Data Evaluation Report on the Toxicity of Florasulam to the Diatom *Navicula pellicosa*

PMRA Submission Number {.....} EPA MRID Number 468083-28

Data Requirement:

PMRA Data Code	9.8.2
EPA DP Barcode	D329529
OECD Data Point	{.....}
EPA MRID	468083-28
EPA Guideline	123-2

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 Tackacs.
PMRA **Date:** 9.19.2000

Primary Reviewer: Brian D. Kiernan, Biologist **Date:** 4.21.2007 10/8/07
EPA

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

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

Date Evaluation Completed: 4.21.2007

CITATION: Milazzo, D.P., A. M. Landre, J.M. Hugo, M.D. Martin (1996) XDE-570 Herbicide: The toxicity to the freshwater diatom, *Navicula pelliculosa*, ES-3045, December 3, 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 nonvascular aquatic plants. 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.

2068757

EXECUTIVE SUMMARY:

In a 5-day static laboratory toxicity test, Four replicates of 50 mL cultures of freshwater diatom *Navicula pelliculosa* at an initial cell density of 10000 cell/mL sterile EPA Algal Assay freshwater growth medium (AAM medium) were exposed to XDE-570, at mean measured concentrations of 0.0133, 0.0493, 0.163, 0.556, 1.84 and 6.04 mg a.i./L. The growth medium control and test solution without algae control were included. The test was carried out under temperature of 24.1 ± 0.18 °C, continuous illumination of 4346 ± 644 lux with continuous agitation. The pH of the test solutions without algae ranged from 7.5 to 7.7 and pH ranged from 7.2 to 7.3 in test media with algal cell growth. The test results were based on the inhibition of algal growth or the total cell count/mL of algae produced at each dose level in comparison to the control group. This study was conducted in accordance with the US EPA FIFRA Subdivision J Guideline 123-2 and the EPA GLP standards. Chemical analysis indicated that XDE-570 was stable for at least 5 days under test condition. Significant inhibition of growth was evident in the daily cell count data at concentrations of 0.163 mg ai/L and above. The 96-hour NOEC, EC_{50} and EC_{25} values, based on cell count were, 0.0493, 0.97 and 0.18 mg a.i./L, respectively. The results were presented based on the mean measured concentration.

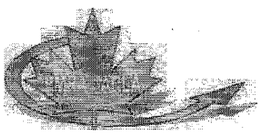
This study is classified acceptable and is consistent with the guideline requirement for a diatom toxicity study.

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

EC_{50}: 0.049 mg a..i./L
NOAEC: 0.97 mg a..i./L
Endpoint(s) Affected: growth



Reviewer: Peter Takacs **Date:** 19-September-2000

STUDY TYPE: Freshwater Diatom *Navicula pelliculosa*

TEST MATERIAL: XDE-570 (Florasulam)

SYNONYMS: DE-570, XR-570

CITATION: Milazzo, D.P., A. M. Landre, J.M. Hugo, M.D. Martin (1996) XDE-570 Herbicide: The toxicity to the freshwater diatom, *Navicula pelliculosa*, ES-3045, December 3, 1996.

STUDY SPONSOR: DowElanco, 9330 Zionsville Road, Indianapolis, Indiana 46268-1054

EXECUTIVE SUMMARY:

In a 5-day static laboratory toxicity test, Four replicates of 50 mL cultures of freshwater diatom *Navicula pelliculosa* at an initial cell density of 10000 cell/mL sterile EPA Algal Assay freshwater growth medium (AAM medium) were exposed to XDE-570, at mean measured concentrations of 0.0133, 0.0493, 0.163, 0.556, 1.84 and 6.04 mg a.i./L. The growth medium control and test solution without algae control were included. The test was carried out under temperature of 24.1 ± 0.18 °C, continuous illumination of 4346 ± 644 lux with continuous agitation. The pH of the test solutions without algae ranged from 7.5 to 7.7 and pH ranged from 7.2 to 7.3 in test media with algal cell growth. The test results were based on the inhibition of algal growth or the total cell count/mL of algae produced at each dose level in comparison to the control group. This study was conducted in accordance with the US EPA FIFRA Subdivision J Guideline 123-2 and the EPA GLP standards.

Chemical analysis indicated that XDE-570 was stable for at least 5 days under test condition. Significant inhibition of growth was evident in the daily cell count data at concentrations of 0.163 mg ai/L and above. The 96-hour NOEC, EC_{50} and EC_{25} values, based on cell count were, 0.0493, 0.97 and 0.18 mg a.i./L, respectively. The results were presented based on the mean measured concentration.

This acute toxicity study satisfies the guideline requirement for a freshwater algal toxicity study. This study is classified as acceptable.

