

US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the Toxicity of Florasulam to Cyanobacteria

PMRA Submission Number {.....}

EPA MRID Number 468083-27

Data Requirement:

PMRA Data Code	9.8.2
EPA DP Barcode	D329529
OECD Data Point	{.....}
EPA MRID	468083-27
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-sulfonilide
 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: Tamara Sheremata, Ph.D.
 PMRA

Date: 9.11.2000

Primary Reviewer: Brian D. Kiernan, Biologist
 EPA

Date: 4.21.2007

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., Landre, A.M., Rick, D.L., and M.D. Martin. (1995) XDE-570 Herbicide: The Toxicity to the Blue-Green Algae, *Anabaene flos-aquae*. The Environmental Toxicology & Chemistry Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI, Study ID ES-3005, Study date (12-Dec.-1995), Dow Elanco, Indianapolis, IN, Volume 5, 47 pages, Unpublished, Submitted to Canada on September 30, 1998, DACO 9.8.2.1.

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.

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PMRA Submission Number {.....}

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EXECUTIVE SUMMARY:

This study is classified as unacceptable because the data reported have unacceptably large standard deviations (many as high as 50 % of the calculated mean). In a 96 hour static laboratory toxicity test, the freshwater blue-green algae *Anabaena flos-aquae*, was exposed to XDE-570, at concentrations from 29.9 :g a.i./L to 499 :g a.i./L in EPA Algal Assay freshwater growth medium. The NOEC, EC50, and EC25, based on cell counts, were 235, 363 (-214 to 940), and 213 (-340 to 767) :g a.i./L, respectively. The results were based on the mean measured concentration..

This study is classified unacceptable and is not 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

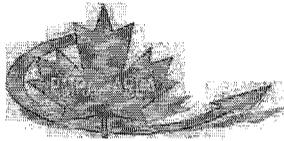
Test Organism Size/Age(mean weight or length):

Test Type: Semi-static

EC₅₀:

NOAEC:

Endpoint(s) Affected:



Reviewer: Tamara Sheremata, Ph.D.

Date: 23-August-2000

STUDY TYPE: Freshwater Algae *Anabaena flos-aquae*, blue-green algae**TEST MATERIAL:** XDE-570, Florasulam**SYNONYMS:**

CITATION: Milazzo, D.P., Landre, A.M., Rick, D.L., and M.D. Martin. (1995) XDE-570 Herbicide: The Toxicity to the Blue-Green Algae, *Anabaene flos-aquae*. The Environmental Toxicology & Chemistry Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI, Study ID ES-3005, Study date (12-Dec.-1995), Dow Elanco, Indianapolis, IN, Volume 5, 47 pages, Unpublished, Submitted to Canada on September 30, 1998, DACO 9.8.2.1.

STUDY SPONSOR: DowElanco, Indainapolis, IN**EXECUTIVE SUMMARY:**

This acute toxicity study **does not** satisfy the guideline requirement for a freshwater algal toxicity study. This study is classified as **unacceptable** because the data reported have unacceptably large standard deviations (many as high as 50 % of the calculated mean).

In a 96 hour static laboratory toxicity test, the freshwater blue-green algae *Anabaena flos-aquae*, was exposed to XDE-570, at concentrations from 29.9 µg a.i./L to 499 µg a.i./L in EPA Algal Assay freshwater growth medium. The NOEC, EC50, and EC25, based on cell counts, were 235, 363 (-214 to 940), and 213 (-340 to 767) µg a.i./L, respectively. The results were based on the mean measured concentration.

Aside from reduced cell counts, there were no other specific compound related phytotoxic effects.

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

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I. MATERIALS AND METHODS:

A. GUIDELINE FOLLOWED: U.S.-EPA-FIFRA Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Non-target Plants, Guidelin 123-2, Growth and Reproduction of Aquatic Plants, Tier2.

