mg a.s./L. Diluter stock solutions were prepared at a target concentration of 7.346 mg a.s./L in dilution water. A Hamilton syringe dispenser introduced 0.375-mL volumes of the diluter stock solution to the diluter system, where the diluter stock solution volume was diluted with approximately 3900 mL of dilution water.

Mysids were fed *ad libitum* brine shrimp nauplii (*Artemia* sp.; 24-48 hours old) at least three times daily except for initiation and termination when mysids were fed twice. Observations of mortality and sub-lethal responses for F₀-mysid generation were made daily for the duration of the testing period. The body lengths of mysids (as measured by total midline body length) were measured to the nearest 0.1 mm with a dissecting microscope.

Ovigerous F₀-female mysids (i.e., females with eggs within the marsupium) were isolated and paired with adult males and transferred to the brood baskets on day 13 of the exposure. Once paired, the mysids in the brood cups were observed for mortality and reproduction (i.e., young per female). The first day young were observed was considered the day of first brood, although release of these young may have occurred over 2 or 3 days.

After 14 days of exposure, the body length of all surviving F₀ mysids present in the growth-retention basket was measured. The growth-retention baskets were terminated following these measurements. The F₁-mysid exposure phase of the test was initiated with the first 15 post-larval F₁ mysids, or fewer when 15 young

were not available. The post-larval F₁ mysids were assigned to retention baskets within the same test chambers as the F₀-mysid exposure. The isolated F₁ mysids were observed daily for mortality during the exposure and at termination of the F₀-mysid exposure (i.e., study day 28). The F₁ mysids were terminated when they reached 9 days of age because this was the maximum achievable age for all but the highest treatment F₁ mysids at termination of the F₀-mysid exposure (i.e., study day 28). The body length of all surviving 9-day old F₁ mysids was measured. Temperature, dissolved oxygen, salinity, and pH were measured in the replicate test chambers of all treatments at test initiation and termination and at least weekly during the definitive test. Test solution salinity was also measured daily in at least one replicate test chamber. Light intensity was measured on day 28.

Results

The mean measured concentrations of XDE-729 Methyl in the 0.090, 0.18, 0.35, 0.70, and 1.4 mg a.s./L treatments were 0.0815, 0.152, 0.325, 0.646 and 1.27 mg a.s./L, which represented 91, 84, 93, 92 and 91% nominal, respectively. Full details are presented below in Table B.9.2.130.

Table B.9.2.130: Analytical results

Measured XDE-729 Methyl concentration as mg a.s./L (% nominal) and XDE-729 Acid concentrations as mg/L										
Day	0.090		0.18		0.35		0.70		1.4	
-	Methyl	Acid	Methyl	Acid	Methyl	Acid	Methyl	Acid	Methyl	Acid
-N	0.0814 (90)	0.00167	0.151 (84)	0.00308	0.339 (97)	0.00590	0.704 (101)	0.0128	1.37 (98)	0.0250
0	0.0976 (108)	0.00192	0.171 (95)	0.00350	0.373 (107)	0.00806	0.730 (104)	0.0167	1.33 (95)	0.0174
7	0.0874 (97)	0.00149	0.160 (89)	0.00258	0.353 (101)	0.00572	0.694 (99)	0.0116	1.32 (94)	0.0246
14	0.0510 (57)	0.00107	0.100 (56)	0.00234	0.228 (65)	0.00436	0.451 (64)	0.00792	0.870 (87)	0.0154
16	0.0832 (92)	0.00109	0.159 (88)	0.00199	0.322 (92)	0.00420	0.622 (89)	0.00762	1.36 (97)	0.0187
21	0.0812 (90)	0.000792	0.164 (91)	0.00175	0.335 (96)	0.00290	0.697 (100)	0.00564	1.37 (98)	0.0133
28	0.0884 (98)	0.000802	0.160 (89)	0.00163	0.337 (96)	0.00288	0.681 (97)	0.00562	1.34 (96)	0.0108
Mean measured	0.0815 (91)	na	0.152 (84)	na	0.325 (93)	na	0.646 (92)	na	1.27 (91)	na

The test acceptability criteria for this study were met. The water-quality characteristics remained within the tolerance limits set forth in the protocol; water temperature was 24.1 – 25.0°C, dissolved oxygen was 80-108% saturation, pH was 7.9-8.6 and salinity was 18.8-21.2 ‰. Survival of the control F₀ mysids was 98% after 14 days (prior to pairing) and 93% after 28 days (after pairing). The percentage of control F₀-female mysids available to produce young that actually

did produce a brood was 100%. The average total number of young produced per control F₀-female mysid was 18.1.

The effects of XDE-729 methyl on survival, growth and reproduction are summarized in the tables below.

Table B.9.2.131: Effects of XDE-729 Methyl on survival of F₀ mysids.

Mean Measured Methyl Concentration (mg a.s./L)	Pre-pairing survival at day 7 mean % survival	Pre-pairing survival at day 7 day 14 mean % survival	Post-pairing survival day 21 mean % survival	Post-pairing survival day 28 Mean % survival
Control	98	98	95	93
Vehicle Control	100	98	100	98
0.0815	100	98	100	95
0.152	98	96	100	97
0.325	98	96	100	100
0.646	96	96	98	98
1.27	84	82*	97	97

* Statistically significant reduction (Fisher's Exact Test; $p \leq 0.05$) in survival as compared to the control.

Table B.9.2.132: Effects of XDE-729 Methyl on growth and reproduction of F₀ mysids.

Mean Measured Methyl Concentration (mg a.s./L)	Body length (mm) of first-generation mysid (f ₀) exposed for 14-days		Body length (mm) of first-generation mysid (f ₀) exposed for 28-days		Days to first brood release by F ₀ mysid	Mean young per female (Total Young)
Control	4.73	4.85	5.79	6.12	17.8	18.1 (381)
Vehicle Control	4.76	4.96	5.85	6.10	17.2	24.4 (485)
0.0815	4.85	4.82	5.81	6.15	18.4	20.8 (436)
0.152	4.83	4.78	5.69	5.95	17.9	20.4 (427)
0.325	4.78	4.76	5.60*	5.85	18.2	20.0 (420)
0.646	4.72	4.78	5.52*	5.85*	19.0	16.9 (333)
1.27	4.04*	4.52	5.02*	5.44*	24.5*	5.82 (90)*

* Statistically significant reduction (Fisher's Exact Test; $p \leq 0.05$) in survival as compared to the control.

Table B.9.2.133: Effects of XDE-729 Methyl on survival and growth of F1 mysids

Mean Measured Methyl Concentration (mg a.s./L)	Observation period				
	% mortality of second generation mysids			Mean length of second generation mysids on day 9 (mm)	
	Day 4	Day 7	Day 9	Males	Females
Control	0	0	0	4.60	4.70
Vehicle Control	0	0	0	4.49	4.61
0.0815	2	13	15	4.53	4.60
0.152	0	0	0	4.48	4.53
0.325	0	0	0	4.36	4.54
0.646	2	2	2	4.39	4.58
1.27	13 a	-b	-b	-b	-b

a Only one of the three replicates reached this day before study termination. The mysids in the remaining two replicates were terminated on study day 28 and so did not have an opportunity to reach this day before study termination.

b The mysids were terminated on study day 28 and so did not have an opportunity to reach this day before study termination.

Table B.9.2.134: Sub-lethal effects of XDE-729 Methyl on appearance or behaviour of F0 mysids.

Mean Measured Methyl Concentration (mg a.s./L)	Observation period			
	Observation - Days 7 and 14 (prior to pairing) (% affected)		Observation 2 – Day 21 and 28 (after pairing) (% affected)	
	Day 7	Day 14	Day 21	Day 28
Control	0	0	0	0
Vehicle Control	0	0	0	0
0.0815	0	0	0	0
0.152	0	0	0	0
0.325	0	0	0	0
0.646	0	0	0	0
1.27	0	0	0	0

Note: There were no sub-lethal effects of XDE-729 Methyl on appearance or behaviour observed during the study.

Conclusions

Based on mean measured XDE-729 Methyl concentrations:

Biological parameter	NOEC mg a.s./L	LOEC mg a.s./L	LC50 mg a.s./L	MATC mg a.s./L
F0 Mysid Survival (7-day; prior to pairing)	1.27	>1.27	>1.27	NA
F0 Mysid Survival (14-day; prior to pairing)	0.646	1.27	>1.27	0.906
F0 Mysid Survival (21-day; post-pairing)	1.27	>1.27	>1.27	NA
F0 Mysid Survival (28-day; post-pairing)	1.27	>1.27	>1.27	NA
F0-Male Mysid Length – Day 14	0.646	1.27	NA	0.906
F0-Female Mysid Length - Day 14	1.27	>1.27	NA	NA
F0-Male Mysid Length – Day 28	0.152	0.325	NA	0.222
F0-Female Mysid Length – Day 28	0.646	1.27	NA	0.458
F0 Mysid Day of First Brood	0.646	1.27	NA	0.906
Mean Total Young per F0-Female Mysid	0.646	1.27	NA	0.906
F1 Mysid Survival (4-day)	0.646	>0.646	>0.646	NA
F1 Mysid Survival (7-day)	0.646	>0.646	>0.646	NA
F1 Mysid Survival (9-day)	0.646	>0.646	>0.646	NA
F1-Male Mysid Length – Day 9	0.646	>0.646	NA	NA
F1-Female Mysid Length - Day 9	0.646	>0.646	NA	NA

RMS Comment: The study is considered to be acceptable and suitable for risk assessment purposes. The proposed endpoint for use in risk assessment is a mean measured NOEC of 0.152 mg a.s./L based on F0-Male Mysid Length at Day 28.

B.9.2.1.2.3 Chronic toxicity to sediment dwelling invertebrates

Gerke, A. (2011): XDE-729 Methyl: Chronic Toxicity in Whole Sediment to Freshwater Midge, *Chironomus riparius*. ABC Laboratories, Columbia, Missouri, ABC 65899. Dow AgroSciences unpublished report, Study Number 101130. 22 June 2011.

Test material (non-radiolabelled)

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Lot No./Batch No. :	E2837-51

Test material (radiolabelled)

Test item:	XDE-729 Methyl
Purity:	97.8 wt/%
Description:	Off-white solid
Inventory No :	INV027098-0002
Specific activity	29.6 mCi/mmol

Test system

Organism (Species):	Midge (<i>Chironomus riparius</i>)
Study Type:	Chronic life cycle study Static
GLP Status:	GLP (except the water characterization)
Guideline followed:	OECD 219: sediment-water chironomid toxicity test using spiked water
Guideline deviations reported by Study Director:	Two replicates contained 21 midge.
Duration of study:	28 days
Method of test item application:	Spiked water
Parameters measured:	Number emerged, number survived, development rate, & time to emergence
Observation intervals:	Daily
Age of test organisms at test initiation:	First instar
Test concentrations:	Nominal: 0 (control), 0 (vehicle control), 0.13, 0.25, 0.50, 0.99, and 2.0 mg a.s./L Initial Measured Overlying water: <MQL, <MQL, 0.143, 0.261, 0.452, 0.910, and 1.26 mg TRR/L Geometric Mean Measured Overlying water: <MQL, <MQL, 0.100, 0.261,

	<p>0.316, 0.910, and 1.20 mg TRR/L</p> <p>(NB concentrations are as overlying water TRR concentrations (^{14}C XDE-729 Methyl equivalents))</p> <p>The primary dosing solution was prepared by combining a non-radio-labelled test solution with a radio-labelled test solution. A radio-labelled solution was prepared by quantitatively transferring 0.5 mCi of ^{14}C-labelled XDE-729 Methyl to a 25-mL volumetric flask and bringing the flask to volume with acetone. A 19.6 mg a.i./mL stock solution was prepared by weighing 0.2020 g of XDE-729 Methyl into a culture tube marked at a 10-mL volume. An 8.3-mL volume of the radio-labelled solution was added to the culture tube. The tube was brought up to the 10-mL volume mark with acetone. Aliquots of the primary stock solution were diluted to a volume of 5.0 mL with acetone to prepare working standard solutions at concentrations of 1.29, 2.48, 4.95, and 9.90 mg a.s./mL. Using a pipette, 60 μL aliquots of the primary stock solution and working standard solutions were added and the overlying water was gently stirred to minimally disturb the sediment. A 60 μL aliquot of acetone was added to the vehicle control replicates, and the overlying water was gently stirred to minimally disturb the sediment.</p> <p>At each sample point, the concentration of ^{14}C activity calculated as total radioactive residue in the overlying water, interstitial water, and sediment was determined by LSC analysis. Each replicate of the overlying water was analyzed from the control and each test substance treatment at test initiation (i.e., replicates A-D of the control and levels 2 and 4 and replicates A-G of the vehicle control and levels 1, 3, and 5) and one sample of overlying water from levels 1, 3, and 5 was analyzed on day 7 and test termination.</p>
Feeding:	Daily
Ratio of sediment layer to depth of overlying water:	1:4
Reference substances:	XDE-729 Methyl and XDE-729 Acid
No. of Chironomid per vessel:	20
No. of vessels per dose group:	4

No. of vessels per control group:	4
Environmental conditions:	Temperature °C: 19.9 to 20.8°C Dissolved oxygen: 5.8 to 8.1 mg/L (67 to 93% saturation) pH: 7.8 to 8.4 Total Hardness: 228 to 278 mg CaCO ₃ /L Un-ionized Ammonia: 0.0000108 to 0.00314 mg/L Photoperiod: 16-hour light and 8-hour dark with two 30 minute transition periods Light intensity: 515 to 633 lux Formulated sediment: 75% fine sand, 20% kaolin clay and 5% sphagnum peat

Methodology

A 28 day test was performed with nominal overlying water concentrations of 0.0 (control), 0 (vehicle control), 0.13, 0.25, 0.50, 0.99, and 2.0 mg a.s./L. One day prior to study initiation, i.e., addition of the test substance to overlying water, a total of 20 midge larvae were added to each vial in a set of labelled containers. Each container was randomly assigned to a treatment replicate by a computer-generated random number table. The individuals within a container were transferred via pipette into each biological replicate and the termination analytical replicates. There were four biological replicates per treatment level, resulting in 80 midge per test treatment.

Aeration was provided at an initial rate of 60-100 bubbles per minute to each test chamber through a glass pipette. The pipette was inserted such that the tip was two to three centimetres from the sediment surface. Observations of the biological replicates were recorded at least every other day during the initial 14 days of the exposure and daily thereafter. Any abnormal activity (i.e., sediment avoidance, inactivity, etc.) was noted, if observed. The larvae were fed daily. Daily emergence observations (i.e., adult flies retained within the emergence traps) were recorded. Evidence of emergence was noted by the presence of exuviae as well as adults. Where possible, the adult flies observed in the emergence traps were identified and enumerated by gender and also for total emergence. If an exuviae was present but there was no adult fly present (i.e., escaped) or if there was a greater number of exuviae present than was accounted for by the number of emergent adults, then these missing adults were recorded to be of an unknown gender. Although gender could not be determined in the missing emergent adults, these organisms were still included in the total development rate calculation. At test termination, the sediments were sieved and surviving larvae or pupae, if any, were retained by the mesh and were recorded. These organisms were included with the total number of emergent adults to determine the 28-day survival values for each treatment level.

Measurements of temperature, dissolved oxygen concentration, and pH of the overlying water were measured at test initiation and at least weekly in each replicate test chamber. The waterbath temperature was continuously measured and

recorded with an electronic data logger. On days 0, 7, and 28, composite samples of overlying water were taken from approximately 1-2 cm above the sediment in each biological replicate for measurement of total hardness and ammonia concentrations.

Results

Presented below in are the mean measured concentrations in terms of radioactive residue.

Table B.9.2.135: Results from analysis of Overlying water samples

Overlying Water Nominal Concentration (mg a.s./L)	Mean Measured Concentrations of ¹⁴ C-labelled XDE-729 Methyl as mg total radioactive residue/L			
	Day 0 Mean	Day 7	Day 28	Geometric Mean (Day 0 Mean, Day 7, and Day 28)
0 (control)	<MQL	---	---	<MQL
0 (vehicle control)	<MQL	<MQL	<MQL	<MQL
0.13	0.143	0.0855	0.0827	0.100
0.25	0.261	---	---	0.261
0.50	0.452	0.254	0.274	0.316
0.99	0.910	---	---	0.910
2.0	1.26	1.06	1.31	1.20
MQL:	Day 0: 0.00140 mg TRR/L; Day 7: 0.00309 mg TRR/L; Day 28: 0.00384 mg TRR/L			

Table B.9.2.136: Results from analysis of pore water/interstitial water samples

Overlying Water Nominal Concentration (mg a.s./L)	Mean Measured Concentrations of ¹⁴ C-labelled XDE-729 Methyl as mg total radioactive residue/L		
	Day 0	Day 7	Day 28
0 (control)	---	---	---
0 (vehicle control)	<MQL	<MQL	<MQL
0.13	<MQL	0.0190	0.0411
0.25	---	---	---
0.50	0.00570	0.0455	0.134
0.99	---	---	---
2.0	0.0173	0.165	0.598
MQL:	Day 0: 0.00271 mg TRR/L; Day 7: 0.00334 mg TRR/L; Day 28: 0.00415 mg TRR/L		

Table B.9.2.137: Results from analysis of sediment samples

Overlying Water Nominal Concentration (mg a.s./L)	Mean Measured Concentrations of 14C-labelled XDE-729 Methyl as mg/kg dry sediment		
	Day 0	Day 7	Day 28
0 (control)	---	---	---
0 (vehicle control)	<MQL	<MQL	<MQL
0.13	<MQL	0.328	0.241
0.25	---	---	---
0.50	0.0648	0.705	0.565
0.99	---	---	---
2.0	0.728	3.89	2.09
MQL:	Day 0: 0.0385 mg TRR/L; Day 7: 0.0379 mg TRR/L; Day 28: 0.0426 mg TRR/L		

Measured concentrations of the XDE-729 Methyl and XDE-729 Acid by chromatographic analysis of the overlying water samples at initiation were 0.155, 0.361, and 1.35 mg XDE-729 Methyl/L (68 to 119% of the nominal concentrations) and 0.000646, 0.00167, and 0.00576 mg XDE-729 Acid/L in the 0.13, 0.50, and 2.0 mg a.s./L nominal test solutions, respectively. Measured overlying water concentrations on day 7 were 0.0176, 0.0593, and 0.608 mg XDE-729 Methyl/L (12 to 30% of the nominal concentrations) and 0.0772, 0.142, and 0.391 mg XDE-729 Acid/L in the 0.13, 0.50, and 1.0 mg a.s./L nominal test solutions, respectively. At termination, the overlying water concentrations of XDE-729 Methyl were all below the MQL of 0.000238 mg XDE-729 Methyl/L. The concentrations of XDE-729 Acid were 0.0874, 0.266, and 1.14 mg XDE-729 Acid/L in the 0.13, 0.50, and 2.0 mg a.s./L nominal test solutions, respectively. No residues of XDE-729 Methyl or XDE-729 Acid were detected in the control or vehicle control samples above the MQL values for the analytes.

Measured concentrations of the XDE-729 Methyl and XDE-729 Acid by chromatographic analysis of the pore water/interstitial water samples at initiation were 0.000438, 0.00164, and 0.0116 mg XDE-729 Methyl/L and <MQL, 0.000484, and 0.00104 mg XDE-729 Acid/L in the 0.13, 0.50, and 2.0 mg a.s./L nominal test solutions, respectively. Measured pore water concentrations on day 7 were 0.00140, 0.00504, and 0.0291 mg XDE-729 Methyl/L and 0.0149, 0.0360, and 0.0948 mg XDE-729 Acid/L in the 0.13, 0.50, and 2.0 mg a.s./L nominal test solutions, respectively. At termination, measured pore water concentrations were <MQL, 0.000568, and 0.00238 mg XDE-729 Methyl/L and 0.0454, 0.138, and 0.524 mg XDE-729 Acid/L in the 0.13, 0.50, and 2.0 mg a.s./L nominal test solutions, respectively. No residues of XDE-729 Methyl or XDE-729 Acid were detected in the control or vehicle control samples above the MQL values for the analytes.

Measured sediment concentrations of XDE-729 Methyl and XDE-729 Acid were less than the MQL values of 1.60 mg XDE-729 Methyl/kg and 1.63 mg XDE-729 Acid/kg on days 0, 7, and 28. No residues of XDE-729 Methyl or XDE-729 Acid were detected in the vehicle control samples above the MQL values for the analytes. Quality control fortifications for XDE-729 Methyl ranged from 93 to

122% of the nominal concentrations and QC fortifications for XDE-729 Acid ranged from 93 to 111% of the nominal concentrations.

Table B.9.2.138: Effect of XDE-729 Methyl on adult emergence and development rate at Day 28

Initial Measured Overlying Water Concentration (mg TRR/L)	Sex of emerged midge	Adult Emergence				
		Rep 1	Rep 2	Rep 3	Rep 4	Mean of all replicates
Control	% Emerged	100	90	85	80	89
	M Dev. Rate	0.0548	0.0532	0.0540	0.0558	0.0545
	F Dev. Rate	0.0499	0.0481	0.0446	0.0496	0.0481
	T Dev. Rate	0.0523	0.0501	0.0485	0.0519	0.0507
	Mean F Emerge Time	20.7	21.5	23.0	20.8	21.5
	Mean M Emerge Time	18.9	19.4	19.1	18.5	19.0
Vehicle Control	% Emerged	90	75	100	80	86
	M Dev. Rate	0.0577	0.0572	0.0559	0.0585	0.0573
	F Dev. Rate	0.0519	0.0541	0.0480	0.0519	0.0515
	T Dev. Rate	0.0538	0.0566	0.0527	0.0535	0.0542
	Mean F Emerge Time	20.0	19.0	21.4	19.9	20.1
	Mean M Emerge Time	18.0	18.1	18.5	17.6	18.1
0.143	% Emerged	90	95	85	80	88
	M Dev. Rate	0.0592	0.0586	0.0554	0.0596	0.0582
	F Dev. Rate	0.0522	0.0505	0.0502	0.0513	0.0511
	T Dev. Rate	0.0553	0.0527	0.0520	0.0549	0.0537
	Mean F Emerge Time	19.9	20.5	20.7	20.0	20.3
	Mean M Emerge Time	17.5	17.6	18.6	17.4	17.8
0.261	% Emerged	90	100	80	85	89
	M Dev. Rate	0.0573	0.0554	0.0561	0.0560	0.0562
	F Dev. Rate	0.0477	0.0505	0.0524	0.0524	0.0508
	T Dev. Rate	0.0518	0.0525	0.0536	0.0545	0.0531
	Mean F Emerge Time	21.6	20.4	19.6	19.7	20.3
	Mean M Emerge Time	18.3	18.6	18.4	18.4	18.4
0.452	% Emerged	95	75	80	80	83
	M Dev. Rate	0.0562	0.0640	0.0593	0.0572	0.0592
	F Dev. Rate	0.0485	0.0552	0.0482	0.0527	0.0512

Initial Measured Overlying Water Concentration (mg TRR/L)	Sex of emerged midge	Adult Emergence				
		Rep 1	Rep 2	Rep 3	Rep 4	Mean of all replicates
	T Dev. Rate	0.0508	0.0583	0.0552	0.0549	0.0548
	Mean F Emerge Time	21.4	19.0	21.3	19.5	20.3
	Mean M Emerge Time	18.3	16.3	17.5	18.1	17.6
0.910	% Emerged	85	95	90	70	85
	M Dev. Rate	0.0602	0.0560	0.0531	0.0559	0.0563
	F Dev. Rate	0.0507	0.0485	0.0484	0.0534	0.0503
	T Dev. Rate	0.0530	0.0512	0.0505	0.0536	0.0521
	Mean F Emerge Time	20.4	21.2	21.3	19.4	20.6
	Mean M Emerge Time	17.3	18.4	19.5	18.4	18.4
1.26	% Emerged	100	80	70	90	85
	M Dev. Rate	0.0563	0.0590	0.0616	0.0576	0.0586
	F Dev. Rate	0.0495	0.0536	0.0542	0.0562	0.0534
	T Dev. Rate	0.0530	0.0563	0.0566	0.0561	0.0555
	Mean F Emerge Time	20.8	19.3	19.0	18.3	19.4
	Mean M Emerge Time	18.4	17.8	16.8	18.1	17.8

M=Male, F=Female, T=Total, Dev. Rate = Development Rate, TRR = Total Radioactive Residues

Note, there was no statistically significant ($p = 0.05$) emergence effect as compared to the controls.

Please note that the individual replicate data for mean female and male emergence time was not presented in the report and has not been verified.

Conclusions

The negative and vehicle control animals met the acceptability criteria for mean percent emergence (i.e., >70%) as specified by the study protocol and the OECD 219 testing guideline. Based on initial measured concentrations and total emergence and survival, the estimated EC50 value was >1.26 mg TRR/L, the highest concentration tested. The no-observable-effect concentration (NOEC) and lowest-observable-effect concentration (LOEC), based on emergence and survival, were 1.26 mg TRR/L and >1.26 mg TRR/L, respectively. Based on male, female, and total adult average emergence time, the 28-day no observed effect concentration (NOEC) was 1.26 mg TRR/L. Based on male, female, and total development rate, the 28-day NOEC was 1.26 mg TRR/L.

RMS Comment: The study is considered to be acceptable and can be used for risk assessment purposes. It is proposed to use the NOEC based on initial measured concentrations of 1.26 mg/L.

Gerke, A. (2011): XDE-729 Methyl: Whole Sediment 10 Day Acute Toxicity Test with Midge Larvae (*Chironomus dilutus*). ABC 64607. Dow AgroSciences unpublished report, Study Number 090183. 27 May 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Lot No./Batch No. :	E2837-51

Test system

Organism (<i>Species</i>):	Midge (<i>Chironomus dilutus</i>)
Study Type:	10 day acute study
GLP Status:	GLP (except dilution water characterization)
Guideline followed:	OPPTS 850.1735
Guideline deviations reported by Study Director:	None.
Duration of study:	10 day
Test conditions:	Static
Parameters measured: (Observation intervals)	Temperature, dissolved oxygen, pH (daily) conductivity, total alkalinity, total hardness, ammonia (day 0 + 10)
Age of test organisms at test initiation:	Third instar
Test concentrations:	Nominal: 0 (Control), 0 (Vehicle Control), 6.4, 12, 25, 49, and 99 mg a.i./kg dry sediment Geometric Mean Measured: <MQL (control), <MQL (vehicle control), 5.05, 11.6, 22.7, 48.7, and 89.3 mg TRR/kg dry sediment
Analytical confirmation of test concentrations:	At the beginning and end of study
Reference substances:	XDE-729 Methyl and XDE-729 Acid
No. of Chironomid per dose group:	80
No. of Chironomid per control group:	80
Environmental conditions:	Temperature: 22.9 to 23.3°C Dissolved Oxygen: 4.2 to 8.5 mg/L (51 to 104% saturation) pH: 7.5 to 8.6 Specific Conductivity: 364 to 499 µS Total Alkalinity: 160 to 220 mg CaCO ₃ /L Total Hardness: 144 to 222 mg CaCO ₃ /L

	Un-ionized Ammonia: 0.00456 to 0.126 mg/L Light Intensity: 621 to 770 lux (16 hrs daily)
--	---

Methodology

A 10 day static test was performed with nominal test concentrations 0 (Control), 0 (Vehicle Control), 6.4, 12, 25, 49, and 99 mg a.i./kg dry sediment. Concentrated solutions of the test item were prepared in acetone solvent and then administered to a sand carrier and vented overnight. After venting the dosed sand aliquots were mixed with bulk amounts of a sandy loam sediment (sampled from an onsite pond, 57% sand content, organic matter of 1.5%). The amount of sediment dosed was enough to prepare 8 replicates for that treatment group. The control group was treated with the sand carrier only and the vehicle control was treated with vented acetone only in a sand carrier.

Ten midge larvae were impartially added to a set of labeled containers with each container representing one treatment replicate. Each container was then randomly assigned to a treatment replicate by random number generator. The individuals within each container were then pipetted from the container into the corresponding test chamber. There were eight biological replicates per treatment level, resulting in 80 midge per test treatment. Each replicate consisted of a 1L glass jar containing 291.5 g (wet weight) of appropriately treated sediment and 700 mL of overlying dilution water.

Aeration was provided from day 1 of the test to each test chamber through a glass pipette set at a depth of 2-3 cm above the sediment and maintained at a rate of 60 to 100 bubbles per minute. Observations for sediment activity, aeration, and water level were made daily for the duration of the test. Temperature, pH, and dissolved oxygen concentration were measured in each biological replicate daily. On days 0 and 10, equal volumes of sample were removed with a pipette from 1 to 2 cm above the sediment surface from each replicate and the replicates were composited by treatment for analysis of specific conductivity, total alkalinity, total hardness, and ammonia concentrations. At test termination, the entire contents of each test chamber were poured through a stainless steel mesh and the live and dead organisms were enumerated. Observations of general health and behaviour of the organisms were also noted. Any midge not accounted for on day 10 (i.e., not found) were considered dead.

ResultsTable B.9.2.139: Results from analysis of Overlying water samples

Nominal Sediment Concentration (mg a.i./kg dry sediment)	Mean Measured Concentrations (mg TRR/L)	
	Day 0	Day 10
0 (Control)	<MQL	<MQL
0 (Vehicle Control)	<MQL	<MQL
6.4	0.125	0.493
12	0.214	0.832
25	0.274	1.24
49	0.394	1.99
99	0.417	2.38
MQL: Day 0: 0.00690 mg TRR/L; Day 10: 0.00781 mg TRR/L		

Table B.9.2.140: Results from analysis of Pore water samples

Nominal Sediment Concentration (mg a.i./kg dry sediment)	Mean Measured Concentrations (mg TRR/L)	
	Day 0	Day 10
0 (Control)	<MQL	<MQL
0 (Vehicle Control)	<MQL	<MQL
6.4	3.11	1.71
12	3.83	2.30
25	4.50	3.06
49	5.33	3.83
99	5.88	5.40
MQL: Day 0: 0.00800 mg TRR/L; Day 10: 0.00791 mg TRR/L		

Table B.9.2.141: Results from analysis of Sediment samples

Nominal Sediment Concentration (mg a.i./kg dry sediment)	Mean Measured Concentrations (mg TRR/kg)			Other parameters
	Day 0	Day 10	Geometric Mean (Days 0-10)	
0 (Control)	<MQL	<MQL	<MQL	Type: Natural
0 (Vehicle Control)	<MQL	<MQL	<MQL	Type: Sandy Loam
6.4	5.28	4.84	5.05	Total organic carbon (%): 0.87
12	12.5	10.8	11.6	Total organic matter (%): 1.5
25	25.0	20.7	22.7	Clay (%): 18
49	52.4	45.7	48.7	Sand (%): 57
99	124	74.2	89.3	Silt (%): 18
MQL:	Day 0: 0.124 mg TRR/kg; Day 10: 0.124 mg TRR/kg			

Measured overlying water concentrations in two replicates of the 99 mg a.i./kg treatment level at initiation were 0.197 and 0.190 mg XDE-729 Methyl/L and 0.160 and 0.152 mg XDE-729 Acid/L. At termination the overlying water concentrations were 0.0362 and 0.0232 mg XDE-729 Methyl/L and 0.512 and 0.408 mg XDE-729 Acid/L. No residues of XDE-729 Methyl or XDE-729 Acid were detected in the control samples above the MQL values for the analytes.

Measured sediment concentrations in two replicates of the 99 mg a.i./kg treatment level at termination were 16.4 and 27.9 mg XDE-729 Methyl/kg and 6.40 and 6.38 mg XDE-729 Acid/kg. No residues of XDE-729 Methyl or Acid were detected in the control at or above the MQL value of 1.60 mg XDE-729 Methyl/kg dry sediment or 1.63 mg XDE-729 Acid/kg dry sediment.

Table B.9.2.142: Effect of XDE-729 Methyl on survival

Geometric Mean Sediment Concentration (mg TRR/kg dry sediment)	Day 10 Survival	
	Number Surviving/Number Tested	Percent Survival (%)
0 (Control)	66/80	83
0 (Vehicle Control)	70/80	88
5.05	68/80	85
11.6	65/80	81
22.7	68/80	85
48.7	67/80	84
89.3	66/80	83
NOEC	89.3 mg TRR/kg dry sediment	
LC50	>89.3 mg TRR/kg dry sediment	

Table B.9.2.143: Sub-lethal effects of XDE-729 Methyl

Geometric Mean Sediment Concentration (mg TRR/kg dry sediment)	Day 10	
	Mean Growth (mg/individual)	Observation (% affected)
0 (Control)	0.888	--
0 (Vehicle Control)	0.987	--
5.05	0.848	--
11.6	0.964	--
22.7	0.911	--
48.7	0.876	--
89.3	0.839	--
Note: No sub-lethal effects noted		

Conclusions

The negative and vehicle control animals met the acceptability criteria for mean survival (i.e., >70%) and for ash-free dry weights (> 0.48 mg per individual) as specified by the study protocol and the OPPTS 850.1735 testing guideline. The estimated 10 day LC_{50} was >89.3 mg TRR/kg dry sediment, the highest concentration tested. The NOEC and LOEC values were 89.3 and >89.3 mg TRR/kg dry sediment, respectively, based upon the lack of statistically significant ($p < 0.05$) mortality at this and all lower test substance concentrations. The NOEC and LOEC values based on growth were 89.3 and >89.3 mg TRR/kg dry sediment, respectively, based upon the lack of statistically significant ($p < 0.05$) growth measurements at this and all lower test substance concentrations.

RMS comments:

This study is considered valid and acceptable for risk assessment purposes. The endpoint for use in this risk assessment is a 10d NOEC of 89.3 mg a.s./kg dry sediment, based on mean measured concentrations of the test item.

B.9.2.1.3 Bioaccumulation

██████████ 2011: XDE-729 methyl: Bioconcentration and Metabolism Study with Bluegill, *Lepomis macrochirus*. ██████████ study number 66001. Dow AgroSciences unpublished report, Study Number 101135. 29 July 2011.

The bioconcentration potential of XDE-729 Methyl in the bluegill sunfish (*Lepomis macrochirus*) was assessed at two separate exposure levels (termed 'low'- and 'high'-level). Non-radiolabelled XDE-729 Methyl had a purity of 97.2% and was lot number E2837-51; radiolabelled XDE-729 Methyl had a purity of 97.8% and a radiochemical purity of 29.6 mCi/mmol.

Guideline followed was OPPTS 850.1730, OECD 305. No deviations that affected the outcome or validity of the study were noted and the study was conducted in line with GLP except the water characterization.

The study was conducted under flow-through conditions for 42 days (28-day exposure followed by 14-day elimination period). One group of 120 fish was exposed to nominal test concentrations of 0.0 (solvent control of DMF), one group to 0.002 mg/L, and one group to 0.02 mg/L.

The Bluegill sunfish were juveniles and were 1.2 to 5.2 g at the study initiation, length was 45 to 70 mm. Biomass loading was 0.86 g. The water hardness was 140 to 148 mg CaCO₃/L, pH was 7.6 to 8.5, dissolved oxygen was 6.9 to 9.2 mg/L (equivalent to 82 to 110% saturation), total organic carbon was 17.7 to 33.9 ppm, and temperature was 21.8 to 22.5°C. The study was a flow through study with 70 L per 24-hours.

Prior to the initiation of the uptake phase (addition of fish), the test solutions were allowed to flow through the test chambers for a period of approximately five days. The concentrations of XDE-729 Methyl were confirmed by measuring both TRR and parent XDE-729 Methyl to ensure the treatment groups had appropriately equilibrated to consistent test concentrations prior to initiation of the uptake phase. The uptake phase of the definitive test was initiated by transferring fish from the culture tank to each of three labelled barrels containing dilution water. Each barrel corresponded to the vehicle control, low, or high test treatment. Fish were transferred until each barrel contained 125 fish. Five fish were impartially selected from each barrel, totalling 15 fish, for weight and length measurements, leaving 120 fish in each barrel. The remaining fish were transferred from the barrels to the appropriate test aquarium. Fish were maintained in the laboratory at the test temperature for a minimum of 14 days before starting the test. The criterion for the length of the uptake phase was that the ¹⁴C-residue concentration in the fish tissues be at steady-state equilibrium or a minimum of 28 days. Steady-state equilibrium was defined as three consecutive sampling days during which the mean ¹⁴C-residue concentrations in the fish tissues showed no statistically significant differences. The fish were observed daily for any mortality and/or adverse behaviour. Water was sampled daily and fish were sampled on days 1, 3, 7, 14, 21 and 28. Only water was sampled on day 0.

On day 28 of the uptake phase, following the collection of all water and fish tissue samples, the depuration phase was initiated. Remaining fish in each uptake phase test chamber were transferred with a soft net to the appropriate depuration aquarium. The fish were then exposed to flowing dilution water, absent of vehicle and test substance, for 14 days. The fish were observed daily for any mortality and/or adverse behaviour. Water was sampled daily during the depuration phase and fish were sampled on days 1, 2, 3, 7 and 14.

The amount of total aqueous ^{14}C radioactivity in the dilution water of the control and exposure aquaria was determined daily using a liquid scintillation counter (LSC). In the low level, the mean measured concentration ranged from 0.00175 to 0.00240 mg TRR/L and in the high-level, the mean measured concentration ranged from 0.0173 to 0.0236 mg TRR/L.

Whole fish, muscle fillet, and viscera tissue (*i.e.*, head, tail, internal organs, and skeleton) were sampled and analyzed for total ^{14}C radioactivity by tissue oxidation using a Harvey OX-500 Biological Oxidizer followed by measurement of total ^{14}C activity by LSC. Whole fish were also homogenized, extracted, and radioassayed for XDE-729 Methyl and metabolites using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Results regarding the concentration of ^{14}C labelled XDE-729 in bluegill fillet, whole fish and viscera are presented in Table B.9.2.144, whilst those related to the depuration phase are presented in Table B.9.2.145.

Table B.9.2.144: Measured concentrations of ^{14}C -labelled XDE-729 in bluegill fillet, whole fish and viscera during the uptake phase

Nominal concentration mg/L	Study day	TRR-fillet Mean mg/kg	TRR-whole fish Mean mg/kg	TRR-viscera Mean mg/kg
0	All sample days	<MQL	<MQL	<MQL
0.0020	2	0.0404	0.354	0.900
	3	0.0804	0.265	1.08
	7	0.217	0.628	0.566
	14	0.126	0.686	0.746
	21	0.0942	0.330	0.809
	28	0.0654	0.382	0.695
0.020	1	0.446	3.62	7.04
	3	1.24	3.38	7.58
	7	0.639	4.93	8.50
	14	0.455	3.78	12.1
	21	0.517	5.03	9.59
	28	0.461	3.94	8.71

Table B.9.2.145: Measured concentrations of ^{14}C -labelled XDE-729 in bluegill fillet, whole fish and viscera during the depuration phase

Nominal concentration mg/L	Study day (depuration phase day)	TRR-fillet Mean mg/kg	TRR-whole fish Mean mg/kg	TRR-viscera Mean mg/kg
0	All sample days	<MQL	<MQL	<MQL
0.0020	29 (1)	0.101	0.0233	0.0970
	30 (2)	<MQL	0.0122	0.0143
	31 (3)	<MQL	0.00906	0.0140
	35 (7)	<MQL	<MQL	0.00531
	42 (14)	0.00499	<MQL	<MQL
0.020	29 (1)	0.0530	0.668	1.39
	30 (2)	<MQL	0.136	0.245
	31 (3)	<MQL	0.0791	0.132
	35 (7)	<MQL	<MQL	0.0702
	42 (14)	<MQL	<MQL	<MQL

Since the BCF of total ^{14}C residues did not exceed 1000 mL/g, no attempt was necessary to identify and quantify XDE-729 Methyl metabolites in fish tissues; as per guideline requirements. However, characterization of the ^{14}C -labelled metabolites was conducted using LC-MS/MS.

For the low-level concentration, the calculated kinetic bioconcentration factors (BCF_K) for total ^{14}C activity in whole fish and fillet tissue were 233 and 57.4 mL/g, respectively. The BCF_K for viscera was not calculated due to the uncertainty in the uptake and depuration rate constants. For the high-level concentration, the calculated BCF_K for total ^{14}C activity in whole fish and viscera tissue were 214 and 467 mL/g, respectively. The BCF_K for fillet was unable to be calculated as the data did not fit the model.

For the low-level concentration, the calculated steady state bioconcentration factors (BCF_{ss}) for total ^{14}C activity in whole fish, fillet and viscera were 186, 49.0 and 361 mL/g, respectively. For the high-level concentration, the calculated BCF_{ss} for total ^{14}C activity in whole fish, fillet and viscera tissue were 217, 26.3 and 465 mL/g, respectively.

XDE-729 Methyl was very rapidly metabolized, yielding XDE-729 Acid and approximately five metabolites. Two of the metabolites were positively identified as X11449757 and X11406790.

There was no discernable difference in percent lipid values between the control and exposure fish. The mean lipid values (g lipid/g whole fish, expressed as a percentage) for day 0 control fish was 4.40%. The mean lipid values during the test (from days 28 of uptake and 14 of depuration) ranged from 7.08 – 7.39% for

control fish, 7.09 – 8.34% for low treatment fish, and 6.78 – 8.25% for high treatment fish.

No significant level of sub-lethal effects was observed during the conduct of the studies.

Conclusion

This study satisfies the guideline requirements for a bioconcentration study with the freshwater fish, *Lepomis macrochirus* (bluegill sunfish).

Whole body ^{14}C - residue concentrations reached steady-state equilibrium between 14 and 21 days; the steady state BCF (i.e. BCF_{ss}) was estimated at 186 for the low concentration and 217 for the high concentration. Based on the ratio of the uptake and depuration rate constants (k_1 and k_2 respectively) the BCF_k was estimated at 214 for the low concentration and 233 for the high concentration. Following transfer to clean water the whole body ^{14}C - residue was rapidly depurated, with the calculated time to 95% depuration of the ^{14}C -residue being 1.6 days for both the low and high treatment.

RMS Comment: The study is considered to be acceptable for risk assessment purposes. It is proposed that the following outputs are used for risk assessment purposes – BCF_{ss} 217, with a time to 95% depuration of 1.6 days.

Endocrine disruption

██████████ (2012). XDE-729 Methyl: Fish Short-Term Reproduction Assay with the Fathead Minnow (*Pimephales promelas*). ██████████
██████████ ██████████ Project number 379A-153. Dow AgroSciences unpublished report, Study Number 102125. Report date: 25 April 2012.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2%
Description:	Solid
Test Substance No./Lot No.:	TSN031117-0004 / E2837-51

Test system

Organism (Species):	Fathead minnow (<i>Pimephales promelas</i>)
Study Type:	Fish Short-Term Reproduction Assay
GLP Status:	GLP (With exception of periodic analyses of water for potential contaminants)
Guidelines followed:	OPPTS Number 890.1350; OECD 229
Guideline deviations reported	Spawning tiles were placed on screening trays, both fecundity and fertility were assessed on the tiles and the

by Study Director:	trays.
Duration of study:	35 days (14 days pre-exposure, 21 days exposure)
Test conditions:	Flow through
Parameters measured:	Adult survival, wet weight, total length, female fecundity, egg fertility, gonadosomatic index, tubercle score, plasma vitellogenin concentration, gonad histopathology. Observations of abnormal behaviour, changes in secondary sexual characteristics (e.g., banding patterns, fatpad, ovipositor) or other symptoms were noted.
Observation intervals:	Daily for fecundity, fertility, adult survival, abnormal behaviour and other symptoms. Test termination for all other parameters.
Stage of development at test initiation:	Adult reproductively mature fathead minnows approximately 6 months of age at start of pre-exposure.
Test concentrations:	Nominal: 0 (negative control), 0 (solvent control, 20µl/l of DMF), 0.024, 0.078, 0.25 mg a.s./L Mean measured: 0 (negative control), 0 (solvent control), 0.022, 0.077, 0.24 mg a.s./L.
Analytical confirmation of test concentrations:	On days: Pretest, 0, 7, 14 and 21 from two alternating replicates. Additional samples were collected on days 8, 15 and 19 to confirm concentrations when previous results were questionable or due to a diluter malfunction.
Acclimation holding conditions:	Fish were held for approximately 6 weeks prior to commencement of the 14 day pre-exposure breeding period under water quality, temperature and lighting conditions similar to experimental conditions.
No. of holding days before dosing:	Approximately 6 weeks prior to pre-exposure breeding period, approximately 8 weeks total before dosing.
Number of fish per dose group:	Pre-Exposure Breeding: 192 (32 replicates of 6 fish, 2 males & 4 females) Exposure: 24 (4 replicates of 6 fish, 2 males & 4 females)
Number of fish per control group:	24 (4 replicates of 6 fish, 2 males & 4 females)
Feeding regime:	Acclimation period: commercial flake food (Sera Vipan) twice per day at a rate sufficient to maintain body condition and promote active reproduction Pre-exposure and exposure periods: commercial flake food (Sera Vipan) and brine shrimp nauplii (<i>Artemia</i> sp.) twice per day at a rate sufficient to maintain body condition and promote active reproduction.

Environmental conditions:	<p>Loading rate: 0.9 g/L instantaneous rate; 0.1 g/L in 24-hour period flow-through rate.</p> <p>Tank dimensions: Pre-exposure period: 19L (10L water); Exposure period: 12L (10L water)</p> <p>Water depth: Pre-exposure: 11 cm; Exposure period: 12.7 cm</p> <p>Spawning substrates: spawning tile (10 cm length inverted semi-circular piece of PVC pipe) on a stainless steel tray with a stainless steel mesh bottom to catch any eggs falling from the tile and so provide an accurate measure of egg production.</p> <p>Temperature: $25 \pm 1^\circ\text{C}$ (slight deviation on day 8 reported in RMS comments).</p> <p>Photoperiod: 16-hour light and 8-hour dark, 30-minute transition period; ranging from 693 - 1659 lux.</p> <p>Dissolved oxygen concentration: at or above 5.0 mg/L ($\geq 61\%$ of saturation. Gentle aeration was supplied to all replicates on Day 1 of exposure period.</p> <p>pH: 8.0 – 8.5.</p> <p>Alkalinity: 178 - 186 mg CaCO_3/L as measured weekly in the negative control and 0.24 mg a.s./L treatment.</p> <p>Total hardness: 144 - 148 mg CaCO_3/L as measured weekly in the negative control and 0.24 mg a.s./L treatment.</p> <p>Conductivity: 362 - 388 $\mu\text{S}/\text{cm}$ as measured weekly in the negative control and 0.24 mg a.s./L treatment.</p>
---------------------------	--

Methodology

Pre-exposure Period

A 14-day pre-exposure period immediately preceded the 21-day exposure period to identify groups of actively spawning fish. Almost twice the number of breeding groups needed for testing were maintained in the pre-exposure period so that breeding groups with a proven history of spawning could be selected for inclusion in the exposure phase of the test. Thirty-two replicate aquaria each containing 6 reproductively mature fathead minnows (2 males & 4 females) and 3 spawning substrates, were maintained under flow-through conditions as similar as possible to those used during the exposure period. All fish selected for the pre-exposure period, had observable secondary sex characteristics. The mean weight of males was 2.1 g and the mean weight of females was 0.8 g. Spawning was monitored and recorded daily. A replicate was considered suitable for use in the exposure period if spawning occurred at least twice during the 7-day period immediately preceding test initiation and if there were at least 15 eggs/female/reproductive day in that aquarium. Any pre-exposure aquaria with mortalities or any injured fish were excluded from use in the test.

Initiation of Exposure

The spawning groups from the pre-exposure period were ranked from low to high fecundity and the top 20 groups were selected. The top 20 spawning groups were

randomly assigned to exposure replicates in each treatment and control group, based on fecundity data collected during the pre-exposure period.

Exposure Period

The toxicity test was conducted using an exposure system consisting of a continuous-flow diluter to deliver each test concentration, control and a solvent control to the test chambers. Delivery of test solutions to the test chambers was initiated five days prior to the introduction of the test organisms in order to achieve equilibrium of the test substance in the test system.

During the exposure period, four replicate test chambers were maintained in each treatment and control group. Each replicate contained a reproductive group of two males and four females, for a total of 24 fish per treatment or control group. During the 21-day exposure period, survival, general observations, and assessments of fecundity and fertility were recorded daily.

Three spawning substrates were maintained in each test chamber during the exposure period. The test chambers were randomly positioned by treatment group in the environmental chamber and were labelled with the project number, test concentration and replicate designation. At least weekly during the exposure period, the fish were transferred to clean test chambers to prevent the build up of microbial growth. Observations of abnormalities (e.g., hemorrhage or discoloration) or abnormal behaviour (e.g., hyperventilation, loss of equilibrium, uncoordinated swimming, atypical quiescence or feeding abstinence) relative to the controls were noted. Spawning substrates were removed from the test chambers daily and any eggs that were present were enumerated. Eggs were evaluated for fertilization success by applying toluidine blue dye to highlight embryo development. The number of fertile eggs was calculated as the difference in the number of infertile eggs and the total number of eggs on the substrate.

The fish were not fed for at least 12 hours prior to test termination to allow for clearance of the digestive tracts before terminal weight measurements were made. The delivery system and test chambers were placed in a temperature-controlled environmental chamber to maintain the target water temperature throughout the test period. Temperature, dissolved oxygen and pH were measured in each test chamber weekly during the exposure period. Temperature was also monitored continuously in one test chamber during the pre-exposure period and in one negative control replicate during the exposure period. Hardness, alkalinity and conductance were measured in one replicate test chamber of the negative control and the highest concentration treatment group weekly during the test. Light intensity was measured weekly during the exposure period in five indiscriminately selected locations within the environmental chamber.

Termination of Exposure

At termination on Day 21, all fish were euthanized with buffered MS-222 and measured for total length and wet weight. Observations of secondary sex characteristics were recorded (i.e., presence of pigmentation patterns, tubercles (presence or absence), fatpad appearance and presence of ovipositor) and the external sex was determined. Blood samples were collected for analysis of serum concentrations of vitellogenin (VTG). VTG concentrations were determined using

a commercially available ELISA kit based on methodology provided by the ELISA system manufacturer and those presented by the US EPA in the study guidelines.

Each fish was dissected and the gonadal sex was determined using a dissecting microscope. The gonads were fixed, removed and weighed for calculation of gonadosomatic index (GSI), and were preserved for gonadal histology. The fixed gonads were examined using a light microscopic. Histomorphologic parameters assessed included relative germ cell numbers, alterations in numbers and sizes of non-germ cells (e.g., testicular interstitial cells and ovarian perfollicular cells) and increased degenerative changes. When appropriate, the pathologist used a scoring system to indicate the severity of these changes and other abnormalities. Any changes not amenable to grading instead were designated as "Present". In addition, the stage of developmental maturity of each gonad pair was indicated.

Results

Mean measured XDE-729 Methyl test concentrations for this study were 0.022, 0.077 and 0.24, representing 92- 99% of nominal concentrations. Measured concentrations of the samples collected weekly during the test were generally within $\pm 20\%$ of nominal concentrations with a few exceptions attributed to equipment malfunctions. At the 0.077 mg a.s./L concentration, analytical samples taken on day 14 were 148 and 149% of the nominal. An additional sample taken on day 15 was 59.5% of the nominal. These deviations were due to a malfunction with the mixer in the diluter mixing cup. At the 0.25 mg a.s./L concentration, analytical samples taken on day 7 were 54.3 and 55.3% of the nominal. These deviations were due to a probably sampling or processing error. These deviations have been discussed further in the RMS comments.

Results of the study are based on the mean measured concentrations. There were no apparent effects on the survival or appearance of the fish, and no statistically significant differences in the final length or weight of males or females at test termination. With the exception of two males in the negative control group that exhibited a bruised operculum and one male in the 0.24 mg a.s./L treatment group that was fin-picked, all fathead minnows in the control and treatment groups appeared normal throughout the test. These sub lethal effects were not considered to be treatment related.

Table B.9.2.146: Effects of XDE-729 Methyl on mortality, mean wet weight and mean length in adult fathead minnow (*Pimephales promelas*) in the 21 day exposure phase of the fish short-term reproduction assay.

Mean Measured Concentration (mg a.s./L)	% Mortality		Wet Weight (g)		Length (mm)	
	Males	Females	Males	Females	Males	Females
Negative control	0	0	2.51± 0.237	1.10± 0.089	58± 2.7	46± 1.9
Solvent control	0	0	2.36± 0.102	1.06± 0.061	57± 1.8	46± 0.6
Pooled control	0	0	2.44± 0.186	1.08± 0.074	57± 2.2	46± 1.3
0.022	0	0	2.47± 0.299	1.16± 0.114	58± 1.9	46± 2.0
0.077	0	0	2.24± 0.355	1.11± 0.117	57± 2.8	46± 1.4
0.24	0	0	2.17± 0.186	1.08± 0.106	57± 1.9	46± 1.8
There were no statistically significant differences (Jonckheere-Terpstra Trend Test; $p > 0.05$) between the test substance treatments and the pooled control.						

Table B.9.2.147: Effects of XDE-729 Methyl on fecundity, fertility, gonadosomatic index and tubercle score in adult fathead minnow (*Pimephales*

Mean Measured Concentration (mg a.s./L)	Fecundity (eggs/female /day)	Egg fertility (%)	Gonadosomatic Index GSI (gonad weight/wet weight)		Male Tubercle Score	Vitellogenin (µg/ml)	
			Male	Female		Male	Female
Negative control	32.7 ± 10.52	98.4 ± 0.5	1.94± 0.432	13.5± 1.49	21.0 ± 3.81	0.15 ± 0.145	1960 ± 371
Solvent control	33.3 ± 0.96	97.5± 1.6	1.73± 0.232	16.0± 1.43	20.9 ± 2.10	0.42 ± 0.607	1687 ± 413
Pooled control	33.0 ± 6.92	97.9± 1.2	1.83± 0.340	-- ^a	20.9 ± 2.85	0.28 ± 0.433	1824 ± 392
0.022	24.8 ± 15.23	95.6± 3.9	2.04± 0.449	17.4± 2.12	20.4 ± 2.02	0.34 ± 0.340	2228 ± 604
0.077	22.0 ± 9.77	95.6 ± 2.0* ^b	1.87± 0.094	15.6± 1.07	21.4 ± 3.12	0.22 ± 0.13	2738 ± 911* ^b
0.24	13.2 ± 6.61*	89.2± 4.3*	2.36± 0.111*	17.5± 2.76	24.4 ± 3.43	0.21 ± 0.290	2618 ± 726* ^b

All parameters reported as mean ± standard deviation,
^aNegative and solvent controls were significantly different for GSI in females. Treatment means were compared to the solvent control.
^bDunnett's test indicated there was no statistically significant difference between the treatment group and the pooled control ($p > 0.05$)
*Indicates a statistically significant trend (Jonckheere-Terpstra Trend Test; $p \leq 0.05$) in comparison to the pooled control.

promelas) in the 21 day exposure phase of the fish short-term reproduction assay

A statistically significant decrease in cumulative number of eggs produced and number of eggs per female per reproductive day was identified in the 0.24 mg a.s./L treatment group. There were also statistically significant decreases in fertility in the 0.077 and 0.24 mg a.s./L treatment groups. However, in the 0.077 mg a.s./L treatment group, the fertility exceeded 95% and the difference from the pooled controls was only slight (2 %).

For the endocrine diagnostic endpoints, there were no statistically significant effects detected for tubercle score among males. There were no apparent treatment-related changes in size or shape of the fatpad in males, and no females in any group were noted with a fatpad. Therefore, it can be concluded that there were no treatment-related effects on secondary sex characteristics among males or females at any concentration tested.

There was a statistically significant increase in GSI among males at 0.24 mg a.s./L, (0.05). However, analysis of gonad weights measured at termination indicated that there were no statistically significant differences. There were no statistically significant effects on GSI among females.

Mean plasma VTG levels among females were significantly higher at 0.077 and 0.24 mg a.s./L than in controls (Jonckheere-Terpstra trend test), but there was no difference in plasma VTG levels at any test concentration among males. There were no treatment-related differences noted in any histological examinations of the ovaries or testes.

