TEXT SEARCHABLE DOCUMENT

Data Evaluation Report on the Chronic Toxicity of Florasulam to *Daphnia magna*

PMRA Submission Number {.....} EPA MRID Number 468083-21

Data Requirement:

PMRA Data Code	9.3.2
EPA DP Barcode	D329529
OECD Data Point	{.....}
EPA MRID	468083-21
EPA Guideline	72-4

Test material: XDE-570 **Purity:** 99.2%
Common name florasulam
Chemical name: IUPAC 2',6',8-trifluoro-5-methoxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonanilide
CAS name *N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonamide
CAS No. 145701-23-1
Synonyms

Primary Reviewer: Peter Takacs
PMRA **Date:** 8.14.2000

Primary Reviewer: Brian D. Kiernan, Biologist **Date:** 3.21.2007
EPA 10/8/07

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	129108	

Date Evaluation Completed: 3.06.2007

CITATION: Kirk, H.D., Landre, A.M., Hugo, J.M. and Stahl, D.C. (1996): Evaluation of the chronic toxicity of XDE-570 herbicide to the daphnid, *Daphnia magna* Straus. Dow AgroSciences, unpublished report No. DECO-ES-2944, 11 January 1996.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the chronic toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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EXECUTIVE SUMMARY:

A chronic 21-day toxicity study (life-cycle) was conducted to determine the effects of XDE-570 on daphnids (*Daphnia magna*), conducted under semi-static condition. The 1st instars were exposed to XDE-570 at nominal concentrations of 0 (control), 14, 23.3, 38.9, 64.8, 108, and 180 mg ai/L (mean analyzed concentrations ranged from 13.7 to 176 mg ai/L). Each exposure concentration consisted of ten replicates of one daphnid to evaluate the effects on reproduction and growth, and three replicates of five daphnids to evaluate effects on survival. The test concentrations were renewed at days 2, 4, 7, 9, 11, 15 and 18. The general health, number of progeny, number of alive and dead progeny, mortality and immobility were recorded. On the last day of the test, the first generation daphnids from reproduction and growth test were counted and measured and the dry weight per adult was determined. The number of young, alive or dead, were also counted. The test was conducted under a 16 h light (672 ± 57 lux) : 8 h dark photoperiod at 19.3-20.9 °C, pH 6.5 to 8 and at dissolved oxygen concentrations of 7.3 mg O_2/L (>82 air saturation). The study was conducted in accordance with GLP standards.

The length of the first generation daphnids at the termination of the study on day 21 was significantly less at the lowest test concentration of 14.0 mg/L ($P<0.05$) compared to control, as determined by the study authors (Dunnet's t-test) and the primary reviewer (one way ANOVA with Tukey's test). The next two concentrations, 23.3 and 38.9 mg/L, did not cause a significant reduction in daphnid length. Effects on length were again significant at the next concentration of 108 mg/L. The study authors "biologically determined" that the negative effects observed at 14.0 mg/L were not biologically significant because no significant adverse effects were observed in other treatment groups or endpoints up to 38.9 mg/L. Therefore, the 21-day NOEC for the length and weight of adult daphnids is 38.9 mg/L, while the LOEC was 64.8 mg/L.

Sublethal effects, such as hormesis, i.e., an increase in the total number of progeny produced per treatment group compared to control, were observed in the groups exposed to 14.0, 23.3 and 64.8 mg/L of XDE-570. According to the study authors, production of offspring in the treated groups indicated that XDE-570 had a significant effect on the reproduction (mean number of progeny produced per adult and mean number of broods per adult). There was a significantly greater number of progeny produced at the concentration of 38.9 mg/L compared to control and a significantly lower number of progeny produced at a concentration of 108 mg/L compared to control.

This study is classified acceptable and is consistent with the guideline requirement for a chronic daphnid toxicity study.

EFED accepts the PMRA DER in lieu of the generation of a new DER.