B. MATERIALS:

1. Test Material: XDE-570

Description: technical grade, white crystalline solid

Purity: 99.2 % a.i.

Lot/Batch #: TSN1002298, Lot # 930910

Storage stability of compound: Not indicated

CAS #: 145701-23-1

Chemical name: 2',6',8'-trifluoro-5-methoxy-s-triazolo[1,5-c]pyrimidine-2-sulphonanilide (IUPAC)

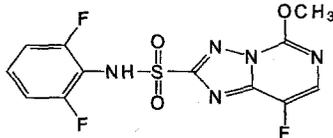
Solubility in water: 121 mg/L

pK_a: 4.54

K_{ow}: at pH 4 = 10, at pH 7 = 0.06, at pH 10 = 0.009

Mode of phytotoxic action: growth inhibition ?

Structure:



2. Test Organisms:

Species and class: *Anabaena flos-aquae*, blue-green algae

Strain number: Not indicated

Axenic¹ culture: Claimed, but not verified

Unialgal culture: Not indicated

Exponential growth: Not Indicated

Source: ATCC

Incubation conditions: 24 ± 2 °C, continuous light (2000 ± 300 lux)

Acclimation period: 4 wks

Culturing procedure: ?

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

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C. STUDY DESIGN:**1. Algal Growth Medium:**

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

Parameter	Details
Standard Growth Medium*	EPA Algal Assay Bottle Test
Chelator	EDTA (as Na ₂ EDTA•2H ₂ O)
Carbon source	NaHCO ₃
Water source and purity	Not indicated, but may be indicated in original protocol?
Method of sterilization	Not indicated, but manipulations were carried out in a laminar flow-through hood.
pH	7.3

2. Experimental conditions:

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Table 2: Experimental design.

Experimental Design Parameters		Details
Storage conditions of freshwater growth medium		Not indicated, but there is mention that the medium should be stored in the dark at 4°C
Volume of freshwater growth medium		50 mL
Controls	Negative	contained the algal medium
	Solvent control	contained the algal medium and dimethylformamide (DMF)
Test organisms	Age of inoculum (d)	0
	Inoculum cell count at Day 0	10,000 cells/mL
Test concentrations (nominal), µg/L		0, 29.9, 59.8, 120, 249, and 499
Pesticide addition method		50 mL medium was spiked with appropriate volume of an 11.4 µg/mL stock solution (in dimethylformamide) to yield the desired nominal concentration.
Method of analytical verification		HPLC analysis of blanks that contained growth medium and herbicide only.
Number of replicates	Control	twice as many as treatment group (6 for DMF and 6 for algal assay medium)
	Treatments	triplicate
Test conditions	Test duration	5 d
	Test vessel (volume, cover, composition (e.g., glass, polystyrene, etc.))	250 mL Erlenmeyer flasks fitted with Shimadzu closures.
	Incubation facility	Environmental growth chamber
	Aeration or agitation	swirled once a day
	Static, static-renewal or flow-through test system	static
	Temperature (°C)	24 ± 2 °C
	Photoperiod	continuous
	Light fluence rate**	2000 ± 200 lux (SD) ?
	Light wavelengths	Not indicated
Light source	Not indicated.	

3. Observations:

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Table 3: Observations

Observation Parameters		Details
Test dates	Initiation	Not indicated
	Termination	Not indicated
Observation or sampling intervals		3, 4, and 5 d
Measurement endpoint parameter(s)		cell count
Measurement technique		Coulter Multisizer

No additional observations were made.

4. Description of analytical procedures:

Extraction: Extraction of XDE-570 from algae was not done.

Identification and quantification of parent compound: XDE-570 was quantified by HPLC in solutions that contained 20 % DMF to enhance solubility.

Detection limits (LOD, LOQ): LOQ of 3.5 µg/L, LOD not specified.

5. Statistical Analysis:

EC25 and EC50 were determined by least squares linear regression using mean total cell counts/mL versus the concentration of XDE-570. The NOEC was determined using analysis of variance and Dunnett's test comparing each dose group to the control.