Table B.9.2.148: Staging of gonads of adult fathead minnow (*Pimephales promelas*) in the 21 day exposure phase of the fish short-term reproduction assay on XDE-729 Methyl

Mean Measured Concentration (mg a.s./L)	Gonadal Stage (Number Observed)																		
	Female (Ovaries)										Male (Testes)								
	# Ex.	Ju v.	St. 0	St. 1	St. 2	St. 2.5	St. 3	St. 3.5	St. 4	St. 5	# Ex.	Ju v.	St. 0	St. 1	St. 1.5	St. 2	St. 2.5	St. 3	St. 4
NC	16	0	0	0	4	7	4	1	0	0	8	0	0	1	0	3	3	1	0
SC	16	0	0	0	3	3	5	2	3	0	8	0	0	0	0	4	2	2	0
0.022	16	0	0	0	2	4	6	3	1	0	8	0	0	0	1	1	3	3	0
0.077	16	0	0	0	6	4	2	2	2	0	8	0	0	0	1	3	0	4	0
0.24	16	0	0	0	2	3	8	2	1	0	8	0	0	0	0	2	3	3	0

Male testes (St.) stages:
 Juvenile (Juv.)=Exclusive spermatogonia;
 St. 0=Undeveloped,
 St. 1=Early spermatogenic;
 St. 2=Mid spermatogenic;
 St. 3=Late spermatogenic;
 St. 4=Spent.

Female ovaries (St.) stages:
 Juvenile (Juv.)=Exclusive oögonia;
 St. 0=Undeveloped;
 St. 1=Early development;
 St. 2=Mid development;
 St. 3=Late development;
 St. 4=Late development/hydrated;
 St. 5=Post-ovulatory.

When the gonadal stage was intermediate between the stages defined, the gonad was assigned a fractional value falling between the 2 stages (i.e., 1.5, 2.5, 3.5). See the USEPA and OECD guideline for full description of gonadal stages.

Ex. = Number of fish examined; NC = negative control; SC = solvent control.

There were no treatment-related changes in the gonadal stages in any of the treatment groups.

Table B.9.2.149: Histopathological findings in testes of males and ovaries of female adult fathead minnow (*Pimephales promelas*) in the 21 day exposure phase of the fish short-term reproduction assay on XDE-729 Methyl.

Sex	Description of Findings ^a	Histopathological Findings Per Treatment Level (Number Observed)				
		Treatment Level (mg a.s./L)				
		Negative Control	Solvent Control	0.022	0.077	0.24
Female	Number examined	16	16	16	16	16
	Normal	15	14	11	14	16
	Increased oocyte necrosis (atresia) - Grade 2	1	2	4	1	0
	Increased oocyte necrosis (atresia) - Grade 3	0	0	1	0	0
	Increased oocyte necrosis (atresia) - Grade 4	0	0	0	1	0
Male	Number examined	8	8	8	8	8
	Normal	7	8	8	8	8
	Increased secondary spermatocytes – Grade 2	1	0	0	0	0
	Decreased spermatozoa – Grade 2	1	0	0	0	0
^a Grades refer to degree of severity: Grade 1=minimal, Grade 2=mild; Grade 3=moderate, Grade 4=severe There were no treatment-related changes in the histopathological findings in any of the treatment groups.						

Conclusions

All breeding groups used in the exposure period met the criteria of use in the test (an average of at least 15 eggs per female per reproductive day and spawning at least twice in the previous 7 days). Greater than 95% fertility of eggs from the control animals was observed during the exposure. Mortality in the water (or solvent) controls did not exceed 10 percent at the end of the exposure period. Dissolved oxygen concentration was at least 60 percent of the air saturation value throughout the exposure period.

There were no statistically significant effects on survival, length or weight. There were clear effects on fertility and fecundity in the high (0.24 mg a.s./L) treatment group. The effects on fecundity coincided with a slight increase in VTG concentrations at 0.077 and 0.24 mg a.s./L. There was a statistically significant

increase in male GSI at the highest test concentration. There were no effects on male or female secondary sex characteristics, male VTG or male and female gonad histopathology.

In conclusion, the applicant has stated:

'The decrease in fecundity appears to correlate with a slight increase in VTG concentrations in females in the treatment groups, as well as with the increase in GSI in males in the high treatment group. While these effects appeared to be correlated and may be treatment-related, it is plausible that the observed increases in VTG in females were due to reduced elimination of VTG during production of eggs, and the increase in GSI in males may have been the result of a slight, non-statistically significant, decrease in body weight at this concentration. Since there were no treatment-related effects on male and female secondary sex characteristics, male VTG, or male and female gonad histopathology in this test, the reductions in the reproductive endpoints suggest that the effects were not endocrine-mediated and more likely resulted from other causes such as overt toxicity.'

RMS comment:

It was noted in the study that a significant increase in Gonadosomatic Index GSI (gonad weight/wet weight) occurred in the solvent control group compared to the untreated control group. The solvent used (DMF) is not currently considered to cause endocrine disruption. To ensure that any observed differences in treatment group GSI were only as a result of the test item, they were compared against the solvent control group only. As the solvent control and each treatment were exposed to an equal rate of DMF solvent this would ensure that any detected differences were due only to the presence of the test item at the applied concentration.

It is worth noting that temperature increased to a maximum of 28.2°C in the solvent control (replicates B, C and D) and in the 0.077 mg a.s./L treatment group (replicates A, B and C) on day 8, due to a reported malfunction in the temperature control system. On Day 13, the min/max thermometer recorded a minimum water temperature of 23.0°C, which was below the target temperature range of 25 ± 1°C. These deviations were for a relatively short duration and therefore are not considered to have adversely impacted the test results, as demonstrated by control group performance. Furthermore these deviations do not exceed the recommended temperature range in OECD 229 guideline, which states that fathead minnow tests should be maintained in a temperature of 25 ± 2°C.

It is also worth noting that the concentrations of the test substance in solution were not maintained within ± 20% of the mean measured values. The coefficient of variance (CV) in the 0.077 mg a.s./L treatment group was 24.86% and therefore exceeded the 20% stated in the validity criteria. It was however reported that this was due to a malfunction with the mixer on day 14 (samples measured 148 and 149% of the nominal). Additional samples were collected from each replicate of the 0.077 mg a.s./L treatment group on day 15 and were 92.34%, 90.52%, 60.26% and 91.82%. The third sample taken on day 15 was further deemed as a malfunction and thus an additional sample was taken on day 19 to confirm this low concentration, the result was an 86.88% recovery.

Despite these deviations at the 0.077 mg a.s./L concentration, an increase of up to 149% of the nominal would not have produced concentrations greater than the highest

tested concentration of 0.25 mg/L. Any study conclusions are driven by the highest concentration rate and so this is not deemed an issue. Furthermore this study was repeated at this concentration in a further dose response study (Schneider *et al.*, 2012, study number 120018), with the test concentrations having been maintained and the CV calculated to be below the 20% threshold.

Furthermore in the 0.24 mg a.s./L test concentration the CV calculated by the evaluator was 18.63%. Two samples taken on day 7 were 56.67% and 57.50%. These two samples were thought to be '*a probable sampling or processing error, and are not included in the calculation of the mean measured concentration*'. The following day, samples were taken from each of the four replicates which confirmed concentrations of 105%, 102.08%, 102.08% and 102.5%. Therefore samples taken on day 7 were excluded from the CV calculation and thus the CV reported in the study was 6.4%. This slight deviation is deemed acceptable, including these day 7 samples into the CV calculated would have still produced a CV of below 20% (18.6%).

Light intensity reached 1659 lux, the stated range in the protocol was a limit of 1000 lux. As control performance met the validity criteria and no unusual behaviour was reported, this deviation is acceptable. The OECD 229 guideline states that the initial wet weight of female fish should be $1.5\text{g} \pm 20\%$. Female fish used in this study were only 0.8g (which is a 53% deviation from 1.5g). This 0.8g is the arithmetic mean weight of 10 female fish which were weighed prior to the start of pre exposure. Fish were only considered suitable for use in the exposure period if spawning occurred at least twice during the 7-day period immediately preceding test initiation and if there were at least 15 eggs/female/reproductive day in that aquarium. The fish selected displayed this spawning criterion and thus the lower initial wet weight had no effect on their reproductive output. Furthermore the US EPA states that initiation weights should be recorded and should not vary by more than 20% but it does not state a range. Considering the information given, this deviation is acceptable.

2012. XDE-729 Methyl: 21-Day Reproduction Test with the Fathead Minnow (*Pimephales promelas*).
Project Number 379A-155. Dow AgroSciences unpublished report, Study Number 120018. REPORT DATE: 10 October 2012

Test material

Test item:	XDE-729 Methyl
Purity:	97.2%
Re-certification date:	30 November 2013
Test Substance No./Lot No.	TSN031117-0004 / E2837-51

Test system

Organism (<i>Species</i>):	Fathead minnow (<i>Pimephales promelas</i>)
Study Type:	Fish Reproduction Test
GLP Status:	GLP (except periodic analysis of water for contaminants)
Guidelines followed:	OPPTS Number 890.1350; OECD 229
Guideline deviations reported by Study Director:	None
Duration of study:	35 days (14 days pre-exposure, 21 days exposure)
Test conditions:	Flow through
Parameters measured:	Adult survival, wet weight, total length, female fecundity and egg fertility, gonad weight and gonadosomatic index (GSI), blood plasma vitellogenin concentration. Observations of secondary sexual characteristics, abnormal behaviour, and any other symptoms were noted.
Observation intervals:	Daily for fecundity, fertility, adult survival, abnormal behaviour and other symptoms. Day 21 of the exposure period for wet weight, total length, gonad weight and GSI index, secondary sexual characteristics, blood plasma vitellogenin concentration.
Stage of development at test initiation:	Adult reproductively mature fathead minnows approximately 6 months of age at start of pre-exposure.
Test concentrations:	Nominal: 0 (negative control), 0 (solvent control as 20 µL/L DMF), 2.5, 5.0, 10, 20, 40 and 80 µg a.s./L Mean measured: <LOQ (negative control), <LOQ (solvent control), 2.1, 4.2, 9.2, 19, 36 and 78 µg a.s./L. LOQ = 2.00 µg a.s./L
Analytical confirmation of test concentrations:	On days: Pre-test (day -1), 0, 7, 14, 21, 22
Reference substances:	XDE-729 Methyl (99.1%) and XDE-729 Acid (99.0%)
Acclimation holding conditions:	6 weeks acclimation followed by a 2 week pre-exposure phase under approximate test conditions (temperature and lighting). Holding conditions during 2 weeks before the test pre-exposure phase were: Temperature: 24.7 - 26.4 °C pH: 8.4 – 8.7 Dissolved oxygen: 7.8 – 8.3 mg/L
Number of fish per dose group:	24 (4 replicates each containing 4 females and 2 males)
Number of fish per control group:	24 (4 replicates each containing 4 females and 2 males)
Feeding regime:	Commercial flake food (Sera Vipan) 0.05g/fish/day and brine shrimp nauplii (<i>Artemia</i> sp.) 1.3 mL/fish/day Feed split over two feeding occasions per day
Environmental conditions:	Loading rate: 1.0 g/L (0.1 g/L/day) (maximum – determined at test termination) Replicate vessel: 19 L glass aquarium Replicate water volume: 10 L

	Spawning substrates: 10 cm length inverted semi-circular piece of PVC pipe, on a stainless steel tray with a stainless steel mesh bottom (3/replicate). Temperature: 23.8 – 25.5 °C Photoperiod: 16:8 Light intensity: 540 – 1080 lux. Dissolved oxygen concentration: 5.8 – 8.3 mg/L pH: 8.0 – 8.6. Alkalinity: 132 – 144 mg/L as CaCO ₃ Total hardness: 172 – 182 mg/L as CaCO ₃ Conductivity: 334 – 412 µS/cm
--	--

Methodology

Pre-exposure Period

A 14-day pre-exposure period immediately preceded the 21-day exposure period to identify groups of actively spawning fish. Sixty vessels (19L glass aquaria) containing 6 reproductively mature fathead minnows (2 males & 4 females) and 3 spawning substrates, were maintained under flow-through conditions as similar as possible to those used during the exposure period. At the start of the pre-exposure period a sample of 10 male and 10 female fish were taken to determine mean body weight. Mean male body weight as 2.6 g and female mean body weight was 1.0 g, with all individual weights within 30% of the mean. Spawning was monitored and recorded daily. A replicate was considered suitable for use in the exposure period if spawning occurred at least twice during the final 7-day period of pre-exposure and if there were at least 15 eggs/female/reproductive day in that aquarium. Any pre-exposure aquaria with mortalities were excluded from use in the test.

Initiation of Exposure

The exposure period was initiated after successful spawning was verified during the 14-day pre-exposure period. Spawning groups were randomly assigned to exposure replicates in each treatment and control group using a stratified random procedure, based on fecundity data collected during the pre-exposure period, to ensure that there was no reproductive bias towards a particular group.

Exposure Period

Delivery of the test item concentrations was via a flow-through diluter system to constantly renew test media. A concentrated Primary stock solution of the test substance (4000 µg a.s./mL in Dimethylformamide/DMF) was used to prepare five secondary stocks by proportional dilution with DMF, to give concentrations of 125, 250, 500, 1000 and 2000 µg a.s./mL. These secondary stocks were loaded into syringe pumps and pumped at set flow rates in to mixing chambers, where they was mixed with dilution water to prepare the target test concentrations (2.5, 5.0, 10, 20, 40 and 80 µg a.s./L nominal concentrations). The flow of dilution water into each mixing chamber was controlled using rotameters, and was adjusted to provide approximately 10 volume additions of test solution in each test chamber per day. After mixing, the test solution in each mixing chamber was pumped into the

appropriate replicate test chamber using a peristaltic pump. The control group received dilution water only, at the same volume replacement rate. A stock solution of DMF was used in the same manner as the treated stock solutions and was mixed with dilution water to prepare a final concentration of 20 µL DMF/L of test media. This was used to expose the solvent control group.

Analytical confirmation of the test media concentrations was performed regularly throughout the exposure period (and on one occasion prior to initiation of the exposure period). Water samples were collected from two alternating replicate test chambers on Days 0, 7, 14 and 21 of exposure to measure concentrations of the test substance. Additional samples were collected on Day 22 from the remaining replicates on this day. Additional samples were taken and analysed on day 23 from 2 replicates of the 40 and 80 µg a.s./L treatment groups to confirm previously detected concentrations of the test substance. Samples were collected from water mid-depth at each interval, placed in glass vials, mixed with 10% phosphoric acid (to ensure stability) and processed immediately for analysis using HPLC. In addition, stock solution concentrations were confirmed (96.2 – 98.4% of nominal) from samples collected one day prior to exposure period initiation.

During the 21-day exposure period, survival, general observations, and assessments of fecundity and fertility were recorded daily. Observations of abnormalities (e.g., hemorrhage or discoloration) or abnormal behavior (e.g., hyperventilation, loss of equilibrium, uncoordinated swimming, atypical quiescence or feeding abstinence) relative to the controls were noted. Spawning substrates were removed from the test chambers daily and any eggs that were present were enumerated. Eggs were evaluated for fertilization success by applying toluidine blue dye to highlight embryo development and counting the number of infertile eggs. The number of fertile eggs was calculated as the difference in the number of infertile eggs and the total number of eggs on the substrate.

Throughout the exposure period temperature, pH and dissolved oxygen was measured weekly in each replicate test vessel. In addition temperature was monitored continuously via a max-min thermometer maintained in one of the control replicates. Water hardness, alkalinity and conductivity were measured weekly in one control replicate and one replicate in the highest treatment group (80 µg a.s./L nominal concentration). The replicate used was alternated on each occasion. Light intensity was measured weekly in five indiscriminate locations within the test area.

Termination of Exposure

Due to the size of the study, termination was conducted across two days; 21 and 22 days after exposure initiation. Two replicates from each treatment were sampled on day 21 and the remaining two replicates per treatment sampled on day 22, so as not to create any treatment-related bias. At termination of exposure all fish were euthanized with buffered MS-222, blotted dry and measured for total length and wet weight (to the nearest millimeter and 0.1 mg respectively). The external sex was determined based on secondary sex characteristics (presence of tubercles and pigmentation for males; presence of an ovipositor for females). Blood samples were collected from the caudal vein/artery of each fish with capillary tubes for

analysis of plasma concentrations of vitellogenin (VTG). Analysis for vitellogenin was conducted with a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit. Each fish was dissected and the liver and digestive tract was removed. The gonadal sex was determined; the gonads were fixed, removed and weighed for calculation of the gonadosomatic index (GSI), and were preserved for potential future analysis of gonadal histology.

Results

End points were expressed in terms of mean measured concentrations, which were calculated to be 2.1, 4.2, 9.2, 19, 36 and 78 $\mu\text{g a.s./L}$, equivalent to 84, 84, 92, 95, 90 and 98% of the nominal concentrations of XDE-729 Methyl.

There were no apparent effects on the survival or general behaviour of the fish, and only isolated instances of abnormal appearance (two occasions of a female with a swollen 'egg-bound' abdomen and one instance of hemorrhage). There were no statistically significant differences in the final length or weight of males or females at test termination. Results of mean treatment mortality, wet weight and total length are displayed in table 1. There were no significant effects on fecundity or egg fertility at any of the mean measured concentrations tested. Table 2 presents mean fecundity and egg fertility for each treatment and control group.

There was a statistically significant increase in GSI among males at 78 $\mu\text{g a.i./L}$, but not in females (as shown in table 2). However, analysis of gonad weights measured at termination indicated that there were no significant increases in gonad weight among males in the 78 $\mu\text{g a.i./L}$ treatment group in comparison to the pooled control ($p > 0.05$). The higher GSI among males in the 78 $\mu\text{g a.i./L}$ treatment group had no apparent effect on reproduction. Additionally, the OPTTS guideline states that male GSI is usually 1-2% and the calculated mean GSI at this top treatment group is only just above this expected range (GSI of 2.08%). There were no statistically significant effects on GSI among females in any treatment group in comparison to the pooled control ($p > 0.05$), with the exception of the middle (19 $\mu\text{g a.i./L}$) treatment group. Although the increase in this treatment group was significantly different from the pooled control, there was not a clear concentration response. OECD guideline 229 does not mention GSI as a relevant test end point. Gonadal histopathology was not performed.

There were no statistically significant effects on VTG among males or females in any treatment group in comparison to the pooled control ($p > 0.05$). The increase in VTG concentration in males in the 78 $\mu\text{g a.i./L}$ treatment group was primarily due to one fish. The mean VTG when excluding that fish was 3.44 $\mu\text{g/mL}$.

Table B.9.2.154: Effects of XDE-729 Methyl on mortality, mean weight and mean length in adult fathead minnow (*Pimephales promelas*) in the 21 day exposure phase.

Mean Measured Concentration XDE-729 Methyl (µg a.s./L)	Mortality			Wet Weight (g)		Length (mm)	
	Males	Females	Total	Males	Females	Males	Females
Negative control	0	1	1	2.58	1.13	57	46
Solvent control	0	1	1	2.50	1.17	56	46
Pooled Control	0	2	2	2.54	1.15	56	46
2.1	0	0	0	2.78	1.25	58	47
4.2	0	0	0	2.62	1.12	58	46
9.2	0	0	0	2.77	1.17	59	46
19	0	0	0	3.02	1.19	60	47
36	0	0	0	2.68	1.14	58	47
78	0	0	0	2.68	1.10	59	46

Table B.9.2.155: Effects of XDE-729 Methyl on mean fecundity, fertility, and gonadosomatic index in adult fathead minnow (*Pimephales promelas*) in the 21 day exposure phase.

Mean Measured Concentration XDE-729 Methyl (µg a.s./L)	Fecundity ^a (eggs/female/day)	Egg Fertility (%)	Gonadosomatic Index (gonad weight/wet weight)*100	
			Male	Female
Negative control	12.0 ± 3.93	96.7	1.44	13.2
Solvent control	16.5 ± 4.00	96.7	1.53	13.8
Pooled control	14.3 ± 4.38	96.7	1.48	13.5
2.1	16.4 ± 1.59	96.0	1.65	15.9
4.2	13.5 ± 3.90	93.5	1.52	14.2
9.2	12.5 ± 3.82	94.8	1.75	13.5
19	15.2 ± 3.95	95.1	1.86	17.2*

36	14.7 ± 3.67	93.9	1.75	14.8
78	11.5 ± 2.31	95.5	2.08*	15.1
^a Mean ± Standard Deviation, N=4 * - statistically significant difference (Dunnett's Test; $p \leq 0.05$) in comparison to the pooled control				

Table B.9.2.156: Effects of XDE-729 Methyl on mean plasma vitellogenin in adult fathead minnow (*Pimephales promelas*) in the 21 day exposure phase.

Mean Measured Concentration XDE-729 Methyl (µg a.s./L)	Mean Plasma Vitellogenin (µg/mL plasma)	
	Male	Female
Negative control	1.00 ± 0.590	4328 ± 1822
Solvent control	1.09 ± 0.953	3577 ± 1554
Pooled control	1.04 ± 0.736	3952 ± 1618
2.1	2.41 ± 2.890	3631 ± 531
4.2	3.60 ± 5.909	4234 ± 2198
9.2	1.19 ± 0.876	4889 ± 1242
19	3.25 ± 4.901	5186 ± 2502
36	0.598 ± 0.558	4712 ± 1738
78	20.54 ± 34.828	4308 ± 353

The mortality in the control group did not exceed 10 percent at the end of the exposure period for a valid test (4% pooled control mortality achieved). The dissolved oxygen concentration was maintained at $\geq 60\%$ of the air saturation value throughout the exposure period in accordance with OECD guideline 229. Minimum dissolved oxygen level observed during the exposure period was 5.8 mg/L. At the water temperature maintained during the test, 60% air saturation value (ASV) is equivalent to 4.9 mg/L, confirming that satisfactory levels of dissolved oxygen were maintained throughout the exposure period. Water temperature did not differ by more than 1.5°C between test vessels at any observed time during the exposure period, with the exception of Day 21 when the difference was 1.7°C. The range of observed water temperature was maintained within $\pm 2^\circ\text{C}$ of the 25°C temperature specified for testing with the fathead minnow (23.8 – 25.5°C, giving a maximum range of 1.7°C).

OECD guideline 229 requires that evidence be provided to demonstrate that the concentrations of the test substance in solution have been satisfactorily maintained within $\pm 20\%$ of the mean measured values. Coefficient of variation (CV) values for the 2.1, 4.2, 9.2, 19, 36 and 78 µg a.s./L mean measured concentrations were calculated to be 14, 7, 8, 10, 9 and 17% respectively, demonstrating an acceptable level of variation from the mean measured concentrations for each treatment group ($<20\%$).

Spawning in all pre-exposure replicates used in the exposure period occurred at least twice

in the 7 days prior to exposure, demonstrating that all replicates used were actively spawning as required by OECD validity criteria.

Conclusions

All OECD guideline 229-specified validity criteria were met, with the exception of the inter-vessel temperature range at day 21 which is not considered to be detrimental to test performance. Breeding groups of fathead minnows (*Pimephales promelas*) exposed to XDE-729 Methyl at mean measured concentrations of 2.1, 4.2, 9.2, 19, 36 and 78 µg a.s./L for 21 days displayed no effects to survival, length, body weight, fecundity, egg fertility or blood vitellogenin concentration when compared to the control group. This would indicate that XDE-729 methyl does not cause adverse effects due to endocrine system interference at the maximum tested concentration of 78 µg a.s./L.

RMS comment:

Although this study demonstrates that all OECD guideline 229 validity criteria were met this study displayed some deviations from the other cited guideline (OPPTS Number 890.1350). It is required according to this guideline that EITHER spawning must occur at least every four days OR fecundity must be at least 15 eggs/female/day, with regard to control groups. During the exposure phase some replicates across the control and solvent control groups had periods of four or more days without egg production. Additionally one replicate (replicate D in the negative control group) only spawned every 5.25 days on average (4 days of egg production across the 21 day exposure period). This inferior fecundity may also explain why the negative control and pooled control mean fecundity were below the OPPTS requirement of ≥ 15 eggs/female./day.

Compared to the OECD guideline methods there were some minor deviations: The maximum recorded light intensity was 1080 lux, in excess of the 1000 lux upper limit suggested for all suitable species. Additionally, pH in one replicate at 4.2 µg a.s./L exceeded the maximum of 8.5 recommended on one occasion. At day 0 it is suggested that female fish should have a mean weight of 1.5 g, and that weight variation should be $\pm 20\%$ of the mean. Female fish used in this study were found to have a mean weight of 1.0g and for both males and females there was a reported weight variation of $<30\%$, suggesting that a variation of $>20\%$ from mean weight may have occurred.

The higher GSI among males in the 78 µg a.i./L treatment group had no apparent effect on reproduction. Additionally, the OPTTS guideline states that male GSI is usually 1-2% and the calculated mean GSI at this top treatment group is only just above this expected range (GSI of 2.08%). As such this observed elevated GSI is not considered to be biologically significant and in isolation not enough to indicate endocrine disrupting effects, in the opinion of the evaluator.

(2012). XDE-729 Methyl: Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances. [REDACTED]
[REDACTED] Project Number 379A-152. Dow AgroSciences unpublished report, Study Number 102126. Report date 13 June 2012.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2%
Re-certification date:	30 November 2013
Test Substance No./Lot No. :	TSN031117-0004 / E2837-51

Test system

Organism (<i>Species</i>):	African clawed frog (<i>Xenopus laevis</i>)
Study Type:	Amphibian Metamorphosis Assay (AMA)
GLP Status:	GLP (except water iodide concentration analysis)
Guidelines followed:	OPPTS Number 890.1100 OECD 231
Guideline deviations reported by Study Director:	Feed rates during acclimation and exposure were reduced to ½ the guideline rates.
Duration of study:	21 days
Test conditions:	Flow-through
Parameters measured:	Daily: Mortality, abnormal behaviour and appearance Day 7: Hind-limb length, Snout to vent length (SVL), developmental stage, wet weight. Day 21: Hind-limb length, SVL, developmental stage, wet weight, thyroid histopathological effects.
Stage of development at test initiation:	Nieuwkoop and Faber (NF) Stage 51 (15 days post-fertilization) tadpoles
Test concentrations:	Nominal: 0 (negative control), 0 (solvent control), 0.020, 0.10, 0.50 mg a.s./L Mean measured: <LOQ* (negative control), <LOQ* (solvent control), 0.020, 0.094, 0.38 mg a.s./L. *LOQ = 0.0100 mg a.s./L
Analytical confirmation of test concentrations:	On days: Pretest (-1), 0, 7, 14, and 21.
Reference substance:	XDE-729 Methyl and XDE-729 Acid
Broodstock holding conditions:	Parental frogs obtained from commercial breeder. Eggs obtained from a single spawning occasion were hatched and the tadpoles reared at 21.3–22.4°C, pH 7.8–8.3, 12:12 light cycle and ≥7.2 mg/L dissolved oxygen.
Number of tadpoles per dose group:	80 (4 replicates of 20 tadpoles each)
Number of tadpoles per control group:	80 (4 replicates of 20 tadpoles)

Feeding regime:	During the exposure phase: Sera Micron® at rates (mg/tadpole/day) of: 15 (day 0-4) 20 (day 5-7) 25 (day 8-10) 35 (day 11-14) 40 (day 15-21) All daily feed amounts were divided over three occasions each day.
Environmental conditions:	Replicate Vessels: 12 L glass aquaria Replicate media volume: 10 L approx Media depth in vessel: 12 cm Loading rate: 0.012 g/L/day Temperature: 21.0-22.8°C Photoperiod: 12-hour light and 12-hour dark, 30-min transition period. Light intensity: 842-1965 lux Dissolved oxygen concentration: 5.1-8.7 mg/L. pH: 7.9-8.3 Alkalinity: 179-185 mg as CaCO ₃ /L Total hardness: 138-148 mg as CaCO ₃ /L Conductivity: 309-370 µS/cm Dilution water iodide concentration: 3-6 µg/L

Methodology

Initiation of Exposure

The exposure period was initiated after successful spawning and rearing of *Xenopus laevis* tadpoles under test-equivalent conditions to developmental growth stage 51 (according to the Nieuwkoop and Faber scale). The exposure phase was initiated on day 15 post fertilization of the spawn used to derive the tadpoles used. On day 0 of the exposure phase tadpoles confirmed as suitable were transferred indiscriminately to transfer vessels in groups of 20. Each transfer vessel was then randomly allocated to a test replicate and the tadpoles were gently transferred into the exposure system.

Exposure Period

Delivery of the test item concentrations was via a flow-through diluter system to constantly renew test media. Concentrated stock solutions of the test substance (1, 5 and 25 g a.s./mL in the solvent carrier, DMF) was loaded into syringe pumps and pumped at set flow rates in to mixing chambers, where they were mixed with dilution water to prepare the target test concentrations (0.02, 0.1, and 0.5 mg a.s./L nominal concentrations). A syringe pump containing dimethylformamide (DMF) only was used to prepare the solvent control media at a final target concentration of 20 µL/L test media (after dilution with water). The flow of dilution water into each mixing chamber was controlled using rotameters, and was adjusted to provide approximately ten volume additions of test solution in each test vessel per day. After mixing, the test solution in each mixing chamber was pumped into the appropriate replicate test vessel using a peristaltic pump. The control group received dilution water only, at the same volume replacement rate.

Analytical confirmation of the test media concentrations was performed regularly throughout the exposure period (and on one occasion prior to initiation of the exposure period). Water samples were collected from all replicate test chambers on Days 0, 7, 14 and 21 of exposure to measure concentrations of the test substance. Samples were collected from mid-depth at each interval, placed in glass vials, and processed immediately for analysis using HPLC. In addition stock solution concentration was confirmed from a sample collected one day prior to exposure period initiation.

During the 21-day exposure period, mortality and observations of physical abnormalities (e.g. lesions or malformations) or abnormal behaviour relative to the controls were assessed daily. On day 7 of the exposure period five tadpoles were sampled from each replicate, euthanized and developmental stage, total length, wet weight, snout-to-vent length, and hind-limb length were evaluated.

Throughout the exposure period temperature, pH and dissolved oxygen were measured weekly in each replicate test vessel. In addition temperature was monitored continuously via a max-min thermometer maintained in one of the control replicates. Water hardness, alkalinity and conductivity were measured weekly in one control replicate and one replicate in the highest treatment group (0.5 mg a.s./L nominal concentration). The replicate used was alternated on each occasion. Light intensity was measured weekly in five indiscriminate locations within the test area.

Termination of Exposure

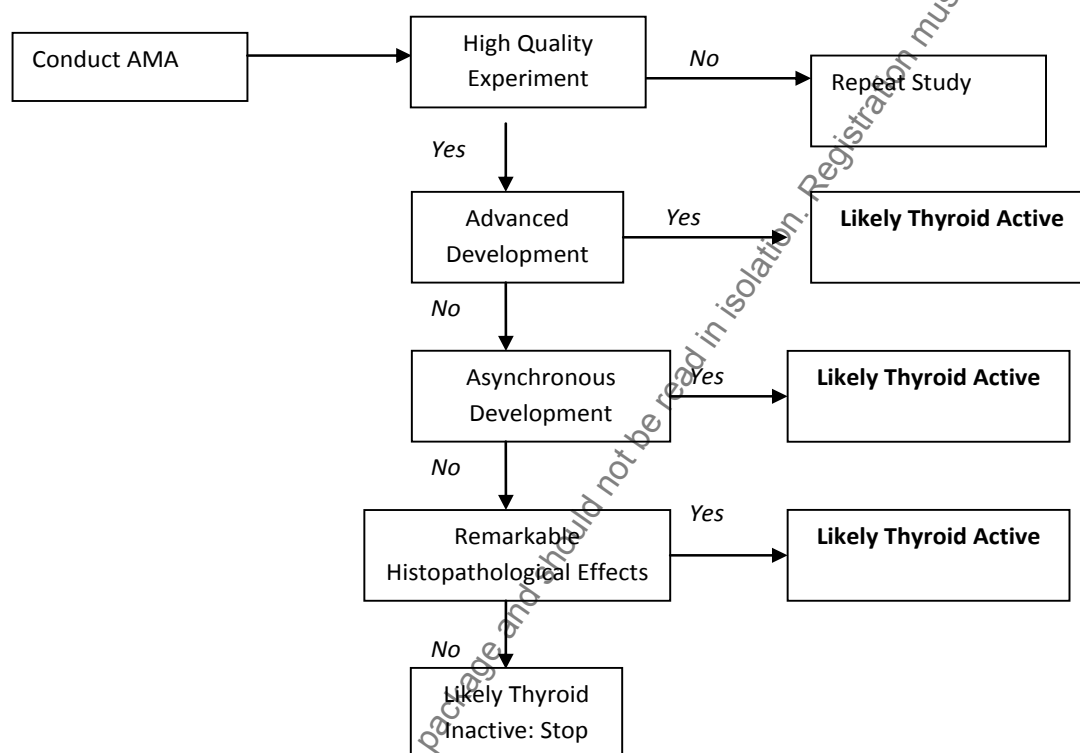
On Day 21, all remaining tadpoles were euthanized and developmental stage, total length, wet weight, snout-to-vent length, and hind-limb length were evaluated. Tadpoles were then preserved in Davidson's solution for at least 48 hours, followed by long-term preservation in formalin. Five tadpoles per replicate (20 tadpoles per treatment) were selected for histopathological examination. The basis of selection of the histopathology examination of the treatment samples rested on matching the median developmental stage of the control group (stage 57). If an insufficient number of treatment tadpoles were available at the median developmental stage of the controls, then random selection from the NF stage above or below the median was employed. The tadpoles were removed from the formalin and tissue samples were cut (consisting of trimmed head tissues containing the lower jaw) and processed for histopathological evaluation.

The pathologist used light microscopy to qualitatively evaluate the thyroid glands for morphological changes. The pathologist identified and reported morphologic changes in the glands of tadpoles in the treatment groups as compared to the controls. Parameters assessed included relative increases or decreases in the overall size of the thyroid glands, changes in follicular epithelial cell numbers or height, and alterations in colloid consistency. When appropriate, the pathologist used a scoring system to indicate the severity of these changes and other abnormalities according to the following scale: Grade 0 = unremarkable, Grade 1 = mild, Grade 2 = moderate, and Grade 3 = severe. Any changes not amenable to grading instead were designated as "Present".

Unless otherwise noted, the unit of statistical analysis was the replicate test chamber. Hind limb length was normalized by snout-to-vent length. Length and weight data for tadpoles reaching stages greater than NF stage 60 were excluded from statistical analyses due to morphological changes at this stage and above. Data from the negative and solvent control groups for each parameter were compared using an appropriate statistical

test. The negative and solvent control data were pooled for comparisons to the treatment group data, as recommended by OECD guideline 231. In accordance with recommendations of OECD 231 and OPPTS 890.1100, analysis of metamorphic stage was performed using the multi-quantile analysis developed by T. Springer and J. Green, and using the step-down Jonckheere-Terpstra trend test on stage quantiles in the replicate test chambers. Statistical tests used to evaluate treatment effects were performed at confidence level of $\alpha = 0.05$ with SAS software.

As this study is intended to determine the potential for the test material to interact with the thyroid system, specific decision logic has been employed following the guideline as shown in the figure below.



Decision logic for the conduct and interpretation of the Amphibian Metamorphosis Assay

Results

Mean measured XDE-729 Methyl test concentrations for this study were 0.020, 0.094 and 0.38 mg a.i./L, representing 100, 94 and 76% of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations. Stock solutions analysed one day prior to beginning the exposure phase yielded test substance concentrations of 93.4-97.6% of nominal.

There were no apparent effects on survival and general behaviour or appearance of the tadpoles during the test. At termination, tail curvature was scored from 0–3 for each tadpole (0 = none; 1 = slight/too close to call; 2 = moderate; 3 = severe). There were 35, 39, 40, 47 and 19% of tadpoles in the negative control, solvent control, 0.020, 0.094 and 0.38 mg a.s./L treatment groups, respectively, that were considered to have tail curvature (score ≥ 2). This was considered to be related to feed rates, rather than a thyroid-related effect. Table 1 summarises any abnormal observations made through the exposure period.

For the endocrine diagnostic endpoints, there were no statistically significant increases in normalized hind-limb length or developmental stage, nor was there evidence of asynchronous development or histological findings on days 7 and 21. One replicate in the negative control group (Replicate D) had four mortalities by test termination (Day 21). This replicate was considered to be compromised as defined by the study guidelines (i.e. had > 3 deaths: OECD guideline 231) and was excluded from statistical analyses of day 21 end points (displayed in table 3).

On day 7, there were slight decreases in developmental stage and snout-to-vent length at 0.38 mg a.s./L that were statistically significant in comparison to the pooled control. However, the differences from the pooled control were slight (only 1 stage difference, and $<5\%$ difference in snout-to-vent length), and were not considered to be endocrine mediated since the differences represented a decrease in the measured parameter, rather than an increase, in comparison to the control. End points from individuals sampled at day 7 are presented in table B.9.2.150.

Table B.9.2.150: Sub-lethal effects of XDE-729 Methyl on appearance or behaviour of *Xenopus laevis*.

Treatment Expressed as Mean Measured Concentration (mg a.s./L)	Observation period (Study Day)	
	Days 1-7	Days 8-21
Negative control	Lethargy in 1 tadpole, Days 3 and 4; all other tadpoles appear normal	Tadpoles appear normal
Solvent control	Tadpoles appear normal	Tadpoles appear normal
0.020	Tadpoles appear normal	Tadpoles appear normal
0.094	Tadpoles appear normal	Tadpoles appear normal
0.38	Tadpoles appear normal	Discoloration of tail in 1 tadpole, Day 21; all other tadpoles appear normal

Table B.9.2.151: Effects of XDE-729 Methyl on 7-day survival, growth and developmental stage in *Xenopus laevis*.

Treatment Expressed as Mean Measured Concentration (mg a.s./L)	Survival (%)	Wet Weight (mg)	Snout-Vent Length (mm)	Hind-Limb Length (mm/mm) ^a	Median Developmental Stage
Negative control	100 ± 0.0	250 ± 22.5	15.0 ± 0.41	0.12 ± 0.010	54
Solvent control	98.8 ± 2.5	288 ± 15.4	15.8 ± 0.27	0.13 ± 0.008	54
Pooled control	99.4 ± 1.8	269 ± 27.0	15.4 ± 0.57	0.13 ± 0.009	54
0.020	98.8 ± 2.5	280 ± 7.8	15.5 ± 0.47	0.14 ± 0.004	54
0.094	100 ± 0.0	263 ± 39.7	15.0 ± 0.81	0.13 ± 0.008	54
0.38	98.8 ± 2.5	233 ± 17.1	14.7 ± 0.36*	0.13 ± 0.008	53*

^a Hind-limb length normalized to snout-to-vent length.
* Statistically significant trend in comparison to the pooled control using the Jonckheere-Terpstra trend test ($p \leq 0.05$).

Table B.9.2.152: Effects of XDE-729 Methyl on 21-day survival, growth and developmental stage in *Xenopus laevis*.

Treatment Expressed as Mean Measured Concentration (mg a.s./L)	Survival (%)	Wet Weight (g)	Snout-Vent Length (mm)	Hind-Limb Length (mm/mm) ^a	Median Developmental Stage
Negative control ^b	96.7 ± 5.8	1.00 ± 0.060	23.4 ± 0.07	0.36 ± 0.009	57
Solvent control	98.8 ± 2.5	1.12 ± 0.046	24.7 ± 0.30	0.44 ± 0.024	57
Pooled control	97.9 ± 3.9	1.07 ± 0.082	24.1 ± 0.74	0.41 ± 0.048	57
0.020	98.8 ± 2.5	1.21 ± 0.067	25.0 ± 0.36	0.39 ± 0.017	57
0.094	100 ± 0.0	1.11 ± 0.076	24.2 ± 0.31	0.41 ± 0.021	57
0.38	98.8 ± 2.5	1.06 ± 0.054	23.8 ± 0.38	0.40 ± 0.025	57

^a Hind-limb length normalized to snout-to-vent length. Tadpoles Stage 60 and above were omitted from this measurement due to overt morphological changes that occur normally at these stages.
^b Negative control replicate D was excluded from statistical analyses; the replicate was considered to be compromised due to four mortalities.

There were no apparent effects observed by histopathological examination of the thyroids of tadpoles in any treatment group at termination of the 21-day test (see table 4). All other histopathologic criteria, such as overall size, follicular luminal area, amount and type of colloid and follicular cell type and arrangement were comparable to those of the controls.

Table B.9.2.153: Histopathological findings in 21-day thyroid glands in *Xenopus laevis* following XDE-729 Methyl exposure.

Treatment Expressed as Mean Measured Concentration (mg a.s./L)	Thyroid Gland Observation ^a (Number Observed/Number Examined)							
	Atrophy			Hypertrophy			Follicular Cell Hypertrophy	Follicular Cell Hyperplasia
	Grade 0	Grade 1	Grade 2	Grade 0	Grade 1	Grade 2	Grade 0	Grade 0
Negative control	15/20	4/20	1/20	15/20	2/20	3/20	20/20	20/20
Solvent control	14/20	5/20	1/20	19/20	0/20	1/20	20/20	20/20
0.020	14/20	5/20	1/20	19/20	0/20	1/20	20/20	20/20
0.094	15/20	5/20	0/20	19/20	1/20	0/20	20/20	20/20
0.38	17/20	2/20	1/20	17/20	2/20	1/20	20/20	20/20

^a Grade 0 = unremarkable, Grade 1 = mild, Grade 2 = moderate, and Grade 3 = severe

The present study met the validity requirements for test performance in accordance with the OECD guideline. Mean measured XDE-729 Methyl test concentrations for this study were 100, 94 and 76% of nominal concentrations, respectively. The variability of the measured test concentrations were maintained at $\leq 20\%$ CV over the 21-day test (5%, 6% and 10% for the 0.02, 0.1 and 0.5 mg a.s./L nominal concentrations respectively). There was at least 90% survival of control animals over the duration of the exposure period in the total of eight control tanks used in the study (four tanks in the negative control and four tanks in the solvent control). Mortality in one control tank exceeded two tadpoles, where there were four mortalities at the end of the study. This replicate was considered compromised and so was excluded from statistical analyses of Day 21 endpoints. There were at least two treatment groups without overt toxicity and only one replicate (which was in the control group) was considered to be compromised (≤ 2 compromised replicates is required by OECD guideline 231 in order for a valid test to demonstrate negative thyroid activity by the test item).

The dissolved oxygen concentration was maintained at $\geq 40\%$ Air Saturation Value/3.5 mg/L throughout the exposure period (minimum of 5.1 mg/L), the water pH was maintained between 6.5 and 8.5. Water temperatures did not differ by more than 1°C between test vessels at any one assessment occasion during the exposure period, and were maintained at 21.0 to 22.8°C, within the $22 \pm 1^\circ\text{C}$ range stated in the relevant OECD guideline.

Overall, the test is considered to be compliant with the associated test guidelines and the experimental findings valid.

Conclusions

According to the OECD and OPPTS guidelines, the key findings for an thyroid active substance would include a significant increase in normalized hind-limb length on Day 7 and/or Day 21, a significant increase in developmental stage on Day 7 and/or Day 21, asynchronous development of tadpoles (i.e., tadpoles that could not be staged), and histological findings in evaluation of the thyroids.

In this study, there were no significant increases in normalized hind-limb length or developmental stage, nor was there evidence of asynchronous development or histological findings. Therefore, in accordance with the decision logic provided in the OECD and OPPTS guidelines, it can be concluded that XDE-729 Methyl is not likely thyroid active at the maximum measured concentration tested of 0.38 mg a.s./L.

RMS Comments:

This study is considered valid. Despite one control replicate being compromised due to high mortality, the seven remaining negative control and solvent control replicates were suitable to demonstrate negative thyroid activity by the test substance and no adverse effect on the organism caused by the solvent carrier alone.

XDE-729 Acid:

(2012). XDE-729 Acid: Fish Short-Term Reproduction Assay with the Fathead Minnow (*Pimephales promelas*). Project Number 379A-154. Dow AgroSciences unpublished report, Study Number 120535. 02 August 2012.

Test material

Test item:	XDE-729 Acid
Purity:	95.3%
Re-certification date:	02 November 2013
Test Substance No./Lot No.:	TSN030751-0006 / E2837-52

Test system

Organism (Species):	Fathead minnow (<i>Pimephales promelas</i>)
Study Type:	Fish Short-Term Reproduction Assay
GLP Status:	GLP
Guidelines followed:	OPPTS Number 890.1350; OECD 229
Guideline deviations reported by Study Director:	None
Duration of study:	35 days (14 days pre-exposure, 21 days exposure)
Test conditions:	Flow through
Parameters measured:	Adult survival, wet weight, total length, female fecundity and egg fertility, gonad weight and gonadosomatic index (GSI). Observations of abnormal behaviour, or other symptoms were noted.

Observation intervals:	Daily for fecundity, fertility, adult survival, abnormal behaviour and other symptoms. Day 21 of the exposure period for wet weight, total length, gonad weight and GSI index.
Stage of development at test initiation:	Adult reproductively mature fathead minnows approximately 6 months of age at start of pre-exposure.
Test concentrations:	Nominal: 0 (negative control), 0.010, 0.10, 1.0 and 10 mg a.s./L Mean measured: not detected (negative control), 0.011, 0.11, 1.1 and 12 mg a.s./L.
Analytical confirmation of test concentrations:	On days: Pretest (-3), 0, 7, 14, 21
Reference substances:	XDE-729 Acid (99.0%)
Acclimation holding conditions:	Fish were held for approximately 7 weeks prior to commencement of the 14 day pre-exposure breeding period under water quality, temperature and lighting conditions similar to experimental conditions.
Number of fish per dose group:	24 (4 replicates of 6 fish, 2 males & 4 females)
Number of fish per control group:	24 (4 replicates of 6 fish, 2 males & 4 females)
Feeding regime:	commercial flake food (Sera Vipan) 0.7g/fish/day and brine shrimp nauplii (<i>Artemia</i> sp.) 1.4 mL/fish/day Feed split over two feeding occasions per day (except day 3, where fish were only fed once in error)
Environmental conditions:	Loading rate: 1.1 g/L (0.17 g/L/day) Replicate vessel: 12L glass tanks Replicate water volume: 10 L Spawning substrates: 10 cm length inverted semi-circular piece of PVC pipe, on a stainless steel tray with a stainless steel mesh bottom to catch any eggs falling from the tile, 3 sites per replicate. Temperature: 24.4 - 25.6°C. Photoperiod: 16-hour light and 8-hour dark, 30-minute transition period. Light intensity: 867 - 1636 lux. Dissolved oxygen concentration: 5.9 – 8.3 mg/L. pH: 7.8 – 8.3. Alkalinity: 170 - 180 mg CaCO ₃ /L Total hardness: 132 - 144 mg CaCO ₃ /L Conductivity: 310 - 398 µS/cm

Methodology

Pre-exposure Period

A 14-day pre-exposure period immediately preceded the 21-day exposure period to identify groups of actively spawning fish. Thirty-two vessels (12L glass aquaria) containing 6 reproductively mature fathead minnows (2 males & 4 females) and 3 spawning substrates, were maintained under flow-through conditions as similar as possible to those used during the exposure period. At the start of the pre-exposure period a sample of 10 male and 10 female fish were taken to determine mean body weight. Mean male body weight as 2.3 g and female mean body weight was 1.1 g, with all individual weights within 20% of the mean. Spawning was monitored and recorded daily. A replicate was considered suitable for use in the exposure period if spawning occurred at least twice during the final 7-day period of pre-exposure and if there were at least 15 eggs/female/reproductive day in that aquarium. Any pre-exposure aquaria with mortalities or an injured fish were excluded from use in the test.

Initiation of Exposure

The exposure period was initiated after successful spawning was verified during the 14-day pre-exposure period. The top 20 spawning groups were randomly assigned to exposure replicates in each treatment and control group using a stratified random procedure, based on fecundity data collected during the pre-exposure period, to ensure that there was no reproductive bias towards a particular group.

Exposure Period

Delivery of the test item concentrations was via a flow-through diluter system to constantly renew test media. A concentrated stock solution of the test substance (80 mg a.s./L in dilution water) was loaded into syringe pumps and pumped at set flow rates in to mixing chambers, where it was mixed with dilution water to prepare the target test concentrations (0.01, 0.1, 1.0 and 10 mg a.s./L nominal concentrations). The flow of dilution water into each mixing chamber was controlled using rotameters, and was adjusted to provide approximately 9 volume additions of test solution in each test chamber per day. After mixing, the test solution in each mixing chamber was pumped into the appropriate replicate test chamber using a peristaltic pump. The control group received dilution water only, at the same volume replacement rate.

Analytical confirmation of the test media concentrations was performed regularly throughout the exposure period (and on one occasion prior to initiation of the exposure period). Water samples were collected from two alternating replicate test chambers on Days 0, 7, 14 and 21 of exposure to measure concentrations of the test substance. Additional samples were collected on Day 18 due to a delivery failure. Samples were collected from mid-depth at each interval, placed in glass vials, and processed immediately for analysis using HPLC. In addition stock solution concentration was confirmed from a sample collected three days prior to exposure period initiation.

During the 21-day exposure period, survival, general observations, and assessments of fecundity and fertility were recorded daily. Observations of abnormalities (e.g., hemorrhage or discoloration) or abnormal behavior (e.g., hyperventilation, loss of equilibrium, uncoordinated swimming, atypical quiescence or feeding abstinence) relative to the controls were noted. Spawning substrates were removed from the test chambers daily and any eggs that were present were enumerated. Eggs were evaluated

for fertilization success by applying toluidine blue dye to highlight embryo development and counting the number of infertile eggs. The number of fertile eggs was calculated as the difference in the number of infertile eggs and the total number of eggs on the substrate.

Throughout the exposure period temperature, pH and dissolved oxygen was measured weekly in each replicate test vessel. In addition temperature was monitored continuously via a max-min thermometer maintained in one of the control replicates. Water hardness, alkalinity and conductivity were measured weekly in one control replicate and one replicate in the highest treatment group (10 mg a.s./L nominal concentration). The replicate used was alternated on each occasion. Light intensity was measured weekly in five indiscriminate locations within the test area.

Termination of Exposure

At termination of exposure on Day 21, all fish were euthanized with buffered MS-222, blotted dry and measured for total length and wet weight (to the nearest millimeter and 0.1 mg respectively). Blood samples were collected from the caudal vein/artery of each fish for possible later analysis of serum concentrations of vitellogenin (VTG). These samples were processed by centrifugal separation of the plasma, which was then added to a protease inhibitor and the mixed samples stored frozen. Each fish was dissected and the gonadal sex was determined. The gonads were removed and weighed before being preserved for gonadal histology. Since there were no treatment-related effects of XDE-729 Acid on reproduction, it was not necessary to process these samples.

Results

End points were expressed in terms of mean measured concentrations, which were calculated to be 0.011, 0.11, 1.1 and 12 mg a.s./L, equivalent to 110, 110, 110 and 120% of the nominal concentrations of XDE-729 Acid.

Survival in the control group and all treatment groups was 100% and there were no significant differences in fish wet weight, total length or fecundity (egg production/female/day) between any treatment group compared with the controls (Dunnett's test; $p > 0.05$). No behavioural or morphological abnormalities were observed during the exposure period. Mean treatment group mortality, weight and length are displayed in table B.9.2.157.

The mean percent fertility in the negative control, 0.011, 0.11, 1.1 and 12 mg a.s./L treatment groups was 98.3, 98.0, 95.7, 97.4 and 98.2% respectively. There was a statistically significant decrease in fertility in the 0.11 mg a.s./L treatment group in comparison to the negative control ($p < 0.05$). However, this was due to lower fertility in one replicate resulting in the difference from the negative control mean being slight (3%) and with no clear dose-response. The slight decrease in fertility in the 0.11 mg a.s./L treatment group was therefore not considered to be treatment related. Mean fecundity and egg fertilisation percentage are displayed in table B.9.2.158. No statistically significant difference in comparison with the control was found with regards to male or female gonad weight and GSI (table B.9.2.159), using Dunnett's test ($p > 0.05$).

Table B.9.2.157: Effects of XDE-729 Acid on mortality, mean weight and mean length (\pm standard deviation) in adult fathead minnow (*Pimephales promelas*) in the 21 day exposure phase of the fish short-term reproduction assay.

Mean Measured Concentration of XDE-729 Acid (mg a.s./L)	% Mortality			Wet Weight (g)		Length (mm)	
	Males	Females	Total	Males	Females	Males	Females
Negative control	0	0	0	2.82 \pm 0.743	1.22 \pm 0.091	59 \pm 5.4	48 \pm 1.4
0.011	0	0	0	2.68 \pm 0.172	1.29 \pm 0.060	59 \pm 0.6	49 \pm 0.7
0.11	0	0	0	2.59 \pm 0.154	1.25 \pm 0.071	57 \pm 1.2	49 \pm 0.8
1.1	0	0	0	2.54 \pm 0.170	1.26 \pm 0.131	59 \pm 1.1	48 \pm 1.0
12	0	0	0	2.67 \pm 0.196	1.22 \pm 0.054	59 \pm 3.0	48 \pm 0.9

Table B.9.2.158: Effects of XDE-729 Acid on mean fecundity and fertility in adult fathead minnow (*Pimephales promelas*) in the 21 day exposure phase of the fish short-term reproduction assay.

Mean Measured Concentration of XDE-729 Acid (mg a.s./L)	Fecundity ^a (eggs/female/day)	Egg Fertility (%)
Negative control	32.1 \pm 3.84	98.3
0.011	26.1 \pm 4.14	98.0
0.11	29.1 \pm 5.63	95.7*
1.1	29.5 \pm 6.73	97.4
12	27.9 \pm 8.23	98.2

^a Mean \pm Standard Deviation

* Statistically significant trend in comparison to the negative control using the Wilcoxon Rank Sum test ($p \leq 0.05$).

Table B.9.2.159: Effects of XDE-729 Acid on mean male and female gonad weight and gonadosomatic index (GSI) in the 21 day exposure phase of the fish short-term reproduction assay.

Mean Measured Concentration of XDE-729 Acid (mg a.s./L)	Gonad Weight ^a (g)		Gonadosomatic Index ^{a, b} (GSI)	
	Males	Females	Males	Females
Negative control	0.052 ± 0.024	0.17 ± 0.023	1.78 ± 0.399	14.2 ± 1.86
0.011	0.049 ± 0.012	0.20 ± 0.035	1.79 ± 0.359	15.0 ± 2.07
0.11	0.042 ± 0.006	0.17 ± 0.014	1.61 ± 0.195	13.7 ± 0.99
1.1	0.048 ± 0.012	0.20 ± 0.041	1.88 ± 0.405	15.4 ± 1.71
12	0.045 ± 0.011	0.16 ± 0.033	1.71 ± 0.354	13.1 ± 2.30
^a Mean ± Standard Deviation				
^b (Gonad weight/Fish wet weight) x 100				

The present study met the following requirements for test validity in accordance with the OECD guideline and general accordance with the USEPA guideline, unless otherwise indicated.

The mortality in the control group did not exceed 10 percent at the end of the exposure period for a valid test (0% control mortality achieved). The dissolved oxygen concentration was at least 60% of the air saturation value throughout the exposure period (OECD 229). The minimum dissolved oxygen level observed during the exposure period was 5.9 mg/L. At the water temperature maintained during the test, 60% air saturation value (ASV) is equivalent to 4.9 mg/L, confirming that satisfactory levels of dissolved oxygen were maintained throughout the exposure period. Water temperature did not differ by more than 1°C between test vessels at any observed time during the exposure period. The continuously monitored water temperature in a control replicate was maintained within ±1°C of the 25°C temperature specified for testing with the fathead minnow (24.7 – 25.2 °C).

OECD guideline 229 requires that evidence be provided to demonstrate that the concentrations of the test substance in solution have been satisfactorily maintained within ± 20% of the mean measured values. Coefficient of variation (CV) values for the 0.011, 0.11, 1.1 and 12 mg a.s./L mean measured concentrations were calculated to be 17, 10, 8 and 7% respectively, demonstrating an acceptable level of variation from the mean measured concentrations for each treatment group.

Successful egg production was observed in all replicates used in the exposure period. Spawning in all pre-exposure replicates used in the exposure period occurred at least twice in the 7 days prior to exposure and fecundity was ≥15 eggs/female/reproductive day/replicate). Successful egg production was observed in each of the four control vessels (e.g. spawning occurred at least every 4 days or at least 15 eggs/female/reproductive day/replicate). Fertility of control-produced eggs was observed to be 98.3% (>95% required by OECD 229).

Conclusions

All OECD guideline 229-specified validity criteria were met and accordance with the corresponding EPA guideline was demonstrated. Breeding groups of fathead minnows (*Pimephales promelas*) exposed to XDE-729 Acid at mean measured concentrations of 0.011, 0.11, 1.1 and 12 mg a.s./L for 21 days displayed no effects to survival, length, body weight, fecundity or fertility when compared to the control group.