Results Synopsis

Test Organism Size/Age(mean weight or length):
Test Type: Semi-static

NOAEC: 38.9 mg a..i./L
Endpoint(s) Affected: weight, length

Appendix 9.3.3

PMRA Reviewer: Peter Takacs **14-August-2000**

STUDY TYPE: Daphnia sp. Chronic (Life Cycle) Study;
PMRA DATA CODE: 9.3.3;
OECD Data Point IIA 8.3.2.1

TEST MATERIAL (PURITY): XDE-570 (Florasulam) (99.2%)

SYNONYMS: DE-570, XR-570

CITATION: Kirk, H.D., Landre, A.M., Hugo, J.M. and Stahl, D.C. (1996): Evaluation of the chronic toxicity of XDE-570 herbicide to the daphnid, *Daphnia magna* Straus. Dow AgroSciences, unpublished report No. DECO-ES-2944, 11 January 1996.

SPONSOR: Dow AgroSciences Canada Inc. Suite 201, 1144 - 29th Avenue, N.E. Calgary, Alberta T2E 7P1

EXECUTIVE SUMMARY:

A chronic 21-day toxicity study (life-cycle) was conducted to determine the effects of XDE-570 on daphnids (*Daphnia magna*), conducted under semi-static condition. The 1st instars were exposed to XDE-570 at nominal concentrations of 0, 14, 23.3, 38.9, 64.8, 108, and 180 mg ai/L (mean analyzed concentrations ranged from 13.7 to 176 mg ai/L). Each exposure concentration consisted of ten replicates of one daphnid to evaluate the effects on reproduction and growth, and three replicates of five daphnids to evaluate effects on survival. The test concentrations were renewed at days 2, 4, 7, 9, 11, 15 and 18. The general health, number of progeny, number of alive and dead progeny, mortality and immobility were recorded. On the last day of the test, the first generation daphnids from reproduction and growth test were counted and measured and the dry weight per adult was determined. The number of young, alive or dead, were also counted. The test was conducted under a 16 h light (672 ± 57 lux) : 8 h dark photoperiod at 19.3-20.9 °C, pH 6.5 to 8 and at dissolved oxygen concentrations of 7.3 mg O_2/L (>82 air saturation). The study was conducted in accordance with OECD Guideline No. 202 Part II. and U.S. EPA FIFRA subdivision E series 72-4 and the EPA GLP standards.

The 21-day LC50 based on mortality was 169.2 mg/L. The maximum concentration of XDE-570 tested (180 mg/L) was equivalent to 72000 times the EEC in water (0.0025 mg a.i./L), based on a single application at rate of 7.5 g a.i./ha. The length of the first generation daphnids at the termination of the study on day 21 was significantly less at the lowest test concentration of 14.0 mg/L ($P<0.05$) compared to control, as determined by the study authors (Dunnet's t-test) and the reviewer (one way ANOVA with Tukey's test). The next two concentrations, 23.3 and 38.9 mg/L, did not cause a significant reduction in daphnid length. Effects on length were again significant at the next concentration of 108 mg/L. The study authors "biologically determined" that the negative effects observed at 14.0 mg/L were not biologically significant because no

significant adverse effects were observed in other treatment groups up to 38.9 mg/L. Therefore, **the 21-day NOEC for the length and weight of adult daphnids was assumed to be 38.9 mg/L**, while the LOEC was 64.8 mg/L. A similar trend was observed for daphnid weight, having the same NOEC and LOEC values as the length endpoint. Sublethal effects, such as hormesis, i.e., an increase in the total number of progeny produced per treatment group compared to control, were observed in the groups exposed to 14.0, 23.3 and 64.8 mg/L of XDE-570. According to the study authors, production of offspring in the treated groups indicated that XDE-570 had a significant effect on the reproduction (mean number of progeny produced per adult and mean number of broods per adult) at the concentration of 108 mg/L. However, the authors used all data, including progeny produced by daphnids that died before the completion of the experiment, and arrived at the mean number of progeny per adult by diving the total number produced per treatment level by 10, the number of adults at the start of the experiment. The raw data (not including progeny produced by individuals that died before the completion of the study) for the former endpoint (mean number of progeny produced per adult) were re-analyzed by the reviewer, using a one way ANOVA with multiple comparisons (Tukey's test, α =0.05). This re-analysis indicated that there was a significantly greater number of progeny produced at the concentration of 38.9 mg/L compared to control and a significantly lower number of progeny produced at a concentration of 108 mg/L compared to control.

This study is classified acceptable and does satisfy the guideline requirement for a chronic *daphnia* sp. toxicity study (DATA CODE: 9.3.3).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

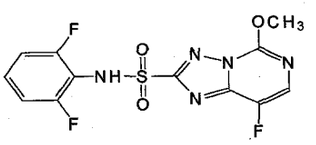
I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OECD Guideline No. 202 Part II. and U.S. EPA FIFRA subdivision E series 72-4.

