Data Evaluation Report on the Toxicity of Florasulam Degradate to the Algae *Selenastrum capricornutum*

PMRA Submission Number {.....} EPA MRID Number 468083-29

Data Requirement:

PMRA Data Code	9.8.2
EPA DP Barcode	D329529
OECD Data Point	{.....}
EPA MRID	468083-29
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: Tamara Sheremata, Ph.D. **Date:** 24-August-2000
PMRA

Primary Reviewer: Brian D. Kiernan, Biologist **Date:** 4.21.2007
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: 4.21.2007

CITATION: Milazzo, D.P. Hugo, J.M., and L. McFadden. (1996) XDE-570 5-Hydroxy: The Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum* PRINTZ. The Environmental Toxicology & Chemistry Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland MI, Study ID: ES-3115, Study completed on August 19, 1996, DowElanco, Indianapolis IN, Volume number 5, 46 pages, Unpublished, Submitted to Canada on September 30, 1998, DACO number 9.8.2.

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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EXECUTIVE SUMMARY:

In a 96-hour static laboratory toxicity test, triplicate 100 mL cultures of a freshwater unicellular green alga, *Selenastrum capricornutum* Printz, at an initial cell density of 10,000 cells/mL sterile EPA Algal Assay freshwater growth medium were exposed to 5-OH-XDE-570 (XDE-570 5- Hydroxy), a major transformation product of XDE-570, at mean measured concentrations of 3.48, 6.64, 13, 25.4, 49.5 and 96.1 mg a.i./L. The cultures were incubated for 96 h at 24.6 ± 0.1 °C under continuous illumination of 4395 ± 421 lux with constant stirring. The growth medium control and test solution without algae control were included. The pH of these test solutions without algae ranged from 7.2 (control) to 4.7 (highest concentration) throughour the study compaired with values of 7.6 (control) to 4.5 (highest concentration) in test media with algae. Samples were taken daily for cell density measurements. The exposed concentrations were verified at 0, 72 and 96 h. Chemical analysis indicated that 5-OH-XDE-570 was stable for at least 4 days under test condition. Significant inhibition of growth was evident in cell count data at measured concentrations of 13 mg ai/L and above. There were no other compound-related phytotoxic effects besides reduction in cell growth. The NOEC, EC_{50}, and EC_{25} values, based on cell count were 6.64, 25.57 (13.36-34.84), and 11.59 (7.16-18.75) mg a.i./L, respectively. The results were presented based on the mean measured concentration.

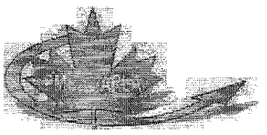
This study is classified ~~acceptable~~ and is consistent with the guideline requirement for a diatom toxicity study. Supplement 60K

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}: 25.57 mg a..i./L
NOAEC: 6.64 mg a..i./L
Endpoint(s) Affected: growth



Reviewer: Tamara Sheremata, Ph.D. **Date:** 24-August-2000

STUDY TYPE: Freshwater Algae, *Selenastrum capricornutum* Printz, Strain ATCC 22662, green algae]

TEST MATERIAL: XDE-570 5-Hydroxy

CITATION: Milazzo, D.P. Hugo, J.M., and L. McFadden. (1996) XDE-570 5-Hydroxy: The Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum* PRINTZ. The Environmental Toxicology & Chemistry Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland MI, Study ID: ES-3115, Study completed on August 19, 1996, DowElanco, Indianapolis IN, Volume number 5, 46 pages, Unpublished, Submitted to Canada on September 30, 1998, DACO number 9.8.2.

STUDY SPONSOR: DowElanco, Indianapolis IN.

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

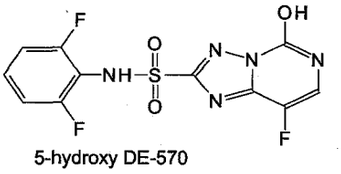
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. GUIDELINEs FOLLOWED: OECD Guideline No. 201, EEC Directive 92/69/EEC C.3: Algal Inhibition Test; U.S. EPA - FIFRA Pesticide Assessment Guidelines Subdivision J, Hazard Evaluation: Non-target Plants Guideline 123-2

B. MATERIALS:

1. **Test Material:** XDE-570 5-Hydroxy
   Description: technical grade, white crystalline powder
   Purity: 100.0 % a.i.
   Lot/Batch #: A1151-3
   Storage stability of compound: Not indicated
   CAS #: not applicable
   Chemical name: 2′,6′,8-trifluoro-5-hydroxy-*s*-triazolo[1,5-*c*]pyrimidine-2-sulphonanilide (IUPAC)
   Solubility in water: 354 mg/L
   pK_a: Not indicated in chemistry review
   K_{ow}: 2.09 at pH 5; pH 7; 1.41×10^{-2} at pH 7; 4.74×10^{-3} at pH 9
   Mode of phytotoxic action: cell growth inhibition
   Structure:



5-hydroxy DE-570

2. **Test Organisms:**
   Species and class: *Selenastrum capricornutum* Printz, green algae
   Strain number: ATCC 22662
   Axenic[1] culture: Indicated, but not verified
   Unialgal culture: Indicated, but not verified
   Exponential growth: Yes

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

Source: Starr Algal Collection at the University of Texas, Austin, Texas.
Incubation conditions: 24 ± 2 °C
Acclimation period: 4 wks
Culturing procedure: ?