RMS comment:

This study is considered valid. Light intensity regularly exceeded the maximum of 1000 lux recommended by the OECD test guideline, but due to the control group performance during the test this is not thought to have had any negative impact.

B.9.2.2 Risk assessment

Toxicity

The critical endpoints employed in the risk assessment for aquatic organisms have been selected from the data-base summarized in the table below:

Table B.9.2.160: Summary table of all aquatic toxicity data

Duration and test compound	Species	Endpoint	Toxicity	Reference
Fish				
Acute toxicity XDE-729 Methyl	Rainbow trout (<i>Oncorhynchus mykiss</i>)	96 hr LC ₅₀	2.01 mg a.s/L	██████████ (2011) IIA 8.2.1.1/1
	Fathead minnow (<i>Pimephales promelas</i>)		>3.22 mg a.s/L	██████████ (2011) IIA 8.2.1.2/1
	Sheepshead minnow (<i>Cyprinodon variegates</i>)		>1.33 mg a.s/L	██████████ (2011) IIA 8.2.1.2/2
Acute toxicity XDE-729 acid	Rainbow trout (<i>Oncorhynchus mykiss</i>)		>107 mg XDE-729 acid/L	██████████ (2011) IIA 8.2.1.3/1
Acute toxicity X11449757	Rainbow trout (<i>Oncorhynchus mykiss</i>)		>120 mg X11449757 /L	██████████ (2011) IIA 8.2.1.3/2
Acute toxicity X11406790	Rainbow trout (<i>Oncorhynchus mykiss</i>)		>30 mg X11406790/L	██████████ (2012) IIA 8.2.1.3/3
Acute toxicity GF-2573	Rainbow trout (<i>Oncorhynchus mykiss</i>)		78.7 mg GF-2573/L (0.661 mg a.s./L)	██████████ (2011) IIIA 10.2.2.1/1
Chronic toxicity XDE-729 Methyl	Fathead minnow (<i>Pimephales promelas</i>)	NOEC	0.259 mg a.s/L	██████████ (2011) IIA 8.2.4/1
	Sheepshead minnow (<i>Cyprinodon variegates</i>)		11.5 µg a.s/L	██████████ (2012) IIA 8.2.4/2
Chronic toxicity XDE-729 acid	Fathead minnow (<i>Pimephales promelas</i>)		11.8 mg XDE-729 acid/L	██████████ (2011) IIA 8.2.4/3
Chronic toxicity X11449757	Fathead minnow (<i>Pimephales promelas</i>)		8.9 mg X11449757/L	██████████ (2012) IIA 8.2.4/4
21-day reproduction assay XDE-729 Methyl	Fathead minnow (<i>Pimephales promelas</i>)		0.078 mg/L	██████████ (2012) IIA 8.16.1.2/2
21-day reproduction assay XDE-729 Acid	Fathead minnow (<i>Pimephales promelas</i>)		12.0 mg XDE-729 acid/L	██████████ (2012) IIA 8.16.1.1/1
Bioconcentration and metabolism XDE-729 Methyl	Bluegill sunfish (<i>Lepomis macrochirus</i>)	Bioaccumulation	BCF _{SS} = 217 CT ₉₅ = 1.6	██████████ (2011) IIA 8.2.6/1

Duration and test compound	Species	Endpoint	Toxicity	Reference
Aquatic Invertebrates				
Acute toxicity XDE-729 Methyl	Water flea (<i>Daphnia magna</i>)	48 hr EC ₅₀	2.12 mg a.s/L	Rebstock, M (2011) IIA 8.3.1.1/1
	Shrimp (Americamysis bahia)	96 hr LC ₅₀	>1.30 mg a.s/L	Bergfield, A (2011) IIA 8.3.1.3/1
	Eastern Oyster (<i>Crassostrea virginica</i>)	96 hr EC ₅₀	>1.21 mg a.s/L	Hicks, S.L. (2011) IIA 8.3.1.4/1
	Marine amphipod	10 d LC ₅₀	>58.1 mg a.s/kg	Gerke, A (2011) IIA 8.11.1
Acute toxicity XDE-729 acid	Water flea (<i>Daphnia magna</i>)	48 hr EC ₅₀	> 106 mg XDE-729 acid /L	Bergfield, A (2011) IIA 8.3.1.1/2
Acute toxicity X11449757			>120 mg X11449757/L	Bergfield, A (2011) IIA 8.3.1.1/3
Acute toxicity X11406790			>30 mg X11406790/L	Gaertner, K (2012) IIA 8.3.1.1/4
Acute toxicity GF-2573	Water flea (<i>Daphnia magna</i>)		14.0 mg GF-2573/L (0.118 mg a.s./L)	Bergfield A (2011) IIIA 10.2.2.2/1
Acute toxicity GF-2573 blank formulation*			9.1 mg GF-2573/L	Gaertner, K (2012) IIIA 10.2.2.2/2
Chronic toxicity XDE-729 Methyl	Water flea (<i>Daphnia magna</i>)	NOEC	0.144 mg a.s/L	Bergfield, A (2011) IIA 8.3.2.1/1
	Shrimp (Americamysis bahia)		0.152 mg a.s/L	Hicks, S.L. (2011) IIA 8.3.2.4/1
Chronic toxicity XDE-729 acid	Water flea (<i>Daphnia magna</i>)		100 mg XDE-729 acid/L	Bergfield, A (2011) IIA 8.3.2.1/2
Sediment Dwellers				
Chronic toxicity XDE-729 Methyl	Freshwater midge (<i>Chironomus riparius</i>)	NOEC	1.26 mg a.s/L	Gerke, A (2011) IIA 8.3.2.2/1
	Freshwater midge (<i>Chironomus dilutus</i>)		89.3 mg a.s./kg	Gerke, A (2011) IIA 8.5.1/1
Algae				

Duration and test compound	Species	Endpoint	Toxicity	Reference
Toxicity XDE-729 Methyl	Green microalgae (<i>Pseudokirchneriella subcapitata</i>)	96 hr E _r C ₅₀ and E _y C ₅₀ 72 hr E _r C ₅₀ and E _y C ₅₀	>0.245 mg a.s./L >0.855 mg a.s./L	Weber, K. (2011) IIA 8.4/1
	<i>Skeleonema costatum</i>	72 hr EC ₅₀ (cell density) 96 hr EC ₅₀ (cell density) 72 hr ErC ₅₀ 96 hr ErC ₅₀	0.904 mg a.s./L 1.07 mg a.s./L 1.80 mg a.s./L >1.85 mg a.s./L	Rebstock, M. (2011) IIA 8.4/4
	Blue-green algae (<i>Anabaena flos-aquae</i>)	96 hr E _r C ₅₀ and E _y C ₅₀ 72 hr E _r C ₅₀ and E _y C ₅₀	>0.775 mg/L 1.13 mg/L	Weber, K. (2011) IIA 8.4/3
	<i>Navicula pelliculosa</i>	96 hr E _y C ₅₀ 96 hr E _r C ₅₀ 72 hr E _y C ₅₀ 72 hr E _r C ₅₀	0.663 mg a.s./L 1.26 mg a.s./L 0.822 mg a.s./L 1.50 mg a.s./L	Rebstock, M. (2011) IIA 8.4/2
Toxicity XDE-729 acid	Green microalgae (<i>Pseudokirchneriella subcapitata</i>)	72 hr E _y C ₅₀ 72 hr E _r C ₅₀	23 mg XDE-729 acid /L 63 mg XDE-729 acid /L	Rebstock, M. (2011) IIA 8.4/5
	<i>Navicula pelliculosa</i>	72 hr E _y C ₅₀ 72 hr E _r C ₅₀	50 mg XDE-729 acid /L 56 mg XDE-729 acid /L	Rebstock, M. (2011) IIA 8.4/2
	Blue-green algae (<i>Anabaena flos-aquae</i>)	72 hr E _y C ₅₀ 72 hr E _r C ₅₀	49 mg XDE-729 acid /L 55 mg XDE-729 acid /L	Rebstock, M. (2011) IIA 8.4/7
	<i>Skeleonema costatum</i>	96 hr EC ₅₀ (cell density) 96 hr ErC ₅₀ 72 hr EC ₅₀ (cell density) 72 hr E _r C ₅₀	66 mg XDE-729 acid /L 77 mg XDE-729 acid /L 68 mg XDE-729 acid /L 78 mg XDE-729 acid /L	Rebstock, M. (2011) IIA 8.4/8
Toxicity X11449757	Green microalgae (<i>Pseudokirchneriella subcapitata</i>)	72hr E _y C ₅₀ 72hr E _r C ₅₀	4.13 mg X11449757/L >15.8 mg X11449757/L	Rebstock, M. (2011) IIA 8.4/9
Toxicity X11406790	Green microalgae (<i>Pseudokirchneriella subcapitata</i>)	72 hr E _y C ₅₀ 72hr E _r C ₅₀	1.8 mg X11406790/L >5.7 mg X11406790/L	Rebstock, M. (2012) IIA 8.4/10

Duration and test compound	Species	Endpoint	Toxicity	Reference
Toxicity GF-2573	Green microalgae (<i>Pseudokirchneriella subcapitata</i>)	72 hr E _y C ₅₀ 72hr E _r C ₅₀	0.29 mg GF-2573/L (2.44 µg a.s./L) 1.1 mg GF-2573/L (9.24 µg a.s./L)	Rebstock, M. (2011) IIIA 10.2.2.3/1
Toxicity [REDACTED]	Green microalgae (<i>Pseudokirchneriella subcapitata</i>)	72 hr E _y C ₅₀ 72 hr E _r C ₅₀	0.37 mg [REDACTED] 2.9 mg [REDACTED]	Rebstock, M. (2012) IIIA 10.2.2.3/2
Aquatic Plants				
Toxicity XDE-729 Methyl	Duckweed (<i>Lemna gibba</i>)	7 d E _y C ₅₀ 7 d E _r C ₅₀	2.13 mg a.s/L >2.27 mg a.s/L	Rebstock, M. (2011) IIA 8.6/1
	Eurasian watermilfoil (<i>Myriophyllum spicatum</i>)	14 d E _y C ₅₀ 14 d E _r C ₅₀	0.149 µg a.s/L 0.393 µg a.s/L	Gonsior, G. (2012) IIA 8.6/5
Toxicity XDE-729 Acid	Duckweed (<i>Lemna gibba</i>)	7 d E _y C ₅₀ 7 d E _r C ₅₀	15 mg XDE-729 acid/L >50 mg XDE-729 acid/L	Rebstock, M. (2011) IIA 8.6/2
	Eurasian watermilfoil (<i>Myriophyllum spicatum</i>)	14 d E _y C ₅₀ 14 d E _r C ₅₀	0.800 µg XDE-729 acid/L 1.58 µg XDE-729 acid/L	Gonsior, G. (2012) IIA 8.6/6
Toxicity X11449757	Duckweed (<i>Lemna gibba</i>)	7 d E _y C ₅₀ 7 d E _r C ₅₀	>92.9 mg X11449757/L >92.9 mg X11449757/L	Rebstock, M. (2011) IIA 8.6/3
	Eurasian watermilfoil (<i>Myriophyllum spicatum</i>)	14 d E _y C ₅₀ 14 d E _r C ₅₀	>100 µg X11449757/L >100 µg X11449757/L	Gonsior, G. (2012) IIA 8.6/7
Toxicity X11406790	Duckweed (<i>Lemna gibba</i>)	7 d E _y C ₅₀ 7 d E _r C ₅₀	>12 mg X11406790/L >12 mg X11406790/L	Rebstock, M. (2012) IIA 8.6/4
	Eurasian watermilfoil (<i>Myriophyllum spicatum</i>)	14 d E _y C ₅₀ 14 d E _r C ₅₀	>100 µg X11449757/L >100 µg X11449757/L	Gonsior, G. (2012) IIA 8.6/8
Toxicity GF-2573	Duckweed (<i>Lemna gibba</i>)	7 d E _y C ₅₀ 7 d E _r C ₅₀	>80 mg GF-2573/L >80 mg GF-2573/L (>0.672 mg a.s./L)	Rebstock, M. (2011) IIIA 10.8.2.1/1
	Eurasian watermilfoil (<i>Myriophyllum spicatum</i>)	14 d E _y C ₅₀ 14 d E _r C ₅₀	40.2 µg GF-2573/L (0.338 µg a.s./L) 84.4 µg GF-2573/L (0.709 µg a.s./L)	Gonsior, G. (2012) IIIA 10.8.2.1/2
Other Aquatic organisms				

Duration and test compound	Species	Endpoint	Toxicity	Reference
Acute toxicity XDE-729 Methyl	African clawed frog (<i>Xenopus laevis</i>) - tadpoles	96-hr LC ₅₀	>2 mg/L	(2011) IIA 8.16.1
21-day metamorphosis toxicity XDE-729 Methyl		NOEC	0.38 mg/L	(2012) IIA 8.16.1/2

*blank formulation - the representative formulation without the active substance

Bold values – used in the risk assessment

With regards to toxicity studies with the formulation: The fish acute formulation toxicity was ca.3-fold greater than would be predicted based on the a.s. content, which is within the range of experimental variability. Therefore, the other formulation ingredients are considered not to make a significant contribution to toxicity.

Daphnia formulation study: recoveries of XDE-729 Methyl ranged between 98 and 105% (initial concentrations, renewed solutions and spent solutions). The mean calculated concentrations in the test substance treatment solutions during the 48-hour exposure represented recoveries of 98 to 100% of the nominal concentrations. It is not possible to comment on the stability of the other formulation ingredients.

In addition, for aquatic invertebrates it was apparent that toxicity of the formulation was not primarily attributable to XDE-729 Methyl. Testing of *Daphnia magna* with the technical active substance gave a 48-hr EC₅₀ of 2.12 mg a.s./L, compared to 0.118 mg a.s./L when exposed to the formulation. This would suggest that other components in the formulation are adding to the toxicity to daphnia. An additional test was conducted with blank formulation (i.e. containing everything except the 0.84% XDE-729 Methyl). This additional test demonstrated, through comparison of endpoints from testing with the formulation and blank formulation, that toxicity was due to other ingredients (suspected to be the methylated seed oil adjuvant, which makes up ca. 85% of the formulation). As such basing concentrations on nominal formulation concentrations is considered reasonable for GF-2573.

Algal study: Initially measured concentrations of XDE-729 Methyl were 100-112% of nominal but due to this being a static study there was rapid degradation of the Methyl over 72 hrs due to photolysis. Thus the concentrations were not maintained at ±20% nominal throughout the study (reduced to ca 5% of nominal a.s. concentration after 3 days).

However, it was evident from the response of the algae that formulation toxicity was not primarily attributable to XDE-729 Methyl. An additional test was conducted with co-formulant [REDACTED], suspected of causing toxicity to algae within the formulation. The resultant 72-h E_yC₅₀ was found to be 0.37 mg/L, compared to a corresponding 72-h E_yC₅₀ of 0.29 mg GF-2573/L. This indicates that within GF-2573, co-formulants (particularly [REDACTED], which makes up 82% of the formulation by weight) and not the active substance XDE-729 methyl are primarily responsible for

algal toxicity. Therefore, results of the formulation study are presented in terms of nominal GF-2573 concentrations.

Exposure

The following three scenarios have been considered in the assessment of effects on aquatic organisms:

- 1 L GF-2573/ha (7.8 g a.s./ha) in autumn to winter cereals. Earliest application at emergence of crop.
- 0.8 L GF-2573/ha (6.25 g a.s./ha) in spring to winter cereals. Earliest application at the 1st January.
- 0.8 L GF-2573/ha (6.25 g a.s./ha) in spring to spring cereals. Earliest application 7 days post-emergence.

GF-2573 is not applied directly to the surface of water bodies; therefore the primary route of exposure of the formulated product to aquatic organisms considered in this risk assessment is via spray drift as a result of normal agricultural applications. This is because it is assumed that the formulation will not remain intact in the environment for long after application, so drainage and runoff from the formulated product is unlikely. The maximum PEC_{sw} values for GF-2573 in the standard FOCUS surface water bodies (ditch, pond and stream) were calculated using the FOCUS drift calculator in SWASH. Based on the maximum application rate of GF-2573 the resulting PEC_{sw} values were calculated:

Table B.9.2.161: GF-2573 maximum PEC_{sw} concentrations

FOCUS Step	Buffer distance	Initial PEC _{sw} (µg GF-2573/L)		
		Ditch	Stream	Pond
Step 3		5.814	4.315	0.198
Step 4	5m	1.576	1.576	0.172

The worse case GAP in terms of total dose applied is winter cereals in the autumn at 7.82 g a.s./ha at crop growth stage BBCH 10-29 followed by a second application in the spring at 6.25 g a.s./ha at growth stage BBCH 13-45 with a minimum 70 day interval. Estimates of the maximum predicted environmental concentrations in surface waters (PEC_{sw}) of XDE-729 Methyl, XDE-729 Acid, X11449757 and X11406790 across all three exposure scenarios (see above) have been calculated according to FOCUS and the critical values detailed below. In addition, estimates of the maximum PEC_{sw} for the major photolysis metabolites observed in the aqueous photolysis study have been calculated on the basis of the maximum formation fractions seen in the study, and corrected for molecular weight.

The PEC_{sw} and PEC_{sed} values at Step 1 and Step 2 for the active substance and the metabolites are summarised below. The PEC_{sw} values at Step 1 and 2 cover all GAP scenarios. Further modelling at FOCUS steps 3 and 4 (to consider different surface water scenarios and possible mitigation measures) was also undertaken to refine the risk to some aquatic organisms. These are also presented in this section.

Table B.9.2.162: FOCUS estimates of maximum concentrations - PEC_{sw}

FOCUS scenarios		Maximum PEC _{sw} (µg/L)			
		XDE-729 Methyl	XDE-729 Acid (X11393729)	X1149757	X11406790
Step 1	All regions	1.197	0.983	0.748	0.051
Step 2	Northern Zone Oct-Feb	0.412	0.536	0.233	0.032
	Southern Zone Oct-Feb	0.332	0.430	0.198	0.026

Table B.9.2.163: FOCUS estimates of maximum concentrations PEC_{sed}

FOCUS scenarios		Maximum PEC _{sed} (µg/kg)			
		XDE-729 Methyl	XDE-729 Acid (X11393729)	X1149757	X11406790
Step 1	All regions	11.11	0.271	0.252	0.177
Step 2	Northern Zone	4.076	0.150	0.040	0.149
	Southern Zone	3.287	0.120	0.034	0.127

Table B.9.2.164: FOCUS Step 3 maximum PEC_{sw} values (µg/L) for XDE-729 methyl

Scenario	PEC _{sw} (µg/L)					
	Source	XDE-729 Methyl (a)	Source	XDE-729 Methyl (b)	Source	XDE-729 Methyl (c)
D1 Ditch	Drift	0.050	Drift	0.040	Drift	0.039
D1 Stream	Drift	0.044	Drift	0.035	Drift	0.031
D2 Ditch	Drift	0.050	Drift	0.039	--	--
D2 Stream	Drift	0.043	Drift	0.030	--	--
D3 Ditch	Drift	0.049	Drift	0.039	Drift	0.039
D4 Pond	Drift	0.002	Drift	0.001	Drift	0.001
D4 Stream	Drift	0.043	Drift	0.031	Drift	0.033
D5 Pond	Drift	0.002	Drift	0.001	Drift	0.001
D5 Stream	Drift	0.046	Drift	0.029	Drift	0.031
D6 Ditch	Drift	0.050	Drift	0.040	--	--
R1 Pond	Runoff	0.003	Drift	0.001	--	--
R1 Stream	Runoff	0.035	Drift	0.026	--	--
R3 Stream	Drift	0.046	Drift	0.037	--	--
R4 Stream	Runoff	0.041	Drift	0.026	Drift	0.026

(a) – Autumn application to winter cereals at 7.82 g a.s./ha (covers double application including subsequent application at 6.25 g a.s./ha spring application)

(b) – Spring application to winter cereals at 6.25 g a.s./ha

(c) – Spring application to spring cereals at 6.25 g a.s./ha

Table B.9.2.165: FOCUS Step 3 maximum PEC_{sw} values (µg/L) for XDE-729 acid

Scenario	PEC _{sw} (µg/L)					
	Source	XDE-729 acid (a)	Source	XDE-729 acid (b)	Source	XDE-729 acid (c)
D1 Ditch	Drainflow	0.217	Drainflow	0.109	Drainflow	0.015
D1 Stream	Drainflow	0.136	Drainflow	0.068	Drainflow	0.017
D2 Ditch	Drainflow	0.293	Drainflow	0.212	--	--
D2 Stream	Drainflow	0.184	Drainflow	0.132	--	--
D3 Ditch	Drainflow	0.0001	Drainflow	0.0001	Drainflow	0.0001
D4 Pond	Drainflow	0.024	Drainflow	0.001	Drainflow	0.001
D4 Stream	Drainflow	0.043	Drainflow	0.002	Drainflow	0.001
D5 Pond	Drainflow	0.028	Drainflow	0.024	Drainflow	0.000
D5 Stream	Drainflow	0.045	Drainflow	0.055	Drainflow	0.000
D6 Ditch	Drainflow	0.112	Drainflow	0.097	--	--
R1 Pond	Runoff	0.001	Runoff	0.001	--	--
R1 Stream	Runoff	0.059	Runoff	0.018	--	--
R3 Stream	Runoff	0.061	Runoff	0.019	--	--
R4 Stream	Runoff	0.032	Runoff	0.006	Runoff	0.027

(a) – Autumn application to winter cereals at 7.82 g a.s./ha (covers double application including subsequent application at 6.25 g a.s./ha spring application)

(b) – Spring application to winter cereals at 6.25 g a.s./ha

(c) – Spring application to spring cereals at 6.25 g a.s./ha

Table B.9.2.166: FOCUS Step 4 maximum PEC_{sw} values (µg/L) for XDE-729 methyl

Scenario	PEC _{sw} (µg/L)					
	Source	XDE-729 Methyl (a)	Source	XDE-729 Methyl (b)	Source	XDE-729 Methyl (c)
5m drift reduction only						
D1 Ditch	Drift	0.014	Drift	0.011	Drift	0.011
D1 Stream	Drift	0.016	Drift	0.013	Drift	0.011
D2 Ditch	Drift	0.014	Drift	0.011	--	--
D2 Stream	Drift	0.016	Drift	0.011	--	--
D3 Ditch	Drift	0.013	Drift	0.010	Drift	0.011
D4 Pond	Drift	0.001	Drift	0.001	Drift	0.001
D4 Stream	Drift	0.016	Drift	0.011	Drift	0.012
D5 Pond	Drift	0.001	Drift	0.001	Drift	0.001
D5 Stream	Drift	0.017	Drift	0.010	Drift	0.011
D6 Ditch	Drift	0.014	Drift	0.011	--	--
R1 Pond	Runoff	0.003	Drift	0.001	--	--
R1 Stream	Runoff	0.035	Runoff	0.020	--	--
R3 Stream	Runoff	0.041	Runoff	0.018	--	--
R4 Stream	Runoff	0.041	Runoff	0.016	Drift	0.018
10m drift and runoff reduction						
D1 Ditch	Drift	0.007	--	--	--	--
D1 Stream	Drift	0.008	--	--	--	--
D2 Ditch	Drift	0.007	--	--	--	--
D2 Stream	Drift	0.008	--	--	--	--
D3 Ditch	Drift	0.007	--	--	--	--
D4 Pond	Drift	0.001	--	--	--	--
D4 Stream	Drift	0.008	--	--	--	--
D5 Pond	Drift	0.001	--	--	--	--
D5 Stream	Drift	0.009	--	--	--	--
D6 Ditch	Drainflow	0.009	--	--	--	--
R1 Pond	Runoff	0.001	Drift	0.001	--	--
R1 Stream	Runoff	0.016	Runoff	0.009	--	--
R3 Stream	Runoff	0.019	Runoff	0.008	--	--
R4 Stream	Runoff	0.018	Runoff	0.007	Runoff	0.008
20m drift and runoff reduction						
D1 Ditch	--	--	--	--	--	--
D1 Stream	--	--	--	--	--	--
D2 Ditch	--	--	--	--	--	--
D2 Stream	--	--	--	--	--	--
D3 Ditch	--	--	--	--	--	--
D4 Pond	--	--	--	--	--	--
D4 Stream	--	--	--	--	--	--
D5 Pond	--	--	--	--	--	--
D5 Stream	--	--	--	--	--	--

Scenario	PEC _{sw} (µg/L)					
	Source	XDE-729 Methyl (a)	Source	XDE-729 Methyl (b)	Source	XDE-729 Methyl (c)
D6 Ditch	--	--	--	--	--	--
R1 Pond	Runoff	0.001	--	--	--	--
R1 Stream	Runoff	0.008	--	--	--	--
R3 Stream	Runoff	0.010	--	--	--	--
R4 Stream	Runoff	0.010	--	--	--	--

(a) – Autumn application to winter cereals at 7.82 g a.s./ha (covers double application including subsequent application at 6.25 g a.s./ha spring application)

(b) – Spring application to winter cereals at 6.25 g a.s./ha

(c) – Spring application to spring cereals at 6.25 g a.s./ha

Table B.9.2.167: FOCUS Step 4 maximum PEC_{sw} values (µg/L) for XDE-729 acid – Autumn application to autumn cereals at 7.82 g a.s./ha

Scenario	Maximum PEC _{sw} (µg/L)	
	Source	XDE-729 acid (X11393729)
10m drift and runoff reduction		
R1 Pond	Runoff	0.000
R1 Stream	Runoff	0.027
R3 Stream	Runoff	0.028
R4 Stream	Runoff	0.014

Bold – critical step 4 scenario

Surface water PECs for the major aqueous photolysis metabolites are summarised below; these are relevant for the assessment of acute risk to fish and aquatic invertebrates. For algae and aquatic plants, any toxicity associated with the aqueous photolysis metabolites is captured by the endpoint derived for the active substance since extensive photolysis occurred during the studies. Therefore the risk from any photolysis metabolites is addressed via parental risk. It was noted in the aqueous photolysis study that Deg 10, Deg 11 and Deg 14 all fell below 10% AR within 4 hours and thus appear to be transient.

Table B.9.2.168: Estimates of maximum concentrations PEC_{sw} for photolysis metabolites

Photoproduct	Molecular weight	Max % in water	Step 1	Step 2 North Oct-Feb	Step 2 South Oct-Feb
XDE-729 methyl	345	-	1.197	0.412	0.332
Deg 10	326	12.6	0.234	0.049	0.039
Deg 11	273	15.7	0.178	0.061	0.049
Deg 14	229	11.5	0.091	0.031	0.025

Toxicity-Exposure Ratios (TERs)**9.2.2.1 TER_A for fish**

Table B.9.2.169: TER's for acute fish toxicity

Application to winter cereals					
Species	Test substance	Critical End-point (µg a.s./L)	Maximum PEC _{sw} (µg/L)		Reference
Sheepshead minnow	XDE-729 Methyl	>1330	Step 1	1.197	>1111 [REDACTED] (2011) IIA 8.2.1.2/2
Rainbow trout	XDE-729 Methyl	2010	Step 1	1.197	1679 [REDACTED] (2011) IIA 8.2.1.1/1
Rainbow trout	XDE-729 Acid	>107000	Step 1	0.983	>108850 [REDACTED] (2011) IIA 8.2.1.3/1
Rainbow trout	X11449757	>120000	Step 1	0.748	>160428 [REDACTED] (2011) IIA 8.2.1.3/2
Rainbow trout	X11406790	>30000	Step 1	0.051	588235 [REDACTED] (2012) IIA 8.2.1.3/3
Rainbow trout	GF-2573	78700 µg GF-2573/L	Step 3	5.814	13536 [REDACTED] (2011) IIIA 10.2.2.1/1

For XDE-729 Methyl, the lowest definitive acute toxicity endpoint (LC₅₀) was obtained with rainbow trout (2.01 mg/L). For the other fish species no definitive acute toxicity endpoint could be obtained since LC₅₀ values were above the achieved limit of solubility under the test conditions. Indeed, the lowest non-definitive endpoint (LC₅₀ >1.33 mg/L) was observed for sheepshead minnow, for which no mortality was observed at the maximum achieved concentration. For the purposes of a conservative Tier 1 risk assessment the lowest non-definitive endpoint for sheepshead minnow was used as well as the lowest definitive endpoint.

The 96h LC₅₀ for XDE-729 Methyl was 2.01 mg/L for rainbow trout; since GF-2573 contains 0.84 % XDE-729 Methyl, the predicted 96h LC₅₀ for GF-2573 to rainbow trout is therefore 239 mg GF-2573/L (i.e. [100/0.84] x 2.01) assuming there is no significant toxicity associated with the other formulation components. Given the very low concentration of the active substance in the formulation, an assessment of the formulation toxicity was made in a laboratory study (Bergfield, A (2011) IIIA 10.2.2.1/1). Since a definitive LC₅₀ was only obtained with rainbow trout, the formulation was tested with this species to allow a direct comparison of relative toxicity to the active substance to be made. The measured LC₅₀ for GF-2573 was 78.7 mg/L, a factor of 3 compared to the predicted formulation toxicity. This is considered to be within the range of experimental variability, indicating that the other formulation ingredients have minimal contribution to the overall toxicity of GF-2573 given their high content (99.16%) relative to that of the active

substance. As the experimentally derived endpoint for acute toxicity of GF-2573 to fish was lower, this value was used to derive a TER with regards to the formulation.

All TER values calculated for acute toxicity of XDE-729 methyl, XDE-729 acid and their metabolites were all greater than the Annex VI trigger value of 100 at FOCUS step 1, meaning no further refinement is required and acute risk to fish from XDE-729 is low.

9.2.2.2 TER_{LT} for fish

Table B.9.2.170: TER's for long-term fish toxicity

Application to winter cereals					
Species	Test substance	Critical End-point (µg a.s./L)	Maximum PECsw (µg/L)		Reference
Sheepshead minnow ELS	XDE-729 Methyl	11.5	Step 1	1.197	[REDACTED] (2012) IIA 8.2.4/2
			Step 2	0.412	
Fathead minnow ELS	XDE-729 Acid	11800	Step 1	0.983	[REDACTED] (2011) IIA 8.2.4/3
Fathead minnow ELS	X11449757	8900	Step 1	0.748	[REDACTED] (2012) IIA 8.2.4/4
Value in bold does not exceed Annex VI TER trigger of 10.					

The marine fish, sheepshead minnow was significantly more sensitive than fathead minnow to XDE-729 Methyl in an early life stage study, and this provided the lowest chronic critical endpoint for fish. For XDE-729 Methyl the TER was below the trigger of 10 at Step 1, but greater than 10 at Step 2. Based on the results obtained for both ELS and short term reproduction assay tests with the fathead minnow testing XDE-729 Methyl (overall NOEC of 77 µg a.s./L), it is reasonable to assume that effects on reproduction could also occur at similar concentrations to effects on the early life stages. Thus the above risk assessment is considered to also demonstrate a low risk of adverse reproductive effects on fish.

XDE-729 Acid and X11449757 presented significantly lower toxicity to fish in chronic studies than the XDE-729 Methyl. In the early-life-stage toxicity test and fish short-term reproduction assay with fathead minnow testing XDE-729 Acid there were no effects on any parameter at the maximum tested concentration (11.8 and 12 mg/L respectively). In the ELS with the fathead minnow testing X11449757 there were no significant effects to any observed parameter at the maximum tested concentration of 8.9 mg/L.

9.2.2.3 TER_A for *Daphnia*Table B.9.2.171: Acute TERs for *Daphnia magna*

Application to winter cereals					
Test substance	Critical End-point (µg a.s./L)	Maximum PEC _{sw} (µg/L)		TER	Reference
XDE-729 Methyl	2120	Step 1	1.197	1771	Rebstock, M (2011) IIA 8.3.1.1/1
XDE-729 Acid	>106000	Step 1	0.983	>107833	Bergfield, A (2011) IIA 8.3.1.1/2
X11449757	>120000	Step 1	0.748	>160428	Bergfield, A (2011) IIA 8.3.1.1/3
X11406790	>30000	Step 1	0.051	>588235	Gaertner, K (2012) IIA 8.3.1.1/4
GF-2573	14000 µg GF-2573/L	Step 3	5.814	2408	Bergfield, A (2011) IIIA 10.2.2.2/1
GF-2573 Blank formulation	9100 µg blank formulation/L	Step 3	5.814	1565	Gaertner, K (2012) IIA 10.2.2.2/2

Daphnia acute toxicity studies were conducted with the active substance, metabolites and the formulation. XDE-729 Acid, X11449757 and X11406790 were found to exhibit lower acute toxicity to *Daphnia* compared to XDE-729 Methyl. Given the very low concentration of the active substance in the formulation (0.84%), an assessment of the formulation toxicity was made. The measured EC₅₀ for GF-2573 was 14.0 mg/L, which represents *ca.* 18 fold greater toxicity than would be predicted ($[100/0.84] \times 2.12 = 252.4$ mg/L predicted toxicity), indicating that the other formulation ingredients have a significant contribution to the overall toxicity of GF-2573. An additional study was therefore conducted on a blank formulation (no active substance present) to assess the impact of the co-formulants in GF-2573. The toxicity of the blank formulation was similar (9.1 mg/L compared to 14.0 mg/L when formulation contained XDE-729 methyl) and confirmed that the toxicity of GF-2573 to *Daphnia* is not primarily attributable to XDE-729 Methyl, but is most likely attributable to co-formulants, e.g. the methylated seed oil adjuvant (), which is *ca.* 80% w/w of the overall formulation.

The TER_A values for XDE-Methyl, its metabolites, and the formulation were all calculated to be greater than the Annex VI trigger value of 100 at FOCUS step 1, demonstrating that acute risk to *Daphnia* from XDE-729 is low.

9.2.2.4 TER_{LT} for *Daphnia*Table B.9.2.172: Long-term TERs for *Daphnia magna*

Application to winter cereals						
Species	Test substance	Critical End-point (µg a.s./L)	Maximum PEC _{sw} (µg/L)		TER	Reference
<i>Daphnia magna</i>	XDE-729 Methyl	144	Step 1	1.197	120	Bergfield, A (2011) IIA 8.3.2.1/1
	XDE-729 Acid	100000	Step 1	0.983	101729	Bergfield, A (2011) IIA 8.3.2.1/2

For XDE-729 Methyl and XDE-729 Acid, 21-day chronic toxicity studies with *Daphnia magna* were undertaken. The results showed a much greater toxicity exhibited by XDE-729 methyl, compared to the acid. TER values at FOCUS step 1 were above the Annex VI trigger value of 10, so no higher tier refinement was necessary.

9.2.2.5 TER_A for an aquatic insect species

An acute study was not conducted and no data is submitted. XDE-729 is not an insecticide and testing with *Daphnia magna* is considered suitably representative.

9.2.2.6 TER_{LT} for an aquatic insect speciesTable B.9.2.173: TER_{LT} for *Chironomus* - surface water and sediment

Application to winter cereals						
Species	Test substance	Critical End-point (µg a.s./L)	Maximum PEC _{sw} or PEC _{sed} (µg/L or µg/kg)		TER	Reference
<i>Chironomus riparius</i> (spiked water)	XDE-729 Methyl	1260	Step 1	1.197	1053	Gerke, A (2011) IIA 8.3.2.2/01
<i>Chironomus dilutus</i> (spiked sediment)	XDE-729 Methyl	89300 µg a.s./kg	Step 1	11.11	8038	Gerke, A (2011) IIA 8.5.1/01

A water-spiked study with *Chironomus riparius* and a sediment-spiked study with *Chironomus dilutus* were conducted with the active substance to assess the toxicity to aquatic insects from different environmental compartments. TERs are calculated using PEC_{sw} and PEC_{sed} FOCUS Step 1 values accordingly. XDE-729 Methyl was not toxic to *Chironomus* at the maximum concentration tested, whether from spiked water or spiked sediment. The TERs exceed the triggers of 10 and no further refinement using higher tier FOCUS modeling is necessary for the water or sediment assessment.

Metabolites were detected in the sediment phase during water/sediment studies conducted (cross ref the environmental fate section of the DAR, B.8.4.4). However, it can be concluded from comparing metabolite toxicity to other aquatic invertebrates (e.g. *Daphnia*) with that of XDE-729 methyl that any relevant sediment metabolites are likely to be of much lower toxicity to sediment-dwelling invertebrates. Additionally, even assuming a 10x metabolite toxicity compared to the parent, and a 100% formation rate, the risk from sediment-relevant metabolites would be acceptably low.

9.2.2.7 TER_A for a marine aquatic crustacean species

Table B.9.2.174: TER_A for Mysid Shrimp

Application to winter cereals					
Species	Test substance	Critical End-point (µg a.s./L)	Maximum PEC _{sw} (µg/L)		Reference
<i>Americamysis bahia</i>	XDE-729 Methyl	>1300	Step 1	1.197	>1086 Bergfield, A (2011) IIA 8.3.1.3/01

A study testing acute toxicity of the active substance to the mysid shrimp *Americamysis bahia* was conducted and a TER calculated using FOCUS step 1 PEC_{sw} value. TERs at step 1 were found to be greater than 100, indicating low risk to this organism group and no need for refinement using higher tier assessment.

9.2.2.8 TER_{LT} for a marine aquatic crustacean species

Table B.9.2.175: TER_{LT} for Mysid Shrimp

Application to winter cereals					
Species	Test substance	Critical End-point (µg a.s./L)	Maximum PEC _{sw} (µg/L)		Reference
<i>Americamysis bahia</i>	XDE-729 Methyl	152	Step 1	1.197	127 Hicks, S.L. (2011) IIA 8.3.2.4/1

A study testing chronic toxicity of the active substance to the mysid shrimp *Americamysis bahia* was conducted and a TER calculated using FOCUS step 1 PEC_{sw} value. TERs at step 1 were found to be greater than 10, indicating low risk to this organism group and no need for refinement using higher tier assessment.

9.2.2.9 TER_A for an aquatic gastropod mollusc speciesTable 9.2.176: TER_A for Oyster

Application to winter cereals					
Species	Test substance	Critical End-point (µg a.s./L)	Maximum PEC _{sw} (µg/L)		TER
Eastern Oyster <i>Crassostrea virginica</i>	XDE-729 Methyl	>1210	Step 1	1.197	>1011
					Hicks, S.L. (2011) IIA 8.3.1.4/01

A shell deposition study with the marine bivalve mollusc, *Crassostrea virginica*, was conducted with the active substance. A TER is calculated using PEC_{sw} FOCUS Step 1 value. For both steps the TER is greater than 100, indicating low acute risk to this organism group with no need for further refinement via FOCUS modelling.

9.2.2.10 TER_{LT} for an aquatic gastropod mollusc species

A long-term gastropod study is not required and no data is submitted.

9.2.2.11 TER_A for algae

Algal toxicity studies were performed using a total of 4 algal species. The most sensitive definitive endpoint determined with the active substance, XDE-729, was an E_yC₅₀ of 0.663 mg a.s./L which was obtained with *Navicula pelliculosa*. However, the lowest EC₅₀ across all tested algal species was found with the green algae *Pseudokirchneriella subcapitata* where the EC₅₀ for both growth rate and yield was found to be 0.245 mg a.s./L. To maintain the conservative nature of the tier 1 risk assessment, the toxicity endpoint with *P.subcapitata* was used. The major aquatic metabolites identified in the aerobic water-sediment studies (XDE-729 Acid, X11449757 and X11406790) were assessed independently for algae. The metabolite XDE-729 Acid was tested with a range of algal species, and again the lowest endpoint was obtained for *P.subcapitata* (72-hour E_yC₅₀ = 23 mg/L). Since this gave the lowest endpoint, the metabolites X11449757 and X11406790 were also tested with this species to provide a direct comparison of relative toxicities to the same species to be made.

With regards to the relevant endpoints from algal studies, current noted aquatic guidance (SANCO/3268/2001 rev.4) states that wherever possible “both biomass and growth rate should be reported. As there is no clear evidence available to indicate which is the most relevant endpoint for the field situation the lower figure should be used in the risk assessment.” In most cases this tends to be the biomass/yield endpoint from a laboratory study with algal species.

However, the European Food Safety Authority (EFSA) have drafted a newer guidance document for conducting a tiered aquatic risk assessment (EFSA Journal 2013;11(7):3290), although it is not yet noted and has no definitive implementation date. The EFSA guidance differs in what it considers the most relevant endpoint of algal laboratory testing: “Growth rate (*r*) is the preferred endpoint. Other, usually more sensitive endpoints such as yield may also be used if growth rate endpoints are not provided.” In the interests of providing a comprehensive, but suitably conservative risk assessment, the RMS will calculate and present TERs based on both growth rate and biomass/yield endpoints. The understanding of the RMS is that the most conservative endpoint will still be used to determine a Regulatory Acceptable Concentration (RAC), and also to define any required mitigation.

Table B.9.2.177: TERs for algae (*Pseudokirchneriella subcapitata*) with regards to Biomass/yield and growth rate

Application to winter cereals						
Test substance	Endpoint (µg a.s./L)		Maximum PEC _{sw} (µg/L)		TER	Reference
XDE-729 Methyl	E _y C ₅₀	>245	Step 1	1.197	>205	Weber, K. (2011) IIA 8.4/1
	E _r C ₅₀	>245	Step 1	1.197	>205	
XDE-729 Acid	E _y C ₅₀	23000	Step 1	0.983	23398	Rebstock, M. (2011) IIA 8.4/5
	E _r C ₅₀	63000	Step 1	0.983	64090	
X11449757	E _y C ₅₀	4130	Step 1	0.748	5521	Rebstock, M. (2011) IIA 8.4/9
	E _r C ₅₀	>15800	Step 1	0.748	>21123	
X11406790	E _y C ₅₀	1800	Step 1	0.051	35294	Rebstock, M. (2012) IIA 8.4/10
	E _r C ₅₀	>5700	Step 1	0.051	>111765	
GF-2573	E _y C ₅₀	290 µg GF-2573/L	Step 3	5.814	50	Rebstock, M. (2011) IIIA 10.2.2.1
	E _r C ₅₀	1100	Step 3	5.814	189	
[REDACTED] (component of GF-2573)	E _y C ₅₀	370 µg/L	Step 3	5.814	64	Rebstock, M. (2012) IIIA 10.2.2.3/2
	E _r C ₅₀	2900 µg/L	Step 3	5.814	499	

As XDE-729 Methyl represents 0.84% of the formulation content (GF-2573), the predicted toxicity of the formulation, assuming that any toxicity is caused by the active substance alone, would be a 72-hour E_yC₅₀ of approximately 29 mg GF-2573/L. A study with *P.subcapitata* undertaken to determine the toxicity of the formulation resulted in a 72-hour E_yC₅₀ of 0.29 mg GF-2573/L. As this is much lower (by a factor of 100) than the predicted formulation toxicity it can be deduced that other components of the formulation contribute significantly to algal toxicity.

This assumption was confirmed in a separate study *P. subcapitata* was exposed to the methylated seed oil adjuvant (), which is ca. 80% w/w of the overall formulation. This study resulted in an EyC_{50} almost the same as that obtained for GF-2573, showing that the toxicity of GF-2573 to *P. subcapitata* is not primarily caused by XDE-729 Methyl, but is most likely attributable to the co-formulants in GF-2573, in particular the methylated seed oil adjuvant.

At FOCUS step 1 all calculated TERs for XDE-729 Methyl and its metabolites, as well as the formulation and main formulation component () were above the Annex VI trigger value of 10. No higher tier refinement of the risk is required and the risk to algae from XDE-729 Methyl and the formulation GF-2573 is low under the proposed GAP. There is no difference in risk assessment outcome using growth rate end points as recommended by draft EFSA guidance (EFSA Journal 2013;11(7):3290).

9.2.2.12 TER for non-target aquatic plants

Studies with *Lemna gibba* and *Myriophyllum spicatum* have been conducted with the active substance, the major metabolites and the formulation.

Under exposure conditions required for testing aquatic plants, rapid () light intensity, spectrum and duration required for these tests. This was demonstrated in a study under annex point KII 8.6/09, determining the photolysis reaction of the active substance under test conditions for a *Myriophyllum* (Hellstem, J., 2012). Because photolysis was the major, and rapid, route of degradation of XDE-729 Methyl in the aquatic plant tests, exposure to photolysis metabolites occurred *in situ*; consequently, any toxic contribution of the photolysis metabolites are reflected in the reported endpoints for XDE-729 Methyl.

With regards to the relevant endpoints from macrophyte (aquatic plant) studies, current noted aquatic guidance (SANCO/3268/2001 rev.4) states that “The number of fronds is the most important endpoint but if for example toxicity values for biomass or other endpoints are lower these may be used in the risk assessment if appropriate”.

However, the European Food Safety Authority (EFSA) have drafted a newer guidance document for conducting a tiered aquatic risk assessment (EFSA Journal 2013;11(7):3290) although it is not yet noted and has no definitive implementation date. The EFSA guidance differs in what it considers the most relevant endpoint of aquatic macrophyte laboratory testing: “Growth rate (*r*) is the preferred endpoint. Other, usually more sensitive endpoints such as yield may also be used if growth rate endpoints are not provided.” In the interests of providing a comprehensive, but suitably conservative risk assessment, the RMS will calculate and present TERs based on both growth rate and biomass/yield endpoints. The understanding of the RMS is that the most conservative endpoint will still be used to determine a

Regulatory Acceptable Concentration (RAC), and also to define any required mitigation.

Table B.9.2.178: TERs for non-target aquatic plants at FOCUS step 1- *Lemna gibba*

Application to cereals						
Test substance	Endpoint (µg a.s./L)		Maximum PEC _{sw} (µg/L)		TER	Reference
XDE-729 Methyl	7 d EyC ₅₀	2130	Step 1	1.197	1779	Rebstock, M. (2011) DAS 090182. IIA 8.6/1
	7 d ErC ₅₀	>2270	Step 1	1.197	>1896	
XDE-729 Acid	7 d EyC ₅₀	15000	Step 1	0.983	15259	Rebstock, M. (2011) DAS 101145. IIA 8.6/2
	7 d ErC ₅₀	>50000	Step 1	0.983	>50865	
X11449757	7 d EyC ₅₀	>92900	Step 1	0.748	>124198	Rebstock, M. (2011) DAS 101159. IIA 8.6/3
	7 d ErC ₅₀	>92900	Step 1	0.748	>124198	
X11406790	7 d EyC ₅₀	>12000	Step 1	0.051	>235294	Rebstock, M. (2012) DAS 120022. IIA 8.6/4
	7 d ErC ₅₀	>12000	Step 1	0.051	>235294	
GF-2573	7 d EyC ₅₀	>80000 µg GF-2573/L	Step 3	5.814	>13760	Rebstock, M. (2011) DAS 101125. IIIA 10.8.2.1/1
	7 d ErC ₅₀	>80000 µg GF-2573/L	Step 3	5.814	>13760	

TER values for *Lemna* were all greater than the annex VI defined trigger values, indicating low risk and no refinement using higher tier FOCUS modeling is necessary. As the formulation contains 0.84% active substance the predicted toxicity to *Lemna* of the formulation, assuming that only the active substance causes significant toxicity, would be 253.6 mg formulation/L. In a separate study with the formulation GF-2573 the 7 day EC₅₀ endpoints were found to be >80 mg formulation/L. The predicted and laboratory test-derived toxicity values are approximately a factor of 3 apart, i.e. within the limits of scientific variability. The conclusion of this is that the co-formulants within GF-2573 do not contribute significantly towards *Lemna* toxicity. There is no difference in risk assessment outcome using growth rate end points as recommended by draft EFSA guidance (EFSA Journal 2013;11(7):3290).

Table B.9.2.179: Risk assessment of non-target aquatic plants at FOCUS steps 1 and 2 – *Myriophyllum spicatum*

Application to cereals						
Test substance	End-point (µg a.s./L)		Maximum PEC _{sw} (µg/L)		TER	Reference
XDE-729 Methyl	14 d EyC ₅₀	0.149	Step 1	1.197	0.12	Gonsior, G. (2012) DAS 102023. IIA 8.6/5
			Step 2	0.412	0.36	
	14 d ErC ₅₀	0.393	Step 1	1.197	0.33	
			Step 2	0.412	0.95	
XDE-729 Acid	14 d EyC ₅₀	0.80	Step 1	0.983	0.81	Gonsior, G. (2012) DAS 120533. IIA 8.6/6
			Step 2	0.536	1.5	
	14 d ErC ₅₀	1.58	Step 1	0.983	1.6	
			Step 2	0.536	2.9	
X11449757	14 d EyC ₅₀	>100	Step 1	0.748	134	Gonsior, G. (2012) DAS 102015. IIA 8.6/7
	14 d ErC ₅₀	>100	Step 1	0.748	134	
X11406790	14 d EyC ₅₀	>100	Step 1	0.051	1961	Gonsior, G. (2012) DAS 120534. IIA 8.6/8
	14 d ErC ₅₀	>100	Step 1	0.051	1961	
GF-2573	14 d EyC ₅₀	40.2	Step 3	5.814	6.9	Gonsior, G. (2012) DAS 120584. IIA 10.8.2.1/2
			5m buffer	1.576	26	
	14 d ErC ₅₀	84.4	Step 3	5.814	15	
			5m buffer	1.576	54	
Values in bold do not exceed Annex VI TER trigger of 10.						

The representative formulation GF-2573 requires a 5m buffer zone (or equivalent mitigation, to reduce exposure via spray drift) in order to pose an acceptably low risk to the aquatic macrophyte *Myriophyllum spicatum*. Step 1 and step 2 PEC_{sw} values resulted in TER values below the Annex VI trigger of 10 for *Myriophyllum* exposures to XDE-729 Methyl and XDE-729 Acid. Therefore, refined TER values were calculated using Step 3 and Step 4 (drift and run-off mitigation options) PEC_{sw} values for the following scenarios:

- Autumn application at 7.82 g XDE-729 Methyl/ha to winter cereals (covers double application including subsequent spring application at 6.25 g a.s./ha)
- Spring application at 6.25 g XDE-729 Methyl/ha to winter cereals
- Spring application at 6.25 g XDE-729 Methyl/ha to spring cereals

XDE-729 methyl (active substance):

Table B.9.2.180: Risk assessment of non-target aquatic plants – *Myriophyllum spicatum* FOCUS Step 3 and 4 – autumn application at 7.82 g a.s./ha to winter cereals

XDE-729 Methyl - Autumn application to winter cereals				
Test substance	Maximum PEC _{sw} (µg/L)		TER (based on EyC ₅₀ 0.149µg a.s./L)	TER (based on ErC ₅₀ 0.393µg a.s./L)
XDE-729 Methyl - Autumn application to winter cereals Step 3				
XDE-729 Methyl	D1 ditch	0.050	3.0	7.9
	D1 stream	0.044	3.4	8.9
	D2 ditch	0.050	3.0	7.9
	D2 stream	0.043	3.5	9.1
	D3 ditch	0.049	3.0	8.0
	D4 pond	0.002	74.5	196.5
	D4 stream	0.043	3.5	9.1
	D5 pond	0.002	74.5	196.5
	D5 stream	0.046	3.2	8.5
	D6 ditch	0.050	3.0	7.9
	R1 pond	0.003	49.7	131.0
	R1 stream	0.035	4.3	11.2
	R3 stream	0.046	3.2	8.5
	R4 stream	0.041	3.6	9.6
XDE-729 Methyl - Autumn application to winter cereals Step 4 (5m buffer for drift)				
Test substance	Maximum PEC _{sw} (µg/L)		TER (based on EyC ₅₀ 0.149µg a.s./L)	TER (based on ErC ₅₀ 0.393µg a.s./L)
XDE-729 Methyl	D1 ditch	0.014	10.6	28.1
	D1 stream	0.016	9.3	24.6
	D2 ditch	0.014	10.6	28.1
	D2 stream	0.016	9.3	24.6
	D3 ditch	0.013	11.5	30.2
	D4 pond	0.001	149.0	393.0
	D4 stream	0.016	9.3	24.6
	D5 pond	0.001	149.0	393.0
	D5 stream	0.017	8.8	23.1
	D6 ditch	0.014	10.6	28.1
	R1 pond	0.003	49.7	131.0
	R1 stream	0.035	4.3	11.2
	R3 stream	0.041	3.6	9.6

XDE-729 Methyl - Autumn application to winter cereals				
	R4 stream	0.041	3.6	9.6
XDE-729 Methyl - Autumn application to winter cereals Step 4 (10m buffer for drift and 10m runoff reduction)				
Test substance	Maximum PEC _{sw} (µg/L)		TER (based on EyC ₅₀ 0.149 µg a.s./L)	TER (based on ErC ₅₀ 0.393 µg a.s./L)
XDE-729 Methyl	D1 ditch	0.007	21.3	56.1
	D1 stream	0.008	18.6	49.1
	D2 ditch	0.007	21.3	56.1
	D2 stream	0.008	18.6	49.1
	D3 ditch	0.007	21.3	56.1
	D4 pond	0.001	149.0	393.0
	D4 stream	0.008	18.6	49.1
	D5 pond	0.001	149.0	393.0
	D5 stream	0.009	16.6	43.7
	D6 ditch	0.009	16.6	43.7
	R1 pond	0.001	149.0	393.0
	R1 stream	0.016	9.3	24.6
	R3 stream	0.019	7.8	20.7
	R4 stream	0.018	8.3	21.8
XDE-729 Methyl - Autumn application to winter cereals Step 4 (20m runoff reduction)				
Test substance	Maximum PEC _{sw} (µg/L)		TER (based on EyC ₅₀ 0.149 µg a.s./L)	TER (based on ErC ₅₀ 0.393 µg a.s./L)
XDE-729 Methyl	R1 pond	0.001	149.0	393.0
	R1 stream	0.008	18.6	49.1
	R3 stream	0.010	14.9	39.3
	R4 stream	0.010	14.9	39.3

Values in **bold** do not exceed Annex VI TER trigger of 10.

When comparing predicted exposure in surface water with a worst-case endpoint in accordance with current SANCO aquatic guidance, a 20m runoff reduction method is required in order to demonstrate acceptable risk in all FOCUS scenarios. All drainage scenarios are addressed by implementation of a 10m buffer for spray and runoff reduction.

When comparing predicted exposure in surface water with a macrophyte growth rate endpoint in accordance with the draft EFSA aquatic guidance document (EFSA Journal 2013;11(7):3290), all drainage and runoff FOCUS scenarios can be demonstrated as resulting in acceptable risk when a 10m buffer for spray and runoff reduction is implemented. Implementation of a 5m buffer (for spray reduction only) results in all scenarios passing except for R3 and R4 stream.

Under the current, more conservative risk assessment methods recognised by the EU, the risk to the sensitive aquatic macrophyte *Myriophyllum spicatum* from XDE-729 methyl is acceptable following autumn application to winter cereals at an application rate of 7.82 g a.s./ha. This is dependent on mitigation equivalent to 20m buffer for drift and runoff reduction. The application of a subsequent spring application at 6.25 g a.s./ha is included under this risk scenario.

Table B.9.2.181: Risk assessment of non-target aquatic plants – *Myriophyllum spicatum* FOCUS Step 3 and 4 – spring application at 6.25 g a.s./ha to winter cereals

XDE-729 Methyl - Spring application to winter cereals				
Test substance	Maximum PEC _{sw} (µg/L)		TER (based on E _{ryC} ₅₀ 0.149µg a.s./L	TER (based on E _{rC} ₅₀ 0.393µg a.s./L
Step 3				
XDE-729 Methyl	D1 ditch	0.040	3.7	9.8
	D1 stream	0.035	4.3	11.2
	D2 ditch	0.039	3.8	10.1
	D2 stream	0.030	5.0	13.1
	D3 ditch	0.039	3.8	10.1
	D4 pond	0.001	149.0	393.0
	D4 stream	0.031	4.8	12.7
	D5 pond	0.001	149.0	393.0
	D5 stream	0.029	5.1	13.6
	D6 ditch	0.040	3.7	9.8
	R1 pond	0.001	149.0	393.0
	R1 stream	0.026	5.7	15.1
	R3 stream	0.037	4.0	10.6
	R4 stream	0.026	5.7	15.1
Step 4 (5m buffer for drift only)				
XDE-729 Methyl	D1 ditch	0.011	13.5	35.7
	D1 stream	0.013	11.5	30.2
	D2 ditch	0.011	13.5	35.7
	D2 stream	0.011	13.5	35.7
	D3 ditch	0.010	14.9	39.3
	D4 pond	0.001	149.0	393.0
	D4 stream	0.011	13.5	35.7
	D5 pond	0.001	149.0	393.0
	D5 stream	0.010	14.9	39.3
	D6 ditch	0.011	13.5	35.7
	R1 pond	0.001	149.0	393.0
	R1 stream	0.020	7.5	19.7
	R3 stream	0.018	8.3	21.8
	R4 stream	0.016	9.3	24.6

Step 4 (10m buffer for drift and runoff reduction)				
Test substance	Maximum PEC _{sw} (µg/L)		TER (based on EyC ₅₀ 0.149 µg a.s./L)	TER (based on ErC ₅₀ 0.393 µg a.s./L)
XDE-729 Methyl	R1 pond	0.001	149.0	393.0
	R1 stream	0.009	16.6	43.7
	R3 stream	0.008	18.6	49.1
	R4 stream	0.007	21.3	56.1
Values in bold do not exceed Annex VI TER trigger of 10.				

