

Data Evaluation Report on the Toxicity of Florasulam to the Diatom *Skeletonema costatum*
 PMRA Submission Number {.....} EPA MRID Number 468083-25

Data Requirement: PMRA Data Code 9.8.2
 EPA DP Barcode D329529
 OECD Data Point {.....}
 EPA MRID 468083-25
 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-sulfonamide
 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: Hughes, J.S; Williams, T.L.; L.A. Conder. (1995) The Toxicity of XDE-570 to *Skeletonema costatum*. Carolina Ecotox, Inc., Durham, NC, Testing Laboratory report number 10-03-1, Study date (7-September-1995), The Dow Chemical Company, Midland MI, Report DECO-ES-3021, Volume 5, 73 pages, unpublished, submitted to Canada on September 30, 1998, MRID number, DACO 9.8.3.

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.



EXECUTIVE SUMMARY:

In a 5d static laboratory toxicity test, the marine diatom, *Skeletonema costatum*, was exposed to XDE-570, at mean measured concentrations of 12.1, 22.8, 42.9, 89.9 and 173 mg a.i./L in a marine algal assay medium. The nutrient medium control was included. The test was conducted under these conditions: temperature of 20.55 °C, 14 h light : 10 h dark photoperiod of 4306 ± 646 lumens/m² and manually shaken daily. The pH of the test solutions ranged from 8.01 (control) to 7.21 (highest test concentration) at the start of the study and 8.72 (control) to 7.65 (highest test concentration) at day 5. The test results were based on the cell density measurements. This study was conducted in accordance with GLP standards.

A dose dependent relationship between increasing concentration and inhibition effect was evident in the cell count data at concentrations of 42.9 mg ai/L and above. Mean cell density at concentrations below 42.9 mg ai/L were significantly lower than control values at 48 h but no significant differences were detected at these concentrations at any other sampling points. Therefore, the NOEC, based on the log of the standing crop values, was 22.8 mg/L and the EC₅₀, based on standing crop values was 47.6 mg a.i./L.

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

EC₅₀: 47.6 mg a..i./L
NOAEC: 22.8 mg a..i./L
Endpoint(s) Affected: cell density



Reviewer: Tamara Sheremata, Ph.D.

Date: 11-September-2000

STUDY TYPE: Marine Algae, *Skeletonema costatum*, diatom

TEST MATERIAL: XDE-570 (florasulam)

SYNONYMS: XR-570 (1990-Jan. 1994), XDE-570 (Jan. 94 - Jan. 97), DE-570 (Feb. 1997-?), Florasulam.

CITATION: Hughes, J.S; Williams, T.L.; L.A. Conder. (1995) The Toxicity of XDE-570 to *Skeletonema costatum*. Carolina Ecotox, Inc., Durham, NC, Testing Laboratory report number 10-03-1, Study date (7-September-1995), The Dow Chemical Company, Midland MI, Report DECO-ES-3021, Volume 5, 73 pages, unpublished, submitted to Canada on September 30, 1998, MRID number, DACO 9.8.3.

STUDY SPONSOR: The Dow Chemical Company, Midland, MI.

EXECUTIVE SUMMARY:

In a 5-d static laboratory toxicity test, the marine diatom, *Skeletonema costatum*, was exposed to XDE-570, at mean measured concentrations of 12.1, 22.8, 42.9, 89.9 and 173 mg a.i./L in a marine algal assay medium. The nutrient medium control was included. The test was carried out under temperature of 20.55 °C, 14 h light : 10 h dark photoperiod of 4306 ± 646 lumens/m² and manually shaken daily. The pH of the test solutions ranged from 8.01 (control) to 7.21 (highest test concentration) at the start of the study and 8.72 (control) to 7.65 (highest test concentration) at day 5. The test results were based on the cell density measurements. This study was conducted in accordance with the U.S. EPA - FIFRA Pesticide Assessment Guidelines Subdivision J, 123-2 and OECD Guideline 201 and the EPA GLP standards.

A dose dependent relationship between increasing concentration and inhibition effect was evident in the cell count data at concentrations of 42.9 mg ai/L and above. Mean cell density at concentrations below 42.9 mg ai/L were significantly lower than control values at 48 h but no significant differences were detected at these concentrations at any other sampling points. Therefore, the NOEC, based on the log of the standing crop values, was 22.8 mg/L on day 1, <12.1 mg/L on day 2, and 22.8 mg/L on days 3, 4, and 5. The EC₂₅ and EC₅₀ on day 5, based on standing crop values, were 32.4 mg a.i./L and 47.6 mg a.i./L, respectively. The results were presented based on the measured concentration.