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 $Na_2EDTA{\cdot}2H_2O$)
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 adjustments	7.3

2. Experimental conditions:

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
Negative Control			contained the algal medium
Test organisms	Age of inoculum (d)		0 d
	Inoculum cell count at Day 0		10,000 cells/mL
Test concentrations (nominal)* mg a.i./L			3.23, 6.46, 12.7, 25.3, 50.5, and 101
Pesticide addition method			101 mg XDE-570 5-Hydroxyl was combined with 1 L of algal medium, this was used to prepare the 6 different solutions.
Method of analytical verification			HPLC
Number of replicates		Control	6
		Treatments	3
Test conditions	Test duration		4 d
	Test vessel (volume, cover, composition (e.g., glass, polystyrene, etc.))		250 mL Erlenmeyer flasks
	Incubation facility		Environmental growth chamber
	Agitation		continuous
	Static, static-renewal or flow-through test system		static
	Temperature (°C)		24 ± 2 °C
	Photoperiod		continuous
	Light fluence rate**		4305 ± 646 lux
	Light wavelengths		Not indicated
	Light source		Not indicated

3. Observations:

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 XDE-570 5-Hydroxyl compound was achieved by HPLC.
Detection limits (LOD, LOQ): Not indicated, and not relevant since the nominal concentration range was high to start with.

5. Statistical Analysis:

EC25 and EC50 were determined by least squares linear regression using mean total cell counts/mL against the concentration. 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.

The effect of XDE-570 5-Hydroxy on algal growth (relative to the negative control) ranged from 13.9 % stimulation (at 3.48 mg/L) to 98.9 % inhibition (at 96.1 mg/L) on day 3. On day 4, 24.6 % stimulation (at 3.48 mg/L) to 99.8 % inhibition (at 96.1 mg/L).

A. RESIDUE ANALYSIS:

Table 4: Concentrations of XDE-570 5-Hydroxyl 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	3 d	4 d	Mean
Negative control	<0.3 mg/L	<0.3 mg/L	<0.3 mg/L	<0.3 mg/L	<0.3 mg/L
Treatment 1	3.13	3.41	3.91	3.13	3.48
Treatment 2	6.25	6.54	7.33	6.05	6.64
Treatment 3	12.5	13.0	13.6	12.5	13.0
Treatment 4	25.0	25.7	25.7	24.7	25.4
Treatment 5	50.0	49.8	50.3	48.5	49.5
Treatment 6	100	99.7	93.6	95.1	96.1

* Validated by HPLC analysis.

B. INHIBITORY EFFECTS:

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

Treatment (analyzed) (mg a.i./L)	Observation period							
	1 d		2 d		3 d		4 d	
	Cell Count, cells/mL	% Inhibition	Cell Count, cells/mL	% Inhibition	Cell Count, cells/mL	% Inhibition	Cell Count, cells/mL	% Inhibition
Negative control	28658 ± 1216	0.0	93256 ± 5923	0.0	340179 ± 22890	0.0	908896 ± 50750	0.0
3.48	29094 ±789	-1.5	103924 ± 6294	-11.4	387391 ± 21579	-13.9	1132655 ± 118029	-24.6
6.64	28650 ± 675	0.0	95591 ± 3353	-2.5	357457 ± 16132	-5.1	935358 ± 25069	-2.9
13.0	23958 ± 1203	16.4	68104 ± 4510	27.0	219304 ± 16019	35.5	602014 ± 32088	33.8
25.4	21141 ± 787	26.2	57025 ± 6375	38.9	144205 ± 9251	57.6	368166 ± 60768	59.5
49.5	17892 ± 1035	37.6	22558 ± 1710	75.8	44577 ± 2187	86.9	60585 ± 6166	93.3
96.1	7585 ± 1852	73.5	2187 ± 5496	97.7	3766 ± 1642	98.9	1567 ± 2303	99.8

Table 6: Statistical endpoint values.

Statistical Endpoint	Value for cell count (day 4)
NOEC (mg a.i./L)	6.64
EC_{50} (mg a.i./L) (95% C.I.)	21.57 (13.6-34.84)
EC_{25} (mg a.i./L) (95% C.I.)	11.59 (7.16-18.75)

C. **OTHER EFFECTS:** Besides reduction in cell growth (in # cells/mL), there were no other compound related phytotoxic effects.

III. STUDY DEFICIENCIES: The purity of the culture was not verified by plating techniques or other verification methods. In addition, sources of water used to prepare the medium should have been included in this study.

IV. REVIEWER'S COMMENTS/CONCLUSIONS: Based on the data provided in this study, it appears that the study results are valid and that the NOEC, EC50, and EC25 are of adequate quality to allow for comparisons with the EEC.

V. 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\9.8.2.1c Freshwater_algae.wpd*