When comparing predicted exposure in surface water with a worst-case endpoint in accordance with current SANCO aquatic guidance, a 10m runoff reduction method is required in order to demonstrate acceptable risk in all FOCUS scenarios. All drainage scenarios are addressed by implementation of a 5m buffer for spray reduction only (as drift is the main route of exposure in these scenarios).

When comparing predicted exposure in surface water with a macrophyte growth rate endpoint in accordance with the draft EFSA aquatic guidance document (EFSA Journal 2013;11(7):3290), all drainage and runoff FOCUS scenarios can be demonstrated as resulting in acceptable risk when a 5m buffer for spray reduction is implemented. All runoff scenarios pass without mitigation requirements (i.e. at FOCUS step 3) under this method of risk assessment.

Under the current, more conservative risk assessment methods recognised by the EU, the risk to the sensitive aquatic macrophyte *Myriophyllum spicatum* from XDE-729 methyl is acceptable following spring application to winter cereals at an application rate of 6.25 g a.s./ha. This is dependent on mitigation equivalent to 10m buffer for drift and runoff reduction.

Table B.9.2.182: Risk assessment of non-target aquatic plants – *Myriophyllum spicatum* FOCUS Step 3 and 4 – spring application at 6.25 g a.s./ha to spring cereals

Spring application of XDE-729 Methyl to spring cereals				
Test substance	Maximum PEC _{sw} (µg/L)		TER (based on EyC ₅₀ 0.8 µg a.s./L	TER (based on ErC ₅₀ 1.58 µg a.s./L
Step 3				
XDE-729 Methyl	D1 ditch	0.039	3.8	10.1
	D1 stream	0.031	4.8	12.7
	D3 ditch	0.039	3.8	10.1
	D4 pond	0.001	149.0	393.0
	D4 stream	0.033	4.5	11.9
	D5 pond	0.001	149.0	393.0
	D5 stream	0.031	4.8	12.7
	R4 stream	0.026	5.7	15.1
Step 4 (5m buffer for drift)				

Spring application of XDE-729 Methyl to spring cereals				
XDE-729 Methyl	D1 ditch	0.011	13.5	35.7
	D1 stream	0.011	13.5	35.7
	D3 ditch	0.011	13.5	35.7
	D4 pond	0.001	149.0	393.0
	D4 stream	0.012	12.4	32.8
	D5 pond	0.001	149.0	393.0
	D5 stream	0.011	13.5	35.7
	R4 stream	0.018	8.3	21.8
Step 4 (10m buffer for drift and runoff reduction)				
XDE-729 Methyl	R4 stream	0.008	18.6	49.1
Values in bold do not exceed Annex VI TER trigger of 10.				

When comparing predicted exposure in surface water with a worst-case endpoint in accordance with current SANCO aquatic guidance, a 10m runoff reduction method is required in order to demonstrate acceptable risk in all FOCUS scenarios. All drainage scenarios are addressed by implementation of a 5m buffer for spray reduction only (as drift is the main route of exposure in these scenarios).

When comparing predicted exposure in surface water with a macrophyte growth rate endpoint in accordance with the draft EFSA aquatic guidance document (EFSA Journal 2013;11(7):3290), all drainage and runoff FOCUS scenarios can be demonstrated as resulting in acceptable risk at step 3 (i.e. without need for mitigation).

Under the current, more conservative risk assessment methods recognised by the EU, the risk to the sensitive aquatic macrophyte *Myriophyllum spicatum* from XDE-729 methyl is acceptable following spring application to spring cereals at an application rate of 6.25 g a.s./ha. This is dependent on mitigation equivalent to 10m buffer for drift and runoff reduction.

XDE-729 Acid (metabolite X11393729):

Table B.9.2.183: Risk assessment of non-target aquatic plants from XDE-729 Acid – *Myriophyllum spicatum* FOCUS Step 3 – autumn application at 7.82 g a.s./ha to winter cereals

XDE-729 Acid - Autumn application to winter cereals Step 3				
Test substance	Maximum PEC _{sw} (µg/L)		TER (based on EyC ₅₀ 0.8 µg a.s./L)	TER (based on ErC ₅₀ 1.58 µg a.s./L)
XDE-729 Acid	D1 ditch	0.217	3.7	7.3
	D1 stream	0.136	5.9	11.6
	D2 ditch	0.293	2.7	5.4
	D2 stream	0.184	4.3	8.6
	D3 ditch	0.0001	8000.0	15800.0
	D4 pond	0.024	33.3	65.8
	D4 stream	0.043	18.6	36.7
	D5 pond	0.028	28.6	56.4
	D5 stream	0.045	17.8	35.1
	D6 ditch	0.112	7.1	14.1
	R1 pond	0.001	800.0	1580.0
	R1 stream	0.059	13.6	26.8
	R3 stream	0.061	13.1	25.9
	R4 stream	0.032	25.0	49.4
Values in bold do not exceed Annex VI TER trigger of 10.				

When comparing predicted exposure in surface water with a worst-case endpoint in accordance with current SANCO aquatic guidance, All FOCUS step 3 runoff scenarios pass without the need for mitigation. However drainage scenarios D1 ditch and stream, D2 ditch and stream and D6 ditch TERs are all below the Annex VI trigger of 10. Mitigation for these scenarios is not possible as exposure to XDE-729 acid will be predominantly via drainflow.

When comparing predicted exposure in surface water with a macrophyte growth rate endpoint in accordance with the draft EFSA aquatic guidance document (EFSA Journal 2013;11(7):3290), all runoff FOCUS scenarios can be demonstrated as resulting in acceptable risk at step 3 (i.e. without need for mitigation). All drainage scenarios pass at step 3 with the exception of D1 ditch and D2 ditch and stream. Mitigation for these scenarios is not possible as exposure to XDE-729 acid will be predominantly via drainflow.

Under the current, more conservative risk assessment methods recognised by the EU, the risk to the sensitive aquatic macrophyte *Myriophyllum spicatum* from XDE-729 acid is acceptable following Autumn application to winter cereals at an application rate of 7.82 g a.s./ha for all FOCUS scenarios except D1 ditch and stream, D2 ditch and stream and D6 ditch. Member states should consider which scenarios are most relevant in their national risk assessments for XDE-729 acid.

The application of a subsequent spring application at 6.25 g a.s./ha is included under this risk scenario.

Table B.9.2.184: Risk assessment of non-target aquatic plants from XDE-729 acid – *Myriophyllum spicatum* FOCUS Step 3 – spring application at 6.25 g a.s./ha to winter cereals

XDE-729 Acid - Spring application to winter cereals Step 3				
Test substance	Maximum PEC _{sw} (µg/L)		TER (based on EyC ₅₀ 0.8 µg a.s./L)	TER (based on ErC ₅₀ 1.58 µg a.s./L)
XDE-729 Acid	D1 ditch	0.109	7.3	14.5
	D1 stream	0.068	11.8	23.2
	D2 ditch	0.212	3.8	7.5
	D2 stream	0.132	6.1	12.0
	D3 ditch	0.0001	8000.0	15800.0
	D4 pond	0.001	800.0	1580.0
	D4 stream	0.002	400.0	790.0
	D5 pond	0.024	33.3	65.8
	D5 stream	0.055	14.5	28.7
	D6 ditch	0.097	8.2	16.3
	R1 pond	0.001	800.0	1580.0
	R1 stream	0.018	44.4	87.8
	R3 stream	0.019	42.1	83.2
	R4 stream	0.006	133.3	263.3
Values in bold do not exceed Annex VI TER trigger of 10.				

When comparing predicted exposure in surface water with a worst-case endpoint in accordance with current SANCO aquatic guidance, All FOCUS step 3 runoff scenarios pass without the need for mitigation. However drainage scenarios D1 ditch, D2 ditch and stream and D6 ditch TERs are all below the Annex VI trigger of 10. Mitigation for these scenarios is not possible as exposure to XDE-729 acid will be predominantly via drainflow.

When comparing predicted exposure in surface water with a macrophyte growth rate endpoint in accordance with the draft EFSA aquatic guidance document (EFSA Journal 2013;11(7):3290), all runoff FOCUS scenarios can be demonstrated as resulting in acceptable risk at step 3 (i.e. without need for mitigation). All drainage scenarios pass at step 3 with the exception of D2 ditch. Mitigation for this scenario is not possible as exposure to XDE-729 acid will be predominantly via drainflow.

Under the current, more conservative risk assessment methods recognised by the EU, the risk to the sensitive aquatic macrophyte *Myriophyllum spicatum* from XDE-729 acid is acceptable following spring application to winter cereals at an application rate of 6.25 g a.s./ha for all FOCUS scenarios except D1 ditch, D2

ditch and stream and D6 ditch. Member states should consider which scenarios are most relevant in their national risk assessments for XDE-729 acid.

Table B.9.2.185: Risk assessment of non-target aquatic plants from XDE-729 acid – *Myriophyllum spicatum* FOCUS Step 3 – spring application at 6.25 g XDE-729 acid a.s./ha to spring cereals

XDE-729 Acid - Spring application to spring cereals Step 3				
Test substance	Maximum PEC _{sw} (µg/L)		TER (based on EyC ₅₀ 0.8 µg a.s./L	TER (based on ErC ₅₀ 1.58 µg a.s./L
XDE-729 Acid	D1 ditch	0.015	53.3	105.3
	D1 stream	0.017	47.1	92.9
	D3 ditch	0.0001	8000.0	15800.0
	D4 pond	0.001	800.0	1580.0
	D4 stream	0.001	800.0	1580.0
	D5 pond	0.0001	8000.0	15800.0
	D5 stream	0.0001	8000.0	15800.0
	R4 stream	0.027	29.6	58.5

When comparing predicted exposure in surface water with either a worst-case endpoint in accordance with current SANCO aquatic guidance or growth rate endpoint in accordance with the draft EFSA aquatic guidance document (EFSA Journal 2013;11(7):3290), all FOCUS step 3 runoff scenarios pass without the need for mitigation. Low risk to aquatic macrophytes from XDE-729 acid for all FOCUS scenarios can be concluded without the need for mitigation, when XDE-729 is spring-applied to spring cereals at 6.25 g a.s./ha.

Table B.9.2.186: Conclusion of the risk to aquatic plants from XDE-729 methyl and XDE-729 acid, according to guidance SANCO/3268/2001 rev.4:

Exposure to:	Application	Scenarios <u>not</u> acceptable risk at FOCUS step 3	Scenarios <u>not</u> acceptable with 5m buffer (spray only)	Scenarios <u>not</u> acceptable with 10m buffer (spray + runoff)	Scenarios <u>not</u> acceptable with 20m buffer (spray + runoff)
XDE-729 methyl	Autumn application to winter cereals – 7.82 g a.s./ha*	D1 ditch D1 stream D2 ditch D2 stream D3 ditch D4 stream D5 stream D6 ditch R1 stream R3 stream R4 stream	D1 stream D2 stream D4 stream D5 stream R1 stream R3 stream R4 stream	R1 stream R3 stream R4 stream	--
	Spring application to winter cereals – 6.25 g a.s./ha	D1 ditch D1 stream D2 ditch D2 stream D3 ditch D4 stream D5 stream D6 ditch R1 stream R3 stream R4 stream	R1 stream R3 stream R4 stream	--	--
	Spring application to spring cereals – 6.25 g a.s./ha	D1 ditch D1 stream D3 ditch D4 stream D5 stream R4 stream	R4 stream	--	--
XDE-729 acid	Autumn application to winter cereals – 7.82 g a.s./ha*	D1 ditch D1 stream D2 ditch D2 stream D6 ditch	NA	NA	NA
	Spring application to winter cereals – 6.25 g a.s./ha	D1 ditch D2 ditch D2 stream D6 ditch	NA	NA	NA
	Spring application to spring cereals – 6.25 g a.s./ha	--	NA	NA	NA

*- includes risk from dual application to winter cereals of 7.82 g a.s./ha in autumn followed by 6.25 g a.s./ha in spring

NA - not applicable – mitigation via FOCUS step 4 not possible for drainage scenarios

- For Autumn application of GF-2573 to winter cereals at the proposed rate of 7.82 g a.s./ha, risk mitigation equivalent to a 20m buffer zone for spray and runoff reduction is required. Acceptable risk from the acid metabolite

cannot be demonstrated for scenarios D1 ditch and stream, D2 ditch and stream and D6 ditch. This also includes the risk from a dual application to winter cereals of 7.82 g a.s./ha in autumn followed by 6.25 g a.s./ha in spring. Acceptable risk from the acid metabolite cannot be demonstrated for scenarios D1 ditch and stream, D2 ditch and stream and D6 ditch.

- For spring application of GF-2573 to winter cereals at the proposed rate of 6.25 g a.s./ha, risk mitigation equivalent to a 10m buffer zone for spray and runoff reduction is required. Acceptable risk from the acid metabolite cannot be demonstrated for scenarios D1 ditch, D2 ditch and stream and D6 ditch.
- For spring application of GF-2573 to spring cereals at the proposed rate of 6.25 g a.s./ha, risk mitigation equivalent to a 10m buffer zone for spray and runoff reduction is required. Acceptable risk from the acid metabolite can also be demonstrated for all scenarios.

B.9.2.2.13 Bioaccumulation

As the log Kow value of XDE-729 methyl is > 3 (3.76), a bioaccumulation and metabolism study was conducted with XDE-729 Methyl in order to demonstrate acceptably low bioaccumulation potential in fish. The study is summarised under annex point IIA 8.2.6/1 (Leake, T., 2011). Whole body ^{14}C - residue concentrations reached steady-state equilibrium between 14 and 21 days; the steady state BCF (i.e. BCF_{ss}) was estimated at 186 for the low concentration and 217 for the high concentration. Following transfer to clean water the whole body ^{14}C - residue was rapidly depurated, with the calculated time to 95% depuration of the ^{14}C -residue being 1.6 days for both the low and high treatment.

SANCO/3268/2001 rev 4 (2002) guidance states that the following 3 criteria should all be met for an evaluation of biomagnification in aquatic food chains to be triggered:

1. Whole body $\text{BCF} > 1000$
2. Elimination of radioactivity during the 14 day depuration phase of the bioconcentration study is $< 95\%$
3. $\text{DT}_{90\text{f}} > 100$ days

As concluded in the study the highest BCF was calculated to be 217 (< 1000) and the time to 95% depuration was 1.6 days (< 14 days). Therefore no further investigation into biomagnification is required.

B.9.2.3 Conclusions: Overall risks to aquatic organisms

With the exception of aquatic plants, the acute and long-term toxicity/exposure ratios (TERs) for GF-2573, XDE-729 Methyl and the major metabolites are in excess of the Annex VI triggers for FOCUS Step 1 and/or Step 2.

XDE-729 Methyl and XDE-729 Acid are significantly more phytotoxic to the aquatic plant *Myriophyllum spicatum* than to *Lemna*; as a result the TER values for XDE-729 Methyl and XDE-729 Acid are below the Annex VI trigger for FOCUS Step 1, Step 2 and Step 3 for most application scenarios. The metabolites X11449757 and X11406790 showed low phytotoxicity towards *Myriophyllum* at the maximum concentration tested (100 µg/L) and as a result the TER values exceed the Annex VI triggers at FOCUS Step 1.

The risk from the formulated product was concluded as acceptable for all organism groups with the implementation of a 5m buffer zone to reduce spray drift (to address the risk to aquatic plants).

XDE-729 Methyl: Exposure of aquatic plants occurs principally via drift immediately following application of GF-2573. Consequently, for applications to winter cereals a 10 m buffer (for spring applications at 6.25 g/ha) or 20 m buffer (for autumn applications at 7.82 g/ha) will provide adequate mitigation for all FOCUS surface water scenarios. For applications, at 6.25 g/ha, to spring sown cereals a 10 m buffer will provide adequate mitigation. All buffers implemented must reduce exposure via spray drift and runoff.

XDE-729 Acid: Exposure of aquatic plants is predicted to occur principally via drainflow and run-off. Consequently, for autumn applications at 7.82 g/ha to winter cereals acceptable risk from the acid to *Myriophyllum* could not be demonstrated for FOCUS scenarios D1 ditch and stream, D2 ditch and stream and D6 ditch. Spring applications to winter sown cereals at a rate of 6.25 g/ha result in unacceptable risk to *Myriophyllum* for scenarios D1 ditch, D2 ditch and stream, and D6 ditch. Application in spring to spring sown cereals at 6.25 g/ha results in acceptably low risk to *Myriophyllum* from the acid for all FOCUS scenarios.

As an overall acceptable risk from the acid cannot be demonstrated using FOCUS modelling and risk mitigation, member states should consider carefully which scenarios are relevant in their national assessments. Likewise appropriate mitigation should be considered at member state level in line with individual national requirements.

The risk to the most sensitive organism tested (*Myriophyllum spicatum*) was also assessed using growth rate endpoints from the lab data, in line with the draft EFSA aquatic guidance document (EFSA Journal 2013;11(7):3290). The outcome is not greatly different, with only the exact level of mitigation required for each proposed use and unresolved FOCUS scenarios differing. Again member states may wish to consider this approach when conducting their national assessments.


The active substance was concluded as having low bioaccumulation potential in fish (BCF < 1000, 95% depuration time of 1.6 days).

B.9.2.4 Hazard Classification/Labelling**Provisional classification of the active substance for environmental effects according to 67/548/EEC**

The acute toxicity of XDE-729 methyl to the standard aquatic species required for a herbicide is summarised in Table B.9.2.141.

The most acutely sensitive species tested was *Myriophyllum spicatum*, with a E_rC_{50} of 0.000393 mg a.s./L (0.393 µg a.s./L). This is below 1 mg a.s./L, indicating that, according to the Dangerous Substances Directive, XDE-729 methyl should be classified with the R50 phrase: 'Very toxic to aquatic organisms'

A ready-biodegradation study has illustrated that XDE-729 acid (metabolite X11393729) should be classed as 'not readily biodegradable' (Fate and Behaviour Section B.8.4.3). As XDE-729 methyl rapidly degrades to the acid form it is considered relevant to extend this conclusion to XDE-729 methyl. The R53 phrase 'May cause long-term adverse effects in the aquatic environment' is also required.

XDE-729 methyl (67/548/EEC)		
Hazard symbol:		Dangerous for the environment
Risk phrases:	R 50/53	Very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment
Safety phrases:	S60	This material and its container must be disposed of as hazardous waste
	S61	Avoid release to the environment. Refer to special instructions/safety data sheets
	S59	Refer to manufacturer/supplier for information on recovery/recycling
Justification for the proposals:		
Risk phrases:	R 50	<i>Myriophyllum spicatum</i> 14 d E_rC_{50} 0.000393 mg a.s./L (< 1 mg/L)
	R53	Not readily biodegradable
Safety phrases:	S60/61	Recommended for substances that may cause effects in the environment
	S59	Recommended for all dangerous substances recommended for recovery/recycling but <u>obligatory</u> for substances classified as dangerous for the ozone layer (R59)

Provisional hazard classification of the active substance for environmental effects according to Regulation (EC) 1272/2008

Pictogram	GHS09
Signal word	Warning
Hazard statements	H400/H410: Very toxic to aquatic life/Very toxic to aquatic life with long lasting effects (acute/chronic category 1).
M-factor	1000 acute; 1000 chronic
Precautionary statements	P273 Avoid release to the environment P391 Collect spillage P501 Dispose of contents/ container to ... (in accordance with local/ regional/ national/ international regulation (to be specified))


Justification for classification according to Regulation (EC) 1272/2008

H410	Required as XDE-729 methyl is an 'acute category 1' and 'chronic category 1' substance, as defined by: i) <i>Myriophyllum spicatum</i> 14 d $E_{rC_{50}}$ 0.000393 mg a.s./L ii) <i>Myriophyllum spicatum</i> 14 d NOEC <0.0000617 mg a.s./L iii) Substance not 'rapidly biodegradable' (cross ref fate section 8.4.3)
M-factor	i) <i>Myriophyllum spicatum</i> 14 d $E_{rC_{50}}$ 0.000393 mg a.s./L (> 0.0001 to ≤ 0.001 mg/L) ii) <i>Myriophyllum spicatum</i> 14 d NOEC <0.0000617 mg a.s./L (> 0.00001 to ≤ 0.0001 mg/L, not rapidly biodegradable)
GHS09 Pictogram	Required for 'acute category 1' and 'chronic category 1' substance
Signal word 'Warning'	Required for 'acute category 1' and 'chronic category 1' substance
P273, P 391, P 501	Required for 'acute category 1' and 'chronic category 1' substance

Provisional hazard Classification/ Labelling of plant protection products according to 1999/45/EEC

The most sensitive organisms to the technical active substance were also tested using the representative formulation. Therefore this formulation data may be used directly for acute hazard classification according to the Dangerous Preparations Directive, rather than by calculation. The lowest relevant acute endpoint for the formulation 'GF-2573' to an aquatic organism is the 14 d E_rC_{50} for *Myriophyllum spicatum* of 0.0844 mg formulation/L. This is below 1 mg/L, indicating that, according to the Dangerous Substances Directive, 'GF-2573' should be classified with the R50 phrase: 'Very toxic to aquatic organisms'.

Since a ready-biodegradation study has illustrated that the active substance XDE-729 methyl should be classed as 'not readily biodegradable' (Fate and Behaviour Section B.8.4.3), the R53 phrase 'May cause long-term adverse effects in the aquatic environment' is also required.

'GF-2573' (1999/45/EEC)		
Hazard symbol:		Dangerous for the environment
Risk phrases:	R 50/53	Very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment
Safety phrases:	S35	This material and its container must be disposed of in a safe way.
	S57	Use appropriate containment to avoid environmental contamination.
	S59	Refer to manufacturer/supplier for information on recovery/recycling
<u>Justification for the proposals:</u>		
Risk phrases:	R 50	<i>Myriophyllum spicatum</i> 14 d E_rC_{50} = 0.0844 mg formulation/L (< 1 mg/L)
	R53	Active substance not readily biodegradable
Safety phrases:	S35/57	Recommended for products that may cause effects in the environment
	S59	Recommended for all dangerous substances recommended for recovery/recycling but <u>obligatory</u> for substances classified as dangerous for the ozone layer (R59)

The product labels should carry the wording from Article 10, section 1.2 of Directive 1999/45/EC:

'To avoid risks to man and the environment, comply with instructions for use'

and the Annex V of Directive 91/414/EEC 'General provisions' phrase, SP1:

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

Provisional Hazard Classification/ Labelling of plant protection products according to Regulation (EC) 1272/2008

Pictogram	GHS09
Signal word	Warning
Hazard statements	H410: Very toxic to aquatic life with long lasting effects (acute/chronic category 1).
Precautionary statements	P391 Collect spillage P501 Dispose of contents/ container to ... (in accordance with local/ regional/ national/ international regulation (to be specified))

Justification for product ('mixture') classification according to Regulation (EC) 1272/2008:

H 410	Required as 'GF-2573' is an 'acute category 1' and 'chronic category 1' formulation, as defined by: i) <i>Myriophyllum spicatum</i> 14 d $E_rC_{50} = 0.0844$ mg/L ii) <i>Myriophyllum spicatum</i> 14 d $NOEC_r = 0.00732$ mg/L iii) Active substance not 'rapidly biodegradable'
GHS09 Pictogram	Required for 'chronic category 1' mixture
No signal word	Required for 'chronic category 1' mixture
P 391, P 501	Required for 'chronic category 1' mixture. 'P273' not required since mixture (product) is intended for use in the environment.

B.9.3 Effects on other terrestrial vertebrates (IIIA 10.3)

XDE-729 Methyl and GF-2573 is intended for use on winter and spring sown cereals. Thus, foliar application is expected to occur to crops during the growing season in autumn at BBCH 10 to 29 and in the spring at BBCH 13 to 45. Therefore, mammals, and the foliage and/or insects that they feed upon, may be present during the application period; thus requiring a risk assessment.

B.9.3.1 Toxicity

Presented below in Table B.9.3.1 is a summary of the mammal toxicity studies on XDE-729 Methyl. Key regulatory endpoints used in the risk assessment are highlighted in bold.

XDE-729 is formulated as a methyl ester; however, the ester is expected to hydrolyse into XDE-729 acid upon application. Therefore following application to

cereals, exposure to both the ester and the acid must be considered. However, for the mammal risk assessment XDE-729 Methyl ester endpoints only will be used. These endpoints are considered 'worse case' as exposure to the methyl ester automatically incorporates exposure to the metabolites X11406790, which may be further metabolised to X11449757. De-esterification of the methyl also produces XDE-729 acid which further metabolises into X11449757. Only using the acid endpoint does not take into consideration the potential exposure from the metabolite X11406790, which is only metabolised directly from the Methyl. A 2-year reproductive study with the rat was conducted testing the effects of XDE-729 methyl (cross ref study IIA 5.6.1/02, [REDACTED] (2011)) which gave a reproductive NOAEL of 443 mg/kg bw/day. As evident in the result of this study, XDE-729 acid is of much lower reproductive toxicity to mammals than the methyl and so a risk assessment based on toxicity of XDE-729 methyl can be considered to cover the risk from long-term exposure to the acid.

A formulation endpoint has also been provided for the mammal risk assessment; this will also be considered and compared with the subsequent risk assessment for the active.

Table B.9.3.1: Summary of endpoints to be used in the risk assessment for mammals

Duration and test compound	Species	Endpoint	Reference
Acute – a.s.	♀ rats	>5000 mg a.s./kg bw	[REDACTED] (2011) IIA 5.2.1/2 DAS 110543
Acute – formulation	♀ rats	>5000mg form ⁿ ./kg bw	[REDACTED] 2011a) IIIA1 7.1.1/01
Reproductive (developmental) – a.s.	Rabbit	5.78 mg a.s /kg bw/day*	[REDACTED] (2012) IIA 5.6.11/04 DAS 111137

*The endpoint stated for reproductive toxicity has only been used in the screening step of the assessment. This endpoint was used in the human risk assessment to set the ADI. Therefore it is a more conservative approach which is not a true reflection of reproductive toxicity, had the TER at screening step fallen below the trigger value of 5 then this endpoint would have been refined at tier 1.

formⁿ-formulation.

B.9.3.2 Risk assessment

An acute mammal risk assessment was conducted according to EFSA guidance using the formula below.

Acute risk to mammals from the proposed use of XDE-729 Methyl on winter and spring cereals

Acute daily dietary dose (DDD) values were calculated for each of the appropriate scenarios using the following equation:

$$\text{Daily dietary dose (DDD)} = \text{application rate (kg a.s./ha)} \times \text{MAF} \times \text{shortcut value}$$

$$\text{TER} = \text{LD}_{50} / \text{DDD}$$

Within EFSA/2009/1438 the screening step uses a shortcut value based on 90th percentile residues and on the crop type and indicator species (Table 8 for mammals).

For use on cereals the indicator species given in the guidance (Table 8) is a small herbivorous mammal. The shortcut value assigned to this is a value of 118.4.

MAF is the Multiple Application Factor. This was used to take into account the potential accumulation of residues on leafy crops between applications. The standard MAF values for acute exposure for mammals are provided in EFSA/2009/1438, table 9.

XDE-729 may be applied up to twice per year, one application in the autumn at 0.00782 kg a.s./ha and then in spring at 0.00625 kg a.s./ha, with a 70 day interval between applications. Table 9 in the EFSA guideline illustrates that the MAF after 70 days would be 1, irrespective of whether 1 or 2 applications occurred. No accumulation of the active substance would be expected to occur between applications with a 70 day interval. Consequently, it is realistic to evaluate the potential risk to mammals from only a single application, at the highest application rate of 0.00782 kg a.s./ha.

The DDD was calculated using the parameters given in table 9.3.2.

Table B.9.3.2: Summary of acute screening step for mammals exposed to XDE-729 Methyl

Indicator species	Shortcut value	Application rate (kg a.s./ha)	MAF	DDD	Toxicity (mg a.s./kg bw)	TER*	Trigger
small herbivorous mammal	118.4	0.00782	1.0	0.93	>5000	>5400	10

*Value rounded up.

The TER is above the trigger value of 10 showing an acceptable acute risk to mammals from the proposed use of XDE-729 Methyl on winter and spring cereals and so no further consideration is required.

Acute risk to mammals from the proposed use of the formulation GF-2573 on winter and spring cereals

It is proposed to use the formulation endpoint of **LD₅₀ >5000mg formⁿ/kg bw** in a standard risk assessment and compare the results with the data and subsequent risk assessment for the a.s.

Daily dietary dose (DDD) = application rate (kg/ha) x MAF (multiple application factor) x shortcut value

$$TER = LD_{50} / DDD$$

The same indicator species and shortcut value apply, as used in the screening step given above.

However, the application rate needs to be express in terms of the formulation. The GAP states that the concentration of the a.s in the formulation is 7.817 g/L of XDE-729 methyl. From the application rate of 7.82 g/ha we can derive that only a litre is used for an application.

Document J (IIIA 1.4.1) states that the relative density of 1 litre of the product is 0.907 g/ml. Therefore 0.907 kg formⁿ/ha is used per application.

Table B.9.3.3 Summary of acute screening step for mammals exposed to GF-2573.

Indicator species	Shortcut value	Application rate (kg form ⁿ /ha)	MAF	DDD	Toxicity (mg form ⁿ /kg bw)	TER*	Trigger
small herbivorous mammal	118.4	0.907	1.0	107.4	>5000	46.56	10

*Value rounded to 2 decimal places.

The TER is above the trigger value showing an acceptable acute risk to mammals from the proposed use of GF-2573 on winter and spring cereals and so no further consideration is required.

Reproductive risk to mammals from the proposed use of XDE-729 Methyl on winter and spring cereals

Within the EFSA 2009 guidance document the screening step is used if mammals will be breeding during the proposed use which is possible under this application.

The guidance states that for the screening step the endpoints should be presented as mg a.s./kg bw/ day. The studies submitted for this application had already presented in these units so no further conversion needs to take place. The toxicity endpoint used is a NOAEL of 5.78 mg a.s./kg bw/ day.

The daily dietary dose (DDD) of a compound is given by the following equation:

$$DDD = \text{application rate (kg/ha)} \times \text{shortcut value} \times \text{TWA (time weighted average)} \times \text{MAF}$$

$$TER = \frac{\text{Lowest endpoint}}{\text{Daily Dietary Dose}}$$

Within EFSA/2009/1438 the screening step (reproductive assessment) uses a shortcut value based on 50th percentile residues and on the crop type and indicator species.

For use on cereals the indicator species given in the guidance (Table 12) is a small herbivorous mammal. The shortcut value assigned to this is a value of 48.3. As stated above no accumulation of the active substance would be expected to occur between applications with a 70 day interval and therefore no MAF needs to be considered.

The TWA is a calculation of residues on leafy crops, which takes into account the degradation of the active substances over time. TWA residues were used as an estimate of long-term exposure only, since it is considered that the use of maximum residues provides an unrealistically extreme worst-case estimate of long-term exposure. For long-term assessments, a default TWA of 0.53 is used (EFSA/2009/1438) assuming exposure over 21 days and a DT₅₀ of 10 days.

The DDD was calculated using the parameters given in table 9.3.2-3 together with the TWA of 0.53.

Table B.9.3.4: Summary of reproductive screening step for mammals exposed to XDE-729 Methyl

Indicator species	Shortcut value	Application rate (kg a.s./ha)	TWA	MAF	DDD	Toxicity (mg/kg bw/day)	TER **	Trigger
small herbivorous mammal	48.3	0.00782	0.53	1.0	0.20	5.78*	28.87	5

*The endpoint stated for reproductive toxicity has only been used in the screening step of the assessment. This endpoint was used in the human risk assessment to set the ADI. Therefore it is a more conservative approach which is not a true reflection of reproductive toxicity, had the TER at screening step fallen below the trigger value of 5 then this endpoint would have been refined at tier 1.

**Value rounded to 2 decimal places.

The TER is above the trigger value showing an acceptable long-term risk to mammals from the proposed use of XDE-729 Methyl on winter and spring cereals and so no further consideration is required.

Risk assessment for substances with endocrine-disrupting properties.

A discussion of the evidence of any endocrine-disrupting properties caused by the active substance was offered by the applicant and is detailed below:

In mammals, XDE-729 Methyl is rapidly metabolized to XDE-729 Acid by esterase enzymes resulting in no systemic exposure of reproductive organs to XDE-729 Methyl at environmentally relevant exposure levels. There were no effects of XDE-729 Acid on reproductive physiology (fertility, histopathology) noted in the rat two-generation reproduction study (IIA 5.6.1).

The evaluator agrees with the points made by the applicant. There was no evidence of endocrine disruption in the mammal toxicity studies (ref DAR Vol.3, section B6). Furthermore, no evidence of endocrine disruption effects were seen in any of the fish or amphibian studies conducted to assess this possibility.

Based on this evidence, XDE-729 Methyl, XDE-729 Acid, X11406790 and X11449757 do not appear to exhibit endocrine-disrupting properties that would affect reproductive physiology or development of reproductive organs in wild mammals or birds. However, member states should note that there are currently no defined criteria for identifying endocrine disruptors under 2009/1407 and as such only a qualitative case can be made.

Risk assessment for metabolites formed in potential food items**Metabolites in plants**

Following uptake of XDE-729 Methyl by plants, the molecule either undergoes de-esterification to the herbicidal moiety XDE-729 Acid and subsequent metabolism to X11449757, or XDE-729 Methyl undergoes de-methylation of the methoxy group on the phenyl ring to form X11406790, which is rapidly conjugated. The metabolites found in plants were all present at low levels (<10% AR). Thus, although it is theoretically possible that insects could ingest plant matter and thereby take up plant-derived metabolites of XDE-729 Methyl, exposure levels would be negligible and covered by the assessment of the parent.

Metabolites in the environment

Under aerobic soil conditions XDE-729 Methyl is rapidly converted to XDE-729 Acid and X11449757 as well as non-extractable residues and CO₂. These soil derived residues may theoretically be taken up by plants and insects living on the soil surface and therefore should be considered. As stated above, metabolites in plants were all present at low levels (<10%) so this risk is deemed acceptable and doesn't need further consideration. However, because metabolites of XDE-729 Methyl have a very low potential for bioaccumulation and biomagnification with log K_{ow} values well below the trigger value of 3, bioaccumulation of XDE-729 Methyl metabolites in soil organisms such as earthworms and aquatic organisms such as fish is negligible and not a major route of exposure to mammals. Exposure to metabolites in surface water is covered by the drinking water assessment below.

Metabolites in mammals

Since XDE-729 Methyl is rapidly metabolized in mammals to XDE-729 Acid and X11449757 the toxicity of these metabolites is already considered within the assessments conducted with XDE-729 Methyl.

Drinking water

The only scenario considered relevant for assessing the risk of pesticides via drinking water to mammals is the puddle scenario.

- Puddle scenario. Mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for acute and long-term exposure.

The leaf scenario is not deemed relevant for small mammals.

An “escape clause” recommended in the EFSA Guidance Document for Birds and Mammals (2009) allows for screening the need for a quantitative risk assessment by a comparison between the application rate and the toxicity of the respective substance. This escape clause specifies that “due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals ..., no specific calculations of exposure and TER are necessary when the ratio of effective application rate (= application rate x MAF) (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 in the case of more sorptive substances ($K_{oc} \geq 500$ L/kg).”.

The mean K_{OC} value for XDE-729 methyl is 987 L/ kg. XDE-729 may be applied up to twice per year, one application in the autumn at 0.00782 kg a.s. /ha and then in spring at 0.00625kg a.s. /ha, with a 70 day interval between applications. Table 7 in the EFSA guideline illustrates that the MAF after 70 days would be 1, irrespective of whether 1 or 2 applications occurred. No accumulation of the active substance would be expected to occur between applications with a 70 day interval. Consequently, it is realistic to evaluate the potential risk to birds from only a single application, at the highest application rate of 0.00782 kg a.s/ha. For more sorptive substances ($K_{oc} > 500$ L/kg) a ratio of application rate to relevant endpoint of 3000 or less indicates a low risk of poisoning via drinking water through the puddle scenario.

Table 9.3.5: Evaluation of potential concern for exposure of mammals via drinking water (escape clause)

Compound	K _{oc} [L/kg]	Application rate [g as/ha] ^{A)}	endpoint	Ratio (Application rate * / end point)	“Escape clause”	Conclusion
					No concern if ratio	
XDE-729 Methyl	987	7.82	5.78 (long-term)	1.35	≤ 3000	No concern
	987	7.82	>5000 (acute)	0.00156	≤ 3000	No concern

^{A)} Worst-case application to winter cereals

An acceptable risk to mammals can therefore be concluded for XDE-729 Methyl and the metabolites potentially arising from drinking water.

Bioaccumulation and food chain behaviour

According to the EFSA Guidance Document, substances with a log K_{ow} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains. XDE-729 Methyl has a **log K_{ow} value of 3.76**. Therefore, the risk from bioaccumulation to fish-eating and worm-eating mammals has been carried out.

Risk to earthworm-eating mammals

Risk assessment is carried out according to EFSA/2009/1438.

Dry soil approach

The bioconcentration factor for the earthworm (BCF_{earthworm}) is calculated by the following equation:

$$BCF_{earthworm} = \frac{0.84 + 0.012 \times K_{ow}}{f_{oc} \times K_{oc}}$$

K_{ow}- Octanol-water partition coefficient

K_{oc}-Organic carbon adsorption coefficient

f_{oc}- Organic carbon content of soil (0.02 taken as a default value)

A value for the predicted environmental concentration for dry soil (PEC_{soil}) needs to be calculated. This is then multiplied with the BCF_{earthworm} to give the PEC_{earthworm}.

$$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$$

The PEC_{earthworm} calculation needs to be converted into daily dietary dose (DDD) by multiplying with 1.28. This value is based on the worst case scenario of a 10g mammal eating 12.8g worms (fresh) per day.

Finally the toxicity-exposure ratio needs to be determined and compared to the respective trigger value.

$$TER = \frac{NOAEL}{DDD}$$

All input parameters and calculated values are given in the table below.

Table B.9.3.6: Long-term risk from secondary poisoning to earthworm-eating mammals from XDE-729

Crop	PEC _{soil} (mg a.s/kg)	K _{ow}	f _{oc}	K _{oc}	BCF	PEC _{worm} (mg/kg)	DDD (mg/kg bw/d)	NO(A)EL (mg/kg bw/d)	TER
Cereals	0.009	5754.4	0.02	987	3.541	0.032	0.041	5.78	142

The TER value is greater than the trigger value of 5 indicating a low risk to mammals feeding on earthworms following use of XDE-729.

The log K_{ow} values for the XDE-729 Methyl metabolites are all < 3 so an acceptable risk can also be concluded for XDE-729 Acid, X11449757 and X11406790.

Risk to fish-eating mammals

Risk assessment is carried out according to EFSA/2009/1438.

Values for the predicted environmental concentration for surface water (PEC_{sw}) and the whole-body BCF_{fish} have been calculated.

Estimated residues in fish can then be calculated using the following equation:

$$PEC_{fish} = PEC_{sw} \times TWA \times BCF_{fish}$$

The PEC_{fish} calculation needs to be converted into daily dietary dose (DDD) by multiplying with 0.142. This value is based on the worst case scenario of a 3000g mammal eating 425g fresh fish per day.

Finally the toxicity exposure ratio needs to be determined and compared to the respective trigger value.

$$TER = \frac{NOAEL}{DDD}$$

All input parameters and calculated values are given in the table below.

Table B.9.3.7: Long-term risk from secondary poisoning to fish-eating mammals from XDE-729 Methyl

Crop	PEC _{sw} (mg/L) *	BCF	PEC _{fish} (mg/kg)	DDD (mg/kg/bw/day)	NO(A)EL (mg/kg bw/d)	TER _{fish}
Cereals	0.001197	217	0.260	0.037	5.78	157

*PEC_{initial} (mg XDE-729 Methyl/L), as a 1st step and thus more conservative calculation, the TWA has not been used.

The TER value is greater than the trigger value of 5 indicating a low risk to mammals feeding on fish following the use of XDE-729 Methyl.

The log K_{OW} values for the XDE-729 Methyl metabolites are all < 3 so an acceptable risk can also be concluded for XDE-729 Acid, X11449757 and X11406790.

Biomagnification

Despite the log octanol water coefficient suggesting that the active is fat soluble, the metabolism studies with livestock show that XDE-729 methyl is excreted rapidly and fat levels analysed were below the limit of quantitation (LOQ). The ADME data with rats and mice showed no evidence of accumulation in tissues / fat with either the sodium salt of the acid or the methyl ester. Excretion is rapid and essentially complete within 72 hours. 7 days after a single dose, levels in fat were non-detectable (<0.03% of the administered dose). Reference is made to tables B.6.1.1-4 and 6.1.1-5 in the mammal toxicology DAR.

B.9.3.4 Conclusions

When applied in accordance with the proposed GAP for the product GF-2573, the active XDE-729 methyl, XDE-729 acid and its metabolites do not pose a significant risk to mammals.

B.9.4 Effects on bees (IIA 8.3.1, IIIA 10.4)

B.9.4.1 Toxicity

B.9.4.1.1 Acute toxicity

Active substance

Schmitzer S. (2011): Effects of XDE-729 Methyl (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. Lab Study ID: 49528035. Dow AgroSciences unpublished report, DAS study No.: 101128 & 101129, Revised Final Report Date: June 01, 2011.

Test material

Test Item:	XDE-729 Methyl
Purity:	XDE-729 Methyl: 97.2% (wt/wt),
Description:	Solid, Off-white
Lot No./Batch No. :	Lot No.: E2837-51; Test Substance Number: TSN031117-0004

Test system

Organism (Species):	Honey bee (<i>Apis mellifera</i> L.)
Study Type:	Acute oral and contact
GLP Status:	GLP
Guidelines followed:	Acute oral toxicity test: OECD 213 (adopted 21st September 1998) Acute contact toxicity test: OECD 214 (adopted 21st September 1998)
Guideline deviations reported by Study Director:	None
Study design: (No. of bees per replicate, observation intervals etc)	Limit test; acute oral and contact toxicity test; duration 48 h; 5 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality after 4, 24 and 48 hours; reference item: Dimethoate 400 g/L (nominal).
Information on bee colony (health etc):	Female worker bees; obtained from a healthy and queen-right colony, bred by IBACON, collected on the morning of use.
Test concentrations:	Contact test: 98.1 µg a.s./bee (nominal was 100 µg a.s./bee, endpoint has been adjusted for purity of active substance.) Oral test: 108.0 µg a.s./bee (measured).
Amount of treated diet consumed:	Consumption of the treated diets ranged from 108 to 113 µg a.s./bee
Feeding method:	Commercial ready-to-use syrup (Apiinvert; 30 % saccharose, 31 % glucose, 39 % fructose) ad libitum; was given directly after treatment using syringes; no replacements of the food was necessary during the experimental time of the experiments (48 h).
Environmental conditions:	Temperature: 25 °C; relative humidity: 50 % - 70 %; photoperiod: 24 h darkness.
Reference substance (nominal):	0.30, 0.20, 0.15 and 0.10 µg Dimethoate per bee (contact test) 0.30, 0.15, 0.08 and 0.05 µg Dimethoate per bee (oral test)
Solvent substance (if applicable):	Acetone

Methodology

Contact study: a single 5 µL droplet of XDE-729 Methyl in an appropriate carrier (acetone) was placed on the dorsal bee thorax using a Burkard – Applicator. For the control one 5 µL droplet of tap water containing 0.5 % Adhäsit (negative control) and one 5 µL droplet of acetone was used (solvent control).

Oral study: after mixing the test solutions in acetone with ready-to-use sugar syrup and water (composition of the sugar component: 30 % saccharose, 31 % glucose, 39 % fructose) the final concentration of sugar syrup in the test item solution offered to the bees was 50 %. For the controls, the same proportion of syrup, water and acetone was used (solvent control) and similarly, 50 % aqueous syrup solution was used for the negative control.

Results

Table B.9.4.1 Toxicity of XDE-729 Methyl to honey bees in contact and oral toxicity test

Treatment µg a.s./bee	Contact Test [48 h]		Oral Test [48 h]	
Observation Period	24 h	48 h	24 h	48 h
Treatment Mortality	6.0 %	6.0 %	0.0 %	0.0 %
LD50	> 98.1 µg a.s./bee		> 108.0 µg a.s./bee	
The contact and oral LD50 (24 h) values of the reference item (dimethoate) were calculated to be 0.16 and 0.11 µg a.s./bee, respectively.				

In addition two bees in one replicate demonstrated coordination problems after 4 hours. No other sub-lethal symptoms were noted in the test replicates.

Conclusions

The toxicity of XDE-729 Methyl was tested in both an acute contact and an oral toxicity test on honey bees. The LD50 (48 h) was > 98.1 µg a.s./bee in the contact toxicity test. The LD50 (48 h) was > 108.0 µg a.s./bee in the oral toxicity test.

RMS Comment: The study is considered to be acceptable and suitable for use in risk assessments. The key endpoints from the study are the contact LD50 of > 98.1 µg a.s./bee and the oral LD50 of > 108.0 µg a.s./bee.

Formulation

Schmitzer S. (2011): Effects of GF-2573 (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. Lab Study ID: 54805035. Dow AgroSciences unpublished report, DAS study No. 101119 & 101120, Revised Final Report Date: May 25, 2011.

Test material

Test Item:	GF-2573
Purity:	XR-729 methyl: 0.84 % wt/wt, equivalent to 7.6 g/L Cloquintocet-mexyl: 0.81 % wt/wt, equivalent to 7.3 g/L
Description:	Liquid
Density:	0.9056 g/mL (20 °C)
Lot No./Batch No. :	Lot No.: E2837-57 Test Substance Number: TSN031424-0002

Test system

Organism (Species):	Honey bee (<i>Apis mellifera</i> L.)
Study Type:	Acute oral and contact
GLP Status:	GLP
Guidelines followed:	Acute oral toxicity test: OECD 213 (adopted 21 st September 1998) Acute contact toxicity test: OECD 214 (adopted 21st September 1998)
Guideline deviations reported by Study Director:	None
Study design: (No. of bees per replicate, observation intervals etc)	Limit test; acute oral and contact toxicity test; duration 48 h; 5 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality after 4, 24 and 48 hours; reference item: Dimethoate 400 g/L (nominal).
Information on bee colony (health etc):	Female worker bees; obtained from a healthy and queen-right colony, bred by IBACON, collected on the morning of use.
Test concentrations:	Nominal dose level of the test item in the contact test: 200.0 µg GF-2573/bee Measured dose level of the test item in the oral test: 215.6 µg GF-2573/bee. The dose levels are presented as µg product/bee without taking into consideration the content of the a.s.
Amount of treated diet consumed:	Consumption of the treated diets ranged from 207 to 222 µg GF-2573/bee
Feeding method:	Commercial ready-to-use syrup (Apiinvert; 30 % saccharose, 31 % glucose, 39 % fructose) <i>ad libitum</i> ; was given directly after treatment using syringes; no replacements of the food was necessary during the experimental time of the experiments (48 h).

Environmental conditions:	Temperature: 25°C; relative humidity: 45% - 75%; photoperiod: 24 h darkness.
Reference substance (nominal):	0.30, 0.20, 0.15 and 0.10 µg Dimethoate per bee (contact test) 0.30, 0.15, 0.08 and 0.05 µg Dimethoate per bee (oral test)
Solvent substance (if applicable):	None.

Methodology

Contact study: a single 5 µL droplet of GF-2573 in an appropriate carrier (tap water + 0.5 % Adhäsit) was placed on the dorsal bee thorax using a Burkard – Applicator. For the control one 5 µL droplet of tap water containing 0.5 % Adhäsit was used.

Oral study: after mixing the test solutions with ready-to-use sugar syrup (composition of the sugar component: 30% saccharose, 31% glucose, 39% fructose) the final concentration of sugar syrup in the test item solution offered to the bees was 50 %. For the controls water and sugar syrup was used at the same ratio (1 + 1).

Table B.9.4.2: Toxicity of GF-2573 to honey bees in contact and oral toxicity test

Treatment µg GF-2573/bee	Contact Test [48 h]		Oral Test [48 h]	
Observation Period	24 h	48 h	24 h	48 h
Treatment Mortality	4.0 %	4.0 %	0.0 %	2.0 %
LD50	> 200.0 µg product/bee		>215.6 µg product/bee	
The contact and oral LD50 (24 h) values of the reference item (dimethoate) were calculated to be 0.18 and 0.14 µg a.s./bee, respectively.				

In addition, one bee was apathetic after 4 hours; all other bees at all other times were normal.

Conclusions

The toxicity of GF-2573 was tested in both an acute contact and an oral toxicity test on honey bees. The LD50 (48 h) was > 200.0 µg product/bee in the contact toxicity test. The LD50 (48 h) was >215.6 µg product/bee in the oral toxicity test.

RMS Comment: The study is considered to be acceptable and suitable for use in risk assessments. The key endpoints from the study are the contact LD50 of > 200 µg product/bee and the oral LD50 of > 215.6 µg product/bee.

B.9.4.2 Risk assessment

The proposed use of XDE-729 methyl is for winter and spring cereals. Risk will be assessed using the hazard quotient approach where exposure is the maximum single application rate according to SANCO/10329/2002.

Applications of pesticides can potentially result in exposure of honeybees either through direct overspray, or by contact with residues on plants whilst bees are foraging for food. GF-2573 is intended for use on crops at growth stages prior to flowering however; it is intended for application at times of the year when bees could be foraging near by on flowering weeds or other crops. Bees could therefore be exposed during spray applications or to residues of XDE-729 methyl on foliage or flowers.

Table B.9.4.3: Honey bee toxicity endpoints for XDE-729 methyl and GF-2573 to be used in the risk assessment

Duration and test compound	Species	Endpoint	Reference
Acute oral toxicity XDE-729 methyl	European honey bee (<i>Apis Mellifera</i>)	LD ₅₀ > 108 µg a.s./bee	Schmitzer, S (2011) IIA 8.7.1 & IIA 8.7.2
Acute contact toxicity XDE-729 methyl	European honey bee (<i>Apis Mellifera</i>)	LD ₅₀ > 98.1 µg a.s./bee	
Acute oral toxicity GF-2573	European honey bee (<i>Apis Mellifera</i>)	LD ₅₀ > 215.6 µg product/bee	Schmitzer, S (2011) IIIA 10.4.2/1
Acute contact toxicity GF-2573	European honey bee (<i>Apis Mellifera</i>)	LD ₅₀ > 200 µg product/bee	

GF-2573 may be applied up to twice, one application in the autumn at 7.82 g a.s./ha and then in spring at 6.25 g a.s./ha, with a 70 day interval between applications. However, no accumulation of the active substance would be expected to occur between applications with a 70 day interval. Consequently, it is realistic and yet still conservative, to assess the potential risk to bees from one application at 7.82 g a.s./ha.

The acute risk to honeybees was assessed using the single application rate (7.82 g a.s./ha) and the LD₅₀ values (oral and contact) to calculate hazard quotients as follows:

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

Results of the acute risk assessment are presented in the table below.

Table B.9.4.4: Hazard quotients for honeybees exposed to XDE-729 methyl and GF-2573

Crop	Test substance	Exposure route	Maximum single application (g/ha)	LD ₅₀ (µg a.s./bee)	HQ
Winter and spring cereals	XDE-729 methyl	Oral	7.82	>108	<0.07
		Contact		>98.1	<0.08
	GF-2573	Oral	907*	>215.6	<4.21
		Contact		>200	<4.54

* corrected for density. Document J (IIIA 1.4.1) states that the relative density of 1 litre of the product is 0.907 g/ml. (i.e. 1000 x 0.907).

All the hazard quotients are considerably less than the trigger of 50, indicating that both XDE-729 methyl and GF-2573 pose an acceptable risk to bees.

B.9.4.3 Conclusions

For exposure to the active substance and representative formulation, the contact and oral hazard quotients derived using toxicity endpoints from laboratory acute toxicity tests are below the Annex VI trigger value of 50. It can therefore be concluded that if used according to the proposed GAP, XDE-729 methyl and the formulation GF-2573 demonstrate an acceptable risk to bees.

B.9.5 Effects on other arthropod species (IIA 8.3.2, IIIA 10.5)

B.9.5.1 Toxicity

Moll, M. (2011): **GF-2573: Effects of GF-2573 on the parasitic wasp (*Aphidius rhopalosiphi*) in a Tier 1 laboratory test.** Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. Lab Study ID: 54801001. Dow AgroSciences, unpublished report, DAS Study No. 090096, Revised Final Report Date: May 30, 2011.

Test material

Test item:	GF-2573
Purity:	XR-729 methyl: 0.84 % wt/wt (7.6 g/L) Cloquintocet-mexyl: 0.81 % wt/wt (7.3 g/L)
Description:	Yellow liquid
Lot No.:	E2837-57

Test system

Organism (Species):	Parasitic wasp (<i>Aphidius rhopalosiphi</i>)
Study Type:	Tier 1 laboratory study, glass plates for mortality and barley plants for fecundity
GLP Status:	GLP
Guidelines followed:	Mead-Briggs <i>et al.</i> 2000 and Mead-Briggs <i>et al.</i> 2009
Guideline deviations reported by Study Director:	None
Study design:	Assessments of mortality measured 48 hrs after treatment and parasitisation 15 days after treatment. 4 replicates, each consisting of 10 wasps in one arena per test concentration for mortality phase. 17 - 19 females wasps for each treatment (control and test item) taken through to the fecundity phase (20 were introduced, but some wasps were dead or not found after 24 hrs)
Test concentrations:	0 (control), 28.0, 250, 500, 1000 and 2000 mL product/ha.
Environmental conditions:	Temperature: 18 - 21 °C Relative Humidity: 74 - 78 % (acclimatisation, exposure period) 74 - 80 % (post-exposure period; within the test units) Photoperiod: 500 - 1400 lux (acclimatisation, exposure, parasitisation period) 9900 - 13020 lux (post-parasitisation period) Feeding: A 10 %-fructose solution (acclimatisation and exposure)
Reference item:	0.3 mL Perfekthion/ha

Methodology

The study comprised 7 treatment groups (5 dose rates of the test item, control and reference item) with 4 replicates each containing 10 parasitoids. The parasitoids were exposed to fresh, dried residues on treated glass plates. Survival of the parasitoids was assessed after approximately 2, 24 and 48 hours. At 48 hours, for treatment groups where there was less than 50.0 % corrected mortality, female wasps were removed and their reproductive capacity was assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The adult parasitoids were removed after 24 hours and the aphid-infested plants left for a further 12 days before the numbers of aphid mummies that had developed were assessed.

ResultsTable B.9.5.1: Effects of GF-2573 on the survival of *Aphidius rhopalosiphi*

Test concentrations (mL GF-2573/ha)	% mortality	Abbott corrected % mortality
Control	0.0	-
28.0	2.5	2.5
250	10.0	10.0
500	5.0	5.0
1000	2.5	2.5
2000	77.5	77.5 *
Toxic Reference	100.0	100.0 *

* statistically different from the control

Table B.9.5.2: Effects of GF-2573 on the parasitism rate of *Aphidius rhopalosiphi*

Test concentrations (mL GF-2573/ha)	Mean No. of mummies per female/parasitisation rate	% difference compared to control
Control	28.9±14.8	-
28.0	37.0±21.8	-27.8
250	27.5±18.1	5.1
500	30.1±14.9	-4.0
1000	35.0±19.9	-20.9

(negative values indicate better performance compared to control)

Conclusions

Under laboratory conditions the 48-hour LR50 of GF-2573 on *Aphidius rhopalosiphi* is 1612 mL product/ha in 200 L water/ha (95%-confidence limits could not be determined due to mathematical reasons). Reproduction was tested at 28.0, 250, 500 and 1000 mL product/ha. There were no effects on the reproduction (parasitisation efficiency) of surviving females up to and including 1000 mL product/ha.