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

FIRST DRAFT - Do not copy or cite.

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

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, Tier 2 and OECD Guideline 201: Algal Growth Inhibition Test.

B. MATERIALS:

1. Test Material: XDE-570

Description: off-white solid, technical product

Purity: 99.2 % a.i.

Lot/Batch #: TSN 100298

Storage stability of compound: Not indicated

CAS #: 145701-23-1

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

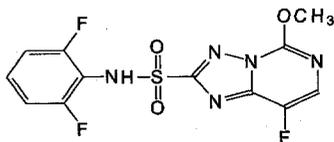
Solubility in water: 121 mg/L

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: *Skeletonema costatum*, diatom

Strain number: Culture Collection of Algae and Protozoa (CCAP) number 278/4

Axenic¹ culture: Not Indicated

Unialgal culture: Not Indicated

Exponential growth: Yes

Source: EPA Environmental Research Laboratory in Gulf Breeze, Florida.

Incubation conditions: Mean temperature of 20.55 °C, shaken manually each day.

Acclimation period: Not indicated.

Culturing procedure: ??

C. STUDY DESIGN:

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

FIRST DRAFT - Do not copy or cite.

1. Algal Growth Medium:

Table 1 Composition of a standard saltwater growth medium.

Parameter	Details
Standard Growth Medium*	Marine algal assay nutrient medium
Chelator	EDTA
Salinity	30 parts per thousand
Water source and purity	ASTM Type I water
Method of sterilization	Filter sterilized (0.22 μ m membrane filter paper)
pH	8.1 \pm 0.1
pH adjustments	0.1 N or 1 N NaOH or HCl

2. Experimental conditions:

FIRST DRAFT - Do not copy or cite.

Table 2: Experimental design.

Experimental Design Parameters		Details
Storage conditions of saltwater growth medium		stored in refrigerator until use
Volume of saltwater growth medium		50 mL solution per flask
Negative Control		medium only
Test organisms	Age of inoculum	7 d
	Inoculum cell count (cell/mL) at Day 0	10 ⁴
Test concentrations (nominal) [mg a.i./L]		12.5, 25.8, 50.0, 100, 200
Pesticide addition method		stock solutions containing 303 mg a.i./L were used to prepare test solutions
Method of analytical verification		HPLC
Number of replicates	Control	6
	Treatments	3
Test conditions	Test duration	5 d
	Test vessel (volume, cover, composition (e.g., glass, polystyrene, etc.))	250 mL Erlenmeyer flask with foam stopper
	Incubation facility	growth chamber (pH Environmental Incubator)
	Aeration or agitation	manual agitation once a day
	Static, static-renewal or flow-through test system	static
	Temperature (°C)	20 ± 2 °C
	Photoperiod	14 h light: 10 h dark
	Light fluence rate	4306 ± 646 lumens/m ²
Light source	overhead fluorescent lights	

3. Observations:

FIRST DRAFT - Do not copy or cite.

Table 3: Observations

Observation Parameters		Details
Test dates	Initiation	April 7, 1995
	Termination	April 12, 1995
Observation or sampling intervals		every day
Measurement endpoint parameter(s)		cell count
Measurement technique		Coulter Counter

No additional observations were made.

4. Description of analytical procedures:

Extraction: No extraction procedures were employed

Identification and quantification of parent compound: HPLC

Detection limits (LOD, LOQ): LOD of 0.527 mg/L

5. Statistical Analysis:

The EC25 and EC50 values and associated 95 % confidence limits were determined by weighted least squares nonlinear regression of the test concentration against the cell counts for each counting day. The EC25 and EC50 values were also calculated on the basis of the area under the growth curve using the same statistical method..

The NOEC values were determined on each counting day using analysis of variance (ANOVA) and Bonferroni's t-test of log-transformed cell counts.

FIRST DRAFT - Do not copy or cite.

II. RESULTS AND DISCUSSION:

The test conditions outlined in the study protocol were maintained throughout the study. Aside from a change in cell count, there were no compound related phytotoxic effects reported in the study.