A. MATERIALS:

1. Test Material: XDE-570
Description: technical herbicide, white powder.
Lot/Batch #: TSN100298
Purity: 99.2% ai.
Stability of compound: not provided
CAS #:145701-23-1
IUPAC name: 2′,6′,8-trifluoro-5-methoxy-s-triazolo[1,5-c]pyrimidine-2-sulphonanilide

Structure:



2. Test organism:

Species: *Daphnia magna*
Source: not provided
Acclimatization: Reared in dilution water at 20 ± 2°C with 16 hr light/8 hr dark periods.
Food: algae (*Selenastrum capricornutum*) were fed to the test organisms at the rate of 3 mL per vessel per day.

B. STUDY DESIGN:

1. Experimental conditions:

a) Range-finding Study: A 21 day chronic probe study was conducted under semi-static conditions with a concentration range of 0, 30 and 300 mg/L. Two daphnids exposed to 300 mg/L and one daphnid exposed to 30 mg/L died during the study. There was a decrease in body weight, length and mean number of progeny at 300 mg/L. At 30 mg/L, there was no apparent decrease in progeny, however, there was a decrease in body weight and length.

b. Definitive Study: The definitive study was conducted using six nominal concentrations (14.0, 23.3, 38.9, 64.8, 108.0, and 180.0 mg/L) and a daphnid dilution water control. Each test concentration and control had 13 replicates. Ten replicates (one organism each) were used to evaluate effects on reproduction and growth. Three replicates (five organisms each) were used to evaluate mortality. Test solutions were renewed every Monday, Wednesday and Friday. Test vessels were not aerated during the study.

Table 1 . Experimental Parameters

Parameter	Value	Remarks
Test vessel and number of replicates	reproduction and growth: 10 mortality and immobilization: 3 250 mL borosilicate jars were used	covered with watch glasses to reduce evaporation
Test concentrations	14.0, 23.3, 38.9, 64.8, 108.0, and 180.0 mg/L.	test concentrations were based on a previous 48 hr acute study and a 21 day probe study. The studies were conducted under static and semi-static conditions, respectively. The 21 day study was set at nominal concentrations of 0, 30, and 300 mg a.i./L.

Number of organisms per replicate	reproduction and growth: one organism/ replicate mortality and immobilization: 5 organisms/ replicate	
Solvent	dilution water	
Photoperiod	16 hr light/8 hr dark	672 ± 56 lux
Temperature	19.3 to 20.9 °C	
Range for pH, dissolved oxygen	pH: 6.5 to 8.0 DO: > 7.3 mg O_2/L	DO was > 82% saturation throughout the study
Water hardness	142-161mg/L $CaCO_3$	

2. Observations:

Test vessels were observed daily for general health and three times a week for the number of progeny. The number of alive and dead progeny were recorded for the 10 replicates identified for the reproduction and growth portion of this test. Mortality and immobility were recorded on days 2, 4, 7, 14, and 21 for the 3 replicates from each level identified for these observations. On the last day of the study the first generation daphnids from the reproduction and growth subgroup were counted and individually measure from the apex of the helmet to the base of the spine. The mean dry weight/adult was also determined. The number of young, both alive and dead, were also counted at that time.

The EC50/LC50 values and 95% confidence intervals were calculated using a computer program which uses probit analysis, moving average angle analysis and binomial probability/non-linear interpolation. The probit analysis and moving average angle analysis calculate both the estimated EC50/LC50 values and their confidence intervals. The appropriateness of a given test was determined by the dose-response data. The raw data were tested for normality and homogeneity and were log, inverse or square root transformed for normalization, as needed. Non-parametric tests (Steel's Many-one rank test and Kruskal-Wallis test) were used for non-normal data. The NOEC was defined as the highest dose group that is not significantly different compared to the control.