RMS Comment: The study is considered to be acceptable and suitable for use in risk assessments. The key endpoint from the study is the LR50 of 1612 mL product/ha.

Schwarz, A. (2011): GF-2573: Effects of GF-2573 on the Predatory Mite *Typhlodromus pyri* in a Tier 1 laboratory test. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. Lab Study ID: 54802063. Dow AgroSciences, unpublished report, DAS Study No. 090097, Revised Final Report Date 01 June 2011.

Test material

Test item:	GF-2573
Purity:	XR-729 methyl: 0.84 % wt/wt (7.6 g/L) Cloquintocet-mexyl: 0.81 % wt/wt (7.3 g/L)
Description:	Yellow liquid
Lot No./Batch No. :	E2837-57

Test system

Organism (Species):	Predatory mite <i>Typhlodromus pyri</i>
Study Type:	Tier 1 laboratory study, glass plates for mortality and fecundity
GLP Status:	GLP
Guidelines followed:	Blümel <i>et al.</i> , 2000
Guideline deviations reported by Study Director:	None
Study design:	Assessments of mortality measured 7 days after treatment and egg production 14 days after treatment. 3 replicates, each consisting of 20 mites in one arena per test concentration.
Test concentrations:	0 (control), 28.0, 250, 500, 1000 and 2000 mL product/ha.
Environmental conditions:	Temperature: 23 – 26 °C Relative Humidity: 68 - 75 % Photoperiod: 230 – 740 lux Feeding: A mixture of pine (<i>Pinus nigra</i>) and birch (<i>Betula</i> sp.) pollen (3:1) <i>ad libitum</i> on the day of the test start and on each assessment day except for the last one (i.e. at least every four days).
Reference substance:	8 mL Perfekthion/ha

Methodology

The study comprised 7 treatment groups (5 dose rates of the test item, control and reference item) with 3 replicates each containing 20 mites. The mites were exposed to fresh, dried residues on treated glass plates. Survival of the mites was assessed after 3 and 7 days. For the reproduction assessment surviving mites from the control and from all test item groups where there was less than 50 % corrected

mortality were sexed and the number of eggs per female was recorded on 3 assessment days within one week.

Results

Table 9.5.3 Effects of GF-2573 on the survival of *Typhlodromus pyri*

Test concentrations (mL GF-2573/ha)	% mortality after 7 days	% escapees	Abbott corrected % mortality
Control	3.3	0.0 ± 0.0	-
28	5.0	3.3 ± 5.8	1.7
250	0.0	0.0 ± 0.0	-3.4
500	6.7	1.7 ± 2.9	3.4
1000	15.0	5.0 ± 5.0	12.1 *
2000	50.0	1.7 ± 2.9	48.3 *
Toxic Reference	98.3	28.3 ± 2.9	98.3 *
(negative values indicate better survivorship compared to control)			
* statistically different from the control			

Table B.9.5.4 Effects of GF-2573 on the fecundity of *Typhlodromus pyri*

Test concentrations (mL GF-2573/ha)	Mean No. of eggs per female	% difference compared to control
Control	9.3	-
28	8.9	4.3
250	9.6	-3.2
500	8.4	9.7
1000	7.2 *	22.6
2000	5.6 *	39.8
(negative values indicate better survivorship compared to control)		
* statistically different from the control		

Conclusions

Under worst case laboratory conditions the 7-day LR50 of GF-2573 on *Typhlodromus pyri* is estimated to be greater than 2000 mL product/ha in 200 L water/ha, the highest rate tested. Reproduction was tested at all dose rates. There were no effects on the reproduction (eggs produced per female) of the mites at 28, 250 and 500 mL product/ha compared to the control. At 1000 and 2000 mL product/ha reproduction was statistically significantly affected compared to the control.

RMS Comment The study is considered to be acceptable and suitable for use in risk assessments. The key endpoint from the study is the LR₅₀ of >2000 mL product/ha.

B.9.5.2 Risk assessment for non-target arthropods

The results from the above standard laboratory studies on non-target arthropods are summarised in the following table. Key regulatory endpoints used in the risk assessment are highlighted in bold.

Table B.9.5.5: Summary of non-target arthropod toxicity data

Species	Test Substance	Endpoint LR ₅₀ (mL GF-2573/ha)	Endpoint LR ₅₀ (g GF-2573/ha)	Reference
<i>Typhlodromus pyri</i>	GF-2573	>2000	1814*	Moll, M., (2010) IIIA 10.5.1/1
<i>Aphidius rhopalosiphi</i>	GF-2573	1612	1462*	Schwarz, A., (2011) IIIA 10.5.1/2

*Initial endpoint in mL product/ha was corrected for density. Document J (IIIA 1.4.1) states that the relative density of 1 litre of the product is 0.907 g/mL. (i.e. >2000 x 0.907)

This risk assessment is based on the guidance document from the ESCORT 2 Workshop (2001).

Tier 1 risk assessment

At Tier I ESCORT 2 proposes the risk assessment to be based on a hazard quotient (HQ) approach. The HQ is derived from the LR₅₀ value generated using *A. rhopalosiphi* and *T. pyri* exposed in glass plate studies and crop-specific application rates (taking into account multiple applications) for in-field assessments as well as drift rates for off-field scenarios. A HQ > 2 for either species indicates the need for further consideration of the risk.

The following equations were used in a Tier 1 assessment.

$$\text{In-field HQ} = \frac{\text{app. rate} \times \text{MAF}}{\text{LR}_{50}}$$

$$\text{Off-field HQ} = \frac{\text{app. rate} \times \text{MAF} \times \text{drift factor} \times \text{CF}}{\text{LR}_{50} \times \text{VDF}}$$

app. rate = application rate (g formⁿ/ha)

MAF = multiple application factor

drift factor = spray drift at field edge (using the 90th percentile drift data according to Ganzelmeier et al., (1995; recalculated by the German BBA and UBA and published by the BBA (2000))

VDF = vegetation distribution factor (default value of 10 used at Tier 1)

CF = correction factor (value of 10 used at first tier to account for inter-species variability in the off-field assessment)

For this risk assessment it is assumed that degradation of the formulated product would occur during the 70 day interval and therefore, only one application at 907 g formⁿ/ha has been considered for this risk assessment. Therefore the MAF value selected is 1.0. A worst-case spray drift value of 2.77 % is selected for applications to field crops at 1m.

In the off-field HQ calculation at Tier 1 a correction factor (CF) of 10 is used to extrapolate from *T. pyri* and *A. rhopalosiphi* as indicator species to all off-field non target arthropods. In addition the potential for exposure to be overestimated by the 90th percentile drift values is corrected for by a vegetation distribution factor (VDF) of 10. At Tier 1 the VDF and CF cancel each other out. In the absence of dissipation data for GF-2573, default MAF values for foliar applications from Appendix II of ESCORT 2 (2000) have been used.

Table B.9.5.6: In-field risk to non-target arthropods from the proposed uses of GF-2573

Crop	Application rate (g form ⁿ /ha)	MAF	Test species	LR ₅₀ (g GF-2573/ha)	HQ	ESCORT 2 trigger
Field crops	907	1.0	<i>Typhlodromus pyri</i>	>1814	<0.50	2
			<i>Aphidius rhopalosiphi</i>	1462	0.62	

Table B.9.5.7: Off-field risk to non-target arthropods from the proposed use of GF-2573

Crop	Application rate (g form ⁿ /ha)	MAF	Drift factor	Exposure (g form ⁿ /ha)	Test species	LR ₅₀ (g GF-2573/ha)	HQ	ESCORT 2 trigger
Field crops	907	1.0	2.77% (1m)	25.12	<i>Typhlodromus pyri</i>	>1814	<0.01	2
					<i>Aphidius rhopalosiphi</i>	1462	0.02	

The HQ values calculated for in-field and off-field risk were less than 2 for both species and indicate that GF-2573 has acceptable risks to non-target arthropods at the maximum in-field application rate, consequently no further testing is necessary. It was noted that the first tier studies on *Aphidius rhopalosiphi* and *Typhlodromus pyri* were extended to include a fecundity phase. For both of these fecundity phases of the experiment, no effects on reproduction >50% were observed at 2000 ml/ha for *T.pyri* and 1000 ml/ha for *A.rhopalosiphi*. Both of these application rates are greater than exposure calculated for both the in-field and off-field risk. These extensions are not considered necessary by the RMS and thus have not been used in the risk assessment however, the results further support the outcome of the tier 1 assessment.

B.9.5.3 Conclusions

Acceptable in-field and off-field risks to non-target arthropods from the proposed use of GF-2573 were demonstrated at first tier. Therefore use of the representative formulation in accordance with the proposed GAP does not pose a significant risk to non-target arthropod species in both the in-field and off-field habitats.

B.9.6 Effects on earthworms (IIA 8.4, IIIA 10.6.1)

B.9.6.1 Toxicity

B.9.6.1.1 Acute toxicity

Witte, B. (2011): XDE-729 Methyl: Acute Toxicity of XDE-729 Methyl to *Eisenia fetida* in Artificial Soil with 5% Peat. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. Lab Study ID: 49524021. Dow AgroSciences unpublished report, Study Number 090099. Study Report Completion Date: January 26, 2010. Revised Final Report No. 1 Date: June 16, 2011

Test material

Test Item:	XDE-729 Methyl
Lot No.	E2837-51
Purity:	97.2%
Test Substance Number:	TSN031117-0004

Test system

Organism (Species):	Earthworm (<i>Eisenia fetida</i>)
Study Type:	14 day acute earthworm study
GLP Status:	Yes
Guidelines followed:	OECD Guideline 207 (Adopted 1984) and ISO 11268-1, 1993
Guideline Deviations reported by Study Director:	<p>None were reported, however according to OECD207 the artificial soil should have an organic content of 10%. 5% peat was used in the artificial soil due to the potential influence of the properties of the active substance in the test item on bioavailability.</p> <p>Soil details were as follows:</p> <p>5.0% Sphagnum-peat, air-dried and finely ground (2 mm); (Floragard, Vertriebs GmbH für Gartenbau, 26138 Oldenburg, Germany)</p> <p>20% Kaolin clay (Erbsloh, 65558 Lohrheim~ Germany)</p> <p>approximately 0.2% calcium carbonate (CaCO₃) added to adjust pH to 6.0 ± 0.5 (Merck, 64293 Darmstadt~ Germany)</p> <p>approximately 74.8% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm.; (Quarzwerte Frechen, Postfach 1780, 50207 Frechen, Germany)</p>
Study Design:	<p>Assessment of the survival, behaviour and weight change of worms.</p> <p>4 replicates, consisting of 10 worms in each vessel per test concentration.</p>
Test Concentrations:	0 (control), 62.5, 125, 250, 500, 1000 mg a.s/kg soil.
Soil Parameters	<p>Artificial soil according to OECD 207 but with reduced organic matter content:</p> <p>pH at initiation: 6.0 to 6.4</p> <p>pH at termination: 5.9 to 6.1</p> <p>Water content at initiation: 21.7% to 22.8% (52.9% to 55.6% of the maximum water holding capacity)</p> <p>Water content at termination: 21.2% to 22.6% (51.7% to 55.1% of the maximum water holding capacity)</p>
Environmental Conditions:	<p>Temperature: 19 to 20°C</p> <p>Light Intensity: Continuous (460 to 800 lux)</p> <p>Feeding: none</p>
Reference Item:	2-chloroacetamide (conducted and reported as a separate study)

Methodology

Five different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the earthworms were introduced on top of the soil. There were 5 concentrations and one control; 4 replicates per concentration with 10 worms each. Assessment of worm mortality and behavioural effects was assessed after 7 and 14 days, measurement of weight change as sub-lethal parameter after 14 days.

Table B.9.6.1: Effects of XDE-729 Methyl on earthworm survival and biomass

Test concentrations (mg/kg)	% mortality after 14 days	Body weight change after 14 days (%) a)
Control	0	-4.0
62.5	0	-5.8
125	0	-5.1
250	0	-6.5
500	0	-7.7
1000	0	-9.5
a) There were no significant differences from the control (Dunnett's t-test, two-sided, $\alpha = 0.05$)		

Conclusions

In a 14-day toxicity study with XDE-729 Methyl to earthworms (*Eisenia fetida*) the 14-day LC₅₀ was determined to be greater than 1000 mg XDE-729 Methyl/kg soil dry weight, e.g. the highest concentration tested. The No Observed Effect Concentration (NOEC) for mortality was determined to be 1000 mg XDE-729 Methyl/kg soil. The No Observed Effect Concentration (NOEC) for body weight was determined to be 1000 mg XDE-729 Methyl/kg soil.

RMS Comment: The study is considered to be acceptable and hence suitable for use in risk assessment. The only deviation is that the artificial soil contained 5% peat compared to the recommended 10%. This was stated due to the characteristics of the a.s. and in particular the fact that it has a logPow greater than 2. Whilst the deviation is not in line with OECD 207, it follows advice presented in OECD 222 and EPPO (2003)⁸. There is further consideration of this issue in the risk assessment section. The key endpoint from this study is the LC₅₀ of >1000 mg a.s./kg soil, which should be corrected to > 500 mg a.s./kg soil, due to the log Pow of the active substance being >2.

⁸ EPPO/OEPP (2003) Bulletin 33, 195-209

Witte, B. (2010): XDE-729 Acid: Acute Toxicity (14 Days) of XDE-729 Acid to the Earthworm *Eisenia fetida* in Artificial Soil with 5% Peat. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. Lab Study ID: 56861021. Dow AgroSciences unpublished report, Study Number 101141. Study Report Completion Date: June 07, 2010.

Test material

Test Item:	XDE-729 Acid
Test Substance Number:	TSN030751-0006
Lot No.:	E2837-52
Active substance(s) / Content:	XDE-729: 95.3% \pm 0.4% (wt/wt)

Test system

Organism (Species):	Earthworm (<i>Eisenia fetida</i>)
Study Type:	14-day acute earthworm study
GLP Status:	Yes
Guidelines followed:	OECD Guideline 207 (Adopted 1984) and ISO 11268-1, 1993
Guideline Deviations reported by Study Director:	<p>None were reported, however according to OECD 207 the artificial soil should have an organic content of 10%. 5% peat was used in the artificial soil due to the potential influence of the properties of the active substance in the test item on bioavailability.</p> <p>Soil details were as follows:</p> <p>5.0% Sphagnum-peat, air-dried and finely ground (2 mm); (Floragard, Vertriebs GmbH für Gartenbau, 26138 Oldenburg, Germany)</p> <p>20% Kaolin clay (Erbsloh, 65558 Lohrheim, Germany)</p> <p>approximately 0.2% calcium carbonate (CaCO_3) added to adjust pH to 6.0 ± 0.5 Merck, 64293 Darmstadt, Germany)</p> <p>approximately 74.8% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm; (Quarzwerte Frechen, Postfach 1780, 50207 Frechen, Germany)</p>
Study Design:	<p>Assessment of the survival, behaviour and weight change of worms.</p> <p>4 replicates, consisting of 10 worms in each vessel per test concentration.</p>
Test Concentrations:	0 (control), 62.5, 125, 250, 500, 1000 mg a.s/kg soil.

Soil Parameters	Artificial soil according to OECD 207 but with reduced organic matter content: pH at initiation: 5.7 to 5.8 pH at termination: 5.5 Water content at initiation: 22.3% to 25.4% (49.6% to 56.4% of the maximum water holding capacity) Water content at termination: 24.0% to 25.1% (53.3% to 55.8% of the maximum water holding capacity)
Environmental Conditions:	Temperature: 20 to 22°C Light Intensity: Continuous (400 to 800 lux) Feeding: none
Reference Item:	2-Chloroacetamide (conducted and reported as a separate study)

Methodology

Five different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the earthworms were introduced on top of the soil. There were 5 concentrations and one control; 4 replicates per concentration with 10 worms each. Assessment of worm mortality and behavioural effects was assessed after 7 and 14 days, measurement of weight change as sub-lethal parameter after 14 days.

Table B.9.6.2: Effects of XDE-729 Acid on earthworm survival and biomass

Test concentrations (mg/kg)	% mortality after 14 days	Body weight change after 14 days (%)
Control	0	11.0
62.5	0	8.4 n.s.
125	0	6.5 n.s.
250	0	8.1 n.s.
500	0	4.3 n.s.
1000	0	-3.2 *
* statistically different from the control (Dunnett's test, two-sided, $\alpha = 0.05$) n.s. not statistically different from the control (Dunnett's test, two-sided, $\alpha = 0.05$)		

Conclusions

In a 14-day toxicity study with XDE-729 Acid to earthworms (*Eisenia fetida*) the 14-day LC50 was estimated to be greater than 1000 mg test item/kg dry soil. The No Observed Effect Concentration (NOEC) for mortality was determined to be 1000 mg test item/kg dry soil. The No Observed Effect Concentration (NOEC) for body weight was determined to be 500 mg test item/kg dry soil.

RMS Comment: The study is considered to be acceptable and hence suitable for use in risk assessment. The only deviation is that the artificial soil contained 5% peat compared to the recommended 10%. This was stated due to the characteristics of the a.s. and in particular the fact that it has a logPow greater than 2. Whilst the deviation is not in line with OECD 207, it follows advice presented in OECD 222 and EPPO (2003)⁹. There is further consideration of this issue in the risk assessment section. The key endpoint from this study is the LC50 of >1000 mg test item/kg soil. There is no need for further adjustment of this endpoint due to log Pow, as XDE-729 Acid has a log Pow of < 2 (-0.83)

Witte, B. (2010): X11449757 (metabolite of XDE-729): Acute Toxicity (14 Days) of X11449757 (metabolite of XDE-729) on the Earthworm *Eisenia fetida* in Artificial Soil with 5% Peat. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. Lab Study ID: 56872021. Dow AgroSciences unpublished report, Study Number 101155. Study Report Completion Date: December 13, 2010.

Test material

Name:	X11449757
Lot No.:	YB1-100780-103
Test Substance Number:	TSN031413-0003
Purity:	99%

Test system

Organism (Species):	Earthworm (<i>Eisenia fetida</i>)
Study Type:	14-day acute earthworm study
GLP Status:	Yes
Guidelines followed:	OECD Guideline 207 (Adopted 1984) and ISO 11268-1, 1993
Guideline Deviations reported by Study Director:	<p>None were reported, however according to OECD207 the artificial soil should have an organic content of 10%. 5% peat was used in the artificial soil due to the potential influence of the properties of the active substance in the test item on bioavailability.</p> <p>Soil details were as follows:</p> <p>5.0% Sphagnum-peat, air-dried and finely ground (2 mm); (Floragard, Vertriebs GmbH für Gartenbau, 26138 Oldenburg, Germany)</p> <p>20% Kaolin clay (Erbsloh, 6555 8 Lohrheirn, Germany)</p> <p>approximately 0.2% calcium carbonate (CaCO₃) added to adjust pH to 6.0 ± 0.5 (Merck, 64293 Darmstadt, Germany)</p> <p>approximately 74.8% fine quartz-sand (F34)</p>

⁹ EPPO/OEPP (2003) Bulletin 33, 195-209

	containing more than 50% by mass of particle size 0.05 mm to 0.2 mm; (Quarzwerte Frechen, Postfach 1780, 50207 Frechen, Germany)
Study Design:	Assessment of the survival, behaviour and weight change of worms. 4 replicates, consisting of 10 worms in each vessel per test concentration.
Test Concentrations:	Control, 1000 mg test item/kg soil dry weight
Soil Parameters:	Artificial soil according to OECD 207 but with reduced organic matter content: pH at initiation: 5.8 to 5.9 pH at termination: 5.8 Water content at initiation: 21.3% to 21.6% (53.3% to 54.0% of the maximum water holding capacity) Water content at termination: 21.1% to 21.5% (52.8% to 53.8% of the maximum water holding capacity)
Environmental Conditions:	Temperature: within the range of 18 to 22°C Light Intensity: Continuous (within the range of 400 to 800 lux) Feeding: none
Reference Item:	2-Chloroacetamide (conducted and reported as a separate study)

Methodology

Five different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the earthworms were introduced on top of the soil. There were 5 concentrations and one control; 4 replicates per concentration with 10 worms each. Assessment of worm mortality and behavioural effects was assessed after 7 and 14 days, measurement of weight change as sub-lethal parameter after 14 days.

Results

Table B.9.6.3: Effects of X11449757 (metabolite of XDE-729) on earthworm survival and biomass

Test concentrations (mg/kg)	% mortality after 14 days	Body weight change after 14 days (%)
Control	0	-1.3
1000	0	0.2 n.s.
n.s. not statistically significantly different from the control (Student t-test, $\alpha = 0.05$)		

Conclusions

In a 14-day toxicity study with X11449757 to earthworms (*Eisenia fetida*) the 14-day LC50 was determined to be greater than 1000 mg test item/kg soil dry weight. The No Observed Effect Concentration (NOEC) for mortality was determined to be 1000 mg test item/kg soil dry weight. The No Observed Effect Concentration (NOEC) for body weight was determined to be 1000 mg test item/kg soil.

RMS Comment The study is considered to be acceptable and hence suitable for use in risk assessment. The only deviation is that the artificial soil contained 5% peat compared to the recommended 10%. This was stated due to the characteristics of the a.s. and in particular the fact that it has a logPow greater than 2. Whilst the deviation is not in line with OECD 207, it follows advice presented in OECD 222 and EPPO (2003)¹⁰. There is further consideration of this issue in the risk assessment section. The key endpoint from this study is the LC50 of >1000 mg test item/kg soil. There is no need for further adjustment of this endpoint due to log Pow, as X11449757 has a log Pow of < 2 (<0.3)

Witte, B. (2011): GF-2573: Acute Toxicity (14 Days) of GF-2573 to the Earthworm *Eisenia fetida* in Artificial Soil with 5% Peat. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. Lab Study ID: 54808021. Dow AgroSciences unpublished report, Study Number 101121. Revised Final Report Completion Date May 25, 2011.

Test material

Test Item:	GF-2573
Test Substance Number:	TSN031424-0002
Active substance(s) / Content:	XR-729 methyl: 0.84 % wt/wt (7.6 g/L) Cloquintocet-mexyl: 0.81 % wt/wt (7.3 g/L)
Batch No. :	E2837-57

Test system

Organism (Species):	Earthworm (<i>Eisenia fetida</i>)
Study Type:	14 day acute earthworm study
GLP Status:	Yes
Guidelines followed:	OECD Guideline 207 (Adopted 1984) and ISO 11268-1, 1993
Guideline Deviations reported by Study Director:	None were reported, however according to OECD207 the artificial soil should have an organic content of 10%. 5% peat was used in the artificial soil due to the potential influence of the properties of the active substance in the test item on bioavailability. Soil details were as follows:

¹⁰ EPPO/OEPP (2003) Bulletin 33, 195-209

	5.0% sphagnum-peat air-dried and finely ground (2 mm); (Floragard, Vertriebs GmbH für Gartenbau, 26138 Oldenburg, Germany) 20% kaolin clay (Erbsloh, 65558 Lohrheim, Germany) Approximately 0.2% calcium carbonate (CaCO_3) added to adjust pH to 6.0 ± 0.5 (Merck, 64293 Darmstadt, Germany) Approximately 74.8% fine quartz-sand (f34) containing more than 50% by mass of particle size 0.05 to 0.2 mm; (Quarzwirke Frechen, Postfach 1780, 50207 Frechen, Germany).
Study Design:	Assessment of the survival, behaviour and weight change of worms. 4 replicates, consisting of 10 worms in each vessel per test concentration.
Test Concentrations:	Control, 62.5, 125, 250, 500 and 1000 mg test item/kg soil
Soil Parameters	Artificial soil according to OECD 207 but with reduced organic matter content: pH at initiation: 5.9 to 6.1 pH at termination: 5.5 to 5.6 Water content at initiation: 23.2% to 24.0% (50.4% to 52.2% of the maximum water holding capacity) Water content at termination: 25.2% to 26.6% (54.8% to 57.8% of the maximum water holding capacity)
Environmental Conditions:	Temperature: 20 to 22°C Light Intensity: Continuous (400 to 800 lux) Feeding: none
Reference Item:	2-Chloroacetamide (conducted and reported as a separate study)

Methodology

Five different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the earthworms were introduced on top of the soil. There were 5 concentrations and one control; 4 replicates per concentration with 10 worms each. Assessment of worm mortality and behavioural effects was assessed after 7 and 14 days, measurement of weight change as sub-lethal parameter after 14 days.

Table B.9.6.4: Effects of GF-2573 on earthworm survival and biomass

Test concentrations (mg/kg)	% mortality after 14 days	Body weight change after 14 days (%) ^{a)}
Control	0	-4.3
62.5	0	-1.6
125	0	-0.6
250	0	1.0
500	0	4.4
1000	0	7.0

^{a)} There were no significant differences from the control (Dunnnett's t-test, one-sided smaller, alpha = 0.05)

Conclusions

In a 14-day toxicity study with GF-2573 to earthworms (*Eisenia fetida*) the 14-day LC50 was determined to be greater than 1000 mg test item/kg soil dry weight. The No Observed Adverse Effect Concentration (NOAEC) for mortality and body weight was determined to be 1000 mg GF-2573/kg soil. The Lowest Observed Adverse Effect Concentration (LOAEC) for body weight was determined to be greater than 1000 mg GF-2573/kg soil.

RMS Comment: The study is considered to be acceptable and hence suitable for use in risk assessment. The only deviation is that the artificial soil contained 5% peat compared to the recommended 10%. This was stated due to the characteristics of the a.s. and in particular the fact that it has a logPow greater than 2. Whilst the deviation is not in line with OECD 207, it follows advice presented in OECD 222 and EPPO (2003)¹¹. There is further consideration of this issue in the risk assessment section. The key endpoint from this study is the LC50 of >1000 mg test item/kg soil, and an LC_{50 corr} > 500 mg/kg.

B.9.6.1.1 Chronic toxicity

Witte, B. (2011): Effects of XDE-729 Methyl on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil with 5% Peat. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. IBACON report 49525022. Dow AgroSciences unpublished report, Study Number 090100. Study Completion Date: March 31, 2010; Revised Final Report No. 1 Date: June 16, 2011.

¹¹ EPPO/OEPP (2003) Bulletin 33, 195-209

Test material

Test item:	XDE-729 Methyl
Purity:	97.2%
Description:	Solid, off-white
Lot No.:	E2837-51

Test system

Organism (Species):	Earthworm (<i>Eisenia fetida andrei</i>)
Study Type:	56 day earthworm reproduction study
GLP Status:	Yes
Guidelines followed:	OECD Guideline 222 (Adopted 2004); ISO-Guideline 11268-2 (1998)
Guideline deviations reported by Study Director:	None
Study design:	Assessment of the survival, behaviour and weight change of worms after 28 days exposure. Assessment of the number of offspring 56 days after treatment. 4 replicates, consisting of 10 worms in each vessel per test concentration. 8 replicates, consisting of 10 worms in each vessel for the control.
Test concentrations:	Control, 0.625, 1.25, 2.5, 5.0 and 10.0 mg XDE-729 Methyl/kg soil dry weight
Soil parameters	Artificial soil according to OECD 222 but with reduced organic matter content: pH at initiation: 6.3 pH at termination: 6.4 to 6.5 Water content at initiation: 22.0% to 23.6% (49.2% to 52.8% of the maximum water holding capacity) Water content at termination: 24.1% to 25.9% (53.9% to 57.9% of the maximum water holding capacity) Soil details were: 5.0% Sphagnum-peat, air-dried and finely ground (2 mm); (Floragard, Vertriebs GmbH fur Gartenbau, 26138 Oldenburg, Germany) 20% Kaolin clay (Erbs16h, 65558 Lohrheim, Germany) approximately 0.2% calcium carbonate (CaCO ₃) added to adjust pH to 6.0 ± 0.5 (Merck, 64293 Darmstadt, Germany) approximately 74.8% fine quartz-sand (F34) containing more than 50% by mass of particle size

	0.05 mm to 0.2 mm; (Quarzwerte Frechen, Postfach 1780, 50207 Frechen, Germany)
Environmental conditions:	Temperature: 18°C to 22°C Light intensity: 420 lux to 800 lux Photoperiod: 16 : 8 h Feeding: Finely ground cattle manure was used as food and was added each week for the first 4 weeks of the experiment.
Reference item:	Luxan Carbendazim 500 FC (Carbendazim, 500 g/L nominal).

Methodology

56-day test in treated artificial soil according to OECD 222; different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioural effects and biomass development were carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after an additional 28 days (assessed 56 days after application).

Table B.9.6.5: Effects of XDE-729 Methyl on earthworm survival, biomass and reproduction

Test concentrations [mg test item/kg soil dry weight]	% mortality after 28 days	% bodyweight change after 28 days ^{a)}	Mean No. of juveniles at day 56 ^{a)}	% change in number of juveniles compared to control
Control	0	13.6±10.4	298±34	-
0.625	0	15.1±3.8	325±45	108.8
1.25	0	13.0±6.1	268±73	89.9
2.5	0	13.3±11.2	272±23	91.2
5.0	0	11.8±4.7	272±36	91.2
10.0	0	20.3±6.7	333±66	111.6
a) Not statistical significant compared to the control (Dunnett's t-test, $\alpha = 0.05$, two-sided for weight change and one-sided smaller for reproduction)				

Conclusions

In an earthworm reproduction and growth study with XDE-729 Methyl the LC50 was determined to be greater than 10.0 mg a.s./kg soil dry weight. The no-observed-effect-concentration (NOEC) for mortality, growth and reproduction of the earthworm *Eisenia fetida* was determined to be 10.0 mg a.s./kg soil dry weight, i.e. the highest concentration tested.

RMS Comment: The study is considered to be acceptable and hence suitable for use in risk assessment. The key endpoint from this study is the NOEC of 10 mg a.s./kg soil.

Witte, B. (2010): Effects of XDE-729 Acid on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil with 5% Peat. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. IBACON report 56862022. Dow AgroSciences unpublished report, Study Number 101142. 13 August 2010.

Test material

Test item:	XDE-729 Acid
Purity:	XDE-729: 95.3% \pm 0.4% (wt/wt)
Description:	Solid, off-white
Lot No.:	E2837-52
Test Substance Number:	TSN030751-0006

Test system

Organism (Species):	Earthworm (<i>Eisenia fetida</i>)
Study Type:	56-day earthworm reproduction study
GLP Status:	Yes
Guidelines followed:	OECD Guideline 222 (Adopted 2004); ISO-Guideline 11268-2 (1998)
Guideline deviations reported by Study Director:	None
Study design:	Assessment of the survival, behaviour and weight change of worms after 28 days exposure. Assessment of the number of offspring 56 days after treatment. 4 replicates, consisting of 10 worms in each vessel per test concentration. 8 replicates, consisting of 10 worms in each vessel for the control.
Test concentrations:	Control, 0.625, 1.25, 2.5, 5.0 and 10.0 mg XDE-729 Acid/kg soil dry weight
Soil parameters	Artificial soil according to OECD 222 but with reduced organic matter content: pH at initiation: 6.2 to 6.3 pH at termination: 5.9 to 6.0 Water content at initiation: 22.3% to 23.8% (49.6% to 52.9% of the maximum water holding capacity) Water content at termination: 26.6% to 28.9% (59.1% to 64.2% of the maximum water holding capacity) Soil details were:

	5.0% Sphagnum-peat, air-dried and finely ground (2 mm); (Floragard, Vertriebs GmbH für Gartenbau, 26138 Oldenburg, Germany) CD 20% Kaolin clay (Erbsloh, 65558 Lohrheim, Germany) approximately 0.2% calcium carbonate (CaCO_3) added to adjust pH to 6.0 ± 0.5 (Merck, 64293 Darmstadt, Germany) approximately 74.8% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm; (Quarzwirke Frechen, Postfach 1780, 50207 Frechen, Germany)
Environmental conditions:	Temperature: 18°C to 22°C Light intensity: 400 lux to 800 lux Photoperiod: 16 : 8 h Feeding: Finely ground cattle manure was used as food and was added each week for the first 4 weeks of the experiment.
Reference item:	Luxan Carbendazim 500 FC (Carbendazim, 500 g/L nominal)

Methodology

56-day test in treated artificial soil according to OECD 222; different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioural effects and biomass development were carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after an additional 28 days (assessed 56 days after application).

Table B.9.6.6: Effects of XDE-729 Acid on earthworm survival, biomass and reproduction

Test concentrations [mg/kg soil dry weight]	% mortality after 28 days	% body weight change after 28 days	Mean No. of juveniles at day 56	% change in number of juveniles compared to control
Control	0	53.4±12.9	272±37	-
0.625	0	55.2±14.2	320±36	117.9
1.25	0	50.5±11.3	273±42	100.4
2.5	0	50.9±3.9	270±15	99.4
5.0	0	55.3±6.6	286±19	105.4
10.0	0	52.3±14.8	320±43	117.7
None of the values was significantly different compared to the control (Dunnett's t-test, $\alpha = 0.05$, two-sided (for body weight), Dunnett's t-test, $\alpha = 0.05$, one-sided smaller (for reproduction))				

Conclusions

In an earthworm reproduction and growth study with XDE-729 Acid the LC50 was estimated to be greater than 10.0 mg test item/kg soil dry weight. The no-observed-effect-concentration (NOEC) for mortality, growth and reproduction of the earthworm *Eisenia fetida* was determined to be 10.0 mg test item/kg soil dry weight, i.e. the highest concentration tested.

RMS Comment: The study is considered to be acceptable and hence suitable for use in risk assessment. The key endpoint from this study is the NOEC of 10 mg a.s./kg soil. There is no need for further adjustment of this endpoint due to log Pow, as XDE-729 Acid has a log Pow of < 2 (-0.83).

Witte, B. (2010): Effects of X11449757 (metabolite of XDE-729) on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil with 5% Peat. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. IBACON report 56873022. Dow AgroSciences unpublished report, Study Number 101156. Study Completion Date: December 13, 2010.

Test material

Name:	X11449757
Lot No.:	YB1-100780-103
Test Substance Number:	TSN031413-0003
Purity:	99%

Test system

Organism (Species):	Earthworm (<i>Eisenia fetida</i>)
Study Type:	56-day earthworm reproduction study
GLP Status:	Yes
Guidelines followed:	OECD Guideline 222 (Adopted 2004); ISO-Guideline 11268-2 (1998)
Guideline deviations reported by Study Director:	None
Study design:	Assessment of the survival, behaviour and weight change of worms after 28 days exposure. Assessment of the number of offspring 56 days after treatment. 4 replicates, consisting of 10 worms in each vessel per test concentration. 8 replicates, consisting of 10 worms in each vessel for the control.
Test concentrations:	Control, 0.625, 1.25, 2.5, 5 and 10 mg X11449757/kg soil dry weight
Soil parameters:	Artificial soil according to OECD 222 but with reduced organic matter content: pH at initiation: 5.6 to 5.7 pH at termination: 5.7 to 5.8 Water content at initiation: 19.3% to 21.6% (48.3% to 54.0% of the maximum water holding capacity) Water content at termination: 23.3% to 25.2% (58.3% to 63.0% of the maximum water holding capacity)
Environmental conditions:	Temperature: within the range of 18°C to 22°C Light intensity: within the range of 400 lux to 800 lux Photoperiod: 16 : 8 h Feeding: Finely ground cattle manure was used as food and was added each week for the first 4 weeks of the experiment. Soil properties were: 5.0% Sphagnum-peat, air-dried and finely ground (2 mm); (Floragard, Vertriebs GmbH fir Gartenbau, 26138 Oldenburg, Germany) 20% Kaolin clay (Erbsloh, 65558 Lohrheim, Germany) approximately 0.2% calcium carbonate (CaCO ₃) added to adjust pH to 6.0 + 0.5 (Merck, 64293 Darmstadt, Germany) approximately 74.8% fine quartz-sand (F34) containing more than 50% by mass of particle size

	0.05 mm to 0.2 mm; (Quarverke Frechen, Postfach 1780, 50207 Frechen, Germany)
Reference item:	Luxan Carbendazim 500 FC (Carbendazim, 500 g/L nominal).

Methodology

56-day test in treated artificial soil according to OECD 222. Different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).

Results

Table B.9.6.7: Effects of X11449757 on earthworm survival, biomass and reproduction

Test concentrations [mg/kg soil dry weight]	% mortality after 28 days	% body weight change after 28 days	Mean No. of juveniles at day 56	% change in number of juveniles compared to control
Control	0	44.6±16.0	183±42	-
0.625	0	34.2±15.6	200±61	109.3
1.25	2.5±5.0	44.1±10.5	192±22	105.1
2.5	0	39.9±9.0	133±12	72.9
5	0	36.1±3.4	182±48	99.3
10	0	39.7±5.6	199±46	108.9
None of the values was significantly different compared to the control (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater (for mortality); Dunnett's t-test, $\alpha = 0.05$, two-sided (for body weight); Dunnett's t-test, $\alpha = 0.05$, one-sided smaller (for reproduction))				

Conclusions

In an earthworm reproduction and growth study with X11449757 the LC50 was estimated to be greater than 10 mg test item/kg soil dry weight. The no-observed-effect-concentration (NOEC) for mortality, growth and reproduction of the earthworm *Eisenia fetida* was determined 10 mg test item/kg soil dry weight, i.e. the highest concentration tested.

RMS Comment: The study is considered to be acceptable and hence suitable for use in risk assessment. The key endpoint from this study is the NOEC of 10 mg a.s./kg soil. There is no need for further adjustment of this endpoint due to log Pow, as X11449757 Acid has a log Pow of < 2 (<0.3)

Witte, B. (2011): Effects of GF-2573 on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil with 5% Peat. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. IBACON report 54809022. Dow AgroSciences unpublished report, Study Number 101122. Revised Final Report Date: May 25, 2011

Test material

Test Item:	GF-2573
Test Substance Number:	TSN031424-0002
Active Substance(s) / Content:	XR-729 methyl: 0.84% wt/wt (7.6 g/L) cloquintocet-mexyl: 0.81% wt/wt (7.3 g/L)
Batch No. :	E2837-57

Test system

Organism (Species):	Earthworm (<i>Eisenia fetida</i>)
Study Type:	56 day earthworm reproduction study
GLP Status:	Yes
Guidelines followed:	OECD Guideline 222 (Adopted 2004); ISO-Guideline 11268-2 (1998)
Guideline deviations reported by Study Director:	None
Study design:	Assessment of the survival, behaviour and weight change of worms after 28 days exposure. Assessment of the number of offspring 56 days after treatment. 4 replicates, consisting of 10 worms in each vessel per test concentration. 8 replicates, consisting of 10 worms in each vessel for the control.
Test concentrations:	75, 150, 300, 600 and 1200 mg form ⁿ /kg soil dry weight
Soil parameters:	Artificial soil according to OECD 222 but with reduced organic matter content: pH at initiation: 5.6 to 5.9 pH at termination: 6.3 to 6.4 Water content at initiation: 18.7% to 23.3% (40.7% to 50.7% of the maximum water holding capacity) Water content at termination: 23.8% to 30.2% (51.7% to 65.7% of the maximum water holding capacity) Soil details were: 5.0% Sphagnum-peat, air-dried and finely ground (2 mm); (Floragard, Vertriebs GmbH für

	Gartenbau, 26138 Oldenburg, Germany) 20% Kaolin clay (Erbsloh, 65558 Lohrheim, Germany) approximately 0.2% calcium carbonate (CaCO ₃) added to adjust pH to 6.0 ± 0.5 (Merck, 64293 Darmstadt, Germany) approximately 74.8% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm; (Quarzwerte Frechen, Postfach 1780, 50207 Frechen, Germany)
Environmental conditions:	Temperature: 20°C to 22°C Light intensity: 400 lux to 800 lux Photoperiod: 16 : 8 h Feeding: Finely ground cattle manure was used as food and was added each week for the first 4 weeks of the experiment.
Reference item:	Luxan Carbendazim 500 FC (Carbendazim, 500 g/L nominal).

Methodology

56-day test in treated artificial soil according to OECD 222; different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of adult worm mortality, behavioural effects and biomass development were carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after an additional 28 days (assessed 56 days after application).

Table B.9.6.8: Effects of GF-2573 on earthworm survival, biomass and reproduction

Test concentrations [mg form ⁿ /kg soil dry weight]	% mortality after 28 days	% bodyweight change after 28 days	Mean No. of juveniles at day 56	% change in number of juveniles compared to control
Control	0	53.9±13.4	197±57	-
75	2.5±5.0	54.6±11.8	226±36	114.8
150	0	48.9±2.5	206±20	104.8
300	0	55.3±4.0	269±51	136.7
600	0	53.8±7.5	245±104	124.6
1200	0	55.0±11.7	256±62	129.9
None of the values was significantly different compared to the control (mortality: Fisher's Exact Test, alpha = 0.05, one –sided greater; body weight and reproduction: Dunnett's t-test, alpha = 0.05, two-sided for body weight and one-sided smaller for reproduction)				

Conclusions

In an earthworm reproduction and growth study with GF-2573 the LC50 was determined to be greater than 1200 mg formⁿ/kg soil dry weight. The No Observed Effect Concentration (NOEC) for mortality, growth and reproduction of the earthworm *Eisenia fetida* was determined to be 1200 mg formⁿ/kg soil dry weight, i.e. the highest concentration tested

RMS Comment: The study is considered to be acceptable and hence suitable for use in risk assessment. The key endpoint from this study is the NOEC of 1200 mg formⁿ/kg soil.

McCormac, A. (2012). Determination of the chronic (sub-lethal) toxicity of aged residues of technical-grade XDE-729 Methyl to the earthworm *Eisenia fetida* in two natural soil substrates. Dow AgroSciences, unpublished report number 110605. Mambo-Tox Study/Report Number DOW-11-38, issued 25 April 2012.

Test material

Name:	XDE-729 Methyl
Lot No.:	E2837-51
Test Substance Number:	TSN030117-0004
Purity:	97.2% w/w
Description:	White solid
recertification date:	30 November 2013

Test system

Organism :	Earthworm (<i>Eisenia fetida</i>) 300-600 mg at study initiation Approx 6.5 months old Visible clitellum
Study Type:	56-day earthworm reproduction study
GLP Status:	Yes
Guidelines followed:	OECD Guideline 222 (Adopted 2004).
Guideline deviations reported by Study Director:	None, but guideline adapted for using natural soils with aged residues.
Assessments:	Assessment of the survival, behaviour and weight change of worms after 28 days exposure. Assessment of the number of offspring after 56 days exposure
Replication:	4 replicates per test concentration. 8 replicates per control group. 10 earthworms per replicate.
Test concentrations:	Control, solvent control, 0.1, 0.32, 1.0, 3.2 and 10

	mg a.s/kg dry soil (repeated for each natural soil used). Aged 29 (UK) and 30 (German) days after treatment before addition of organisms
Soil parameters:	2 x freshly collected natural soils – M802 (German) and M803 (UK) pH at treatment: 5.5 (UK), 5.7 (German) pH at test termination: 5.3-5.5 (UK), 5.2-5.3 (German) Water content at treatment: 50% WHC Water content at test termination: mean 55.3% WHC (33-57%) (UK) 57.3% WHC (56-59%) (German)
Test Vessels:	Polystyrene box 17.1x11.2x6.0 cm. Sealed lid with mesh-covered ventilation holes. 500 g dry weight soil/rep - 5cm soil depth.
Environmental conditions:	Temperature: Within the range of 20-21°C Light intensity: 630-770 lux Photoperiod: 16:8 (light:dark) Feed: finely ground oats moistened with purified water (weekly)
Reference item:	Carbendazim (500g/L SC)

Methodology

The aim of this study was to determine under laboratory test conditions whether the high levels of non-extractable residue (NER) formed in some soils following treatment with XDE-729 Methyl have harmful effects on the survival, growth and reproductive capacity of adult earthworms, *Eisenia fetida* Savigny (Oligochaeta: Lumbricidae).

Technical-grade XDE-729 Methyl was applied to batches of the freshly-collected field soils at a range of concentrations; 10, 3.2, 1.0, 0.32 and 0.1 mg a.s./kg soil dry weight. Solutions of the test item were prepared in acetone and appropriate volumes were applied to aliquots of the test soil. When dry, the treated aliquots were mixed with the remaining portions of soil to achieve final target concentrations. Treatments were incorporated into two natural soil substrates, in two independent bioassays.

The soils were obtained from sites in the UK (Site E1 Farditch Farm, M803) and Germany (RefeSol 03-G soil, M802), respectively, and were the same two soils which resulted in the highest NER within 30 days of treatment with XDE-729 Methyl, as documented by Yoder *et al.* (2011). Furthermore, in a complementary study using the same two natural soils, Lui and Croffie (2012) confirmed that the NER formed following application of XDE-729 Methyl, reached a maximum

between day 32 and 89. These soils will hereafter be referred to by their country of origin.

In each bioassay, these variants were compared with a control treatment of purified water, and a set of acetone-treated arenas was also evaluated as a control for the method of test item delivery.

The soils were placed into chambers comprising polystyrene boxes 17.4 cm x 11.3 cm in area (i.e. 193.2 cm²) x 6 cm deep, with ventilated lids. In each bioassay, there were 8 replicate boxes for the untreated control, 8 for the solvent-treated control, and 4 per test-item treatment concentration. The treated UK and German soils were then stored for a period of 29 and 30 days, respectively, prior to the introduction of the adult earthworms for the bioassays, to allow sufficient time for the NER to be formed. After this period, ten adult *E. fetida* (approx. 6.5 months old, 300-600 mg fresh weight and with a visible clitellum) were introduced into each chamber. The weight of each worm introduced to a replicate was recorded and a replicate mean worm weight calculated prior to introduction. One day after introduction of the worms (1 DAI), hydrated oat flakes were provided as food and this food was replenished weekly. At 28 days after introduction (28 DAI) the numbers of the original worms still surviving were removed from each replicate and their numbers and fresh weights were recorded. Any apparent change in the behaviour or physical condition of the confined worms was also recorded. The test soil, along with any egg cocoons and juvenile worms were returned to the test chambers and a final supply of food was provided. After a further 28 days (56 DAI) the number of juvenile worms that had been produced in each replicate chamber was recorded.

Results

In the test using UK soil control and solvent control group mean parental mortality was 1%, less than the $\leq 10\%$ validity criterion set in the guideline. Mean juvenile production per replicate in this soil was 67 and 56 for the control and solvent control groups respectively, with all replicates in these groups producing more than the minimum of 30 juveniles required by the validity criterion of OECD guideline 222. Both control and solvent control groups resulted in a CV (coefficient of variance) for juvenile production of $\leq 30\%$ (28.7% and 24.3% respectively). Therefore all guideline validity criteria were met and the study can be considered valid when using the UK soil.

In the test using German-collected soil control and solvent control group mean parental mortality was 0%, $\leq 10\%$. Mean juvenile production per replicate in this soil was 153 and 145 for the control and solvent control groups respectively, with all replicates in these groups producing more than the minimum of 30 juveniles required by the validity criterion of the test guideline. Both control and solvent control groups resulted in a CV (coefficient of variance) for juvenile production of $\leq 30\%$ (22.0% and 21.4% respectively). Therefore all guideline validity criteria were met and the study can be considered valid with the German soil.

The results for mortality, weight change and juvenile production in the bioassays with each soil are presented in table B.9.6.9 (UK soil) and table B.9.6.10 (German soil) below. No behavioural or sub-lethal injuries were observed at 28 DAI.

Table B.9.6.9: Effects of aged residues of XDE-729 Methyl on earthworm survival, biomass and reproduction in UK soil

Test concentration [mg a.s./kg soil dry weight]	Mean % mortality after 28 days	Mean% body weight change after 28 days	Mean No. of juveniles (CV) at day 56	% change in number of juveniles compared to control
Control	1	+32	67 (28.7)	-
Solvent control	1	+48	56 (24.3)	-16
0.1	0	+41	54 (27.6)	-19
0.32	0	+41	62 (18.9)	-8
1.0	0	+46	56 (50.2)	-17
3.2	10*	+46	69 (39.8)	+3
10	0	+38	53 (17.8)	-20
*Significantly different to control group. Fishers exact test ($\alpha = 0.05$)				

Table B.9.6.10: Effects of aged residues of XDE-729 Methyl on earthworm survival, biomass and reproduction in German soil

Test concentration [mg a.s./kg soil dry weight]	Mean % mortality after 28 days	Mean% body weight change after 28 days	Mean No. of juveniles (CV) at day 56	% change in number of juveniles compared to control
Control	0	+56	153 (22.0)	-
Solvent control	0	+56	145 (21.4)	-5
0.1	0	+66	137 (16.1)	-11
0.32	0	+74	156 (42.2)	+2
1.0	0	+70	142 (24.6)	-7
3.2	10*	+55	146 (16.0)	-6
10	0	+57	132 (32.0)	-13
*Significantly different to control group – Fishers exact test ($\alpha = 0.05$)				

Conclusions

Based on assessments of mortality, biomass, behaviour and reproduction rate, the NOEC for aged-residues within natural soils collected from the UK (Site E1 Farditch Farm, M803) and Germany (RefSol 03-G, M802) was 10 mg XDE-729 Methyl/kg soil dry weight.

For both soil types, the median lethal concentration (LC_{50}) was > 10 mg a.s./kg dry soil. In addition, no unacceptable effects on earthworm biomass or behaviour were detected at concentrations up to and including 10 mg a.s./kg soil, the maximum tested. For both soil types, the aged soil residues had no significant effects on worm reproduction at concentrations up to and including 10 mg a.s./kg dry soil. The method of test-item delivery, in an acetone solvent, had no significant impact on the outcome of any of the assessments.

In the corresponding fate study (IIA B.8.1.1 c) the mean levels of unextracted radioactivity between 32 and 89 DAT in the 10 mg a.s./kg treatments were 48.6% on the Site E1 soil and 71.0% on the RefSol 03-G soil. Therefore the Applicant considered that the unextracted residue concentrations in the soils during the period of exposure 4.86 mg a.s. equivalent/kg and 7.10 mg a.s. equivalent/kg in the Site E and RefSol 03-G soils respectively. For the purpose of risk assessment therefore a NOEC of 7.10 mg/kg has been used since no effects of NER were observed at this concentration.

RMS Comment: The study is considered to be acceptable and hence suitable for use in risk assessment. The key endpoint from this study is the NOEC of 7.10 mg NER/kg dry soil.

B.9.6.2 Risk assessment

PEC_{soil} values and details on PEC calculations are provided in Annex B.8. Assuming the GAP details stated in table 9.1, mixing over 5 cm depth and bulk density of 1.5 cm³, the initial PEC_{soil} values after the second application for XDE-729 Methyl and after a single application for the metabolites X11393729 (XDE-729 acid), X11449757 and the formulation GF-2573 are listed below

Table 9.6.11: Initial predicted environmental concentrations (PEC_{soil}) for XDE-729 Methyl, X11393729 (XDE-729 acid), X11449757

Compound	Maximum initial PEC soil (mg/kg)
XDE-729 Methyl	0.009
GF-2573	0.905
X11393729 (XDE-729 acid)	0.005
X11449757	0.002

In laboratory aerobic degradation studies, XDE-729 methyl degraded relatively rapidly, but exhibited slower degradation in field dissipation studies with a worst case SFO DT₅₀ of 43 days. For X11393729 (XDE-729 acid) and X11449757, these metabolites showed a worst-case top-down DT₅₀ of 264 and 197 days, respectively. Given the apparent persistence of these substances, a maximum total dose approach has been used for the PEC_{soil} calculation and an accumulation calculation has also been conducted.

Table 9.6.12: Peak accumulation predicted environmental concentrations (PEC_{soil}) for X11393729 and X11449757

Compound	Peak accumulation PEC soil (mg/kg)
X11393729 (XDE-729 acid)	0.009
X11449757	0.0025

For the risk assessment, the peak accumulation PEC_{soil} values have been used for X11393729 (XDE-729 acid) and X11449757.

Table B.9.6.13: Summary of available toxicity data for earthworms

Group	Type of study and test item	Species	Endpoint	Endpoint _{corr} [*]	Reference
Earthworms	Acute XDE-729 Methyl	<i>Eisenia fetida</i>	LC ₅₀ > 1000 mg a.s./kg d.w soil	LC _{50 corr} > 500 mg a.s./kg d.w soil	Witte, B., (2010a) IIA 8.9.1/1
	Acute XDE-729 acid		LC ₅₀ > 1000	--	Witte, B., (2010b) IIA 8.9.1/2
	Acute X11449757		LC ₅₀ > 1000 mg/kg d.w soil		Witte, B., (2010c) IIA 8.9.1/3
	Acute GF-2573		LC ₅₀ > 1000 mg/kg d.w soil	LC _{50 corr} > 500 mg/kg d.w soil	Witte, B., (2011a) IIA 10.6.2/1
	Chronic XDE-729 Methyl		NOEC = 10 mg a.s./kg d.w soil	NOEC _{corr} = 5 mg/kg d.w soil	Witte, B., (2010d) IIA 8.9.2/1
	Chronic XDE-729 acid		NOEC = 10 mg/kg d.w soil	--	Witte, B., (2010e) IIA 8.9.2/2
	Chronic X11449757		NOEC = 10 mg/kg d.w soil	--	Witte, B., (2010f) IIA 8.9.2/3
	Chronic GF-2573		NOEC = 1200 mg/kg d.w soil	NOEC _{corr} = 600 mg/kg d.w soil	Witte, B., (2011b) IIA 10.6.3/1

*All soil used in the laboratory experiments contained 5% organic matter rather than the 10% stated in the OECD guideline. However corrected endpoints are provided for studies with XDE-729 methyl and GF-2573, as the active substance has a log K_{ow} > 2.

Usually, the artificial substrate of the earthworm laboratory test has a higher organic carbon content than many natural soils, the LC₅₀ or NOEC could potentially be lower if the test were conducted in natural soil (Van Gestel 1992). To account for this difference the LC₅₀ and NOEC are usually divided by 2 (to give an LC_{50 corr}/NOEC_{corr} endpoint) when the log K_{ow} is greater than 2 (EPPO 2002a). The laboratory studies conducted above used an artificial soil with a lower organic carbon content than the OECD guideline suggested (5% rather than 10%).

It is the opinion of EFSA, that for substances with a log K_{ow} > 2, the correction factor should be applied to toxicity endpoints unless it has been demonstrated that lowering the organic carbon content allows for the correction factor to not be used. As this has not been demonstrated, corrected endpoints will be used in the risk assessment below for those test items with a log K_{ow} of > 2 (XDE-729 Methyl log K_{ow} of 3.76). Corrected endpoints have also been used for the representative formulation GF-2573. X11393729 (XDE-729 acid) and soil metabolite X11449757 both have a log K_{ow} value of < 2 (-0.83 and < 0.3 respectively) and so no correction

factor has been applied to the toxicity endpoints. This approach is in accordance with the decision of an expert meeting (PRAPeR, April 2012). For completeness, uncorrected endpoints have also been presented in the table below.

This risk assessment is based on the SANCO/10329/2002 guidance document for terrestrial ecotoxicology.

Table B.9.6.14: Acute and chronic risk assessment for earthworms

Study type	Test substance	Toxicity	PEC _{soil} (mg/kg soil)	TER**	Trigger value
Acute	XDE-729 Methyl	LC ₅₀ corr >500mg/kg d.w soil	0.009	55556	10
	XDE-729 acid (X11393729)	LC ₅₀ > 1000 mg/kg d.w soil	0.009	111111	10
	X11449757	LC ₅₀ > 1000 mg/kg d.w soil	0.0025	400000	10
	GF-2573	LC ₅₀ corr >500mg/kg d.w soil	0.905	552	10
Chronic	XDE-729 Methyl	NOEC _{corr} 5.0 mg/kg d.w soil	0.009	556	5
	XDE-729 acid (X11393729)	10 mg/kg d.w soil	0.009	1111	5
	X11449757	10 mg/kg d.w soil	0.0025	4000	5
	GF-2573	NOEC _{corr} 600 mg/kg d.w soil	0.905	663	5

**Values have been rounded.

All acute TER's are above the trigger value of 10, furthermore all chronic TER's are above the trigger value of 5. Consequently, the acute and chronic risk to earthworms posed by GF-2573, XDE-729 Methyl, XDE-729 Acid, X11449757 and any non-extractable residues is acceptable.

B.9.6.3 Conclusions

The acute and chronic risk to earthworms posed by GF-2573, XDE-729 Methyl and the major metabolites XDE-729 Acid and X11449757 is acceptable and so requires no further consideration.

B.9.7 Effects on soil non-target macro-organisms (IIIA 10.6.2)**Active substance**

Lührs, U. (2011): Effects of XDE-729 Methyl on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat. IBACON 64641089. Dow AgroSciences unpublished report, DAS Study Number 110280. Study Report Completion Date: December 13, 2011.