A. RESIDUE ANALYSIS:

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

Treatment	Nominal Concentration (mg a.i./L)	Actual Concentration (mg a.i./L) (Validated by HPLC)		
		Initial	5 d	Mean
Negative control	0	0	0	0
Treatment 1	12.5	12.0	12.1	12.1
Treatment 2	25.8	22.8	22.7	22.8
Treatment 3	50.0	42.7	43.0	42.9
Treatment 4	100.0	89.7	89.8	89.8
Treatment 5	200.0	172	173	173

Measured concentrations were stable over the 5 d test period

B. INHIBITORY EFFECTS:

FIRST DRAFT - Do not copy or cite.

Table 5: Effect of XDE-570 on marine algal growth (% inhibition based on cell count).

Treatment (measured concentration) (mg a.i./L medium)	Observation period									
	day 1		day 2		day 3		day 4		day 5	
	Cell Count	% Inhibition	Cell Count	% Inhibition	Cell Count	% inhibition	Cell Count	% inhibition	Cell Count	% Inhibition
0 (negative control)	35,043	0	145,267	0	241,838	0	399,887	0	562,267	0
12.1	31,520	10.1	100,153	31.1	187,720	22.4	331,887	17.0	528,787	5.95
22.8	31,483	10.2	99,550	31.5	189,140	21.8	341,107	14.7	576,907	-2.60
42.9	19,690	43.8	47,640	67.2	84,357	65.1	230,367	42.4	317,907	43.5
89.8	15,030	57.1	20,197	86.1	26,593	89.0	41,030	89.7	60,540	89.2
173.0	12,263	65.0	12,050	91.7	12,390	94.9	12,813	96.8	13,173	97.7

Table 6: Effect of XDE-570 on marine algal growth (% inhibition based on area under the curve).

Treatment (measured concentration) (mg a.i./L medium)	Observation period									
	day 1		day 2		day 3		day 4		day 5	
	area	% Inhibition	area	% Inhibition	area	% inhibition	area	% inhibition	area	% Inhibition
0 (negative control)	12522	0	92677	0	276229	0	587092	0	1058169	0
12.1	10760	14.1	66597	28.1	200533	27.4	450337	23.3	870674	17.7
22.8	10742	14.2	66258	28.5	200603	27.4	455727	22.4	904734	14.5
42.9	4845	61.3	28510	69.2	84509	69.4	231871	60.5	496008	53.1
89.8	2515	79.9	10129	89.1	23524	91.5	47335	91.9	88120	91.7
173.0	1132	91.0	3288	96.5	5508	98.0	8110	98.6	11103	99.0

At treatment levels of 12.1 mg/L, 22.8 mg/L, and 42.9 mg/L, there is increasing % inhibition on the basis of cell count between day 1 and day 3; after which, the % inhibition waned on day 4 and day 5 (Table 5). However, for the higher treatments (42.9, 89.8, and 173.0 mg/L), % inhibition increased with time, with no observed recovery. Similar trends can be observed for % inhibition that is based on the area under the growth curve (Table 6).

FIRST DRAFT - Do not copy or cite.

Table 7: Statistical endpoint values.

Statistical Endpoint	Value for cell count	Value for area under the growth curve
NOEC (mg a.i./L)	Day 1: 22.8 mg/L Day 2: <12.8 mg/L Day 3: 22.8 mg/L Day 4: 22.8 mg/L Day 5: 22.8 mg/L	
EC ₅₀ (mg a.i./L) (95% C.I.)	Day 5: 47.6 (41.4-54.6)	Day 3: 31.3 (18.0-54.3)
EC ₂₅ (mg a.i./L) (95% C.I.)	Day 5: 32.4 (26.7-39.4)	Day 3: 18.1 (8.38-39.2)

Based on the NOEC values, XDE-570 does not appear to be extremely toxic to *Skeletonema costatum*.

C. OTHER EFFECTS: Aside from inhibition of growth, there were no compound related phytotoxic effects reported in this study.

III. STUDY DEFICIENCIES: No deficiencies were noted.

IV. REVIEWER'S COMMENTS/CONCLUSIONS: The values for NOEC, EC₂₅, and EC₅₀ (Table 7) indicate low toxicity of XDE-570 to *Skeletonema costatum*.

V. REFERENCES: No references were cited.

Template dated: April 8, 1999

Template name: 9_8_2_D_Marine_algae.wpd

Study review filename: X:\EDO\CRO\OECD\Review Exchange\MISC REVIEWS\Florasulam for EPA by DOW Request\Environment\9.8.3 Marine_algae.WPD

FIRST DRAFT - Do not copy or cite.