II. RESULTS AND DISCUSSION:

The test conditions outlined in the study protocol were maintained throughout the study, with the following two exceptions:

1. The protocol stated that the pH readings were taken daily; however, they were taken on day zero and the last day of the study in a representative test flask at each concentration without algae.
2. Protocol stated that the stock culture was obtained from ATCC on September 30, 1995, when in fact it was received on October 5, 1994.

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A. RESIDUE ANALYSIS:

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

Treatment	Nominal Concentration (µg a.i./L)	Measured Concentration (µg a.i./L) (Validated by chemical analyses)*		
		Initial	Day 5	Mean
Negative control	0	<LOQ	<LOQ	<LOQ
Solvent control (DMF)	0	<LOQ	<LOQ	<LOQ
Treatment 1	29.9	27.6	30.3	29.0
Treatment 2	59.8	55.7	60.9	58.3
Treatment 3	120	112	120	116
Treatment 4	249	224	246	235
Treatment 5	499	454	501	478

* Validated by HPLC analysis.

The slight increase in concentration with time may have been due to evaporation.

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B. INHIBITORY EFFECTS:Table 5: Effect of XDE-570 on freshwater algal growth (\pm SD).

Treatment (Analyzed concentration) ($\mu\text{g a.i./L}$)	Observation period					
	3 d		4 d		5 d	
	Cell Count	% Inhibition*	Cell Count	% Inhibition*	Cell Count	% Inhibition*
Negative control	27458 \pm 18740	8.2	57089 \pm 24632	5.4	2332467 \pm 1459212	-51.5
Solvent control (DMF)	29911 \pm 14697	0	60333 \pm 26334	0	1540109 \pm 1412867	0
29.0	35884 \pm 14106	-20.0	49489 \pm 25344	18.0	1673867 \pm 1450744	-8.7
58.3	30604 \pm 1046	-2.3	63600 \pm 18463	-5.4	441956 \pm 593892	71.3
116	32809 \pm 8375	-9.7	81733 \pm 45304	-35.5	2309956 \pm 475686	-50.0
235	13878 \pm 3488	53.6	50111 \pm 19590	16.9	889822 \pm 693964	42.2
478	6676 \pm 66	77.7	9222 \pm 5521	84.7	-3422 \pm 2552	100.0

*solvent control as reference.

Table 6: Statistical endpoint values (95 % confidence intervals in parenthesis).

Statistical Endpoint	Value for cell count (day 4)
NOEC ($\mu\text{g a.i./L}$)	235
EC ₅₀ ($\mu\text{g a.i./L}$)	363 (-214 to 940)
EC ₂₅ ($\mu\text{g a.i./L}$)	213 (-340 to 767)

The authors of this study reported that they discarded data from day 5 because there was excessive variation in algal growth (Table 5). Unstead, day 4 results were reported (Table 6).

III. STUDY DEFICIENCIES/REVIEWER COMMENTS/CONCLUSIONS:

The authors of this study try to make the case that the NOEC is greater than the EC25 because of the difference in the statistical methods used in their estimation. In particular, the NOEC is derived from hypothesis testing and the EC25 is a point estimate derived by regression analysis.

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Hence, the NOEC has no confidence limits. However, as can be seen from Table 6, the confidence intervals around the EC25 are extremely broad (-340 to 767 µg a.i./L); hence, it is not likely that meaningful statistical analysis of this data is possible.

Furthermore, the relatively large standard deviations for the cell counts (Table 5) make it unlikely that the EC25 and EC50 values can be meaningfully compared to the EEC in a risk assessment.

Another deficiency of this study is that the purity of the culture was not verified by plating techniques. Contamination of the culture by other organisms would explain the high variation in cell counts that was observed.

The reviewer recommends that this study be repeated.

IV. 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\9.8.2.1b Freshwater_algae.wpd

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