Test material

Test Item:	XDE-729 Methyl
Lot No.:	E2837-51
Test Substance Number:	TSN031117-0004
Purity:	97.2%

Test system

Organism (<i>Species</i>):	Predatory Mite <i>Hypoaspis aculeifer</i>
Study Type:	Reproduction inhibition study
GLP Status:	Yes
Guidelines Followed:	OECD 226 (2008)
Guideline Deviations reported by Study Director:	Water loss was not compensated in 2 replicates, although weight deviated by more than 2% from the initial weight. This had no effects on the outcome of the study.
Duration of study:	14 days
Parameters measured:	Survival, reproductive output i.e. number of juveniles and morphological differences.
No. of mite per dose group:	4 replicates (10 females in each)
No. of mite per control group:	8 replicates (10 females in each)
Age of test organisms at test initiation:	Adults (approximately 10 days after reaching the adult stage)
Test Concentrations:	Nominal- 0 (vehicle control), 1.56, 3.13, 6.25, 12.5 and 25 mg/kg soil.
Soil Parameters:	Artificial soil according to OECD 226: pH at initiation: 6.4 pH at termination: 6.4 to 6.5 Water content at initiation: 19.0% to 19.6% (46.4% to 47.9% of the maximum water holding capacity) Water content at termination: 17.7% to 18.7% (43.1% to 45.7% of the maximum water holding capacity)
Environmental Conditions:	Temperature: 18 to 22°C Light: 400 to 800 lux (16 h light : 8 h dark)
Feeding:	One spatula of cheese mites (<i>Tyrophagus putrescentiae</i> cultured by IBACON) at initiation and on day 2, 4, 7, 9 and 11.

Reference Item:	BAS 152 11 I (a.i. dimethoate, 400 g/L, nominal). The effects of the reference item are investigated at least once a year in a separate study.
-----------------	---

Methodology

A 14-day exposure test was performed with nominal test concentrations 0 (control), 1.56, 3.13, 6.25, 12.5 and 25 mg/kg dry soil. The stock solution was prepared by dissolving the test item in acetone. Artificial soil was dosed by mixing appropriate volumes of the test item in solution with fine quartz sand. The treated sand was left for 3 hours until the solvent had evaporated and then mixed homogeneously into the artificial soil. The control was treated with the same amount of acetone and sand per g substrate as the test item groups. The soil was added to glass vessels before the predatory mites were introduced on top of the soil. Each vessel contained $20 \text{ g} \pm 1.0 \text{ g}$ artificial soil. The control was replicated eight times with 10 female mites in each, a total of 80 individuals. Each treatment was replicated four times, with 10 female mites in each, a total of 40 individuals. Predatory mites were fed with one spatula of with cheese mites (*Tyrophagus putrescentiae*) at test initiation and on days 2, 4, 7, 9 and 11. All test chambers were aerated periodically by opening the test chamber lids briefly.

After 14 days of incubation the mites were extracted from the soil using a Kempson extractor. The soil including the mites was exposed to temperatures of approximately 25°C and 30°C for approximately 2 days. Escaping mites were collected in a fixing liquid cooled to a temperature of approximately 16°C. The fixing liquid contained glycol and a detergent. Adult animals were counted once visually, juvenile animals were counted twice under binocular microscopes.

Water content was checked at initiation and on day 7 by reweighing each test container. The soil pH was measured in samples taken from the parent soils at test initiation and in samples collected from an additional replicate test chamber from the control and all treatment levels at test termination. Soil temperature was measured continuously.

Table B.9.7.1: Summary of effects on mortality and reproduction of *Hypoaspis aculeifer* following 14 days exposure to XDE-729 methyl.

Parameter	0 (Vehicle control)	XDE-729 (mg/kg dry soil)				
		1.56	3.13	6.25	12.5	25
Mean mortality after 14 days (%)	8	20	10	5	0	10
Mean reproduction (no. juveniles)	272	256	252	265	244	250
% reproduction compared to control	-	94	93	98	90	92

Table B.9.7.2: summary of statistical endpoints from testing *Hypoaspis aculeifer* with XDE-729 methyl

Biological Parameter	NOEC (mg/kg)	LOEC (mg/kg)	EC ₅₀ (95% confidence limits)	LC ₅₀ (95% confidence limits)
Adult Survival	25	>25	-	>25
Total number of young	25	>25	>25	-

Conclusions

The control organisms met the acceptability criteria for mean adult survival (>80%), mean number of juveniles per replicate (>50) and for reproduction (CV less than 30%) as specified by the study protocol. There were no statistically significant effects on mortality or reproduction as compared to the control. No differences in morphology of the mites between the test item treated groups and the control were observed.

The estimated 14 day LC₅₀ and EC₅₀ values for adult survival and total young produced was >25 mg/kg dry soil, the highest concentration tested.

The NOEC and LOEC values for both adult survival and reproduction were 25 and >25 mg/kg dry soil, respectively.

RMS Comment: The study is considered to be acceptable and suitable for use in risk assessments. The key endpoint from the study is a NOEC of 25 mg/kg.

Gerke, A. 2011: XDE-729 Methyl: Inhibition of Reproduction of Collembola, *Folsomia candida*, in Artificial Soil. ABC 64611. Dow AgroSciences unpublished report, Study Number 090181. 25 October 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Lot No./Batch No. :	E2837-51

Test system

Organism (<i>Species</i>):	Collembola (<i>Folsomia candida</i>)
Study Type:	28 day reproduction inhibition study
GLP Status:	GLP
Guideline followed:	OECD Method 232
Guideline deviations reported by Study Director:	None
Duration of study:	28 day
Test conditions:	Static
Parameters measured:	Survival and reproductive output i.e. number of juveniles.
Observation intervals:	Day 28
Age of test organisms at test initiation:	9-12 days
Test concentrations:	Nominal: 0 (control), 0 (vehicle control; 10.3 mL acetone per kg dry soil), 0.31, 0.63, 1.3, 2.5, 5.0, 10, and 1,000 mg a.s./kg dry soil
Analytical confirmation of test concentrations:	1 mL was collected from the vehicle control and each test substance treatment stock solution. The samples were diluted as necessary with 50:50 methanol:water to provide final sample concentrations within the analytical standard concentrations range. The test solution samples were analyzed using HPLC-UV.
Reference substances:	Boric acid
No. of holding days before dosing:	9-12 days
No. of Collembola per dose group:	50 (5 replicates of 10 collembola)
No. of Collembola per control group:	90 (9 replicates of 10 collembola)
Environmental conditions:	pH: 5.9 to 6.3 Photoperiod: 16 hrs light and 8 hrs dark Light Intensity: 464 lux

Methodology

A 28-day test was performed with nominal test concentrations 0 (control), 0 (vehicle control), 0.31, 0.63, 1.3, 2.5, 5.0, 10, and 1,000 mg a.s./kg dry soil. The control and vehicle control were replicated nine times with 10 springtails per replicate, for a total of 90 organisms. Each treatment was replicated five times, and each replicate contained 10 springtails for a total of 50 organisms per treatment. A total of 10 collembola juveniles were impartially added to labelled glass containers. Each container was randomly assigned to a given treatment-replicate test jar or control. Collembola were fed approximately 6 mg of granulated dry yeast at initiation and on day 14 of the study. All test chambers were aerated periodically by opening the test chamber lids briefly.

The stock solution was prepared by dissolving the test item in acetone. Artificial soil was dosed by mixing appropriate volumes of the test item in solution with fine quartz sand. The vehicle control soil thus contained the relevant volume of acetone. Treated sand was left overnight to allow the solvent to evaporate. The treated sand was added to artificial soil and mixed to ensure homogenous distribution. Approximately 30 g of this hydrated soil was added to each replicate test vessel.

At test termination, the artificial soil from each replicate jar was placed into a polypropylene dish and suspended with blended freshwater. Approximately six drops of black ink were added to the suspension of soil and water. The suspension was stirred gently with a glass pipette and allowed to settle for approximately two minutes. The adult and juvenile collembola, if present, floated to the surface and were counted. Adults were counted once and juveniles were counted twice. Any adults not found at termination were considered dead. The counts were made using Leica dissecting microscopes at 0.63 times magnification.

The moisture content was determined from samples of test medium collected from the control, vehicle control, and all treatment levels at test initiation and termination. The soil pH was measured in samples taken from the parent soils at test initiation and in samples collected from an additional replicate test chamber from the control and all treatment levels at test termination. Soil temperature was measured continuously.

ResultsTable B.9.7.3: Summary of effects on mortality and reproduction of *Folsomia candida* following exposure to XDE-729.

Parameter	XDE-729 Methyl (mg a.s./kg dry soil)								
	0 (Control)	0 (Vehicle control)	0.31	0.63	1.3	2.5	5.0	10	1000
Mean percent Survival after 28 days (%)	88	91	88	88	82	84	86	82	88
Mean percent mortality after 28 days (%)	12	9	12	12	18	16	14	18	12
Mean reproduction (no. juveniles) and %CV	232 (10)	241 (9)	249 (18)	251 (13)	237 (19)	189* (24)	193* (13)	205 (10)	211 (8)

*Statistically significant ($p = 0.05$) reduction in total number of young produced as compared to the controls. Although not expected to be test item related based on lack of statistically significant reduction of young produced at higher test concentrations.

Table B.9.7.4: Summary of study endpoints from testing XDE-729 methyl with *F.candida*

Biological Parameter	NOEC (mg a.s./kg)	LOEC (mg a.s./kg)	EC ₅₀ (95% confidence limits)
Adult Survival	1,000	>1,000	>1,000 (not calculated)
Total number of young	1,000	>1,000	>1,000

Conclusions

The control organisms met the acceptability criteria for mean adult survival (i.e., >80%) and for reproduction (CV less than 30%) as specified by the study protocol.

It is noted that a 16 h light: 8 h dark photoperiod has been employed in the study, whereas OECD guideline 232 indicates that 12 h light: 12 h dark is preferable. However, ISO 11267 (1999) indicates that a 16 h light: 8 h dark photoperiod is acceptable.

No significant effects were observed up to and including the maximum tested concentration of 1000 mg XDE-729 methyl/kg soil dry weight. Therefore the EC₅₀ and LC₅₀ for all parameters were >1,000 mg a.s./kg dry soil.

The NOEC and LOEC values for all parameters were 1,000 and >1,000 mg a.s./kg dry soil, respectively.

RMS Comment: It is worth noting that the geometric increase between the two highest concentrations greatly exceeds the factor of 1.8 stated in the guidelines. Furthermore, the study displays elements of a limit test however; the number of replicates used is not in accordance with the OECD guidelines for replicates used in a limit test. Despite these slight deviations, the study is considered to be acceptable and suitable for use in risk assessments. The key endpoint from the study is a NOEC of 1,000 mg a.s./kg.

Witte, B. (2011): XDE-729 acid: Effects of XDE-729 acid on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat. IBACON 56864089. Dow AgroSciences unpublished report, Study Number 102025. Study Report Completion Date: March 15, 2011.

Test material

Test Item:	XDE-729 Acid
Test Substance Number:	TSN030751-0006
Purity:	XDE-729: 95.3% ±0.4% (wt/wt)
Lot No. :	E2837-52

Test system

Organism (Species):	<i>Hypoaspis aculeifer</i> (predatory mite)
Study Type:	14-day reproduction test in artificial substrate
GLP Status:	Yes (excluding range-finding test)
Guidelines followed:	OECD Guideline 226 (Adopted 2008)
Guideline deviations reported by Study Director:	None
Parameters measured:	Mortality of adult predatory mites, behavioural effects, number of juveniles.
Test concentrations:	Control, 1.56, 3.13, 6.25, 12.5 and 25 mg XDE-729 Acid/kg soil dry weight.
Substrate type and constituents:	Artificial substrate according to OECD 226: <ul style="list-style-type: none"> • 5.0% Sphagnum-peat, air-dried and finely ground (2mm) • 20% Kaolin clay • Approximately 74.7% fine quartz-sand (F34) (depending on the amount of CaCO₃ needed, more than 50% by mass of particle size 0.05 mm to 0.2 mm)

	<ul style="list-style-type: none"> Approximately 0.3% calcium carbonate (CaCO₃) added to adjust pH to 6.0 ± 0.5 <p>The artificial substrate was moistened to approximately half of the final water content 2 days before the application. The additional water required to achieve the final water content was added when applying the test item.</p>
Substrate parameters	<p>pH at initiation: 6.4 to 6.5 pH at termination: 5.9 to 6.0 Water content at initiation: 22.6% to 23.5% (48.1% to 49.9% of the maximum water holding capacity*) Water content at termination: 21.1% to 22.3% (44.9% to 47.4% of the maximum water holding capacity*)</p> <p>*WHC = 47% of substrate dry weight</p>
Environmental conditions:	<p>Temperature: within 18°C to 22°C Light intensity: within 400 lux to 800 lux Photoperiod: 16:8 (light:dark)</p>
Feeding:	<p>Cheese mite (<i>Tyrophagus putrescentiae</i> cultured by IBACON) <i>ad libitum</i> after the introduction of the test organisms and on day 2, 5, 7, 9 and 12.</p>
Reference item:	<p>Perfekthion (a.s. Dimethoate, 400 g/L, nominal).</p>

Methodology

28.0 mg of XDE-729 Acid was dissolved in 20 mL acetone. A dilution series was prepared by adding 10 mL of acetone to 10 mL of the stock solution or corresponding dilution. 5 mL of the stock solution and the corresponding dilutions were added to 70 g aliquots of fine quartz sand (one per treatment and control group, control group treated with 5 mL acetone only). The treated sand was left for at least one hour under a fume hood until the solvent had evaporated. Each treated sand aliquot was added to 210 g artificial substrate (with reduced sand fraction). This composition was moistened with deionised water to achieve the required target percentage water holding capacity and mixed with a laboratory mixer for at least one minute to ensure a homogenous distribution.

20.0 g dry weight (± 1.0 g) of treated substrate was measured into appropriately labelled test vessels. The vessels were glass with a volume of 100 mL and a diameter of 5 cm. Four replicate vessels were prepared per treatment group and eight for the control group. For each group an additional replicate was prepared and used to measure pH and substrate water content after 14 days.

For each replicate 10 female *H. aculeifer* (aged 30 days from initial egg-laying) were collected with a fine brush, transferred to a glass tube and introduced to the substrate surface. Food in the form of cheese mites provided *ad libitum* were added and the vessel lid closed tightly. All replicates were ventilated and fed on day 2, 5,

7, 9 and 12. Vessel moisture content was checked on day 7 and replenishment of moisture found to not be required.

After 14 days of incubation the substrate from each replicate was filled into a Millipore pots with attached plastic container for collecting the escaping mites. These extraction units were placed in a Kempson extractor. The soil, including the mites, was exposed to temperatures of approximately 25°C and 30°C for approximately 2 days. Escaping mites were collected in a fixing liquid cooled to a temperature of approximately 16°C. The fixation liquid contained glycol and a detergent. Adult animals were counted once visually, juvenile animals were counted twice under binocular microscopes. The counting method had been previously validated with an efficiency of 92.2%.

Results

Mortality of adult female *Hypoaspis aculeifer* in the test item treated groups ranged from 13% (at 3.13 and 6.25 mg XDE-729 Acid/kg) to 20% (1.56 and 25 mg XDE-729 Acid/kg). The values were not significantly different compared to the control, where 8% of the adult mites were dead. Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be 25 mg XDE-729 Acid/kg substrate. The LOEC and LC₅₀ of XDE-729 Acid in artificial soil was estimated to be >25 mg XDE-729 Acid/kg dry substrate.

The No Observed Effect Concentration (NOEC) for reproduction was determined to be 12.5 mg XDE-729 Acid/kg substrate. The Lowest Observed Effect Concentration (LOEC) was therefore estimated to be 25 mg XDE-729 Acid/kg substrate. An EC₅₀ value of 23.31 mg XDE-729 Acid /kg substrate (Probit analysis, 95% confidence limits of 17.44 to 46.85 mg XDE-729 Acid/kg substrate) was calculated from the results.

Mean control group mortality was 8% (OECD validity criteria requires $\leq 20\%$). Mean control group juvenile production per replicate after 14 days was 181 (validity criteria requirement ≥ 50). The Coefficient of Variation for control juvenile numbers was 28.6% after 14 days (validity criteria $< 30\%$). Therefore all validity criteria were met and the test can be considered valid.

Table B.9.7.5: Effects of XDE-729 Acid on *H.aculeifer* mortality and reproduction

Concentration of XDE-729 Acid [mg/kg substrate dry weight]	Mean % mortality after 14 days	Mean No. of juveniles after 14 days	% change in number of juveniles compared to control
Control	8	181	-
1.56	20	171	94
3.13	13	180	99
6.25	13	159	87
12.5	15	145	80
25	20	82	45*
* significantly different compared to the control: Mortality: Fisher's Exact Test, $\alpha = 0.05$, one-sided greater Reproduction: Dunnett's t-test, $\alpha = 0.05$, one-sided smaller			

Conclusions

All guideline-stipulated validity criteria were met. XDE-729 Acid caused no significant effects on mortality of *Hypoaspis aculeifer* up to and including to 25 mg XDE-729 Acid/kg substrate, the highest concentration tested. The reproductive NOEC for this test was found to be 12.5 mg XDE-729 Acid/kg substrate. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 12.5 mg XDE-729 Acid/kg substrate. The positive control data provided with Perfekthion supports the suitability of the organism cultures used (reproduction $EC_{50} = 3.24$ mg a.s./kg substrate, between the expected levels of 3 and 7 mg a.s./kg substrate).

RMS Comment

This study is considered to be acceptable and hence suitable for use in risk assessment. The endpoint from this study to be used for risk assessment purposes is a NOEC of 12.5 mg XDE-729 Acid/kg substrate, which applies to reproduction, the more sensitive endpoint. Although only "recommended" by the guideline, a non-vehicle control would ideally have been included to demonstrate that solvent venting time was adequate and therefore any effects seen were test item toxicity and not residual solvent effects. As the control group also included solvent at the same rate there is no way to distinguish this. This is not considered to impact of any conclusions drawn as the vehicle control group of the test met all validity criteria.

Witte, B. (2011): Effects of XDE-729 Acid on Reproduction of the Collembola *Folsomia candida* in Artificial Soil with 5% Peat. IBACON 56865016. Dow AgroSciences unpublished report, Study Number 102024. March 25, 2011.

Test material

Test Item:	XDE-729 Acid
Test Substance Number:	TSN030751-0006
Purity:	XDE-729: 95.3% \pm 0.4% (wt/wt)
Lot No. :	E2837-52

Test system

Organism (Species):	Collembola (<i>Folsomia candida</i>) aged 10-12 days at test initiation
Study Type:	28-day reproduction test in artificial substrate
GLP Status:	Yes
Guidelines followed:	OECD Guideline 232 (Adopted 2009); ISO-Guideline 11267 (1999)
Guideline deviations reported by Study Director:	None
Parameters measured:	Mortality, behavioural effects, number of juveniles.
Test concentrations:	Control, 1.56, 3.13, 6.25, 12.5 and 25 mg XDE-729 Acid/kg soil dry weight.
Substrate type and constituents:	<p>Artificial substrate according to OECD 232:</p> <ul style="list-style-type: none"> • 5.0% Sphagnum-peat, air-dried and finely ground (with no visible plant remains) • 20% Kaolin clay (Kaolinite content > 30%) • Approximately 74.8% fine quartz-sand (F34) (depending on the amount of CaCO₃ needed, more than 50% by mass of particle size 0.05 mm to 0.2 mm) • Approximately 0.2% calcium carbonate (CaCO₃) added to adjust pH to 6.0 \pm 0.5 <p>The artificial substrate was moistened to approximately half of the final water content 2 days before the application. The additional water required to achieve the final water content was added when applying the test item.</p>
Substrate parameters	<p>pH at initiation: 6.4 to 6.5</p> <p>pH at termination: 6.4 to 6.5</p> <p>Water content at initiation: 22.6% to 23.5% (48.1% to 49.9% of the maximum water holding capacity*)</p> <p>Water content at termination: 19.6% to 21.3% (41.7% to 45.2% of the maximum water holding capacity*)</p>

	*WHC = 47% of substrate dry weight
Environmental conditions:	Temperature: within 18°C to 22°C Light intensity: within 400 lux to 800 lux Photoperiod: 16:8 (light:dark)
Feeding:	Ca 2 mg of granulated dry yeast per replicate on days 0 and 14
Reference item:	Boric Acid

Methodology

28.0 mg of XDE-729 Acid was dissolved in 20 mL acetone. A dilution series was prepared by adding 10 mL of acetone to 10 mL of the stock solution or corresponding dilution. 5 mL of the stock solution and the corresponding dilutions were added to 70 g aliquots of fine quartz sand (one per treatment and control group, control group treated with 5 mL acetone only). The treated sand was left for at least one hour under a fume hood until the solvent had evaporated. Each treated sand aliquot was added to 210 g artificial substrate (with reduced sand fraction). This composition was moistened with deionised water to achieve the required target percentage water holding capacity and mixed with a laboratory mixer for at least one minute to ensure a homogenous distribution.

30.0 g (\pm 1.0 g) of treated substrate was measured into appropriately labelled test vessels. The vessels were glass with a volume of 100 mL and a diameter of 5 cm. Four replicate vessels were prepared per treatment group and eight for the control group. For each group an additional replicate was prepared and used to measure pH and substrate water content after 28 days.

For each replicate 10 *f.candida* were collected with an aspirator, transferred to a glass tube and introduced to the substrate surface. Approximately 2 mg of dried granular yeast was added and the vessel lid closed tightly. Replicates were fed again at day 14 and their moisture content checked. All replicates were ventilated twice weekly.

At test completion (day 28) test vessel contents were suspended in water, dark ink added and the contents stirred. All *F.candida* present floated to the surface and were counted, adults by eye and juveniles by two counts under binocular microscope.

Results

Mortality of *Folsomia candida* in the test item treated groups ranged from 10% to 23%. The values were not significantly different compared to the control, where 14% of the adult Collembola were dead. Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be 25 mg XDE-729 Acid/kg substrate. The LC₅₀ of XDE-729 Acid for *Folsomia candida* in artificial soil was estimated to be >25 mg XDE-729 Acid/kg substrate.

For reproduction, the No Observed Effect Concentration (NOEC) was also determined to be 25 mg XDE-729 Acid/kg substrate and the EC₅₀ and the Lowest Observed Effect Concentration (LOEC) were estimated to be >25 mg XDE-729 Acid/kg substrate. No sub-lethal effects were observed.

Mean control group mortality was 14% (OECD validity criteria requires < 20%). Mean control group juvenile production per replicate after 28 days was 402 (validity criteria requirement ≥ 100). The Coefficient of Variation for control juvenile numbers was 29.1% after 28 days (validity criteria < 30%). Therefore all validity criteria were met and the test can be considered valid.

Table B.9.7.6: Effects of XDE-729 Acid on *Collembola* mortality and reproduction

Concentration of XDE-729 Acid [mg/kg substrate dry weight]	Mean % mortality after 28 days	Mean No. of juveniles after 28 days	% change in number of juveniles compared to control
Control	14	402	-
1.56	10	451	112
3.13	10	492	122
6.25	15	473	118
12.5	23	391	97
25	10	440	109
None of the values was significantly different compared to the control: Mortality: Fisher's Exact Test, $\alpha = 0.05$, one-sided greater Reproduction: Dunnett's t-test, $\alpha = 0.05$, one-sided smaller)			

Conclusions

All guideline-stipulated validity criteria were met. XDE-729 Acid caused no significant effects on mortality or reproduction of *Folsomia candida* up to and including to the concentration of 25 mg XDE-729 Acid/kg substrate. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be 25 mg XDE-729 Acid/kg substrate. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >25 mg XDE-729 Acid/kg substrate. The positive control data provided with Boric acid supports the suitability of the organism cultures used.

RMS Comment

This study is considered to be acceptable and hence suitable for use in risk assessment. The endpoint from this study to be used for risk assessment purposes is a NOEC of 25 mg XDE-729 Acid/kg substrate, which applies to both mortality and reproduction. Although not required by the guidelines, a non-vehicle control would ideally have been included to demonstrate that solvent venting time was adequate and therefore any effects seen were test item toxicity and not residual solvent effects. As the control group also included solvent at the same rate there is

no way to distinguish this. This is not considered to impact of any conclusions drawn as the vehicle control group of the test met all validity criteria.

Metabolites

Witte, B. (2011): Effects of X11449757 (metabolite of XDE-729) on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5% Peat. IBACON 56871089. Dow AgroSciences unpublished report, DAS Study Number 101154. June 27, 2011.

Test material

Test Item:	X11449757
Test Substance Number:	TSN031413-0003
Purity:	99%
Lot No. :	YB1-100780-103

Test system

Organism (Species):	<i>Hypoaspis aculeifer</i> (predatory mite)
Study Type:	14-day reproduction test in artificial substrate
GLP Status:	Yes
Guidelines followed:	OECD Guideline 226 (Adopted 2008)
Guideline deviations reported by Study Director:	None
Parameters measured:	Mortality of adult predatory mites, sub-lethal effects, number of juveniles.
Test concentrations:	Control, 1.56, 3.13, 6.25, 12.5 and 25 mg X11449757/kg soil dry weight.
Substrate type and constituents:	<p>Artificial substrate according to OECD 226:</p> <ul style="list-style-type: none"> • 5.0% Sphagnum-peat, air-dried and finely ground (2mm) • 20% Kaolin clay • Approximately 74.7% fine quartz-sand (F34) (depending on the amount of CaCO₃ needed, more than 50% by mass of particle size 0.05 mm to 0.2 mm) • Approximately 0.3% calcium carbonate (CaCO₃) added to adjust pH to 6.0 ± 0.5 <p>The artificial substrate was moistened to approximately half of the final water content 2 days before the application. The additional water required to achieve the final water content was added when applying the test item.</p>
Substrate parameters	pH at initiation: 6.4 to 6.5

	<p>pH at termination: 6.3 to 6.4</p> <p>Water content at initiation: 23.0% to 23.6% (48.9% to 50.3% of the maximum water holding capacity*)</p> <p>Water content at termination: 21.8% to 22.6% (46.4% to 48.0% of the maximum water holding capacity*)</p> <p>*WHC = 47% of substrate dry weight</p>
Environmental conditions:	<p>Temperature: within 18°C to 22°C</p> <p>Light intensity: within 400 lux to 800 lux</p> <p>Photoperiod: 16:8 (light:dark)</p>
Feeding:	Cheese mite (<i>Tyrophagus putrescentiae</i> cultured by IBACON) <i>ad libitum</i> after the introduction of the test organisms and on days 2, 5, 7, 9 and 12.
Reference item:	Perfekthion (a.s. Dimethoate, 400 g/L, nominal).

Methodology

Appropriate weighings of X11449757 (metabolite of XDE-729) were prepared separately for each concentration using an analytical balance. Fine quartz sand was added until a final weight of 125 g was reached. After mixing with a spoon to ensure a homogeneous distribution of the test item within the sand, the mixture was added to artificial substrate with reduced sand fraction equivalent to 500 g dry weight.

20.0 g dry weight (± 1.0 g) of treated substrate was measured into appropriately labelled test vessels. The vessels were glass with a volume of 100 mL and a diameter of 5 cm. Four replicate vessels were prepared per treatment group and eight for the control group. For each group an additional replicate was prepared and used to measure pH and substrate water content after 14 days.

For each replicate 10 female *H. aculeifer* (aged 30 days from initial egg-laying) were collected with a fine brush, transferred to a glass tube and introduced to the substrate surface. Food in the form of cheese mites provided *ad libitum* were added and the vessel lid closed tightly. All replicates were ventilated and fed on day 2, 5, 7, 9 and 12. Vessel moisture content was checked on day 7 and replenishment of moisture found to not be required.

After 14 days of incubation the substrate from each replicate was filled into a Millipore pots with attached plastic container for collecting the escaping mites. These extraction units were placed in a Kempson extractor. The soil, including the mites, was exposed to temperatures of approximately 25°C and 30°C for approximately 2.5 days. Escaping mites were collected in a fixing liquid cooled to a temperature of approximately 16°C. The fixation liquid contained glycol and a detergent. Adult animals were counted once visually, juvenile animals were counted twice under binocular microscopes. The counting method had been previously validated with an efficiency of 92.2%.

Results

Mortality of adult female *Hypoaspis aculeifer* in the test item treated groups ranged from 8% (at 6.25, 12.5 and 25 mg X11449757/kg) to 15% (1.56 mg X11449757/kg). The values were not significantly different compared to the control, where 5% of the adult mites were dead. Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be 25 mg X11449757/kg substrate. The LOEC and LC₅₀ of X11449757 in artificial substrate was estimated to be >25 mg X11449757/kg dry substrate.

The No Observed Effect Concentration (NOEC) for reproduction was also determined to be 25 mg X11449757/kg substrate. The Lowest Observed Effect Concentration (LOEC) was therefore estimated to be >25 mg X11449757/kg substrate.

Mean control group mortality was 5% (OECD validity criteria requires $\leq 20\%$). Mean control group juvenile production per replicate after 14 days was 325 (validity criteria requirement ≥ 50). The Coefficient of Variation for control juvenile numbers was 13% after 14 days (validity criteria $< 30\%$). Therefore all validity criteria were met and the test can be considered valid.

Table B.9.7.7: Effects of X11449757 on *H. aculeifer* mortality and reproduction

Concentration of X11449757 [mg/kg substrate dry weight]	Mean % mortality after 14 days	Mean No. of juveniles after 14 days	% change in number of juveniles compared to control
Control	5	325	-
1.56	15	323	99
3.13	13	270	83
6.25	8	304	93
12.5	8	258	79
25	8	288	89
* significantly different compared to the control Mortality: Fisher's Exact Test, $\alpha = 0.05$, one-sided greater Reproduction: Dunnett's t-test, $\alpha = 0.05$, one-sided smaller			

Conclusions

All guideline-stipulated validity criteria were met. XDE-729 Acid caused no significant effects on mortality or reproduction of *Hypoaspis aculeifer* up to and including to 25 mg X11449757/kg substrate, the highest concentration tested. The positive control data provided with Perfekthion supports the suitability of the organism cultures used (reproduction EC₅₀ = 3.24 mg a.s./kg substrate, between the expected levels of 3 and 7 mg a.s./kg substrate).

RMS Comment

This study is considered to be acceptable and hence suitable for use in risk assessment. The endpoint from this study to be used for risk assessment purposes is a NOEC of 25 mg X11449757/kg substrate, which applies to both mortality and reproduction.

Gerke, A. 2011: X11449757: Inhibition of Reproduction of Collembola, *Folsomia candida*, in Artificial Soil. ABC 66452. Dow AgroSciences unpublished report, Study Number 101153. 25 October 2011.

Test material

Test item:	X11449757
Purity:	98.6%
Description:	Off-white solid
Lot No./Batch No. :	YB1-100780-103

Test system

Organism (<i>Species</i>):	Collembola (<i>Folsomia candida</i>)
Study Type:	Reproduction inhibition study
GLP Status:	GLP
Guideline followed:	OECD Method 232
Guideline deviations reported by Study Director:	None.
Duration of study:	28 day
Test conditions:	Static
Parameters measured:	Survival and reproductive output i.e. number of juveniles.
Observation intervals:	Day 28
Age of test organisms at test initiation:	12 days
Test concentrations:	Nominal: 0 (Control), 0.31, 0.63, 1.3, 2.5, 5.0, and 10 mg X11449757/kg dry soil
Analytical confirmation of test concentrations:	None
Reference substances:	Boric acid
No. of holding days before dosing:	12 days
No. of Collembola per dose group:	50 (5 replicates of 10 collembola)
No. of Collembola per control group:	90 (9 replicates of 10 collembola)
Environmental conditions:	pH: 5.7 to 5.9 Photoperiod- 16 hrs light and 8 hours dark Light Intensity: 504 to 511 lux

Methodology

A 28-day test was performed with nominal test concentrations 0 (control), 0.34, 0.63, 1.3, 2.5, 5.0, and 10 mg X11449757/kg dry soil. The control was replicated nine times with 10 springtails per replicate, for a total of 90 organisms. Each treatment was replicated five times, and each replicate contained 10 springtails for a total of 50 organisms per treatment. A total of 10 collembola juveniles were impartially added to labelled glass containers. Each container was randomly assigned to a given treatment-replicate test jar or control. Collembola were fed approximately 6 mg of granulated dry yeast at initiation and on day 14 of the study. All test chambers were aerated periodically by opening the test chamber lids briefly.

Artificial pre-moistened soil was dosed with each treatment solution and mixed by hand to ensure homogenous distribution. The control consisted of pre-moistened artificial soil and deionised water. Approximately 30 g of this hydrated soil was added to each replicate test vessel.

At test termination, the artificial soil from each replicate jar was placed into a polypropylene dish and suspended with blended freshwater. Approximately six drops of black ink were added to the suspension of soil and water. The suspension was stirred gently with a glass pipette and allowed to settle for approximately two minutes. The adult and juvenile collembola, if present, floated to the surface and were counted. Adults were counted once and juveniles were counted twice. Any adults not found at termination were considered dead. The counts were made using Leica dissecting microscopes at 0.63 times magnification.

The moisture content was determined from samples of test medium collected from the control and all treatment levels at test initiation and termination. The soil pH was measured in samples taken from the parent soils at test initiation and in samples collected from an additional replicate test chamber from the control and all treatment levels at test termination. Soil temperature was measured continuously. Light intensity was measured at initiation.

Results**Table B.9.7.8: Summary of effects on mortality and reproduction of *Folsomia candida* following exposure to X11449757**

Parameter	X11449757 (mg X11449757/kg dry soil)						
	0 (Control)	0.31	0.63	1.3	2.5	5.0	10
Mean percent Survival after 28 days (%)	87	82	76	88	76	78	84
Mean percent mortality after 28 days (%)	13	18	24	12	24	22	16
Mean reproduction (no. juveniles) and %CV	133 (16)	128 (14)	112 (22)	112 (17)	112 (25)	101* (13)	110 (15)

* Statistically significant ($p = 0.05$) reduction in total number of young as compared to the controls. This reduction was not considered to be concentration dependent as there was no significant reduction in the higher 10 mg/kg dry soil treatment.

Table B.9.7.9: Summary of study endpoints from testing X11449757 with *F.candida*

Biological Parameter	NOEC (mg X11449757/ kg dry soil)	LOEC (mg X11449757/ kg dry soil)	EC ₅₀ (95% confidence limits)	LC ₅₀ (95% confidence limits)
Adult Survival	10	>10	-	>10 (not calculated)
Total number of young	10	>10	>10 (not calculated)	-

Conclusions

The control organisms met the acceptability criteria for mean adult survival (>80%) and for reproduction (CV less than 30%) as specified by the study protocol.

There was no statistically significant effects ($p = 0.05$) on mortality as compared to the control. There was a statistically significant ($p = 0.05$) reduction in total number of young produced in the 5.0 mg X11449757/kg dry soil treatment group as compared to the control. However, this is not expected to be test item related based on lack of statistically significant reduction of young produced at the higher concentration. The estimated 28 day LC₅₀ and EC₅₀ values for adult survival and

total young produced was >10 mg a X11449757/kg dry soil, the highest concentration tested. The NOEC and LOEC values for both adult survival and reproduction were 10 and >10 mg X11449757/kg dry soil, respectively.

RMS Comment: The study is considered to be acceptable and suitable for use in risk assessments. The key endpoint from the study is a NOEC of 10 mg X11449757/kg dry soil.

B.9.7.1 Toxicity

Usually, the artificial substrate of the earthworm laboratory test has a higher organic carbon content than many natural soils, the LC_{50} or NOEC could potentially be lower if the test were conducted in natural soil (Van Gestel 1992). To account for this difference the LC_{50} and NOEC are usually divided by 2 (to give an $LC_{50\text{ corr}}$ /NOEC_{corr} endpoint) when the $\log P_{ow}$ is greater than 2 (EPPO 2002a). The laboratory studies conducted above used an artificial soil with a lower organic carbon content than the OECD guideline suggested (5% rather than 10%).

It is the opinion of EFSA, that for substances with a $\log P_{ow} > 2$, the correction factor should be applied to toxicity endpoints unless it has been demonstrated that lowering the organic carbon content allows for the correction factor to not be used. As this has not been demonstrated, corrected endpoints will be used in the risk assessment below for those test items with a $\log P_{ow}$ of >2 (XDE-729 Methyl $\log P_{ow}$ of 3.76). X11393729 (XDE-729 acid) and soil metabolite X11449757 both have a $\log P_{ow}$ value of < 2 (-0.83 and < 0.3 respectively) and so no correction factor has been applied to the toxicity endpoints. For completeness, uncorrected endpoints have also been presented in the table below.

Table B.9.7.10: Summary of available toxicity data for soil macro-organisms

Type of study and test compound	Species	Toxicity	Corrected Toxicity*	Reference
XDE-729 methyl	Collembola (<i>Folsomia candida</i>)	NOEC = 1000 mg a.s./kg d.w soil	NOEC _{corr} 500 mg a.s./kg d.w soil	Gerke, A. (2011g) IIA 8.9.2/05
	Predatory mite (<i>Hypoaspis aculeifer</i>)	NOEC = 25 mg a.s./kg d.w soil	NOEC _{corr} 12.5 mg a.s./kg d.w soil	Luhers, Ulf, (2011) IIA 8.9.2/04
XDE-729 acid	Collembola (<i>Folsomia candida</i>)	NOEC = 25 mg/kg d.w soil		Witte, B. , (2011b) IIA 8.9.2/07
	Predatory mite (<i>Hypoaspis aculeifer</i>)	NOEC 12.5 mg/kg d.w soil	--	Witte, B. , (2011a) IIA 8.9.2
X11449757	Collembola (<i>Folsomia candida</i>)	NOEC 10 mg/kg d.w soil	--	Gerke, A., (2011h) IIA 8.9.2/09
	Predatory mite (<i>Hypoaspis aculeifer</i>)	NOEC 25 mg/kg d.w soil	--	Witte, B. , (2011c) IIA 8.9.2/08

* All soil used in laboratory experiments contained 5% organic matter. However corrected endpoints are provided for studies with XDE-729 methyl as the active substance has a log Pow > 2.

B.9.7.2 Risk assessment

PEC_{soil} values and details on PEC calculations are provided in Annex B.8. Assuming the GAP details stated in table 9.1, mixing over 5 cm depth and bulk density of 1.5 cm³, the initial PEC_{soil} values after the second application for XDE-729 Methyl and after a single application for the metabolites X11393729 (XDE-729 acid) and X11449757 are listed in table 9.7.11.

Table 9.7.11: Initial predicted environmental concentrations (PEC_{soil}) for XDE-729 Methyl, X11393729 (XDE-729 acid), X11449757

Compound	Maximum initial PEC soil (mg/kg dry soil)
XDE-729 Methyl	0.009
X11393729 (XDE-729 acid)	0.005
X11449757	0.002

In laboratory aerobic degradation studies, XDE-729 methyl degraded relatively rapidly, but exhibited slower degradation in field dissipation studies with a worst case SFO DT₅₀ of 43 days. For X11393729 (XDE-729 acid) and X11449757, these metabolites showed a worst-case top-down DT₅₀ of 264 and 197 days, respectively. Given the apparent persistence of these substances, a maximum total dose approach has been used for the PEC_{soil} calculation and an accumulation calculation has also been conducted.

Table 9.7.12: Peak accumulation predicted environmental concentrations (PEC_{soil}) for X11393729 and X11449757

Compound	Peak accumulation PEC soil (mg/kg dry soil)
X11393729 (XDE-729 acid)	0.009
X11449757	0.0025

For the risk assessment, the peak accumulation PEC_{soil} values have been used for X11393729 (XDE-729 acid) and X11449757.

This risk assessment is based on the SANCO/10329/2002 guidance document for terrestrial ecotoxicology.

Table B.9.7.13: Chronic risk assessment to soil macro-organisms

Test substance	Species	NOEC	Maximum PEC _{soil} (mg/kg dry soil)	TER
XDE-729 Methyl	Collembola (<i>Folsomia candida</i>)	NOEC _{corr} 500 mg a.s./kg d.w soil	0.009	55556
	Predatory mite (<i>Hypoaspis aculeifer</i>)	NOEC _{corr} 12.5 mg a.s./kg d.w soil		1389
XDE-729 acid (X11393729)	Collembola (<i>Folsomia candida</i>)	NOEC 25 mg/kg d.w soil	0.009	2778
	Predatory mite (<i>Hypoaspis aculeifer</i>)	NOEC 12.5 mg/kg d.w soil		1389
X11449757	Collembola (<i>Folsomia candida</i>)	NOEC 10 mg/kg d.w soil	0.0025	4000
	Predatory mite (<i>Hypoaspis aculeifer</i>)	NOEC 25 mg/kg d.w soil		10000

All TER values are above the trigger value of 5. Consequently, the chronic risk to soil macro-organisms posed by XDE-729 Methyl and the major metabolites XDE-729 Acid (X11393729) and X11449757 is acceptable.

B.9.7.3 Conclusions

An acceptable chronic risk to non target soil macro organisms has been demonstrated for XDE-729 Methyl and the major metabolites XDE-729 Acid (X11393729) and X11449757, no further consideration is required.

B.9.8 Effects on soil non-target micro-organisms (IIA 8.5, IIIA 10.7)**B.9.8.1 Toxicity**

Feil, N. (2011): Effects of XR-729 Methyl on the Activity of the Soil Microflora in the Laboratory; Institut für Biologische Analytik, und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. IBACON report No. 49527080, Dow Study No. 101127. Revised Final Report Date: June 27, 2011.

Test material

Test item:	XR-729 Methyl
Active Substance / Content:	XDE-729 Methyl: 97.2%
Description:	Solid
Lot No./Batch No. :	E2837-51

Test system

Organism (Species):	Soil micro organisms
Study Type:	Laboratory study with OECD guideline natural soil, assessed for: Nitrate formation Microbial respiration
GLP Status:	GLP
Guidelines followed:	OECD Guideline 216 – Soil microorganisms – Nitrogen Transformation Test OECD Guideline 217 – Soil microorganisms – Carbon Transformation Test
Guideline deviations reported by Study Director	None
Duration of study:	28 days
Parameters measured:	Nitrogen transformation: analysis of nitrate, nitrite and ammonium in extracted soil samples, via ion chromatography; limits of quantification: NO ₃ -N: 0.117mg/kg soil dry weight NO ₂ -N: 0.075 mg/kg soil dry weight NH ₄ -N: 0.708 mg/kg soil dry weight soil water content 49% to 50% pH 7.1 to 7.2 Microbial respiration : soil respiration rates after addition of glucose 2 g/kg soil water content 44% to 48% pH 7.0 -7.1
Observation intervals:	0, 7, 14 and 28 days
Test concentrations:	Low Dose: 0.0107 mg/kg XR-729 Methyl soil dry weight

	High Dose: 0.0535 mg/kg XR-729 Methyl soil dry weight
Toxic reference	Sodium chloride The inhibition of soil respiration and nitrogen transformation by sodium chloride at a concentration of 16 g/kg soil dry weight will be determined once a year as a means of assuring that the laboratory test conditions are adequate and have not changed significantly.
Method of test item application	Incorporation into the soil
Environmental conditions:	Conducted in the dark. Temperature: 20-22°C (SD) pH: 7.0 – 7.2 Soil moisture: 44 to 50% of its maximum water holding capacity.
Soil properties	Soil source: The soil batch used in this study was according to the Guidelines and was taken from fallow grassland. District authority: Darmstadt-Dieburg Municipality: 64380 Rossdorf, Germany Geographical position: Longitude 8° 44' 38.70" E; Latitude 49° 51' 59.59" N Moisture content of soil at start: 44% - 49% of MWHC Moisture content of soil at end: 45% - 50% of MWHC Clay (%): 9.4 Silt (%): 33.1 Sand (%): 57.5 Organic Carbon(%): 1.00 Textural classification: Mid loamy sand

Methodology

Determination of soil respiration in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A BSB-Sensomat System® was used to determine the CO₂-production over a period of up to 24 hours at different sampling intervals.

Determination of nitrogen-transformation (ammonium-, nitrite- and nitrate-nitrogen levels) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. NH₄-, NO₂- and NO₃-nitrogen formed from the nitrification process were determined by means of a Dionex ion chromatography system (DX-120 IC, AS 50 autosampler, ECD and UVD 340S UV photometer).

Disposable plastic boxes (discarded at test end); each box contained different soil masses for the two tests: soil respiration test: 750 g to 1000 g soil (dry weight), box size approximately 1 L, (height:width:depth = 0.12:0.165:0.065 m), filled up to 6 cm 411 nitrogen turnover test: 250 g to 500 g (dry weight), box size approximately 0.5 L, (height:width:depth = 0.1:0.1:0.065 m), filled up to 6 cm The soil was loosely filled into the boxes, which were covered by perforated lids to enable a slight, but sufficient air exchange (to ensure aerobic incubation conditions).

Results

Table B.9.8.1 Effects of XR-729 Methyl on the Nitrate formation rate

Interval sampling days	Control	0.0107 mg/kg XR-729 Methyl soil dw			0.0535 mg/kg XR-729 Methyl soil dw		
	[mg/kg/day ¹]	[mg/kg/day ¹]	[% ²]	[sig ³]	[mg/kg/day ¹]	[% ²]	[sig ³]
0-7	-2.84	-2.63	-7.39	*	-2.85	0.35	n. s.
7-14	1.29	1.30	0.78	n. s.	1.20	-6.98	n. s.
14-28	1.74	1.43	-17.82	*	1.41	-18.97	*
1: mean mg NO ₃ -N/kg soil dry weight per day between sampling dates (incremental) 2: deviation from control 3: statistical significance *: significant n.s.: not significant							

Table B.9.8.2 Effects of XR-729 Methyl on the Respiration rate

Interval sampling days	Control	0.0107 mg/kg XR-729 Methyl soil dw			0.0535 mg/kg XR-729 Methyl soil dw		
	Respiration Rate ¹	Respiration Rate ¹	[% ²]	[sig ³]	Respiration Rate ¹	[% ²]	[sig ³]
0	11.700	11.788	0.75	n. s.	11.249	-3.85	n. s.
7	9.790	9.555	-2.40	n. s.	9.396	-4.02	n. s.
14	7.475	7.685	2.81	n. s.	7.480	0.07	n. s.
28	9.493	9.450	-0.45	n. s.	9.070	-4.46	n. s.
1: respiration, mean of 3 replicates 2: deviation from control 3: statistical significance *: significant n.s.: not significant							

Conclusions

The variation between the replicate control samples clearly matched the validity criterion of 15% for both the carbon and nitrogen transformation test.

The soil respiration rates were within the trigger value of $\pm 25\%$ set by OECD guideline 217 at day 28. The soil nitrate content was terminated on day 28 when the difference between the control and XR-729 Methyl treatments were below the 25% trigger value given by the OECD 216 guideline. Based on the results of this study, XR-729 Methyl has no impact on respiration activity, soil nitrate content and soil nitrate formation rate of soil microflora when applied up to and including 0.0535 mg/kg soil dry weight. It can be concluded that XR-729 Methyl will not have any long term influence on soil microflora.

RMS Comment: The study is considered to be acceptable and suitable for use for risk assessment purposes. The endpoint from this study is that there were less than 25% effects compared to the control on both respiration and nitrogen formation rate at 0.0535 mg a.s./kg dry weight soil.

Feil, N. (2010): Effects of XDE-729 acid on the Activity of the Soil Microflora in the Laboratory; Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. IBACON report No. 56863080, Dow Study No. 101143. November 10, 2010.

Test material

Test item:	XDE-729 acid
Active substance / Content:	XDE-729 acid: 95.3% \pm 0.4% (wt/wt)
Description:	Solid
Lot No./Batch No. :	E2837-52

Test system

Organism (Species):	Soil micro organisms
Study Type:	Laboratory study with OECD guideline natural soil, assessed for: Nitrate formation Microbial respiration
GLP Status:	GLP
Guidelines followed:	OECD Guideline 216 – Soil microorganisms – Nitrogen Transformation Test OECD Guideline 217 – Soil microorganisms – Carbon Transformation Test
Guideline deviations reported by Study Director:	None
Duration of study:	28 days
Parameters measured:	Nitrogen transformation: analysis of nitrate, nitrite and ammonium in extracted soil samples, via ion chromatography; limits of quantification: NO ₃ -N: 0.144 mg/kg soil dry weight NO ₂ -N: 0.100 mg/kg soil dry weight NH ₄ -N: 0.578 mg/kg soil dry weight

	<p>soil water content 45% to 47% pH 7.1 to 7.2</p> <p>Microbial respiration:</p> <p>soil respiration rates after addition of glucose 3 g/kg soil water content 43% to 46% pH 7.0 -7.1</p>
Observation intervals:	0, 7, 14 and 28 days
Test concentrations:	<p>Low Dose:</p> <p>0.01 mg XDE-729 acid/kg soil dry weight</p> <p>High Dose:</p> <p>0.05 mg XDE-729 acid/kg soil dry weight</p>
Toxic reference	<p>Sodium chloride</p> <p>The inhibition of soil respiration and nitrogen transformation by sodium chloride at a concentration of 16 g/kg soil dry weight will be determined at least once a year as a means of assuring that the laboratory test conditions are adequate and have not changed significantly.</p>
Method of test item application	Incorporation into the soil
Environmental conditions:	<p>Conducted in the dark.</p> <p>Temperature: 20-22°C (SD)</p> <p>pH: 7.0 – 7.2</p> <p>Soil moisture: 43 to 47% of its maximum water holding capacity.</p>
Soil properties	<p>Soil source: The soil batch used in this study was according to the Guidelines and was taken from fallow grassland:</p> <p>District authority: Darmstadt-Dieburg Municipality: 64380 Rossdorf, Germany</p> <p>Geographical position: longitude 8° 44' 38.70" E latitude 49° 51' 59.59" N</p> <p>Moisture content of soil at start: 43% - 45% of MWHC Moisture content of soil at end: 45% - 47% of MWHC</p> <p>Clay (%): 9.4</p>

	<p>Silt (%): 33.1</p> <p>Sand (%): 57.5</p> <p>Organic Carbon (%): 0.93</p> <p>Textural classification: Mid loamy sand</p>
--	--

Determination of soil respiration in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A BSB-Sensomat System® was used to determine the CO₂-production over a period of up to 24 hours at different sampling intervals.

Determination of nitrogen-transformation (ammonium-, nitrite- and nitrate-nitrogen levels) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. NH_4^- , NO_2^- and NO_3^- -nitrogen formed from the nitrification process were determined by means of a Dionex ion chromatography system (DX-120 IC, AS 50 autosampler, ECD and UVD 340S UV photometer).

Disposable plastic boxes (discarded at test end); each box contained different soil masses for the two tests: soil respiration test: 750 g to 1000 g soil (dry weight), box size approximately 1 L, (height:width:depth = 0.12:0.165:0.065 m), filled up to 6 cm 411 nitrogen turnover test: 250 g to 500 g (dry weight), box size approximately 0.5 L, (height:width:depth = 0.1:0.1:0.065 m), filled up to 6 cm The soil was loosely filled into the boxes, which were covered by perforated lids to enable a slight, but sufficient air exchange (to ensure aerobic incubation conditions).

Results

Table B.9.8.3 Effects of XDE-729 acid on the Nitrate formation rate

Interval sampling days	Control	0.01 mg/kg XDE-729 acid soil dw			0.05 mg/kg XDE-729 acid soil dw		
	[mg/kg/day ¹]	[mg/kg/day ¹]	[% ²]	[sig ³]	[mg/kg/day ¹]	[% ²]	[sig ³]
0-7	-1.37	-1.54	12.41	n.s.	-1.54	12.41	n.s.
7-14	1.14	1.27	11.40	*	1.23	7.89	n.s.
14-28	1.44	1.51	4.86	n.s.	1.39	-3.47	n.s.

1: mean mg NO₃-N/kg soil dry weight per day
2: deviation from control
3: statistical significance
*: significant
n.s.: not significant

Table B.9.8.4 Effects of XDE-729 acid on the Respiration rate

Interval sampling days	Control	0.01 mg/kg XDE-729 acid soil dw			0.05 mg/kg XDE-729 acid soil dw		
	Respiration Rate ¹	Respiration Rate ¹	[% ²]	[sig ³]	Respiration Rate ¹	[% ²]	[sig ³]
0	14.214	14.067	-1.03	n.s.	13.467	-5.26	n.s.
7	12.532	14.372	14.68	*	13.450	7.33	n.s.
14	12.576	12.985	3.25	n.s.	12.660	0.67	n.s.
28	10.674	10.689	0.14	n.s.	10.811	1.28	n.s.
1: respiration, mean of 3 replicates 2: deviation from control 3: statistical significance *: significant n.s.: not significant							

Conclusions

The soil respiration rates were within the trigger value of $\pm 25\%$ set by OECD guideline 217 at day 28. The soil nitrate content was terminated on day 28 when the difference between the control and XDE-729 acid treatments were below the 25% trigger value given by the OECD 216 guideline. Based on the results of this study, XDE-729 acid has no impact on respiration activity, soil nitrate content and soil nitrate formation rate of soil microflora when applied up to and including 0.05 mg/kg soil dry weight. It can be concluded that XDE-729 acid will not have any long term influence on soil microflora.

RMS Comment The study is considered to be acceptable and suitable for use for risk assessment purposes. The endpoint from this study is that there were less than 25% effects compared to the control on both respiration and nitrogen formation rate at 0.05 mg XDE-acid/kg dry weight soil.

Feil, N. (2011): Effects of X11449757 on the Activity of the Soil Microflora in the Laboratory; Institut für Biologische Analytik, und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. IBACON report No. 56874080, Dow Study No. 101157. May 25, 2011.

Test material

Test item:	X11449757
Content:	4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl) picolinic acid 99.0%
Description:	Solid
Lot No./Batch No. :	YB1-100780-103

Test system

Organism (Species):	Soil micro organisms
Study Type:	Laboratory study with OECD guideline natural soil, assessed for: Nitrate formation Microbial respiration
GLP Status:	GLP
Guidelines followed:	OECD Guideline 216 – Soil microorganisms – Nitrogen Transformation Test OECD Guideline 217 – Soil microorganisms – Carbon Transformation Test
Guideline deviations reported by Study Director:	None
Duration of study:	28 days
Parameters measured:	Nitrogen transformation: analysis of nitrate, nitrite and ammonium in extracted soil samples, via ion chromatography; limits of quantification: NO ₃ -N: 0.121 mg/kg soil dry weight NO ₂ -N: 0.086 mg/kg soil dry weight NH ₄ -N: 0.186 mg/kg soil dry weight soil water content 49 to 51% pH 7.1 to 7.2 Microbial respiration : soil respiration rates after addition of glucose 2 g/kg moist soil soil water content 50% to 53% pH 7.0 -7.1
Observation intervals:	0, 7, 14 and 28 days
Test concentrations:	Low Dose: 0.01 mg/kg X11449757 soil dry weight High Dose: 0.052 mg/kg X11449757 soil dry weight
Toxic reference:	Sodium chloride The inhibition of soil respiration and nitrogen transformation by sodium chloride at a concentration of 16 g/kg soil dry weight is determined at least once a year as a means of assuring that the laboratory test conditions are adequate and have not changed significantly.
Method of test item application:	Incorporation into the soil
Environmental conditions:	Conducted in the dark.

	Temperature: 20-22°C (SD) pH: 7.0 – 7.2 Soil moisture: 50 to 53% of its maximum water holding capacity.
Soil properties:	Soil source: The soil batch used in this study was according to the Guidelines and was taken from fallow grassland: District authority: Darmstadt-Dieburg Municipality: 64380 Rossdorf, Germany Geographical position: Longitude 8° 44' 38.70" E Latitude 49° 51' 59.59" N Moisture content of soil at start: 50% - 51% of MWHC Moisture content of soil at end: 50% of MWHC Clay (%): 8.3 Silt (%): 34.6 Sand (%): 57.1 Organic Carbon (%): 1.42 Textural classification: Silty loamy sand

Methodology

Determination of soil respiration in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A BSB-Sensomat System® was used to determine the CO₂-production over a period of up to 24 hours at different sampling intervals.

Determination of nitrogen-transformation (ammonium-, nitrite- and nitrate-nitrogen levels) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. NH₄-, NO₂- and NO₃-nitrogen formed from the nitrification process were determined by means of a Dionex ion chromatography system (DX-120 IC, AS 50 autosampler, ECD and UVD 340S UV photometer).