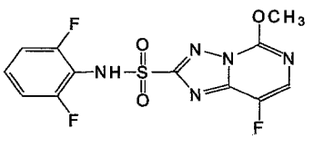
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. GUIDELINE FOLLOWED: Pesticide Assessment Guidelines Subdivision J Hazard Evaluation: Non-target Plants, EPA 540/9-82-020. Holst, R.W. and T.C. Ellwanger, 1982. Hazard Evaluation Division: Standard Evaluation Procedure Non-target plants: Growth and reproduction of aquatic plants Tiers 1 and 2. EPA 540/9-86-134. Holst, R.W., 1986.

B. MATERIALS:

1. Test Material: XDE-570
Description: technical herbicide, white powder.
Purity: 99.2 % a.i.
Lot/Batch #:TSN100298
Storage stability of compound: found to be stable in the test medium
CAS #: 145701-23-1
IUPAC name: 2′,6′,8-trifluoro-5-methoxy-s-triazolo[1,5-c]pyrimidine-2-sulphonanilide
Solubility in water: 121 mg/L
pK_a: 4.54
K_{ow}: 0.06
Mode of phytotoxic action: Acetolactate synthase (ALS) inhibitor
Structure:



2. Test Organisms:
Species and class: *Navicula pelliculosa*
Strain number: not provided
Axenic[1] culture: Yes, not verified.
Unialgal culture: Yes
Exponential growth: Yes
Source: Carolina Biological Supply Company, Burlington, NC.
Incubation conditions: 24 ± 2°C, continuous light (4305 lux)
Acclimation period: 4 weeks
Culturing procedure: not specified

C. STUDY DESIGN:

[1] Axenic = free from other organisms, both active and dormant

1. Algal Growth Medium:

Table 1: Composition of a standard freshwater algal growth medium.

Parameter		**Details**
Standard Growth Medium*		US EPA Algal Assay Medium (AAM)
Chelator		Na_2EDTA (not used in the actual test medium)
Carbon source		$NaHCO_3$
Water source and purity		not provided
Method of sterilization		not provided
pH	Prior to sterilization	not specified
	After sterilization	7.0-7.5
pH adjustments		not specified

2. Experimental conditions:

Table 2: Experimental design.

Experimental Design Parameters			**Details**
Storage conditions of freshwater growth medium			not specified
Volume of freshwater growth medium			50 mL medium/replicate
Controls	Negative		algal assay medium
	Positive		not used
	Solvent control		dimethylformamide (DMF)
Test organisms	Age of inoculum (d)		< 1
	Inoculum cell count at Day 0		~10,000 cells/mL
Test concentrations (nominal)* *[mg a.i./L]*			0, 0.0141, 0.0492, 0.162, 0.541, 1.77, and 5.90
Pesticide addition method			standard solutions were mixed in test flasks with dilution water
Method of analytical verification			HPLC with UV detector
Number of replicates		Control	4
		Treatments	4
Test conditions	Test duration		5 days
	Test vessel (volume, cover, composition (e.g., glass, polystyrene, etc.))		250 mL borosilicate Erlenmeyer flasks with Shimadzu closures
	Incubation facility		incubator
	Aeration or agitation		not specified
	Static, static-renewal or flow-through test system		static
	Temperature (°C)		24.1 ± 0.18
	Photoperiod		continuous light
	Light fluence rate**		4305 ± 646
	Light wavelengths		not specified
	Light source		not specified

**If one test concentration was used, specify whether that rate corresponds to the maximum label application rate.*
***Fluence rate = flow rate of light, flux of light, or the amount of light per unit area per unit time. It is sometimes referred to as light intensity, although this is not a desirable term. The photon fluence rate is given in µmol m^{-2} s^{-1}.*

3. Observations:

Table 3: Observations

Observation Parameters		**Details**
Test dates	Initiation	23-October-1995
	Termination	28-October-1995
Observation or sampling intervals		day 0, 3, 4, 5 for cell counts, day 0 and 5 for pH and test chemical concentration
Measurement endpoint parameter(s)		cell count, (inhibition of growth)
Measurement technique		Coulter counter

No additional observations were made.

4. Description of analytical procedures:

Extraction: Not done.
Identification and quantification of parent compound: HPLC/UV analysis was used (Hitachi L-6200A pump with LDC Spectomonitor 3200 UV detector at 254 nm. The analytical column used was a Waters RCM 8x10 with 8 mm x 100 mm Nova Pak phenyl cartridge.
Detection limits (LOD, LOQ): Not specified.

5. Statistical Analysis: The results are expressed in terms of algal growth (total cell count/mL). The EC50 and EC25 values for algal growth were determined by least squares linear regression of the mean cell counts against the concentration and the common log of the concentration on days 3, 4 and 5. The no observed effect concentration (NOEC) for algal growth was calculated using the analysis of variance and Dunnett's test. The method of calculation of the 95% confidence intervals of the EC50 and EC25 values was not specified.