Table 2: Observations

Criteria	Details	Remarks
Test duration	21 days	
Test dates: start end	6-June-1994 27-June-1994	
Observation intervals	day 0, 2, 4, 7, 9, 11, 15, and 18	
Renewal schedule	day 2, 4, 7, 9, 11, 15, and 18	
Observations at each time interval	test vessels were observed daily for general health and three times a week for the number of progeny. Mortality was recorded daily in the 10 replicates selected for the reproduction portion of the test. For the 3 replicates selected for mortality and immobility, observations were made on days 2, 4, 7, 14, and 21.	
Others		

II. RESULTS AND DISCUSSION:

A. Mortality: The 24 hr LC50 and EC50 values (mortality and immobility, respectively) were both > 180 mg a.i./L. The 21 day LC50 and EC50 values were both 169.2 mg/L (95% CI: 108.0-180.0 mg/L, calculated via binomial method, using linear interpolation of the arcsine transformed data). No mortality was observed at the highest test concentration before day 7 of the study, while 80% mortality occurred by day 21. Ten percent mortality was recorded in the control group.

B. Length of first generation: The effects on daphnid length was among the most sensitive endpoints tested. A one tailed Dunnet's t-test was used. Exposure to the lowest test concentration of 14.0 mg/L produced significantly ($\alpha = 0.05$) shorter length in daphnids compared to control. However, the next two concentrations (23.3 and 38.9 mg/L) did not, while concentrations ≥64.8 mg/L again caused reduced length.
The study authors "biologically determined" 64.8 mg/L as the LOEC and 38.9 mg/L as the NOEC, based on the lack of significant effects between 14.0 and 64.8 mg/L.

C. Reproduction: All test organisms died prior to day 9 in the 180 mg/L group. Two of those organisms exhibited sublethal effects prior to death. There was no mortality in the 14.0 and 23.3 mg/L treatment groups, however, mortality was observed in the control group, 38.9, 64.8, and 108 mg/L. Significant decreases were observed in total number of progeny and total number of broods, both at a concentration of 108 mg/L. The LOEC, NOEC and MATC for total number of progeny were determined to be 108, 64.8 and 83.7 mg/L, respectively. Similarly, the LOEC, NOEC and MATC for total number of broods were108, 64.8 and 83.7 mg/L, respectively. A significant reduction in weight of offspring was noted at 14.0 mg/L, however, as with length, concentrations of 23.3 and

38.9 mg/L did not cause such a response.

Table 3: Effect of XDE-570 on mortality of the first generation

Treatment (nominal concentration: mg a.i./L)	**Observation period**									
	Day 2		**Day 4**		**Day 7**		**Day 14**		**Day 21**	
	No Dead	**% Dead**	**No Dead**	**% Dead**	**No Dead**	**% Dead**	**No Dead**	**% Dead**	**No Dead**	**% Dead**
Negative control	0	0	0	0	0	0	1	10	1	10
14.0	0	0	0	0	0	0	0	0	0	0
23.3	0	0	1	10	1	10	2	20	2	20
38.9	0	0	0	0	0	0	0	0	0	0
64.8	0	0	1	10	1	10	1	10	1	10
108.0	0	0	1	10	3	30	3	30	4	40
180.0	0	0	0	0	5	50	5	50	8	80

Table 4: Effect of XDE-570 on the length of the first generation and number of offspring

Treatment (nominal concentration: mg a.i./L)	**Observation period**	
	Day 21	
	Length (mm)	**No. of offspring alive/dead**
Negative control	3.92	1383
14.0	3.58	1601
23.3	3.83	1635
38.9	3.78	1541

64.8	3.72	1186
108.0	3.42	398
180.0	-	-

-: no data

III. Study deficiencies: In the initial report summary, the nominal test concentrations listed (14.0, 23.3, 38.9, 64.8 and 100 mg/L) differed from those listed elsewhere in the study (14.0, 23.3, 38.9, 64.8, 108 and 180 mg/L). A sub-sample of only 10 out of >1300 F_1 progeny was used to determine mean weight and length. The mean number of progeny per adult was determined as the total number of progeny produced per treatment level divided by 10, the number of adults used per treatment for the reproduction portion of the study. This may have biased the results because several parental organisms died before the end of the study at various time intervals.

IV. Comments: The number of offspring per adult was higher at 14.0, 23.3 and 38.9 mg/L compared to control, while the mean length and weight were lower for all of these treatment concentrations, which could potentially indicate that a less viable population would have resulted from exposure to these concentrations. However, given the application rate of this herbicide, 7.5 g/ha, the EEC in a 15 cm deep body of surface water is estimated to be only 0.005 mg/L.