Disposable plastic boxes (discarded at test end); each box contained different soil masses for the two tests: soil respiration test: 750 g to 1000 g soil (dry weight), box size approximately 1 L, (height:width:depth = 0.12:0.165:0.065 m), filled up to 6 cm 41; nitrogen turnover test: 250 g to 500 g (dry weight), box size approximately 0.5 L, (height:width:depth = 0.1:0.1:0.065 m), filled up to 6 cm The soil was loosely filled into the boxes, which were covered by perforated lids to enable a slight, but sufficient air exchange (to ensure aerobic incubation conditions).

Results

Table B.9.8.5: Effects of X11449757 on the Nitrate formation rate

Interval sampling days	Control	0.01 mg/kg X11449757 soil dw			0.052 mg/kg X11449757 soil dw		
	[mg/kg/day ¹]	[mg/kg/day ¹]	[% ²]	[sig ³]	[mg/kg/day ¹]	[% ²]	[sig ³]
0-7	-2.07	-2.03	-1.9	n.s.	-2.06	-0.5	n.s.
7-14	1.24	1.28	3.2	n.s.	1.39	12.1	*
14-28	2.03	1.97	-3.0	n.s.	1.99	-2.0	n.s.
1: mean mg NO ₃ -N/kg soil dry weight per day 2: deviation from control 3: statistical significance *: significant n.s.: not significant dw: dry weight							

Table B.9.8.6: Effects of X11449757 on the Respiration rate

Interval sampling days	Control	0.01 mg/kg X11449757 soil dw			0.052 mg/kg X11449757 soil dw		
	Respiration Rate ¹	Respiration Rate ¹	[% ²]	[sig ³]	Respiration Rate ¹	[% ²]	[sig ³]
0	9.615	9.844	2.38	n.s.	10.009	4.10	n.s.
7	8.339	8.926	7.04	n.s.	9.048	8.50	n.s.
14	9.170	8.678	-5.37	n.s.	9.791	6.77	n.s.
28	10.805	11.150	3.19	n.s.	11.093	2.67	n.s.
1: respiration, mean of 3 replicates 2: deviation from control 3: statistical significance *: significant n.s.: not significant dw: dry weight							

Conclusions

The soil respiration rates were within the trigger value of $\pm 25\%$ set by OECD guideline 217 at day 28. The soil nitrate content was terminated on day 28 when the difference between the control and X11449757 treatments were below the 25% trigger value given by the OECD 216 guideline. Based on the results of this study, X11449757 has no impact on respiration activity, soil nitrate content and soil nitrate formation rate of soil microflora when applied up to and including 0.052 mg/kg soil dry weight. It can be concluded that X11449757 will not have any long term influence on soil microflora.

RMS Comment The study is considered to be acceptable and suitable for use for risk assessment purposes. The endpoint from this study is that there were less

than 25% effects compared to the control on both respiration and nitrogen formation rate at 0.052 mg X11449757 /kg dry weight soil.

Feil, N. (2011): Effects of GF-2573 on the Activity of the Soil Microflora in the Laboratory; Institut für Biologische Analytik, und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany. IBACON report No. 54804080, Dow Study No. 090101. Revised Final Report Date: June 21, 2011.

Test material

Test item:	GF-2573
Active substance / Content:	XR-729 methyl: 0.84% wt/wt (7.6 g/L) cloquintocet-mexyl: 0.81% wt/wt (7.3 g/L)
Description:	Liquid
Lot No./Batch No. :	E2837-57

Test system

Organism (Species):	Soil micro organisms
Study Type:	Laboratory study with OECD guideline natural soil, assessed for: Nitrate formation Microbial respiration
GLP Status:	GLP
Guidelines followed:	OECD Guideline 216 – Soil microorganisms – Nitrogen Transformation Test OECD Guideline 217 – Soil microorganisms – Carbon Transformation Test
Guideline deviations reported by Study Director:	None
Duration of study:	28 days
Parameters measured:	Nitrogen transformation: analysis of nitrate, nitrite and ammonium in extracted soil samples, via ion chromatography; limits of quantification: NO ₃ -N: 0.123 mg/kg soil dry weight NO ₂ -N: 0.084 mg/kg soil dry weight NH ₄ -N: 0.562 mg/kg soil dry weight soil water content 47% to 50% pH 7.1 to 7.2 Microbial respiration : soil respiration rates after addition of glucose 3 g/kg soil water content 46% to 49%

	pH 7.0 -7.1
Observation intervals:	0, 7, 14 (15) and 28 days
Test concentrations:	Low Dose: 1.207 mg/kg GF-2573 soil dry weight High Dose: 6.037 mg/kg GF-2573 soil dry weight
Toxic reference	Sodium chloride The inhibition of soil respiration and nitrogen transformation by sodium chloride at a concentration of 16 g/kg soil dry weight will be determined at least once a year as a means of assuring that the laboratory test conditions are adequate and have not changed significantly.
Method of test item application	Incorporation into the soil
Environmental conditions:	Conducted in the dark. Temperature: 20-22°C (SD) pH: 7.0 – 7.1 Soil moisture: 46% to 49% of its maximum water holding capacity.
Soil properties	Soil source: The soil batch used in this study was according to the Guidelines and was taken from fallow grassland: District authority: Darmstadt-Dieburg Municipality: 64380 Rossdorf, Germany Geographical position: Longitude 8° 44' 38.70" E Latitude 49° 51' 59.59" N Moisture content of soil at start: 46% - 47% of MWHC Moisture content of soil at end: 46% - 50% of MWHC Clay (%): 9.4 Silt (%): 33.1 Sand (%): 57.5 Organic Carbon (%): 0.93 Textural classification: Mid loamy sand

Methodology

Determination of soil respiration in soil after addition of glucose. Comparison of test item treated soil with a non-treated soil. Three replicates per treatment and concentration. A BSB-Sensomat System® was used to determine the CO₂-production over a period of up to 24 hours at different sampling intervals.

Determination of nitrogen-transformation (ammonium-, nitrite- and nitrate-nitrogen levels) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. Three replicates per

Disposable plastic boxes (discarded at test end); each box contained different soil masses for the two tests: soil respiration test: 750 g to 1000 g soil (dry weight), box size approximately 1 L, (height:width:depth = 0.12:0.165:0.065 m), filled up to 6 cm 411 nitrogen turnover test: 250 g to 500 g (dry weight), box size approximately 0.5 L, (height:width:depth = 0.1:0.1:0.065 m), filled up to 6 cm. The soil was loosely filled into the boxes, which were covered by perforated lids to enable a slight, but sufficient air exchange (to ensure aerobic incubation conditions).

Table B.9.8.7: Effects of GF-2573 on the Nitrate formation rate

Interval sampling days	Control	1.207 mg/kg GF-2573 soil dw			6.037 mg/kg GF-2573 soil dw		
	[mg/kg/day ¹]	[mg/kg/day ¹]	[% ²]	[sig ³]	[mg/kg/day ¹]	[% ²]	[sig ³]
0-7	-2.31	-2.13	-7.79	*	-2.03	-12.12	n.s.
7-14	1.11	1.17	5.41	n.s.	1.19	7.21	n.s.
14-28	1.72	1.55	-9.88	n.s.	1.68	-2.33	n.s.

1: mean mg NO₃-N/kg soil dry weight per day
2: deviation from control
3: statistical significance
*: significant
n.s.: not significant

Table B.9.8.8: Effects of GF-2573 on the Respiration rate

Interval sampling days	Control	1.207 mg/kg GF-2573 soil dw			6.037 mg/kg GF-2573 soil dw		
	Respiration Rate ¹	Respiration Rate ¹	[% ²]	[sig ³]	Respiration Rate ¹	[% ²]	[sig ³]
0	14.109	14.887	5.51	n.s.	13.798	-2.20	n.s.
7	14.103	14.169	0.47	n.s.	13.293	-5.74	*
15+	12.008	12.119	0.92	n.s.	11.538	-3.91	n.s.
28	11.423	12.021	5.24	n.s.	11.124	-2.62	n.s.

1: respiration, mean of 3 replicates (except day 7 only 2 replicates in the higher rate)
 2: deviation from control
 3: statistical significance
 *: significant
 n.s.: not significant
 + day 15 due to a measurement failure on day 14

Conclusions

The soil respiration rates were within the trigger value of $\pm 25\%$ set by OECD guideline 217 at day 28. The soil nitrate content was terminated on day 28 when the difference between the control and GF-2573 treatments were below the 25% trigger value given by the OECD 216 guideline. Based on the results of this study, GF-2573 has no impact on respiration activity, soil nitrate content and soil nitrate formation rate of soil microflora when applied up to and including 6.037 mg/kg soil dry weight. It can be concluded that GF-2573 will not have any long term influence on soil microflora.

RMS Comment: The study is considered to be acceptable and suitable for use for risk assessment purposes. The endpoint from this study is that there were less than 25% effects compared to the control on both respiration and nitrogen formation rate at 6.037 mg Formⁿ/kg dry weight soil.

B.9.8.2 Risk assessment

PEC_{soil} values and details on PEC calculations are provided in Annex B.8. Assuming the GAP details stated in table 9.1 (mixing over 5 cm depth and bulk density of 1.5 cm³), the initial PEC_{soil} values after the second application for XDE-729 Methyl and after a single application for the metabolites X11393729 (XDE-729 acid), X11449757 and the formulation GF-2573 are listed in table 9.8.9.

Table 9.8.9: Initial predicted environmental concentrations (PEC_{soil}) for XDE-729 Methyl, X11393729 (XDE-729 acid), X11449757

Compound	Maximum initial PEC soil (mg/kg dry soil)
XDE-729 Methyl	0.009
GF-2573	0.905
X11393729 (XDE-729 acid)	0.005
X11449757	0.002

In laboratory aerobic degradation studies, XDE-729 methyl degraded relatively rapidly, but exhibited slower degradation in field dissipation studies with a worst case SFO DT₅₀ of 43 days. For X11393729 (XDE-729 acid) and X11449757, these metabolites showed a worst-case top-down DT₅₀ of 264 and 197 days, respectively. Given the apparent persistence of these substances, a maximum total dose approach has been used for the PEC_{soil} calculation and an accumulation calculation has also been conducted.

Table 9.8.10: Peak accumulation predicted environmental concentrations (PEC_{soil}) for X11393729 and X11449757

Compound	Peak accumulation PEC soil (mg/kg dry soil)
X11393729 (XDE-729 acid)	0.009
X11449757	0.0025

For the risk assessment, the peak accumulation PEC_{soil} values have been used for X11393729 (XDE-729 acid) and X11449757.

This risk assessment is based on the SANCO/10329/2002 guidance document for terrestrial ecotoxicology.

Table B.9.8.11: Summary of endpoints to be used in the risk assessment for soil micro-organisms

Test substance	Test substance	Time scale	Toxicity endpoint
XDE-729 Methyl	Nitrogen mineralisation	28 days	<25% effect at day 28 at 0.0535 mg a.s./kg d.w.soil
	Carbon mineralisation		
XDE-729 acid (X11393729)	Nitrogen mineralisation		<25% effect at day 28 at 0.05 mg XDE-729 acid/kg d.w.soil
	Carbon mineralisation		
X11449757	Nitrogen mineralisation		<25% effect at day 28 at 0.052 mg a.s./kg d.w.soil
	Carbon mineralisation		
GF-2573	Nitrogen mineralisation		<25% effect at day 28 at 6.037 mg product/kg d.w.soil
	Carbon mineralisation		

Table B.9.8.12: Comparison of the PEC_{soil} values with the maximum soil concentrations tested

Test substance	Test substance	NOEC (<25% effects) (mg a.s./kg d.w soil)	PEC _{soil} (mg a.s./kg d.w soil)
XDE-729 Methyl	Nitrogen mineralisation	0.0535	0.009
	Carbon mineralisation		
XDE-729 acid (X11393729)	Nitrogen mineralisation	0.05	0.009
	Carbon mineralisation		
X11449757	Nitrogen mineralisation	0.052	0.0025
	Carbon mineralisation		
GF-2573	Nitrogen mineralisation	6.037	0.905
	Carbon mineralisation		

B.9.8.3 Conclusions

The concentrations for which <25% effects were observed, are all in excess of the PEC_{soil} values. Therefore it can be concluded that the risk to soil micro-organisms is acceptable for the use of GF-2573 on cereals if applied according to the proposed GAP.

B 9.8.4 Non-extractable residues (NER's)

In reference to the fate section (IIA B 8.3), aerobic soil degradation studies resulted in formation of greater than 70% non-extractable residue (NER) in some soils. Following treatment with XDE-729 Methyl, levels of NER increased for approximately 30 days before reaching plateau and then declined towards the end of the study period. A chronic earthworm study was conducted to assess the toxicity of NER's to earthworms. The soils used in this study corresponded to the two soils identified in the fate study for which the highest levels of NER's

were observed. The soils were treated with XDE-729 Methyl at three soil concentrations of 0.1, 1.0 and 10 mg a.s/kg soil. XDE-methyl and its major soil metabolites have been demonstrated to show an acceptable risk to soil organisms with TER values substantially above the trigger values. As there was no clear differentiation in the sensitivity observed in soil organisms (earthworms and soil macro/micro-organisms), only an earthworm chronic study was conducted and deemed sufficient to cover the risk to other soil organism groups. Furthermore, in the corresponding fate study (B.8.1.1 c) NER's were produced at the highest levels between 30 and 86 days after treatment. In the chronic earthworm study, test organisms were exposed to the treated soil 30 days after treatment when NER levels were expected to be reaching plateau. A chronic earthworm study has a duration of 56 days and so this is equivalent to exposing the earthworms from day 30 to day 86. Exposure to the peak concentrations of NER's were sufficiently covered by the length of this study.

The study resulted in no effects on earthworm survival, growth or reproduction up to and including the highest test concentration, 10 mg a.s/kg soil dry weight. In the corresponding fate study (IIA B.8.1.1 c) the mean levels of unextracted radioactivity between 32 and 89 DAT in the 10 mg a.s./kg treatments were 48.6% in the Site E1 soil and 71.0% in the RefSol 03-G soil. Therefore the Applicant considered that the unextracted residue concentrations in the soils during the period of exposure were 4.86 mg a.s. equivalent/kg and 7.10 mg a.s. equivalent/kg in the Site E and RefSol 03-G soils respectively. For the purpose of risk assessment, therefore, a NOEC of 7.10 mg/kg has been used since no effects of NER were observed at this concentration.

The total dose of XDE-729 methyl applied in one year is 14.07 g/ha. Using standard soil assumptions and 25% crop interception, this equates to a single year total dose PECsoil of 0.014 mg a.s./kg for NER's expressed as the parent equivalent. The range of dosing in the earthworm study in section B 9.6.1.1, page 330 (0.1 and 10 mg a.s/kg dry soil) are therefore equivalent to 7.1x - 714x the annual total dose of XDE-729 methyl.

Taking account of the unextracted residue formed in the aerobic route and rate of degradation study at B.8.1.1 a), the peak amount of unextracted residue seen was 82.5% AR. Considering the annual total dose PECsoil above (0.014 mg/kg), the unextracted residue PECsoil for a single year would be 0.012 mg a.s. equivalent/ha. The applicant considered that on average during the earthworm study, the worms would have been exposed to an unextracted residue of 4.86 – 7.10 mg a.s. equivalent/kg, this has been confirmed by environmental fate. Therefore these exposure levels are equivalent to 405x – 592x the expected single year unextracted residue (0.012 mg/kg).

PEC_{soil} values and details on PEC calculations are provided in Annex B.8.3.

Table 9.8.13: Predicted environmental concentrations (PEC_{soil}) for NER's expressed as parent equivalent for a single year dose.

Compound	PEC soil (mg/kg dry soil)
Non extractable residues	0.012

Table B.9.8.14: Summary of NER available toxicity data for earthworms

Group	Type of study and test item	Species	Endpoint	Reference
Earthworms	NER of XDE-729 Methyl	<i>Eisenia fetida</i>	NOEC = 7.10 mg NER/kg soil	McCormac, A., (2012) IIA 8.9.2/10

NER = Non-extractable residues.

This risk assessment is based on the SANCO/10329/2002 guidance document for terrestrial ecotoxicology.

Table B.9.8.15: Chronic risk assessment for earthworms exposed to NER

Study type	Test substance	Toxicity	PEC_{soil} (mg/kg soil)	TER**	Trigger value
Chronic	NER* of XDE 729 methyl	NOEC 7.10 mg NER*/kg d.w soil	0.012	592	5

* Non-extractable residues.

** Values have been rounded

The TER value is above the trigger value of 5, therefore an acceptable chronic risk has been demonstrated for soil organisms exposed to the accumulation of non-extractable residues.

B.9.8.5 Conclusions

The chronic risk to soil organisms from exposure to NER's is acceptable and so requires no further consideration.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (IIA 8.6)**B.9.9.1 Toxicity to non-target fauna/flora**

Rockliff, C. (2011): Evaluation of the Phytotoxicity of the XDE-729 acid GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants. Stockbridge Technology Centre Ltd, UK. Project Number: STC/11/E601. Dow AgroSciences unpublished report, Study Number: 101955, 17 October 2011.

Test material

Test item:	XDE-729 acid
Purity:	XDE-729 (95.3%)
Description:	Technical
Lot No./Batch No. :	E2837-52

Test system

Monocotyledon species:-	<i>Lolium perenne</i> (Ryegrass) <i>Avena sativa</i> (Oats) <i>Allium cepa</i> (Onion)
Dicotyledon species:-	<i>Glycine max</i> (Soybean) <i>Vicia faba</i> (Field Bean) <i>Brassica napus</i> (Oilseed Rape) <i>Beta vulgaris</i> (Sugar Beet) <i>Daucus carota</i> (Carrot) <i>Cucumis sativa</i> (Cucumber) <i>Helianthus annuus</i> (Sunflower) <i>Lycopersicon esculentum</i> (Tomato)
Study Type:	Glasshouse study assessing seedling emergence and Seedling growth
GLP Status:	GLP (No claim of GLP compliance for seed details, compost analysis, meteorological data, glasshouse environmental data and photographs.)
Guidelines followed:	OECD Guideline 208
Guideline deviations reported by Study Director:	Please see RMS comments.
Duration of study:	21 days after 50% emergence of the untreated control
Observation intervals:	14 days and 21 days after 50% emergence of the untreated control
Parameters measured:	Emergence counts Mortality Foliar fresh weight Phytotoxicity
Growth conditions:	Temperature (range): 15.5°C minimum (Range 15.5-38.6°C)

	<p>Photoperiod: natural radiation plus supplementary lighting for 16 hours</p> <p>Light intensity (range) – minimum 5000 lux</p> <p>Relative humidity: 29-84%</p> <p>Water regime and schedules: During the period between sowing and treatment application, the pots were lightly watered with a watering can. On the day of treatment application, pots were placed on a plastic saucer and lightly watered overhead with a hosepipe. All subsequent water was applied to the saucers. Final watering was applied the day before/ 2 days before harvest assessment.</p> <p>Water source/type: Mains water</p> <p>Pest control method /fertilization, if used: Nitrogen fertilizer feed when required</p>
Growth medium	<p>Soil type: Sandy loam</p> <p>Details of nutrient medium, if used: None</p> <p>pH: 7.8</p>
Test concentrations:	<p>0 (control), 0.24, 0.47, 0.94, 1.88, 3.75, 7.5 and 15 g as/ha – all crops except Carrot</p> <p>0 (control), 0.12, 0.24, 0.47, 0.94, 1.88, 3.75, 7.5 and 15 g as/ha – Carrot</p>
Analytical verification:	HPLC (96% recovery) analysis by Huntington Life Sciences.
Test material application:	Method: Track sprayer: Pre-emergence application, using an overhead track sprayer fitted with fan nozzles (01F80).
Seed/plant:	<p>Source: Commercial seed batches</p> <p>Prior seed treatment/sterilization: None</p>
Number of control replicates:	5
Number of test concentration replicates:	5 (10 seeds per pot, except cucumber for which there were 5)

Methodology

Seeds from 11 species (8 dicotyledon and 3 monocotyledon) were obtained from commercial seed companies. These were sown on the day before treatment application. Treatments were applied starting with the water only control followed by the highest rate. The test substance was dissolved in Acetone: DMSO (ratio of 97:3) before adding to the mains tap water. The highest rate was then diluted in sequence and applied for all subsequent treatments. All treatment applications were made using a track sprayer calibrated to deliver 200l/ha water. After treatment the pots were removed to a glasshouse and laid out in randomized blocks. All pots were placed in saucers with watering applied directly onto the soil surface to assist germination and then into the saucers to avoid leaching. Seedlings were assessed for emergence, visual

injury and plant survival. Fresh weight was recorded at harvest – 21 days after 50% emergence of the untreated control. Temperature, sunshine, humidity and natural radiation were recorded daily.

The ER₅₀ values for susceptible species were calculated using foliar fresh weight data, expressed as a % of the untreated control. Where a 50% reduction in foliar fresh weight did not occur, or regression analysis could not be run because of the high tolerance of species to XDE-729 acid, the ER₅₀ values were considered to be greater than the highest rate tested, 15 g as/ha.

Results

*% Visual Injury: 100 = dead; 0 = no injury

Table B.9.9.1: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Monocot Species-Ryegrass

Treatment (g a.s./ha)	<i>Lolium perenne</i> (Ryegrass)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	94	-	0	100	0
0.24	82	12.8	0	101	0
0.47	80	14.9	0	85	0
0.94	84	10.6	0	91	0
1.88	86	8.5	0	101	0
3.75	94	0	0	106	0
7.5	90	4.3	0	94	0
15	82	12.8	0	79	0

Table B.9.9.2: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest): Monocot Species-Oats

Treatment (g a.s./ha)	<i>Avena sativa</i> (Oats)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	98	-	0	100	0
0.24	98	0	0	98.3	0
0.47	96	2.1	0	90.7	0
0.94	100	-2	0	93.7	0
1.88	98	0	0	85.7	0
3.75	98	0	0	98.3	0
7.5	98	0	0	87.9	0
15	100	-2	0	91.3	0

Table B.9.9.3: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest): Monocot Species-Onion

Treatment (g a.s./ha)	<i>Allium cepa</i> (Onion)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.24	98	2	0	96.5	0
0.47	100	0	0	97.7	0
0.94	98	2	0	88.4	0
1.88	98	2	0	85.5	7
3.75	100	0	0	80.9	20
7.5	100	0	6	67.6	49
15	94	6	29.8	27.7	72

Table B.9.9.4: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Soybean

Treatment (g a.s./ha)	<i>Glycine max</i> (Soybean)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	96	-	0	100	0
0.24	94	2.1	0	93.1	0
0.47	92	4.2	0	95.6	0
0.94	96	0	4.2	86.4	0
1.88	96	0	0	93	1
3.75	90	6.2	0	74.3	16
7.5	82	14.6	12.2	54.6	37
15	74	22.9	5.4	35.7	50

Table B.9.9.5: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Field bean

Treatment (g a.s./ha)	<i>Vicia faba</i> (Field bean)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	96	-	0	100	0
0.24	90	6.2	0	87	4
0.47	98	-2.1	0	89.3	4
0.94	94	2.1	0	85.8	15
1.88	92	4.2	2.2	79.4	10
3.75	90	6.2	0	63.4	21
7.5	88	8.3	18.2	39.4	26
15	76	20.8	10.5	31.7	38

Table B.9.9.6: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Oilseed rape

Treatment (g a.s./ha)	<i>Brassica napus</i> (Oilseed rape)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	98	-	0	100	0
0.24	100	-2	0	100	4
0.47	96	2.1	0	103.7	4
0.94	100	-2	0	99.5	13
1.88	98	0	0	95	22
3.75	98	0	0	105.3	28
7.5	96	2.1	0	108.9	32
15	82	16.3	0	62.1	48

Table B.9.9.7: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Sugar beet

Treatment (g a.s./ha)	<i>Beta vulgaris</i> (Sugar beet)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	94	-	0	100	0
0.24	94	0	0	95.2	2
0.47	100	-6.4	0	101.1	4
0.94	94	0	0	88.6	10
1.88	100	-6.4	2	99.3	25
3.75	100	-6.4	0	98.1	15
7.5	98	-4.3	0	96.2	37
15	86	8.5	11.6	33.6	61

Table B.9.9.8: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Carrot

Treatment (g a.s./ha)	<i>Daucus carota</i> (Carrot)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	92	-	0	100	0
0.12	92	0	0	98.4	0
0.24	86	6.5	2.3	90.3	12
0.47	90	2.2	17.8	96.8	9
0.94	70	23.9	31.4	45.2	45
1.88	52	43.5	61.5	17.7	63
3.75	26	71.7	69.2	4.8	80
7.5	6	93.2	66.2	1.6	95
15	0	0	0	0	100

Table B.9.9.9: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Cucumber

Treatment (g a.s./ha)	<i>Cucumis sativa</i> (Cucumber)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual Injury*
Control	100	-	0	100	0
0.12	-	-	-	-	-
0.24	100	0	0	98.4	0
0.47	96	4	0	93.3	0
0.94	100	0	0	87.1	2
1.88	100	0	0	90.8	5
3.75	100	0	0	85	12
7.5	100	0	0	66.2	44
15	64	36	37.5	15.2	80

Table B.9.9.10: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Sunflower

Treatment (g a.s./ha)	<i>Helianthus annuus</i> (Sunflower)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.24	98	2	0	95.5	0
0.47	100	0	0	97.6	0
0.94	100	0	0	96.7	3
1.88	98	2	0	92.9	3
3.75	100	0	4	95.9	5
7.5	94	6	2.1	82.1	15
15	86	14	4.7	64.1	32

Table B.9.9.11: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Tomato

Treatment (g a.s./ha)	<i>Lycopersicon esculentum</i> (Tomato)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.24	100	0	0	86.5	0
0.47	100	0	0	87.4	0
0.94	100	0	0	88.5	0
1.88	100	0	0	82.1	0
3.75	100	0	0	80.8	0
7.5	100	0	0	69.3	7
15	90	10	4.4	41.5	32

Ryegrass and oats did not display visual injury at any treatment rate. Visual injury on onion consisted of curled leaves. Affected plants tended to lean over or lie flat to the soil surface and were pale green in colour. Visual injury on soybean, field beans, oilseed rape, cucumber, sunflower and tomato consisted of a check in growth or

stunting with some plants also displayed wrinkled leaves. Carrots also displayed this check in growth and stunting together with curled leaves and stems. Sugarbeet displayed thin leaves, wrinkled and slight leaf cupping.

Sugar beet, field bean, oilseed rape, cucumber and sunflower displayed these symptoms at all treatment rates with symptoms increasing in line with treatment rate. Furthermore, carrot also displayed visual injury at all treatment rates apart from the lowest (0.12 g as/ha). Whereas soybean and onion displayed these visual injury at the four highest treatment rates (1.88 to 15 g as/ha) again with symptoms increased as the treatment rate increased. Tomato displayed visual injury at the two highest treatment rates (7.5 and 15 g as/ha).

Table B.9.9.12: Reported ER₅₀ values for XDE-729 acid based on mean foliar fresh weight reduction as compared to the untreated control

Species		ER ₅₀ (g as/ha)	Regression R ² Square
Monocot	<i>Lolium perenne</i> (Ryegrass)	>15	N/A
	<i>Avena sativa</i> (Oats)	>15	N/A
	<i>Allium cepa</i> (Onion)	10.382	0.78
Dicot	<i>Glycine max</i> (Soybean)	9.371	0.77
	<i>Vicia faba</i> (Field bean)	8.559	0.65
	<i>Brassica napus</i> (Oilseed rape)	>15	N/A
	<i>Beta vulgaris</i> (Sugar beet)	13.483	0.59
	<i>Daucus carota</i> (Carrot)	0.3835	0.79
	<i>Cucumis sativa</i> (Cucumber)	9.173	0.75
	<i>Helianthus annuus</i> (Sunflower)	>15	N/A
	<i>Lycopersicon esculentum</i> (Tomato)	11.162	0.65
N/A – not applicable as no reduction in fresh foliar weight of ≤50% in comparison to the control.			

Conclusions

The majority of the plant species displayed phytotoxicity effects with the exception of ryegrass and oats. Based on reduction in foliar fresh weight, the most sensitive monocotyledon species was onion with an ER₅₀ of 10.382 g a.s./ha. The lowest ER₅₀ for reduction in fresh foliar weight was for the dicotyledon species, carrot with an ER₅₀ of 0.3835g a.s./ha. The least sensitive species were oilseed rape, sunflower, ryegrass and oats.

RMS comment: The relative humidity ranged from 29-84%, the guideline states that humidity should be 75% ± 25%. 29% is lower then the range specified however, this

drop in humidity was for a short duration and values within this range was maintained for the majority of the study. Therefore this minor deviation is not thought to have had any affect on the outcome of the study.

End points were reported as EC (effect concentrations) in the report, but as treatment was via spray at an application rate, ER (effect rate) would be the appropriate end point. This is purely a typographical report error and does not impact the value calculated.

The current endpoint to be used for risk assessment purposes is an ER_{50} of 0.3835 g a.s /ha based on a reduction in foliar fresh weight for carrots. Ideally ER_{50} values for phytotoxicity would have been calculated. For some plant species the % visual injury score could have resulted in a lower ER_{50} value than for fresh foliar weight e.g. onion, sugarbeet and cucumber. However these values would not be as low as the ER_{50} for carrot which is 0.3835 g as /ha and so would not drive the risk assessment.

Although GLP compliance is not claimed for compost analysis, seed details, meteorological data, glasshouse environmental data or photographs it has been confirmed that soil analysis, seed records and environmental conditions were adequately maintained/performed. With regards to environmental conditions (temperature, humidity, light intensity) any significant discrepancies would likely have impacted upon the control plants. As control groups of all 11 species satisfied the related OECD guideline criteria the environmental conditions are considered to have been suitable for a successful study. Meteorological data and the photographs included in the report do not contribute directly to the conclusions drawn from the study and are for supplementary information only.

Following applicant correspondence the amount of soil used per replicate was confirmed as equal for all species, as well as confirmation that the most recent GLP certificate at the time of study commencement was provided.

Rockliff, C. (2011): Evaluation of the Phytotoxicity of the XDE-729 M-757 metabolite GLP Seedling Emergence and Seedling Growth Test. Stockbridge Technology Centre Ltd, UK. Project Number: STC/11/E602. Dow AgroSciences unpublished report, Study Number: 101956. 17 October 2011.

Test material

Test item:	XDE-729 M-757 metabolite (X11449757)
Purity:	99%
Description:	Technical
Lot No./Batch No. :	YB1-100780-103

Test system

Monocotyledon species:-	<i>Lolium perenne</i> (Ryegrass) <i>Avena sativa</i> (Oats) <i>Allium cepa</i> (Onion)
Dicotyledon species:-	<i>Glycine max</i> (Soybean) <i>Vicia faba</i> (Field Bean) <i>Brassica napus</i> (Oilseed Rape) <i>Beta vulgaris</i> (Sugar Beet) <i>Daucus carota</i> (Carrot) <i>Cucumis sativa</i> (Cucumber) <i>Helianthus annuus</i> (Sunflower) <i>Lycopersicon esculentum</i> (Tomato)
Study Type:	Glasshouse study assessing seedling emergence and seedling growth
GLP Status:	GLP (No claim of GLP compliance for seed details, compost analysis, meteorological data, glasshouse environmental data and photographs.)
Guidelines followed:	OECD Guideline 208
Guideline deviations reported by Study Director:	None
Duration of study:	21 days after 50% emergence of the untreated control
Observation intervals:	14 days and 21 days after 50% emergence of the untreated control
Parameters measured:	Emergence counts Mortality Foliar fresh weight Phytotoxicity
Growth conditions:	Temperature (range): 15.4°C minimum (Range 15.4-36.5°C) Photoperiod: natural daylength for 16 hours Natural daily radiation- 4664- 14289 Watts/m ² Relative humidity: 26-94% Water regime and schedules: During the period between sowing and treatment application, the pots were lightly watered with a watering can. On the day of treatment application, pots were placed on a plastic saucer and lightly watered overhead with a hosepipe. All subsequent water was applied to the saucers. Final watering was applied the day before/ 2 days before harvest assessment. Water source/type: Mains water Pest control method /fertilization, if used: Nitrogen fertilizer feed when required
Growth medium	Soil type: Sandy loam Details of nutrient medium, if used: None pH: 7.8

Test concentrations:	0 (control), 0.94, 1.88, 3.75, 7.5 and 15 g X11449757/ha
Analytical verification:	HPLC (88% recovery) analysis by Huntington Life Sciences.
Test material application:	Method: Track sprayer: Pre-emergence application, using an overhead track sprayer fitted with fan nozzles (01F80).
Seed/plant:	Source: Commercial seed batches Prior seed treatment/sterilization: None
Number of control replicates:	5
Number of test concentration replicates:	5 (10 plants per pot, except cucumber which had 5 seeds per pot)

Methodology

Seeds from 11 species (8dicotyledon and 3monocotyledon) were obtained from commercial seed companies. Seeds were sown on the day before treatment application. Treatments were applied starting with the water only control followed by the highest rate. The test substance was dissolved in Acetone: DMSO (97:3 ratio) before adding to the water. The highest rate was then diluted in sequence and applied for all subsequent treatments. All treatment applications were made using a track sprayer calibrated to deliver 200l/ha water. After treatment the pots were removed to a glasshouse and laid out in randomized blocks. All pots were placed in saucers with watering applied directly onto the soil surface to assist germination and then into the saucers to avoid leaching. Seedlings were assessed for emergence, visual injury and plant survival. Fresh weight was recorded at harvest – 21 days after 50% emergence of the untreated control. Temperature, sunshine, humidity and natural radiation were recorded daily.

The ER₅₀ values for susceptible species were calculated using foliar fresh weight data, expressed as a % of the untreated control. Where a 50% reduction in foliar fresh weight did not occur, or regression analysis could not be run because of the high tolerance of species to XDE-729 M-757 metabolite the ER₅₀ values were considered to be greater than the highest rate tested (15 g X11449757/ha).

Results

*% Visual Injury: 100 = dead; 0 = no injury

Table B.9.9.13: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Monocot Species-Ryegrass

Treatment (g X11449757/ha)	<i>Lolium perenne</i> (Ryegrass)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	98	-	0	100	0
0.94	94	4.1	0	97.3	0
1.88	96	2.0	0	111.5	0
3.75	92	6.1	0	102.7	0
7.5	86	12.2	0	99.1	0
15	96	2.0	0	92.9	0

Table B.9.9.14: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Monocot Species- Onion

Treatment (g X11449757/ha)	<i>Allium cepa</i> (Onion)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	94		0	100	0
0.94	98	-4.3	0	92.8	0
1.88	100	-6.4	0	88.4	0
3.75	96	-2.1	0	98.9	0
7.5	98	-4.3	0	96.1	0
15	98	-4.3	0	103.9	0

Table B.9.9.14: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest): Monocot Species- Oats

Treatment (g X11449757/ha)	<i>Avena sativa</i> (Oats)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	96	-	0	100	0
0.94	100	-4.2	0	105.5	0
1.88	98	-2.1	0	101.1	0
3.75	98	-2.1	0	110.2	0
7.5	100	-4.2	0	103.7	0
15	98	-2.1	0	106.3	0

Table B.9.9.15: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest): Dicot Species- Soybean

Treatment (g X11449757/ha)	<i>Glycine max</i> (Soybean)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	96	-	0	100	0
0.94	98	-2.1	0	99	0
1.88	94	2.1	0	95.1	0
3.75	96	0	0	95.5	0
7.5	98	-2.1	0	101.4	0
15	92	4.2	0	93.9	0

Table B.9.9.16: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species- Field bean

Treatment (g X11449757/ha)	<i>Vicia faba</i> (Field bean)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.94	98	2	0	104	0
1.88	98	2	0	104.8	0
3.75	98	2	0	104.7	0
7.5	100	0	0	101.7	0
15	98	2	0	98.8	0

Table B.9.9.17: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species- Oilseed rape

Treatment (g X11449757/ha)	<i>Brassica napus</i> (Oilseed rape)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.94	98	2	0	98.5	0
1.88	100	0	0	105.6	0
3.75	98	2	0	104.7	0
7.5	100	0	0	105.3	0
15	100	0	0	99.1	0

Table B.9.9.18: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species- Sugarbeet

Treatment (g X11449757/ha)	<i>Beta vulgaris</i> (Sugarbeet)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	96	-	0	100	0
0.94	100	-4.2	0	101.8	0
1.88	96	0	0	94.1	0
3.75	96	0	0	98	0
7.5	98	-2.1	0	103.7	0
15	100	-4.2	0	109.2	0

Table B.9.9.19: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Sunflower

Treatment (g X11449757/ha)	<i>Helianthus annuus</i> (Sunflower)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	96	-	0	100	0
0.94	96	0	0	96.8	0
1.88	96	0	0	103.7	0
3.75	98	-2.1	0	101.4	0
7.5	94	2.1	0	93.8	0
15	100	-4.2	0	105.4	0

Table B.9.9.20: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Tomato

Treatment (g X11449757/ha)	<i>Lycopersicon esculentum</i> (Tomato)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.94	98	2	0	107.3	0
1.88	98	2	0	102.2	0
3.75	100	0	0	99.9	0
7.5	96	4	0	101.2	0
15	98	2	0	106.2	0

Table B.9.9.21: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest):
Dicot Species-Carrot

Treatment (g X11449757/ha)	<i>Daucus carota</i> (Carrot)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	94	-	0	100	0
0.94	96	-2.1	0	87.3	0
1.88	98	-4.3	0	101	0
3.75	90	4.3	0	74.5	0
7.5	96	-2.1	0	83.3	0
15	88	6.4	0	84.3	0

Table B.9.9.22: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21 days (harvest); Dicot Species-Cucumber

Treatment (g X11449757/ha)	<i>Cucumis sativa</i> (Cucumber)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.94	100	0	0	95.6	0
1.88	96	4	0	94.1	0
3.75	100	0	0	97.2	0
7.5	100	0	0	104.2	0
15	100	0	0	100.9	0

Table B.9.9.23: Reported ER₅₀ values for X11449757 based on mean foliar fresh weight reduction as compared to the untreated control

Species		ER ₅₀ (g X11449757/ha)	Regression R_Square
Monocot	<i>Lolium perenne</i>	>15	N/A
	<i>Avena sativa</i>	>15	N/A
	<i>Allium cepa</i>	>15	N/A
Dicot	<i>Glycine max</i>	>15	N/A
	<i>Vicia faba</i>	>15	N/A
	<i>Brassica napus</i>	>15	N/A
	<i>Beta vulgaris</i>	>15	N/A
	<i>Daucus carota</i>	>15	N/A
	<i>Cucumis sativa</i>	>15	N/A
	<i>Helianthus annuus</i>	>15	N/A
	<i>Lycopersicon esculentum</i>	>15	N/A
N/A – not applicable as no reduction in fresh foliar weight of ≤50% in comparison to the control, for any of the crop species tested.			

Conclusions

None of the eleven species displayed any visual injury or mortality.

When applied at a maximum rate of 15 g/ha, X11449757 did not reduce the fresh weight of any of the eleven test species by 50 % relative to the untreated control. Therefore, it was not possible to carry out regression analysis and the ER₅₀ value for each of the eleven test species is considered to be > 15 g X11449757/ha (the highest rate tested).

RMS comment:

End points were reported as EC (effect concentrations) in the report, but as treatment was via spray at an application rate, ER (effect rate) would be the appropriate end point. This is purely a typographical report error and does not impact the value calculated.

Following applicant correspondence the amount of soil used per replicate was confirmed as equal for all species, as well as confirmation that the most recent GLP certificate at the time of study commencement was provided.

Although GLP compliance is not claimed for compost analysis, seed details, meteorological data, glasshouse environmental data or photographs it has been confirmed that soil analysis, seed records and environmental conditions were adequately maintained/performed. With regards to environmental conditions (temperature, humidity, light intensity) any significant discrepancies would likely have impacted upon the control plants. As control groups of all 11 species satisfied the related OECD guideline criteria the environmental conditions are considered to have been suitable for a successful study. Meteorological data and the photographs included in the report do not contribute directly to the conclusions drawn from the study and are for supplementary information only.

Rockliff, C. (2011): Evaluation of the Phytotoxicity of GF-2573 (XDE-729, 7.5g ae/l , EC) GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants. Stockbridge Technology Centre Ltd. Project Number: STC/11/E606. Dow AgroSciences unpublished report, Study Number: 101970. 22 August 2011.

Test material

Test item:	GF-2573
Purity:	XDE-729, 7.5g acid equivalent (ae)/l
Description:	EC (emulsifiable concentrate)
Lot No./Batch No.	E2837-83

Test system

Monocotyledon species:-	<i>Lolium perenne</i> (Ryegrass) <i>Avena sativa</i> (Oats) <i>Allium cepa</i> (Onion)
Dicotyledon species:-	<i>Glycine max</i> (Soybean) <i>Vicia faba</i> (Field Bean) <i>Brassica napus</i> (Oilseed Rape) <i>Beta vulgaris</i> (Sugar Beet) <i>Daucus carota</i> (Carrot) <i>Cucumis sativa</i> (Cucumber) <i>Helianthus annuus</i> (Sunflower) <i>Lycopersicon esculentum</i> (Tomato)
Study Type:	Glasshouse study assessing seedling emergence and seedling growth
GLP Status:	GLP (No claim of GLP compliance for seed details, compost analysis, meteorological data, glasshouse environmental data and photographs.)
Guidelines followed:	OECD Guideline 208
Guideline deviations reported by Study Director:	None
Duration of study:	21 days after 50% of the untreated plants had emerged.
Observation intervals:	Emergence, visual injury and plant mortality at 14 and 21 day intervals after 50% of the untreated plants had emerged.
Parameters measured:	Emergence counts Mortality Foliar fresh weight Phytotoxicity
Growth conditions:	Temperature (range): 14.8°C minimum (Range 14.8-30.7°C) Photoperiod: natural radiation plus supplementary lighting for 16 hours. Light intensity (range) – minimum 5000 lux Relative humidity: 29-93% Water regime and schedules: Pots placed on a plastic saucer and lightly watered overhead with a hosepipe on day of treatment application. All subsequent water was applied to the saucers. Final watering was applied the day before/ 2 days before harvest assessment. Water source/type: Mains water Pest control method /fertilization, if used: Nitrogen fertilizer feed when required.
Growth medium	Soil type: Sandy loam

	Details of nutrient medium, if used: None pH: 8.1 Organic matter- 0.7
Test concentrations:	0 (control), 0.06, 0.12, 0.235, 0.47, 0.94, 1.875, 3.75 and 7.5 g ae/ha – Soybean, Field Bean and Carrot 0 (control), 0.12, 0.235, 0.47, 0.94, 1.875, 3.75 and 7.5 g ae/ha – Sunflower 0 (control), 0.12, 0.235, 0.47, 0.94, 1.875, 3.75, 7.5 and 15 g ae/ha – Ryegrass, Oats, Onion, Oilseed Rape, Sugar Beet, Cucumber and Tomato
Analytical verification:	HPLC (97% recovery) analysis by Huntington Life Sciences.
Test material application:	Method: Track sprayer: Pre-emergence application, using an overhead track sprayer fitted with fan nozzles (01F80).
Seed/plant:	Source: Commercial seed batches Prior seed treatment/sterilization: None
Number of control replicates:	5
Number of test concentration replicates:	5 (10 plants per replicate pot- except cucumber which only had 5)

Methodology

Seeds from 11 species (8 dicotyledon and 3 monocotyledon) were obtained from commercial seed companies. These were sown on the day before treatment application. Treatments were applied starting with the water only control followed by the highest rate. The highest rate was then diluted in sequence and applied for all subsequent treatments. All treatment applications were made using a track sprayer calibrated to deliver 200l/ha water. After treatment the pots were removed to a glasshouse and laid out in randomized blocks. All pots were placed in saucers with watering applied directly onto the soil surface to assist germination and then applied into the saucers to avoid leaching. Seedlings were assessed for emergence, visual injury and plant survival. Fresh weight was recorded at harvest – 21 days after 50% emergence on the untreated control. Temperature, sunshine, humidity and natural radiation were recorded daily.

The ER₅₀ values for susceptible species were calculated using foliar fresh weight data, expressed as a % of the untreated control. Where a 50% reduction in foliar fresh weight did not occur and therefore regression analysis could not be run, the ER₅₀ values were considered to be greater than the highest rate tested, 7.5 or 15 g acid equivalent (ae) /ha.

Results

*% Visual Injury: 100 = dead; 0 = no injury

Tables B.9.9.24: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest): Monocot Species- Ryegrass.

Treatment (g ae /ha)	<i>Lolium perenne</i> (Ryegrass)				
	% Emergence	%inhibition of emergence	%Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	90	-	0	100	0
0.12	66	26.7	0	66.7	0
0.235	54	40	0	67.8	0
0.47	88	2.2	0	72.4	0
0.94	92	-2.2	0	73.6	0
1.875	82	8.9	0	85.6	0
3.75	74	17.8	0	90.8	0
7.5	82	8.9	0	93.7	6
15	76	15.6	7.9	60.3	14

Tables B.9.9.25: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest): Monocot Species- Onion

Treatment (g ae /ha)	<i>Allium cepa</i> (Onion)				
	% Emergence	%inhibition of emergence	%Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.12	100	0	0	94.2	0
0.235	100	0	0	90.7	0
0.47	100	0	2	94.2	0
0.94	98	2	0	83.2	4
1.875	98	2	2	67.3	34
3.75	96	4	6.3	53.1	67
7.5	96	4	10.4	40.3	75
15	88	12	11.4	23.9	86

Tables B.9.9.26: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest): Monocot Species- Oats

Treatment (g ae /ha)	<i>Avena sativa</i> (Oats)				
	% Emergence	%inhibition of emergence	%Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.12	100	0	0	92.7	0
0.235	100	0	0	95.8	0
0.47	100	0	0	91.6	0
0.94	100	0	0	108.9	0
1.875	100	0	0	81.1	0
3.75	100	0	0	94.4	0
7.5	100	0	0	97.9	0
15	100	0	0	90	0

Table B.9.9.27: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest): Dicot Species-Soybean

Treatment (g ae /ha)	<i>Glycine max</i> (Soybean)				
	% Emergence	%inhibition of emergence	%Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.06	100	0	0	93.6	0
0.12	100	0	0	96	0
0.235	100	0	0	84.2	0
0.47	100	0	0	93.8	0
0.94	96	4	0	91.7	0
1.875	100	0	0	95.7	0
3.75	92	8	2.2	73.3	21
7.5	80	20	0	60.8	37

Table B.9.9.28: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest):
Dicot Species-Field bean

Treatment (g ae /ha)	<i>Vicia faba</i> (Field bean)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.06	100	0	0	98.4	0
0.12	100	0	0	96.5	0
0.235	100	0	0	96	0
0.47	100	0	0	89.7	0
0.94	98	2	0	89.6	0
1.875	98	2	0	88.7	9
3.75	100	0	0	92.6	12
7.5	100	0	0	75.7	17

Table B.9.9.29: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest):
Dicot Species-Sunflower

Treatment (g ae /ha)	<i>Helianthus annuus</i> (Sunflower)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.12	96	4	0	95.8	0
0.235	96	4	0	95.6	0
0.47	100	0	0	107.6	0
0.94	100	0	0	95.3	0
1.875	100	0	0	87.8	0
3.75	100	0	0	88.9	0
7.5	100	0	0	80.7	12
15	-	-	-	-	

Table B.9.9.30: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest):
Dicot Species-Tomato

Treatment (g ae /ha)	<i>Lycopersicon esculentum</i> (Tomato)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.12	100	0	0	97	0
0.235	100	0	0	100.7	0
0.47	100	0	0	101.5	0
0.94	100	0	0	71.4	9
1.875	98	2	0	76.6	8
3.75	100	0	0	65.1	17
7.5	100	0	12	52.4	47
15	88	12	20.5	41.3	53

Table B.9.9.31: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest):
Dicot Species-Oilseed rape

Treatment (g ae /ha)	<i>Brassica napus</i> (Oilseed rape)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	0	100	0
0.12	100	0	0	96.8	0
0.235	100	0	0	95	0
0.47	100	0	0	97	0
0.94	100	0	0	94.3	4
1.875	100	0	0	98.8	9
3.75	100	0	0	108.4	12
7.5	98	2	0	111.2	25
15	100	0	0	100.2	48

Table B.9.9.32: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest):
Dicot Species-Sugar beet

Treatment (g ae /ha)	<i>Beta vulgaris</i> (Sugar beet)				
	% Emergence	%inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	100	-	2	100	0
0.12	100	0	0	86.1	0
0.235	100	0	0	79.6	0
0.47	100	0	0	77.5	0
0.94	100	0	0	79.3	2
1.875	100	0	0	82.5	5
3.75	100	0	0	72.3	14
7.5	100	0	0	62	37
15	100	0	2	42.1	56

Table B.9.9.33: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest):
Dicot Species-Carrot

Treatment (g ae /ha)	<i>Daucus carota</i> (Carrot)				
	% Emergence	%inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual injury*
Control	98	-	0	100	0
0.06	98	0	0	103.9	0
0.12	92	6.1	0	93.8	6
0.235	98	0	0	80.5	32
0.47	88	10.2	13.6	47.7	36
0.94	50	49	48	10.2	81
1.875	42	57.1	42.9	2.3	96
3.75	40	59.2	75	4.7	87
7.5	22	77.6	90.9	0.8	99
15	-	-	-	-	-

Table B.9.9.34: Observations of plant mortality, % emergence, mean foliar fresh weight as % of control and visual phytotoxicity ratings after 21/22 days (harvest):
Dicot Species-Cucumber

Treatment (g ae /ha)	<i>Cucumis sativa</i> (Cucumber)				
	% Emergence	% inhibition of emergence	% Mortality of emerged	Mean foliar fresh weight as % control	% visual Injury*
Control	100	-	0	100	0
0.06	-	-	-	-	-
0.12	96	4	0	83.6	0
0.235	96	4	0	102.1	0
0.47	100	0	0	103.9	0
0.94	96	4	0	92.4	2
1.875	92	8	0	79.8	11
3.75	100	0	0	79.8	9
7.5	96	4	0	64.5	30
15	84	16	19.1	34.3	66

Visual injury was present in 10 of the 11 tested species. Oats did not display any visual injury or mortality at any treatment rate. Visual injury on soybean, field bean, oilseed rape, carrot, tomato, cucumber and sunflower all consisted of a check in growth. For field bean and oilseed rape, slight cupping of the leaves also occurred. Additional stunting occurred for carrots and tomatoes, with carrots further displaying slight distortion. Cucumber plants also displayed curled, wrinkled leaves and curled, lumpy stems. The worst affected plants became brown in colour.

Onion plants displayed curled leaves and were pale green in colour. Ryegrass and sugarbeet consisted of slightly thinner leaves. Sugarbeet plants also had slightly discoloured stem with affected plants tending to lean over. The most severely affected plants also displayed leaves that curled inwards.

At harvest Ryegrass and soybean both displayed symptoms at the two highest treatment rates (7.5 and 15 g ae/ha). Field bean displayed symptoms at the three highest treatment rates (1.875, 3.75 and 7.5 g ae/ha) with visual injury of 9 %, 12 % and 17 % respectively. Carrot displayed visual injury at all treatment rates except for the lowest (0.06 g ae/ha). Sunflower displayed symptoms at the highest treatment rate (7.5 g ae/ha) only with visual injury of 12 %.

For onion, oilseed rape, sugar beet, cucumber and tomato visual injury was observed at the five highest treatment rates (0.94 to 15g ae/ha) and symptoms increased as the treatment rate increased.

Table B.9.9.35: Reported ER₅₀ values for GF-2573 based on mean foliar fresh weight reduction as compared to the untreated control

Species		ER ₅₀ (g ae /ha)	Regression R_Square
Monocot	<i>Lolium perenne</i> (Ryegrass)	>15	N/A
	<i>Avena sativa</i> (Oats)	>15	N/A
	<i>Allium cepa</i> (Onion)	4.455	0.82
Dicot	<i>Glycine max</i> (Soybean)	>7.5	N/A
	<i>Vicia faba</i> (Field bean)	>7.5	N/A
	<i>Brassica napus</i> (Oilseed rape)	>15	N/A
	<i>Beta vulgaris</i> (Sugar beet)	12.796	0.42
	<i>Daucus carota</i> (Carrot)	0.417	0.79
	<i>Cucumis sativa</i> (Cucumber)	11.025	0.79
	<i>Helianthus annuus</i> (Sunflower)	>7.5	N/A
	<i>Lycopersicon esculentum</i> (Tomato)	11.331	0.57
N/A – not applicable as no reduction in fresh foliar weight of $\leq 50\%$ in comparison to the control.			

Conclusions

The majority of the plant species displayed phytotoxicity effects with the exception of oats. Based on reduction in foliar fresh weight, the most sensitive monocotyledon species was onion with an ER₅₀ of 4.455 g ae /ha. The lowest ER₅₀ for reduction in fresh foliar weight was for the dicotyledon species, carrot with an ER₅₀ of 0.417 g ae /ha. The least sensitive species were oilseed rape, ryegrass and oats.

RMS comment: The relative humidity ranged from 29-93%, the guideline states that humidity should be $75\% \pm 25\%$. 29% is lower than the range specified, however this drop in humidity was for a short duration and values within this range were maintained for the majority of the study. Therefore this minor deviation is not thought to have had any effect on the outcome of the study.

End points were reported as EC (effect concentrations) in the report, but as treatment was via spray at an application rate, ER (effect rate) would be the appropriate end point. This is purely a typographical report error and does not impact the value calculated.

The current endpoint to be used for risk assessment purposes is an ER₅₀ of 0.417 g ae /ha based on a reduction in foliar fresh weight for carrots. Ideally ER₅₀ values for phytotoxicity would have been calculated. For some plants the % visual injury score could have resulted in a lower ER₅₀ value than for fresh foliar weight e.g. sugarbeet, cucumber, tomato and onion (ER₅₀ for onion phytotoxicity lies between concentrations of 1.875 and 3.75 g ae /ha). However these values would not be as low

as the ER₅₀ for carrot which is 0.417 g ae /ha and so would not drive the risk assessment.

Following applicant correspondence the amount of soil used per replicate was confirmed as equal for all species, as well as confirmation that the most recent GLP certificate at the time of study commencement was provided.

Although GLP compliance is not claimed for compost analysis, seed details, meteorological data, glasshouse environmental data or photographs it has been confirmed that soil analysis, seed records and environmental conditions were adequately maintained/performed. With regards to environmental conditions (temperature, humidity, light intensity) any significant discrepancies would likely have impacted upon the control plants. As control groups of all 11 species satisfied the related OECD guideline criteria the environmental conditions are considered to have been suitable for a successful study. Meteorological data and the photographs included in the report do not contribute directly to the conclusions drawn from the study and are for supplementary information only.

Rockliff, C. (2011): Evaluation of the Phytotoxicity of GF-2573 (XDE-729, 7.5g ae/l, EC). GLP Vegetative Vigour Test Terrestrial Non Target Plants Stockbridge Technology Centre Ltd. Project Number: STC/11/E605. Dow AgroSciences unpublished report, Study Number: 101969. 22 August 2011.