II. RESULTS AND DISCUSSION:

The test conditions outlined in the study protocol were maintained throughout the study. Cell counts increased (hormesis) compared to controls at the two lowest test concentrations (0.0133 and 0.0493 mg/L) up to day 4 (13% increase at 0.0493 mg/L), however, by day 5, cell counts at all concentrations were less than control values. The 4 and 5 day EC50 vales and the 95% confidence intervals for algal growth were 0.97 (0.03 to 35.14) mg/L and 1.38 (0.03 to 71.01) mg/L, respectively. The EC25 values for day 4 and 5 were 0.18 (0.01 to 6.44) mg/L and 0.15 (0-7.67) mg/L, respectively. The 4 and 5 day NOEC was 0.0493 mg/L. These data were based on mean measured concentrations and treatment effects were compared to the pooled control data (i.e. mean of negative + solvent control groups).

A. RESIDUE ANALYSIS:

Table 4: Concentrations of XDE-570 used in the acute freshwater algal toxicity test.

Treatment	Nominal Concentration (mg a.i./L)	Measured Concentration (mg a.i./L) (Validated by chemical analyses)*		
		Initial	120 Hours	Mean
Negative control	0	0	0	0
Solvent control	0	0	0	0
Treatment 1	0.0141	0.0115	0.0149	0.0133
Treatment 2	0.0492	0.044	0.0546	0.0493
Treatment 3	0.162	0.147	0.179	0.163
Treatment 4	0.541	0.508	0.603	0.556
Treatment 5	1.77	1.67	2.01	1.84
Treatment 6	5.90	5.48	6.6	6.04

* Validated by HPLC/UV analysis.

B. INHIBITORY EFFECTS:

The effect of XDE-570 on cell growth was such that a clear dose response was not established, i.e., cell growth inhibition did not increase from one treatment level to the next. The percent inhibition compared to the pooled control response (solvent + negative control) at 96 hours was -8.3, -13, 51.4, 29.3, 48.6 and 86.4% for the mean measured concentrations shown above in Table 4, (also see Table 5). The variability in response between the four replicates generally increased at all time intervals with increasing concentration as indicated by the increase in the coefficient of variation.

Table 5: Effect of XDE-570 on freshwater algal growth.

Treatment (measured concentration) (mg a.i./L)	**96 Hours**		**120 Hours**	
	Cell Count	**% Inhibition**	**Cell Count**	**% Inhibition**
Negative control	970466	-	2089300	-
Solvent control	709066	-	1938333	-
0.0133	909600	-8.3	1958400	2.8
0.0493	953533	-13	1988733	1.3
0.163	408100	51.4	1103667	45.2
0.556	593933	29.3	1538667	23.6
1.84	431433	48.6	1158767	42.5
6.04	114233	86.4	411233	79.6

Note: % inhibition was calculated using the mean of the pooled control data (solvent + negative control)

Table 6: Statistical endpoint values.*

Statistical Endpoint	**Value for cell count (96 hr)**
NOEC (mg a.i./L)	0.0493
LOEC (mg a.i./L)	0.163
EC_{50} (mg a.i./L) (95% C.I.)	0.97 (0.03-35.14)
EC_{25} (mg a.i./L) (95% C.I.)	0.18 (0.01-6.44)

C. <u>OTHER EFFECTS</u>: Besides cell growth, no other compound related phytotoxic effects were evaluated.

III. <u>STUDY DEFICIENCIES</u>:

Cell counts were provided for days 3-5 only, which does not allow for estimation of growth (i.e. exponential or not). Dose response regression curves were not shown. It was not stated whether the dose response correlation was significant. The cell count data fit a linear model poorly as indicated by the r^2 values (0.58) which resulted in a very wide 95% confidence interval spanning 3 orders of magnitude (0.03-71 mg/L). The coefficient of variation was very high (standard deviation as much as 67% of the mean) for the cell count data, which produces a low powered ANOVA, thereby reducing the likelihood of obtaining a significant effect. The reviewer compared the means of the cell counts of the two control groups at day 4 using a t-test and found that they are not statistically significant, however, the power of the

test was very low (0.13) and well below the desired 0.8. The 96 hour percent decrease in cell counts was not calculated. The method of calculation of the 95% confidence intervals of the EC50 and EC25 values was not specified. In light of the fact that the maximum EEC of XDE-570 is 2.5 µg/L in an agricultural setting and that the diatom *Navicula pelliculosa* is not the most sensitive freshwater algae to the pesticide studied (LOEC = 163 µg/L), these deficiencies are considered to be minor deficiencies.

IV. REVIEWER'S COMMENTS/CONCLUSIONS: The response of *Navicula pelliculosa* to XDE-570 at the test concentrations used in the study did not allow for adequate power of statistical analysis. However, there were no significant effects at the two lowest concentrations and the EEC is an order of magnitude lower than the NOEC. Therefore the results are acceptable.

VI. REFERENCES: No references were cited.

Template dated: April 8, 1999
Template name: 9_8_2_D_Freshwater_algae.wpd

Study review filename: *X:\EDO\CRO\OECD\Review Exchange\MISC REVIEWS\Florasulam for EPA by DOW Request\Environment\9.8.2.1d florasulam diatom.wpd*