Test material

Test item:	GF-2573
Purity:	XDE-729, 7.5g a.e./L
Description:	EC (emulsifiable concentrate)
Lot No./Batch No. :	E2837-83

Test system

Monocotyledon species:-	<i>Lolium perenne</i> (Ryegrass) <i>Avena sativa</i> (Oats) <i>Allium cepa</i> (Onion)
Dicotyledon species:-	<i>Glycine max</i> (Soybean) <i>Viica faba</i> (Field Bean) <i>Brassica napus</i> (Oilseed Rape) <i>Beta vulgaris</i> (Sugar Beet) <i>Daucus carota</i> (Carrot) <i>Cucumis sativa</i> (Cucumber) <i>Helianthus annuus</i> (Sunflower) <i>Lycopersicon esculentum</i> (Tomato)

Study Type:	Glasshouse study assessing vegetative vigour
GLP Status:	GLP (No claim of GLP compliance for seed details, compost analysis, meteorological data, glasshouse environmental data and photographs.)
Guidelines followed:	OECD Guideline 227
Guideline deviations reported by Study Director:	None
Duration of study:	21 days after treatment application
Observation intervals:	Day 7, 14 and 21 (visual injury, mortality) Day 21 (foliar fresh weight)
Parameters measured:	Plant mortality Foliar fresh weight Visual injury (phytotoxicity)
Growth conditions:	Temperature: 14.8- 30.7°C Photoperiod: natural plus supplementary lighting for 16 hours/day Light intensity (range) – minimum 5000 lux Relative humidity: 29-93% Water source/type: Mains water Watering regime: Overhead with a hosepipe on day of treatment application. All subsequent water was applied to the saucer at base of vessel. Final watering was applied one day before harvest assessment. Fertilization: Nitrogen fertilizer feed when required.
Growth medium	Soil type: Sandy loam (4:4:2 mix of sand:loam:grit) pH: 8.1 Organic Carbon: 0.4%
Test concentrations:	0 (control), 0.06, 0.12, 0.235, 0.47, 0.94, 1.875, 3.75 and 7.5 g acid equivalent (ae)/ha – Soybean, Field Bean and Carrot 0 (control), 0.12, 0.235, 0.47, 0.94, 1.875, 3.75 and 7.5 g ae/ha – Sunflower 0 (control), 0.12, 0.235, 0.47, 0.94, 1.875, 3.75, 7.5 and 15 g ae/ha – Ryegrass, Oats, Onion, Oilseed Rape, Sugar Beet, Cucumber and Tomato
Analytical verification:	HPLC (97% recovery) analysis by Huntington Life Sciences.
Test material application:	Method: Track sprayer fitted with fan nozzles (01F80).
Seed/plant:	Source: Commercial seed batches Growth stage at application: BBCH 12-14 (2 to 4 true leaves).
Number of control replicates:	5
Number of test	5

concentration replicates:	
Number of plants/replicate	5 3 for cucumber 10 for onion

Methodology

Seeds from 11 species (8 dicotyledon and 3 monocotyledon) were obtained from commercial seed companies. Seeds were sown on a range of dates in compost and reared to produce plants at the required growth stage (BBCH 12-14) at spray application. Prior to application all replicates were set up by transferring seedlings to a 16 cm plant pot filled to within 1 cm of the top with a sandy loam soil mix (approximately 1.8L soil mix per pot). Treatments were applied starting with the water only control followed by the highest rate which was then diluted in sequence and applied for all subsequent treatments. All treatment applications were made using a track sprayer calibrated to deliver 200 L/ha water. After treatment the pots were removed to a glasshouse and laid out in randomized blocks, with each pot placed on a saucer. Plants were assessed weekly for visual injury and plant mortality. Fresh weight was recorded at harvest, 21 days after treatment application. Temperature, sunlight hours, humidity and natural radiation were recorded daily. Watering was performed as required for each species and was applied as mains water to the saucer of each replicate to avoid leaching. Nitrate fertiliser was offered to some of the species tested as required.

Results

All seed batches used to grow the plants used in the test displayed historical germination of 89.58 – 100%, which is greater than the guideline-stated minimum of 70%. Control plant survival for all species was 100% (guideline validity criterion > 90%) and no visual phytotoxicity was observed in the control plants for any of the tested species. All replicates for each species were maintained under the same environmental conditions and in the same soil batch.

Analysis of the initial solution (corresponding to the highest treatment rate) was performed on one occasion to quantify active substance content and confirm correct treatment of the plants, as all application solutions at lower treatment rates were prepared from the initial solution. Analysis of this solution showed 97% of the nominal XDE-729 methyl content was present and as such it can be considered that plants were treated at the target application rates.

The ER_{50} values for susceptible species were calculated using foliar fresh weight data, expressed as a % of the untreated control. Where a 50% reduction in foliar fresh weight did not occur, the ER_{50} values were considered to be greater than the highest rate tested, 7.5 or 15g acid equivalent (ae)/ha. Visual injury (phytotoxicity) and plant mortality were calculated as a mean percentage of each treatment on day 21 for all species tested.

Table B.9.9.36: Day 21 observations of mean plant mortality, fresh weight and visual phytotoxicity ratings: Monocot Species

Treatment (g a.e./ha)	<i>Lolium perenne</i> (Ryegrass)			<i>Avena sativa</i> (Oats)			<i>Allium cepa</i> (Onion)		
	% Mortality	Fresh weight (g)	% Phytotox Rating	% Mortality	Fresh weight (g)	Phytotox Rating	% Mortality	Fresh weight (g)	% Phytotox Rating
Control	0	5.98	0	0	12.86	0	0	39.02	0
0.12	0	5.2	0	0	13.16	0	0	35.96	0
0.235	0	6.7	0	0	12.37	0	0	30.5	0
0.47	0	6.36	0	0	12.28	0	0	31.26	0
0.94	0	6.13	0	0	11.37	0	0	29.44	0
1.875	0	6.36	0	0	11.77	0	0	23.19	34
3.75	0	5.85	0	0	12	0	0	21.03	48
7.5	0	7.19	0	0	13.14	0	0	17.5	60
15	0	5.06	22	0	12.85	9	2	10.18	66

Table B.9.9.37: Day 21 observations of mean plant mortality, fresh weight and visual phytotoxicity ratings: Dicot Species

Treatment (g a.e./ha)	<i>Glycine max</i> (Soybean)			<i>Vicia faba</i> (Field bean)			<i>Brassica napus</i> (Oilseed rape)		
	% Mortality	Fresh weight (g)	% Phytotox Rating	% Mortality	Fresh weight (g)	% Phytotox Rating	% Mortality	Fresh weight (g)	% Phytotox Rating
Control	0	33.49	0	0	46.5	0	0	17.98	0
0.06	0	25.64	8	0	48.08	34	-	-	-
0.12	0	24.86	17	0	49.01	31	0	18.75	0
0.235	0	19.92	46	0	35.77	45	0	20.6	0
0.47	0	14.26	61	0	23.88	60	0	18.56	0
0.94	0	15.54	65	4	14.52	69	0	18.1	0
1.875	36	5.22	79	28	9.92	76	0	15.87	0
3.75	48	4.25	83	100	0	100	8	18.02	0
7.5	84	0.43	96	100	0	100	4	22.23	0
15	-	-	-	-	-	-	0	22.57	0

Treatment (g a.e./ha)	<i>Beta vulgaris</i> (Sugar beet)			<i>Daucus carota</i> (Carrot)			<i>Cucumis sativa</i> (Cucumber)		
	% Mortality	Fresh weight (g)	% Phytotox Rating	% Mortality	Fresh weight (g)	% Phytotox Rating	% Mortality	Fresh weight (g)	% Phytotox Rating
Control	0	49.89	0	0	16.77	0	0	76.69	0
0.06	-	-	-	0	15.37	0	-	-	-
0.12	0	48.69	0	0	14.32	0	0	73.5	0
0.235	0	46.73	0	0	14.15	0	0	79.4	16
0.47	0	47.39	3	0	11.41	23	0	70.1	48
0.94	0	47.26	17	0	4.99	67	0	53.67	65
1.875	0	44.76	38	36	2.53	79	0	53.28	71
3.75	0	45.04	54	8	2	85	13	46.65	72
7.5	0	39.30	67	48	1.11	89.6	67	8.97	95
15	0	29.67	71	-	-	-	100	0	100

Treatment (g a.e./ha)	<i>Helianthus annuus</i> (Sunflower)			<i>Lycopersicon esculentum</i> (Tomato)		
	% Mortality	Fresh weight (g)	% Phytotox Rating	% Mortality	Fresh weight (g)	% Phytotox Rating
Control	0	58.48	0	0	36.36	0
0.06	-	-	-	-	-	-
0.12	0	54.99	0	0	36.03	7
0.235	0	53.74	0	0	38.43	12
0.47	0	53.94	1	0	31.5	36
0.94	0	55.65	13	0	32.46	21
1.875	0	54.12	25	0	31.2	40
3.75	0	54.89	43	0	30.65	45
7.5	0	43.62	59	0	27.44	55
15	-	-	-	0	24.66	67

Visual injury was present in 10 of the 11 tested species, with soybean and field bean most sensitive (50% effects lying between 0.235 and 0.47 g ae/ha), showing signs of distorted or stunted growth and shrivelling in affected plants. Only oilseed rape plants did not display signs of visual injury at the highest treatment rate applied, which was 15 g ae/ha. Tomato and cucumber plants were also relatively sensitive and plants displayed twisted, curled and lumpy stems at the majority of treatment rates. Sugar beet, carrot and sunflower plants sprayed at rates of 0.47 g ae/ha and higher exhibited a range of visual damage including curled or curved leaves and stems. Sunflower plants also had some instances of wrinkled leaves while some carrot plants displayed 'leaf strapping'. Onion Plants treated at rates of 1.875 g ae/ha and above developed curled leaves and affected plants were pale green in colour. Only oat and ryegrass plants treated at the highest rate (15 g ae/ha) of XDE-729 displayed visual damage after 21 days, with affected ryegrass plants being duller green in colour and having thinner leaves. Oat plants affected displayed a check in growth and were also duller green compared to unaffected plants.

Table B.9.9.38: Reported ER50 values based on foliar fresh weight reduction

Species	ER50 (g a.e./ha)	Regression R_Square
Monocot		
<i>Lolium perenne</i> (Ryegrass)	>15	N/A
<i>Avena sativa</i> (Oats)	>15	N/A
<i>Allium cepa</i> (Onion)	4.127	0.76
Dicot		
<i>Glycine max</i> (Soybean)	0.400	0.90
<i>Vicia faba</i> (Field bean)	0.5826	0.94
<i>Brassica napus</i> (Oilseed rape)	>15	N/A
<i>Beta vulgaris</i> (Sugar beet)	>15	N/A
<i>Daucus carota</i> (Carrot)	0.6376	0.88
<i>Cucumis sativa</i> (cucumber)	5.553	0.78
<i>Helianthus annuus</i> (Sunflower)	>7.5	N/A
<i>Lycopersicon esculentum</i> (Tomato)	>15	N/A
N/A – not applicable as crop species were tolerant to the test substance		

Conclusions

The most sensitive species in the Vegetative Vigour Test, based on foliar fresh weight reduction were *Glycine max* (Soybean), *Vicia faba* (Field bean) and *Daucus carota* (Carrot) with ER₅₀ values of 0.400, 0.5826 and 0.6376 g a.e./ha respectively. The ER₅₀ values of all the other species tested (*Lolium perenne*, *Avena sativa*, *Allium cepa*, *Brassica napus*, *Beta vulgaris*, *Cucumis sativa*, *Helianthus annuus* and *Lycopersicon esculentum*) ranged from 4.127 g a.e./ha to >15 g a.e./ha (the highest rate tested).

RMS comments

The current endpoint to be used for risk assessment purposes is an ER₅₀ of 0.4 g a.e./ha based on a reduction of foliar fresh weight on soybean plants. Ideally ER₅₀ values for phytotoxicity would have been calculated; in the case of 8/11 species tested, including the most sensitive (Soybean), there is the potential that phytotoxic effects may be a more sensitive endpoint than foliar fresh weight reduction.

End points were reported as EC (effect concentrations) in the report, but as treatment was via spray at an application rate, ER (effect rate) would be the appropriate end point. This is purely a typographical report error and does not impact the value calculated.

Although GLP compliance is not claimed for compost analysis, seed details, meteorological data, glasshouse environmental data or photographs it has been confirmed that soil analysis, seed records and environmental conditions were adequately maintained/performed. With regards to environmental conditions (temperature, humidity, light intensity) any significant discrepancies would likely

have impacted upon the control plants. As control groups of all 11 species satisfied the related OECD guideline criteria the environmental conditions are considered to have been suitable for a successful study. Meteorological data and the photographs included in the report do not contribute directly to the conclusions drawn from the study and are for supplementary information only.

Following applicant correspondence the amount of soil used per replicate was confirmed as equal for all species, as well as confirmation that the most recent GLP certificate at the time of study commencement was provided.

B.9.9.2 Risk assessment

The results from the above standard laboratory studies on non-target plants are summarised in the following table. Key regulatory endpoints used in the risk assessment are highlighted in bold.

Table B.9.9.38: Summary of endpoints for non target plant species

Most sensitive species	Test substance	ER ₅₀ (g/ha) vegetative vigour	ER ₅₀ (g/ha) Emergence	Reference
Carrot	XDE-729 acid		0.3835 g a.e./ha*	Rockcliff, C., (2011c) IIA 8.12/03
All 11 species showed no inhibition <50%	X11449757	-	>15 g/ha	Rockcliff, C., (2011d) IIA 8.12/04
Carrot	GF2573	-	0.417 g ae /ha*	Rockcliff, C., (2011b) IIIA 10.8.1.3/01
Soybean		0.4 g a.e /ha*	-	Rockcliff, C., (2011a) IIIA 10.8.1.2/01

*g ae/ha- grams acid equivalent per hectare.

It is worth noting that for the vegetative vigour study phytotoxic effects were widespread and for the most sensitive species tested (soybean) could have resulted in a lower endpoint than that calculated with regards to foliar fresh weight. 50% effects would be expected to occur at between 0.235 and 0.47 g a.e./ha (46 and 61% phytotoxicity score recorded respectively, consisting of a check in growth, distorted growing points and curled leaves and stems. Affected plants were dark green in colour and drooped over. The most severely affected plants became shrivelled and were yellow or brown in colour).

However, as the measurement of phytotoxicity effect is subjective, the biomass reduction measurement is used in preference as a more quantitative assessment of the toxicological

effect on non-target plant species. Thus, the RMS has conducted the following risk assessment using the lowest calculated ER_{50} from the vegetative vigour study (for Soybean biomass reduction).

As > 50% effects were observed at the proposed field application rate, a tier 2 deterministic risk assessment is performed according to the *EC Guidance Document on Terrestrial Ecotoxicology 91/414, SANCO/1032/ 2002* using the following equation:

Exposure = Maximum proposed application rate x % drift

$TER = \frac{\text{lowest } ER_{50}}{\text{Exposure}}$

Vegetative Vigour Tier 2

GF-2573

Table 9.9.39: Risk assessment for non-target plants – GF-2573 vegetative vigour

Crop	Test substance	Maximum application (g a.e./ha)*	Spray drift	Exposure (g ae/ha)	ER_{50} (g ae/ha)	TER
Cereals	GF-2573	7.50	0.0277 (1 m)	0.21	0.400	1.93
			0.0057 (5 m)	0.043	0.400	9.36

*Maximum single application rate of 7.82 g a.s./ha converted to acid equivalent (molecular mass of Methyl 345 g/mol, molecular mass for Acid is 331. Ratio $331/345 = 0.959$, $7.82 \times 0.959 = 7.50$ g ae/ha)

As effects are to be determined for terrestrial plants in the vicinity of the treated area, spray drift has to be considered for a distance of 1 m from the field edge for field crops, which is 2.77% for a single application (90th percentile). With this drift value, the maximum exposure after one application on winter cereal is 0.21 g a.e/ha, the TER is 1.93. As the TER is < 5, the risk to non-target plants at a distance of 1 m from the treated area, is not acceptable. A buffer zone of 5m, or equivalent risk mitigation, is advisable; a risk assessment taking this into consideration is presented above. With this drift value, the maximum exposure after one application on winter cereal is 0.043 g a.e/ha, the TER is 9.36. This TER is > Annex VI trigger of 5 for non-target plants. Consequently, there is an acceptable risk for non-target plants resulting from application of GF-2573 to cereals at the field rate of 7.82 g a.s./ha assuming a 5 m buffer zone is implemented. Therefore this risk mitigation procedure is acceptable but member states should consider appropriate country-specific mitigation in order to provide the required level of protection to post-emergence non-target plants.

Seedling emergence**GF-2573**

Table 9.9.40: Risk to non-target plants - GF-2573– seedling emergence

Crop	Test substance	Maximum application rate (g a.e/ha)*	Spray drift	Exposure (g/ha)	ER ₅₀ (g/ha)	TER
Cereals	GF-2573	7.5	0.0277 (1m)	0.21	0.417	2.01
			0.0057 (5m)	0.043	0.417	9.75

*Maximum single application rate of 7.82 g a.s./ha converted to acid equivalent (molecular mass of Methyl 345 g/mol, molecular mass for acid is 331. Ratio 331/345 = 0.959, 7.82 x 0.959 = 7.50 g ae/ha

As effects are to be determined for terrestrial plants in the vicinity of the treated area, spray drift has to be considered for a distance of 1 m from the field edge for field crops, which is 2.77% for a single application (90th percentile). With this drift value, the maximum exposure after one application on winter cereal is 0.21 g/ha, the TER is 2.01. As the TER is < 5, the risk to non-target plants at a distance of 1 m from the treated area, is not acceptable. A buffer zone of 5m is advisable; a risk assessment taking this into consideration is presented above. With this drift value, the maximum exposure after one application on winter cereal is 0.043 g/ha, the TER is 9.75. This TER is > Annex VI trigger of 5 for non-target plants. Consequently, there is an acceptable risk for non-target plants resulting from application of GF-2573 to cereals at the field rate of 7.82 g a.s./ha assuming a 5 m buffer zone or equivalent risk mitigation is implemented. Therefore this risk mitigation procedure is acceptable but its use is at the discretion of each individual member state.

XDE-729 Acid

Following uptake of XDE-729 Methyl by plants, the molecule undergoes de-esterification to XDE-729 Acid. Thus, the toxicity and risk associated with XDE-729 Acid has been assessed in the vegetative vigour studies and assessment conducted for XDE-729 Methyl (with the formulation GF-2573). However, a seedling emergence and seedling growth study was submitted with XDE-729 Acid and therefore the risk assessment has been conducted and is summarised below.

Table 9.9.41: Risk to non-target plants – XDE-729 acid– seedling emergence

Crop	Maximum application rate (g a.e./ha)	Spray drift	Exposure (g/ha)	ER ₅₀ (g/ha)	TER
Cereals	7.5	0.0277 (1m)	0.21	0.3835	1.85
		0.0057 (5m)	0.043	0.3835	8.97

*Maximum single application rate of 7.82 g a.s./ha converted to acid equivalent (molecular mass of Methyl 345 g/mol, molecular mass for acid is 331. Ratio 331/345 = 0.959, 7.82 x 0.959 = 7.50 g ae/ha)

As effects are to be determined for terrestrial plants in the vicinity of the treated area, spray drift has to be considered for a distance of 1 m from the field edge for field crops, which is 2.77% for a single application (90th percentile). With this drift value, the maximum exposure after one application on winter cereal is 0.21 g/ha, the TER is 1.85. As the TER is < 5, the risk to non-target plants at a distance of 1 m from the treated area, is not acceptable. A buffer zone of 5m or equivalent risk mitigation is advisable; a risk assessment taking this into consideration is presented above. With this drift value, the maximum exposure after one application on winter cereal is 0.043 g/ha, the TER is 8.97. This TER is > Annex VI trigger of 5 for non-target plants. Consequently, there is an acceptable risk for non-target plants resulting from application of GF-2573 to cereals at the field rate of 7.5 g a.e./ha assuming a 5 m buffer zone is implemented. Therefore this risk mitigation procedure is acceptable but its use is at the discretion of each individual member state.

X11449757

Following uptake of XDE-729 Methyl by plants, the molecule undergoes de-esterification to XDE-729 Acid and subsequent metabolism to X11449757. Thus, the toxicity and risk associated with XDE-729 Acid has been assessed in the vegetative vigour studies and assessment conducted for XDE-729 Methyl (with the formulation GF-2573) However, a seedling emergence and seedling growth study was submitted with X11449757 and therefore the risk assessment has been conducted and is summarised below.

The maximum application for X11449757 is unknown however a worse case assumption of 7.5 g/ha has been used in the risk assessment. As 7.5 g a.e./ha of XDE-729 acid is metabolised after application of 7.82 g a.s/ha, it is likely that the amount of X11449757 present will be less than this.

Table 9.9.42: Risk to non-target plants –X11449757– seedling emergence

Crop	Maximum application rate (g/ha)	Spray drift	Exposure (g/ha)	ER ₅₀ (g/ha)	TER
Cereals	7.5	0.0277 (1m)	0.21	>15	>72.20

The TER for seedling emergence, based on the ER₅₀ of >15 g/ha was >72.20, with a 1 m buffer from the field edge. This TER is > the Annex VI trigger of 5 for non-target plants. Consequently, there is an acceptable risk for non-target plants resulting from application of GF-2573 to cereals at the field rate of 7.5 g/ha (worst case).

B.9.9.3 Conclusions

For the formulation GF-2573 the TER's for vegetative vigour and seedling emergence exceeded the Annex VI trigger of 5 with a 5m buffer zone. Consequently, there are acceptable risks for non target plants resulting from the application of GF-2573 at maximum field rate of 7.82 g a.s./ha **assuming a 5 m buffer zone or equivalent risk mitigation.**

For the metabolite XDE-729 Acid the TER for seedling emergence exceeds the proposed Annex VI trigger of 5 with a 5m buffer zone. Consequently, there are acceptable risks for non target plants resulting from exposure to XDE-729 Acid following application of GF-2573 at 7.5 g ae/ha **assuming a 5 m buffer zone or equivalent risk mitigation.** The result from this risk assessment demonstrates that XDE-729 Acid poses no additional risk to terrestrial non-target plants than that from GF-2573 and as such XDE-729 Methyl, which also resulted in a 5m buffer zone.

For the soil metabolite, X11449757 the TER for seedling emergence exceeds the proposed Annex VI trigger of 5 at first tier.

Overall, there are acceptable risks for non target plants resulting from exposure following applications of GF-2573 at 7.8 g a.s./ha **assuming a 5m buffer zone or equivalent risk mitigation is implemented.**

B.9.10 Effects on biological methods for sewage treatment (IIA 8.7)

B.9.10.1 Effects

Lee, Brian. (2010): XDE-729 Methyl: Activated Sludge, Respiration Inhibition Test. ABC Laboratories, 7200 E. ABC Lane, Columbia, Missouri 65202, ABC Laboratories Project Number 65898. Dow AgroSciences unpublished report, Study Number 101140. 23 December 2010. Amended 28 June 2011.

Test material

Test item:	XDE-729 Methyl
Purity:	97.2 wt/%
Description:	Off-white solid
Test Substance No./Lot No.	E2837-51

Test system

Organism:	Micro-organisms in activated sludge from domestic wastewater treatment plant
Study Type:	Laboratory study assessing oxygen consumption of activated sludge
GLP Status:	GLP
Guidelines followed:	OECD Guideline 209 – Activated Sludge, Respiration Inhibition Test. Adopted 2010.
Guideline deviations reported by Study Director:	None
Incubation period:	3 hours
Parameters measured:	Microbial respiration :- oxygen consumption or respiration rate (mg O ₂ L ⁻¹ minute ⁻¹) dissolved oxygen (mg O ₂ /L) temperature pH
Observation intervals:	Oxygen concentrations were measured following the incubation period by a Strathkelvin Instruments Activated Sludge Respirometer. Oxygen consumption (respiration rate) was calculated by the ASR software from the periodic oxygen concentration readings during the measurement period. Oxygen consumption (respiration rate) percent inhibition was in turn calculated by the ASR software using the untreated control respiration rate versus test or reference substance respiration rate.
Test concentrations:	0.1, 0.97, 9.80, 98.1, and 981 mg active substance (a.s.) XDE-729 methyl/L
Toxic reference:	3,5-Dichlorophenol 1.0, 10 and 30 mg 3,5-Dichlorophenol/L
Conditioning of activated sludge:	The activated sludge was concentrated by centrifugation, the supernatant was decanted, and the bottles were refilled with activated sludge and re-centrifuged until all collected sludge was concentrated. The microbial inoculum was rinsed by adding ABC well water, shaking, and centrifuging as above. The sludge was kept at test temperature under continuous aeration until use.

	Immediately before use, the dry weight suspended solids of the activated sludge was determined, and diluted with well water to 3.0 g/L.
Method of test item application	Test item was added directly to the test vessels and stirring commenced prior to the incubation period. A well-mixed sample of each test medium was poured into a glass tube after exactly 3 hours incubation time and stirred but not further aerated.
Environmental conditions:	Temperature of the environmental chamber ranged from 19.3 to 19.7°C during the incubation and measurement phase of the exposure and during storage of the microbial inoculum prior to test initiation. The pH values for the control, reference, and test contact flasks ranged from 8.26 to 8.48. The pH of the abiotic control contact flask, which contained no activated sludge, was 7.23. Exposure flasks were continuously aerated during the incubation period at a 0.5-1.0 L/minute rate.
Synthetic sewage feed:	Guideline standard sewage feed dissolved in 1 L of water.

Methodology

The purpose of the 3-hour toxicity test was to evaluate the influence of the test item on the activity of activated sludge by measuring the respiration rate under defined conditions. The respiration rate (oxygen consumption) of an aerobic activated sludge fed with a standard amount of synthetic sewage was measured in the presence of various concentrations of the test item after an incubation period of 3 hours. The inhibitory effect of the test item at the particular concentrations was expressed as the percent inhibition in respiration rate versus the mean respiration rate of two controls. This type of study is recognized by the OECD guideline and provides a rational basis to assess the respiration inhibition properties of the test item when incubated with activated sludge.

Results

Table B.9.10.1: Effects of test item on the Respiration rate

Flask No.	Treatment	Concentration [mg/L]	Oxygen consumption [mg O ₂ L ⁻¹ hr ⁻¹]	Inhibition %	pH-values
1	control	---	40.1	---	8.30
10	control	---	45.7	---	8.30
	mean		42.9	---	8.30
	C.V.		9.23	---	0
2	3,5-DCP	1.0	42.6	0.7	8.27
3	3,5-DCP	10	32.2	25.0	8.84
4	3,5-DCP	30	15.3	64.3	8.48
3,5-Dichlorophenol 3hour EC ₅₀ = 20.1 mg/L					
5	XDE-729 methyl	0.1	45.0	0.0	8.26
6	XDE-729 methyl	0.97	44.5	0.0	8.26
7	XDE-729 methyl	9.80	40.1	8.7	8.27
8	XDE-729 methyl	98.1	48.4	0.0	8.27
9	XDE-729 methyl	981	43.8	0.1	8.33
3hour EC ₅₀ = >981 mg a.s./L					

Conclusions

Since the percent inhibition at the highest concentration tested for XDE-729 methyl was less than 50% during the 3-hour exposure, the estimated EC₅₀ was >981 mg a.s./L. The respiration rates of control flasks as well as the estimated EC₅₀ values of the reference toxicant testing met the acceptability criteria outlined in OECD 209. Based on these results XDE-729 methyl has no impact on respiration activity of activated sludge when tested at concentrations up to and including 981 mg a.s./L.

RMS Comment The study is considered to be acceptable and suitable for use for risk assessment purposes. The endpoint from this study is EC₅₀ >981 mg a.s./L.

B.9.10.2 Risk assessment

Table B.9.10.2: Summary of endpoints for effects on biological methods for sewage treatment

Test type/organism	Endpoint	Reference
Activated sludge	EC ₅₀ >981 mg a.s./L	Lee, B. , (2011) IIA 8.15/01

The results from the Activated Sludge, Respiration Inhibition Test (Lee, Brian, 2011, study number 101140) propose an EC₅₀ for XDE-729 methyl of > 981 mg a.s./L. When compared to the worst-case FOCUS step 1 PEC_{sw} for all proposed applications (1.197 µg/L), it can be concluded that an acceptable risk from XDE-729 methyl has been demonstrated for microbial activity in these systems.

B.9.11 Overall summary of ecotoxicological risk assessment

Effects on birds and mammals

At screening step, in a risk assessment conducted to the EFSA (2009) guidance, the acute and reproductive TERs for birds and mammals were above the trigger values for the representative use on winter and spring cereals.

Acute toxicity data for the representative formulation, GE 2573 was also submitted for birds and mammals. An acute risk assessment using these endpoints was carried out and the results were compared to the risk assessment with the active, XDE-729 Methyl. The TER's calculated were above the trigger values.

A low risk to birds and mammals via drinking water, secondary poisoning (from consumption of potentially contaminated fish and earthworms) and from biomagnification was also concluded.

It was considered that there were no relevant metabolites for XDE-729 Methyl that required further evaluation in the bird and mammal risk assessments. Any metabolites present in plants were also present in bird/mammal metabolism studies. These metabolites were all present at low levels (<10%) and were considered to be covered by the active risk assessment. Metabolites of XDE-729 Methyl have a very low potential for bioaccumulation and biomagnification with log K_{ow} values below the trigger value of 3. Therefore bioaccumulation of XDE-729 Methyl metabolites in soil organisms such as earthworms and aquatic organisms such as fish is negligible and not a major route of exposure to birds.

XDE-729 Methyl, XDE-729 Acid, X11406790 and X11449757 do not appear to exhibit endocrine-disrupting properties that would affect reproductive physiology or development of reproductive organs in wild mammals or birds. However, member states should note that there are currently no defined criteria for identifying endocrine disruptors under 2009/1107 and as such only a qualitative case can be made.

Effects on aquatic species

With the exception of aquatic plants, the acute and long-term toxicity/exposure ratios (TERs) for GF-2573, XDE-729 Methyl and the major metabolites are in excess of the Annex VI triggers for FOCUS Step 1 and/or Step 2.

XDE-729 Methyl and XDE-729 Acid are significantly more phytotoxic to the aquatic plant *Myriophyllum spicatum* than to *Lemna*; as a result the TER values for XDE-729 Methyl and XDE-729 Acid are below the Annex VI trigger for FOCUS Step 1, Step 2 and Step 3 for most application scenarios. The metabolites X11449757 and X11406790 showed low phytotoxicity towards *Myriophyllum* at the maximum concentration tested (100 µg/L) and as a result the TER values exceed the Annex VI triggers at FOCUS Step 1.

The risk from the formulated product was concluded as acceptable for all organism groups with the implementation of a 5m buffer zone to reduce spray drift (to address the risk to aquatic plants).

XDE-729 Methyl: Exposure of aquatic plants occurs principally via drift immediately following application of GF-2573. Consequently, for applications to winter cereals a 10 m buffer (for spring applications at 6.25 g/ha) or 20 m buffer (for autumn applications at 7.82 g/ha) will provide adequate mitigation for all FOCUS surface water scenarios. For applications, at 6.25 g/ha, to spring sown cereals a 10 m buffer will provide adequate mitigation. All buffers implemented must reduce exposure via spray drift and runoff.

XDE-729 Acid: Exposure of aquatic plants is predicted to occur principally via drainflow and run-off. Consequently, for autumn applications at 7.82 g/ha to winter cereals acceptable risk from the acid to *Myriophyllum* could not be demonstrated for FOCUS scenarios D1 ditch and stream, D2 ditch and stream and D6 ditch. Spring applications to winter sown cereals at a rate of 6.25 g/ha result in unacceptable risk to *Myriophyllum* for scenarios D1 ditch, D2 ditch and stream, and D6 ditch. Application in spring to spring sown cereals at 6.25 g/ha results in acceptably low risk to *Myriophyllum* from the acid for all FOCUS scenarios.

As an overall acceptable risk from the acid cannot be demonstrated using FOCUS modelling and risk mitigation, member states should consider carefully which scenarios are relevant in their national assessments. Likewise appropriate mitigation should be considered at member state level in line with individual national requirements.

The risk to the most sensitive organism tested (*Myriophyllum spicatum*) was also assessed using growth rate endpoints from the lab data, in line with the draft EFSA aquatic guidance document (EFSA Journal 2013;11(7):3290). The outcome is not greatly different, with only the exact level of mitigation required for each proposed use and unresolved FOCUS scenarios differing. Again member states may wish to consider this approach when conducting their national assessments.

The active substance was concluded as having low bioaccumulation potential in fish (BCF < 1000, 95% depuration time of 1.6 days).

Effects on bees

An acceptable risk was demonstrated at first tier for the active substance, XDE-729 Methyl and the representative formulation, GF-2573. The contact and oral hazard quotients derived using the toxicity endpoints from laboratory acute toxicity tests are below the Annex VI trigger value. It can therefore be concluded that if used according to the proposed GAP, XDE-729 methyl and the formulation GF-2573 demonstrate an acceptable risk to bees.

Effects on other arthropod species

An acceptable in-field and off-field risk to non-target arthropods from the proposed use of GF-2573 was demonstrated at first tier. Based on an ESCORT 2 first tier risk assessment the off-field and in-field hazard quotients were below the Annex VI trigger value. Therefore use of the representative formulation in accordance with the proposed GAP does not pose a significant risk to non-target arthropod species in both in-field and off-field habitats.

Effects on earthworms

An acceptable acute and chronic risk to earthworms has been demonstrated for the representative formulation GF-2573, the active XDE-729 Methyl, the major metabolites XDE-729 Acid (X11393729) and X11449757 and all non-extractable residues. All TER values calculated were above the acute and chronic Annex VI trigger values.

Effects on other soil non-target macro-organisms

An acceptable chronic risk to non target soil macro-organisms has been demonstrated for XDE-729 Methyl and the major metabolites XDE-729 Acid (X11393729) and X11449757. All TER values calculated were above the chronic Annex VI trigger values.

For soil micro-organisms, the concentrations for which <25% effects were observed, were all in excess of the PEC_{soil} values. Therefore it can be concluded that the risk to soil micro-organisms is acceptable for the use of GF-2573 on cereals if applied according to the proposed GAP.

Effects on other non-target organisms (flora and fauna) believed to be at risk

For the formulation GF-2573 the TER's for vegetative vigour and seedling emergence exceeded the Annex VI trigger value, with a 5m buffer zone. Therefore, an acceptable risk has been demonstrated from exposure to the representative formulation, GF-2573 providing a 5 m buffer zone or equivalent risk mitigation is implemented.

For the metabolite XDE-729 Acid the TER for seedling emergence exceeds the proposed Annex VI trigger value, with a 5m buffer zone. Therefore, an acceptable

risk has been demonstrated from exposure to XDE-729 acid, providing a 5 m buffer zone or equivalent risk mitigation is implemented. The result from this risk assessment demonstrates that XDE-729 Acid poses no additional risk than that already demonstrated from exposure to GF-2573 and as such the active XDE-729 Methyl, which also results in a 5m buffer zone or equivalent risk mitigation.

For the soil metabolite, X11449757 the TER for seedling emergence exceeds the proposed Annex VI trigger value at first tier and as such, no additional buffer zone or risk mitigation is required.

Overall, acceptable risks for non target plants has been demonstrated following exposure to the representative formulation, GF-2573 assuming **a 5m buffer zone or equivalent risk mitigation is implemented.**

Effects on biological methods of sewage treatment

The proposed EC_{50} for XDE-729 methyl is $> 981 \text{ mg a.s./L}$. When compared to the worst-case FOCUS step 1 PEC_{sw} for all proposed applications ($1.197 \text{ } \mu\text{g/L}$), it can be concluded that an acceptable risk from XDE-729 methyl has been demonstrated for microbial activity in these systems.

B.9.12 References relied on**Active substance -By Annex Point**

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.1.1/01	██████████ ██████████ ██████████	2011a	XDE-729 Methyl: An Acute Oral Toxicity Study with the Northern Bobwhite. ██ DAS Report No.: 090026, 379-211 (Accession Number) 2004303 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.1.1/02	██████████ ██████████ ██████████	2011b	XDE-729 Methyl: An Acute Oral Toxicity Study with the Zebra Finch (<i>Poephila guttata</i>). ██ DAS Report No.: 090027, 379-212 (Accession Number) 2006420 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.1.2/01	██████████ ██████████ ██████████ ██████████ ██████████	2010 (amended) 2011	XDE-729 Methyl: A dietary LC50 study with the Northern Bobwhite. ██ DAS Report No.: 090028, 379-213 (Accession Number) 2005328 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.1.3/01	██████████ ██████████ ██████████ ██████████ ██████████	2011	XDE-729 Methyl: A dietary LC50 study with the Mallard ██ DAS Report No.: 090029, 379-214 (Accession Number) 2005329 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.1.4/01	██████ ██████	2011a	XDE-729 Methyl: A reproduction study with the Northern Bobwhite ████████████████████. DAS Report No.: 101137, 379-246 (Accession Number) 2008564 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.1.4/02	██████ ██████	2011b	XDE-729 Methyl: A reproduction study with the Mallard ████████████████████. DAS Report No.: 101139, 379-247 (Accession Number) 2008565 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.2.1.1/01	██████	2011a	XDE-729 Methyl: Acute toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions ████████████████████. DAS Report No.: 090187, 64605 (Accession Number) 2008529 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.2.1.2/01	██████ ██	2011a	XDE-729 Methyl: Acute Toxicity to the Fathead Minnow, <i>Pimephales promelas</i> , Determined Under Static-Renewal Test Conditions ████████████████████. DAS Report No.: 090186, 64604 (Accession Number) 2008581 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.2.1.2/02	██████████	2011b	XDE-729 Methyl: Acute Toxicity to the Sheepshead Minnow, <i>Cyprinodon variegatus</i> , Determined Under Flow-Through Conditions ██████████. DAS Report No.: 090188, 64606 (Accession Number) 2008714 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.2.1.3/01	██████████ ████	2011b	XDE-729 Acid: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions ██████████ DAS Report No.: 101152, 65970 (Accession Number) 2008976 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.2.1.3/02	██████████ ████	2011a	X11449757: Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions ██████████ DAS Report No.: 101166, 66008 (Accession Number) 2010748 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.2.1.3/03	██████████	2012a	X11406790 (XDE-729 Metabolite): Acute Toxicity to the Rainbow Trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions ██████████. DAS Report No.: 120020, 68212 (Accession Number) 2013228 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.2.4/01	██████████	2011c	XDE-729 Methyl: Early Life-stage Toxicity Test with the Fathead minnow, <i>Pimephales promelas</i> , Under Flow Through Test Conditions. ██████████ DAS Report No.: 101134, 65896 (Accession Number) 2008689 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.2.4/02	██████████ ██████████	2012a	XDE-729 Methyl: Early Life-Stage Toxicity Test with the Sheepshead Minnow, <i>Cyprinodon variegatus</i> , Under Flow-Through Conditions ██████████ DAS Report No.: 120017, 68313 (Accession Number) 2013712 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.2.4/03	██████████	2011d	XDE-729 Acid: An early Life-stage Toxicity Test with the Fathead minnow, <i>Pimephales promelas</i> , Under Flow Through Conditions. ██████████ DAS Report No.: 101151, 65971 (Accession Number) 2008942 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.2.4/04	[REDACTED]	2012b	X11449757: Early life stage toxicity test with the Fathead Minnow, <i>Pimephales promelas</i> , under flow through conditions [REDACTED]. DAS Report No.: 101165, 66009 (Accession Number) 2014171 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.2.6.1/01	[REDACTED]	2011	XDE-729 Methyl: Bioconcentration and Metabolism Study with Bluegill, <i>Lepomis macrochirus</i> [REDACTED] DAS Report No.: 101135, 66001 (Accession Number) 2009609 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.3.1.1/01	Rebstock, M.	2011c	XDE-729 Methyl: Acute toxicity to the Water Flea, <i>Daphnia magna</i> , Determined Under Static Test Conditions ABC Laboratories, Inc. DAS Report No.: 090185, 64603 (Accession Number) 2008576 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.3.1.1/02	Bergfield, A.	2011b	XDE-729 Acid: Acute toxicity to the Water Flea, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions. ABC Laboratories, Inc. DAS Report No.: 101149, 65969 (Accession Number) 2009021 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.3.1.1/03	Bergfield, A.	2011c	X11449757: Acute toxicity to the Water Flea, <i>Daphnia magna</i> , Determined Under Static-Renewal Test Conditions ABC Laboratories, Inc. DAS Report No.: 101163, 66007 (Accession Number) 2009047 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.3.1.1/04	Gaertner, K.	2012b	X11406790 (XDE-729 Metabolite): Acute Toxicity to the Cladoceran, <i>Daphnia magna</i> , Determined Under Static Test Conditions ABC Laboratories, Inc. DAS Report No.: 120019, 68211 (Accession Number) 2013227 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.3.1.3/01	Bergfield, A.	2011d	XDE-729 Methyl: Acute toxicity Test with the Mysid shrimp, <i>Americamysis bahia</i> , Determined Under Flow-Through Conditions. ABC Laboratories, Inc. DAS Report No.: 090184, 64608 (Accession Number) 2009033 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.3.1.4/01	Hicks, S.L.	2011a	XDE-729 Methyl: Effect on New Shell Growth of the Eastern Oyster (<i>Crassostrea virginica</i>) ABC Laboratories, Inc. DAS Report No.: 090120, 64609 (Accession Number) 2009003 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.3.2.1/01	Bergfield, A.	2011e	XDE-729 Methyl: Chronic Toxicity with the Water Flea, <i>Daphnia magna</i> , Exposed Under Static-Renewal Test Conditions ABC Laboratories, Inc. DAS Report No.: 101133, 65897 (Accession Number) 2009012 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.3.2.1/02	Bergfield, A.	2011f	XDE-729 Acid: Chronic Toxicity Test with the Water Flea, <i>Daphnia magna</i> , Exposed Under Static-Renewal Conditions ABC Laboratories, Inc. DAS Report No.: 101150, 65972 (Accession Number) 2011063 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.3.2.2/01	Gerke, A.	2011e	XDE-729 Methyl: Chronic Toxicity in Whole Sediment to Freshwater Midge, <i>Chironomus riparius</i> ABC Laboratories, Inc. DAS Report No.: 101130, 65899 (Accession Number) 2008991 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.3.2.4/01	Hicks, S.L.	2011b	XDE-729 Methyl: Life-Cycle Toxicity Test of the Saltwater Mysid, <i>Americamysis bahia</i> , Conducted Under Flow-Through Test Conditions ABC Laboratories, Inc. DAS Report No.: 101131, 65895 (Accession Number) 2009346 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.4/01	Weber, K.	2011a	Testing Effects of XDE-729 Methyl on the Single Cell Green Alga, <i>Pseudokirchneriella subcapitata</i> , in a 96 h Static Test EurofinsAgroScience Services GmbH DAS Report No.: 090173, S09-00613 (Accession Number) 2009370 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.4/02	Rebstock, M.	2011d	XDE-729 Methyl: Growth Inhibition Test with the Freshwater Diatom, <i>Navicula pelliculosa</i> ABC Laboratories, Inc. DAS Report No.: 090174, 67182 (Accession Number) 2008977 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.4/03	Weber, K.	2011b	Testing of Effects of XDE-729 Methyl on the Blue-Green Alga, <i>Anabaena flos-aquae</i> , in a 96 h Static Test EurofinsAgroScience Services GmbH DAS Report No.: 090175, S09-00615 (Accession Number) 2009371 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.4/04	Rebstock, M.	2011e	XDE-729 Methyl: Static Growth Inhibition Test with the Marine Diatom, <i>Skeletonema costatum</i> ABC Laboratories, Inc. DAS Report No.: 090176, 64717 (Accession Number) 2008573 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.4/05	Rebstock, M.	2011f	XDE-729 Acid: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc. DAS Report No.: 102027, 66685 (Accession Number) 2009894 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.4/06	Rebstock, M.	2011g	XDE-729 Acid: Growth Inhibition Test with the Freshwater Diatom, <i>Navicula pelliculosa</i> ABC Laboratories, Inc. DAS Report No.: 102029, 66687 (Accession Number) 2009892 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.4/07	Rebstock, M.	2011h	XDE-729 Acid: Growth Inhibition Test with the Blue-Green Alga, <i>Anabaena flos-aquae</i> ABC Laboratories, Inc. DAS Report No.: 101144, 65967 (Accession Number) 2009893 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.4/08	Rebstock, M.	2011i	XDE-729 Acid: Static Growth Inhibition Test with the Marine Diatom, <i>Skeletonema costatum</i> ABC Laboratories, Inc. DAS Report No.: 102028, 66686 (Accession Number) 2010034 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.4/09	Rebstock, M.	2011j	Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> - X11449757 ABC Laboratories, Inc. DAS Report No.: 101158, 66006 (Accession Number) 2009391 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.4 /10	Rebstock, M.	2012a	X11406790: Growth Inhibition Test with the Unicellular Green Alga, <i>Pseudokirchneriella subcapitata</i> ABC Laboratories, Inc. DAS Report No.: 120021, 68210 (Accession Number) 2013237 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.5.1/01	Gerke, A.	2011f	XDE-729 Methyl: Whole sediment 10 day Acute Toxicity test with Midge Larvae (<i>Chironomus dilutus</i>) ABC Laboratories, Inc. DAS Report No.: 090183, 64607 (Accession Number) 2008713 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.6 /01	Rebstock, M.	2011k	XDE-729 Methyl: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, <i>Lemna gibba</i> ABC Laboratories, Inc. DAS Report No.: 090182, 64595 (Accession Number) 2008575 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.6/02	Rebstock, M.	2011	XDE-729 Acid: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, <i>Lemna gibba</i> ABC Laboratories, Inc. DAS Report No.: 101145, 65968 (Accession Number) 2008986 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.6/03	Rebstock, M.	2011 m	X11449757: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, <i>Lemna gibba</i> ABC Laboratories, Inc. DAS Report No.: 101159, 66011 (Accession Number) 2009942 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.6/04	Rebstock, M.	2012b	X11406790: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, <i>Lemna gibba</i> ABC Laboratories, Inc. DAS Report No.: 120022, 68209 (Accession Number) 2013238 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.6/05	Gonsior, G.	2012a	XDE-729 Methyl - Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System Eurofins Agroscience Services EcoChem GmbH DAS Report No.: 102023, S11-02965 (Accession Number) 2013388 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.6/06	Gonsior, G.	2012b	XDE-729 Acid - Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System Eurofins Agroscience Services EcoChem GmbH DAS Report No.: 120533, S12-00215 (Accession Number) 2013766 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.6/07	Gonsior, G.	2012c	X11449757 - Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System Eurofins Agroscience Services EcoChem GmbH DAS Report No.: 102015, S12-00216 (Accession Number) 2013767 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.6/08	Gonsior, G.	2012d	X11406790 - Growth Inhibition of <i>Myriophyllum spicatum</i> in a Water/Sediment System Eurofins Agroscience Services EcoChem GmbH DAS Report No.: 120534, S12-00217 (Accession Number) 2013772 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.6/09 (Evaluated under KIIA 7.6/01)	Hellstern, J.	2012	Determination of Photolysis Reaction of XDE 729 Methyl under Test Conditions used in a <i>Myriophyllum</i> Eurofins Agroscience Services EcoChem GmbH DAS Report No.: 120547, S12-01352 (Accession Number) 2014337 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.7.1/01	Schmitzer S.	2011	Effects of XDE-729 Methyl (Acute Contact and Oral) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory. Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 101128/ 101129, 49528035 (Accession Number) 2006191 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.9.1/01	Witte, B	2011	Acute Toxicity (14 Days) of XDE-729 Methyl to the Earthworm, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 090099, 49524021 (Accession Number) 2004293 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.9.1/02	Witte, B.	2010b	XDE-729 Acid: Acute Toxicity (14 Days) of XDE-729 Acid to the Earthworm, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat. Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 101141, 56861021 (Accession Number) 2005790 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.9.1/03	Witte, B	2010c	Acute Toxicity (14 days) of X11449757 (metabolite of XDE-729) to the Earthworm, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 101155, 56872021 (Accession Number) 2007521 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.9.2/01	Witte, B	2011	Effects of XDE-729 Methyl on Reproduction and Growth of Earthworms, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat (Revised) Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 090100, 49525022 (Accession Number) 2005119 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.9.2/02	Witte, B.	2010e	Effects of XDE-729 Acid on Reproduction and Growth of Earthworms, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 101142, 56862022 (Accession Number) 2006242 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.9.2/03	Witte, B.	2010f	Effects of X11449757 (metabolite of XDE-729) on Reproduction and Growth of Earthworms, <i>Eisenia fetida</i> , in Artificial Soil with 5% Peat Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 101156, 56873022 (Accession Number) 2007522 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.9.2/04	Luhrs, Ulf	2011	Effects of XDE-729 Methyl on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 110280, 64641089 (Accession Number) 2011217 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.9.2/05	Gerke, A.	2011g	XDE-729 Methyl: Inhibition of Reproduction of Collembola, <i>Folsomia candida</i> , in Artificial Soil ABC Laboratories, Inc. DAS Report No.: 090181, 64611 (Accession Number) 2010327 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.9.2/06	Witte, B.	2011a	Effects of XDE-729 Acid on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 102025, DR-0402-7809-066 (Accession Number) 2008202 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.9.2/07	Witte, B.	2011b	Effects of XDE-729 Acid on Reproduction of the Collembola <i>Folsomia candida</i> in Artificial Soil with 5% Peat. Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 102024, DR-0402-7809-067 (Accession Number) 2008319 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.9.2/08	Witte, B.	2011c	Effects of X11449757 (metabolite of XDE-729) on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil with 5% Peat Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 101154, DR-0417-6492-005 (Accession Number) 2009067 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.9.2/09	Gerke, A.	2011h	X11449757: Inhibition of Reproduction of Collembola, <i>Folsomia candida</i> , in Artificial Soil ABC Laboratories, Inc. DAS Report No.: 101153, DR-0417-6492-009 (Accession Number) 2010361 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.9.2/10	McCormac, A.	2012	Determination of the chronic (sub-lethal) toxicity of aged residues of technical-grade XDE-729 Methyl to the earthworm <i>Eisenia fetida</i> in two natural soil substrates Mambo-Tox Ltd. DAS Report No.: 110605, DOW-11-38 (Accession Number) 2012444 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.9.2/11 (Evaluated under KIIA 7.1.1/03)	Liu, D. And Croffie, J.	2012	Determination of Non-Extractable Residues of XDE-729 Methyl in Soil under Aerobic Conditions Dow AgroSciences LLC DAS Report No.: 110767, 110767 (Accession Number) 2012111 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.10.1/01	Feil, N.	2011a	Effects of XR-729 methyl on the activity of the soil microflora in the laboratory Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 101127, 49527080 (Accession Number) 2006265 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.10.1/02	Feil, N.	2010b	Effects of XDE-729 acid on the activity of the soil microflora in the laboratory. Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 101143, 56863080 (Accession Number) 2006956 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.10.1/03	Feil, N.	2011	Effects of X11449757 on the Activity of the Soil microflora in the Laboratory. Institut für Biologische Analytik, und Consulting IBACON GmbH DAS Report No.: 101157, 56874080 (Accession Number) 2008715 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.11.1/01	Gerke, A.	2011i	XDE-729 Methyl: Whole sediment acute toxicity to a marine amphipod (<i>Leptocheirus plumulosus</i>) ABC Laboratories, Inc. DAS Report No.: 101132, 66366 (Accession Number) 2010299 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.12/03	Rockcliff, C.	2011c	Evaluation of the Phytotoxicity of the XDE-729 acid GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants (Based on OECD Guideline 208) - Europe 2011 Stockbridge Technology Centre Ltd DAS Report No.: 101955, STC/11/E601 (Accession Number) 2010184 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.12/04	Rockcliff, C.	2011d	Evaluation of the Phytotoxicity of the XDE-729 M-757 metabolite GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants (Based on OECD Guideline 208) - Europe 2011 Stockbridge Technology Centre Ltd DAS Report No.: 101956, STC/11/E602 (Accession Number) 2010185 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.15/01	Lee, B.	2010 (Amendment 2011)	XDE-729 Methyl: Activated Sludge, Respiration Inhibition Test ABC Laboratories, Inc. DAS Report No.: 101140, 65898 (Accession Number) 2007391 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.16/01	[REDACTED]	2012c	XDE-729 Methyl: Acute toxicity to the Tadpole (<i>Xenopus laevis</i>) determined under flow through test conditions [REDACTED] DAS Report No.: 090121, 64610 (Accession Number) 2011633 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.16/02	[REDACTED]	2012	XDE-729 Methyl: Fish Short-Term Reproduction Assay with the Fathead Minnow (<i>Pimephales promelas</i>) [REDACTED] DAS Report No.: 102125, 379A-153 (Accession Number) 2012508 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.16/03	[REDACTED]	2012	XDE-729 Acid: Fish Short-Term Reproduction Assay with the Fathead Minnow (<i>Pimephales promelas</i>). [REDACTED] DAS Report No.: 120535, 379A-154 (Accession Number) 2013765 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 8.16/04	[REDACTED]	2012	XDE-729 Methyl: Amphibian Etamorphosis Assay for the Detection of Thyroid Active Substances [REDACTED] DAS Report No.: 102126, 379A-152 (Accession Number) 2013201 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 8.16/05	[REDACTED]	2012	XDE-729 Methyl: 21-day Reproduction test with the Fathead Minnow (Pimephales promelas) [REDACTED] DAS Report No.: 379A-155, 120018 (Accession Number) 2015068 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Plant Protection Product 'GF-2573'-By Annex Point

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIIA 10.1.6/01	[REDACTED]	2011	GF-2573: An acute oral toxicity study with the northern bobwhite [REDACTED] DAS Report No.: 102030, 379-277 (Accession Number) 2008554 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.2.2.1/01	[REDACTED]	2011a	GF-2573: Acute Toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , Determined Under Static-Renewal Test Conditions [REDACTED] DAS Report No.: 101126, 65998 (Accession Number) 2009109 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIIA 10.2.2.2/01	Bergfield, A.	2011b	GF-2573: Acute Toxicity to the Water Flea, Daphnia magna, Determined Under Static-Renewal Test Conditions ABC Laboratories, Inc. DAS Report No.: 101123, 65997 (Accession Number) 2009108 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.2.2.2/02	Gaertner, K.	2012	GF-2573 Blank Formulations Acute Toxicity to the Cladoceran, Daphnia magna, Determined Under Static Renewal Test Conditions ABC Laboratories, Inc. DAS Report No.: 120032, 68244 (Accession Number) 2012596 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.2.2.3/01	Rebstock, M.	2011a	GF-2573: Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata ABC Laboratories, Inc. DAS Report No.: 101124, 65996 (Accession Number) 2009048 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.2.2.3/02	Rebstock, M.	2012	██████████ Growth Inhibition Test with the Unicellular Green Alga, Pseudokirchneriella subcapitata. ABC Laboratories, Inc. DAS Report No.: 120031, 68245 (Accession Number) 2012595 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIIA 10.4.2.1/01	Schmitzer, S.	2011	Effects of GF-2573 (Acute Contact and Oral) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory Institut fur Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 101119 / 101120, 54805035 (Accession Number) 2006185 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.5.1/01	Moll, M.	2011	Effects of GF-2573 on the Parasitoid <i>Aphidius rhopalosiphii</i> in the Laboratory (Tier I) Dose Response Test Institut fur Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 090096, 54801001 (Accession Number) 2005874 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.5.1/02	Schwarz, A.	2011	Effects of GF-2573 on the Predatory Mite <i>Typhlodromus pyri</i> in the Laboratory (Tier I) Dose Response Test Institut fur Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 090097, 54802063 (Accession Number) 2006118 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.6.2/01	Witte, B.	2011	Acute Toxicity (14 days) of GF-2573 to the Earthworm, <i>Eisenia fetida</i> in Artificial Soil with 5% Peat. Institut fur Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 101121, 54808021 (Accession Number) 2008987 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIIA 10.6.3/01	Witte, B.	2011	Effects of GF-2573 on Reproduction and Growth of Earthworms Eisenia fetida in Artificial Soil with 5% Peat Institut fur Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 101122, 54809022 (Accession Number) 2006114 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.7.1/01	Feil, N.	2011	Effects of GF-2573 on the Activity of the Soil Microflora in the Laboratory Institut fur Biologische Analytik und Consulting IBACON GmbH DAS Report No.: 090101, 54804080 (Accession Number) 2006776 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.8.1.2/01	Rockcliff, C.	2011a	Evaluation of the Phytotoxicity of GF-2573 (XDE-729, 7.5 g ae/l, EC) GLP Vegetative Vigour Test Terrestrial Non Target Plants (Based on OECD Guideline 227) – Europe, 2011 Stockbridge Technology Centre Ltd. DAS Report No.: 101969, STC/11/E605 (Accession Number) 2009887 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.8.1.3/01	Rockcliff, C.	2011b	Evaluation of the Phytotoxicity of GF-2573 (XDE-729, 7.5 g ae/l, EC) GLP Seedling Emergence and Seedling Growth Test Terrestrial Non Target Plants (Based on OECD Guideline 208) – Europe, 2011 Stockbridge Technology Centre Ltd. DAS Report No.: 101970, STC/11/E606 (Accession Number) 2009888 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIIA 10.8.2.1/01	Rebstock, M.	2011b	GF-2573: Growth Inhibition Test with the Freshwater Aquatic Plant, Duckweed, Lemna gibba ABC Laboratories, Inc. DAS Report No.: 101125, 65999 (Accession Number) 2009409 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 10.8.2.1/02	Gonsior, G.	2012	GF-2573 - Growth Inhibition of Myriophyllum spicatum in a Water/Sediment System Eurofins Agrosience Services EcoChem GmbH DAS Report No.: 120584, S12-02164 (Accession Number) 2014174 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS