Data Evaluation Report on the Toxicity of Florasulam to *Lemna gibba*

PMRA Submission Number {.....} EPA MRID Number 468083-26

Data Requirement:

PMRA Data Code	9.8.2
EPA DP Barcode	D329529
OECD Data Point	{.....}
EPA MRID	468083-26
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.
PMRA **Date:** 9.11.2000

Primary Reviewer: Brian D. Kiernan, Biologist **Date:** 4.21.2007
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., H. D. Kirk, and J. M. Hugo. 1995. The Toxicity of XDE-570 Herbicide to the Aquatic Plant, Duckweed, *Lemna gibba* L. G-3. The Environmental Toxicology & Chemistry Research Laboratory, Midland, MI. ES-2988. November 20, 1995. Dow AgroSciences Canada Inc. ES-2988. Volume No. 5. 58 Pages. Calgary, Canada. Unpublished.

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:

(Lemna gibba), was exposed to XDE-570 at measured concentrations of 0.137, 0.306, 0.616, 1.35, 2.3 and 4.59 :g a.i./L in modified EPA liquid growth medium. Culture medium and test solution without plants were included as controls. The duckweed were incubated at 23.8 ± 0.25 °C under continuous illumination of 4100 - 6400 lux. The pH of the test solutions without plants ranged from 8.5 ± 0.14 (control) to 8.5 ± 0.11 (highest test concentration) compared with values of 8.8 ± 0.24 (control) to 8.7 ± 0.11 (the highest test concentration) in test media with plants. The number of fronds produced in comparison to the control group was recorded. This study was conducted in accordance with the US EPA FIFRA Subdivision J, Guideline 123-2 and the EPA GLP standards.

After 14 days exposure, inhibition of growth ranged from -5.4% at 0.137 •g ai/L to 93.3% at 4.59 •g ai/L when compared to the solvent control. The **NOEC**, EC_{50} and EC_{25} values, based on frond number, were **0.62**, 1.18 and 0.57 ug a.i./L, respectively. The results were presented based on the measured concentration. This acute toxicity study satisfies the guideline requirement for a freshwater aquatic vascular plant toxicity study.

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}: 1.18 ug a..i./L
NOAEC: 0.62 ug a..i./L
Endpoint(s) Affected: frond numbers

Reviewer: Peter Takacs **Date:** 4-October-2000

STUDY TYPE: Aquatic Vascular Plant: *Lemna gibba* L. G3, floating aquatic vascular plant;

TEST MATERIAL: XDE-570 (Florasulam)

SYNONYMS: DE-570, XR-570

CITATION: Milazzo, D. P., H. D. Kirk, and J. M. Hugo. 1995. The Toxicity of XDE-570 Herbicide to the Aquatic Plant, Duckweed, *Lemna gibba* L. G-3. The Environmental Toxicology & Chemistry Research Laboratory, Midland, MI. ES-2988. November 20, 1995. Dow AgroSciences Canada Inc. ES-2988. Volume No. 5. 58 Pages. Calgary, Canada. Unpublished.

Milazzo, D.P., H.D. Kirk and J.M. Hugo (1995). The toxicity of XDE-570 Herbicide to the aquatic plant, Duckweed, *Lemna gibba* L. G3, DECO-ES-2946, February 3, 1995.

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

EXECUTIVE SUMMARY:

In a 14-day static laboratory toxicity test, the freshwater floating vascular plant, the duckweed *(Lemna gibba)*, was exposed to XDE-570 at measured concentrations of 0.137, 0.306, 0.616, 1.35, 2.3 and 4.59 µg a.i./L in modified EPA liquid growth medium. Culture medium and test solution without plants were included as controls. The duckweed were incubated at 23.8 ± 0.25 °C under continuous illumination of 4100 - 6400 lux. The pH of the test solutions without plants ranged from 8.5 ± 0.14 (control) to 8.5 ± 0.11 (highest test concentration) compared with values of 8.8 ± 0.24 (control) to 8.7 ± 0.11 (the highest test concentration) in test media with plants. The number of fronds produced in comparison to the control group was recorded. This study was conducted in accordance with the US EPA FIFRA Subdivision J, Guideline 123-2 and the EPA GLP standards.

After 14 days exposure, inhibition of growth ranged from -5.4% at 0.137 µg ai/L to 93.3% at 4.59 µg ai/L when compared to the solvent control. The **NOEC**, EC_{50} and EC_{25} values, based on frond number, were **0.62**, 1.18 and 0.57 µg a.i./L, respectively. The results were presented based on the measured concentration.

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

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

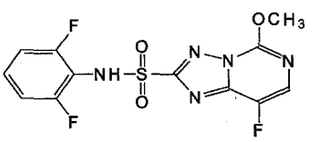
FIRST DRAFT - Do not copy or cite.

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 type: *Lemna gibba* L. G-3, floating aquatic vascular plant
Strain number: G3
Axenic[1] culture: Yes, but not verified
Culture with single plant species: Yes
Exponential growth: Yes,
Source: Dr. C.F. Cleland, Smithsonian Institution, Radiation Biology Laboratory, Rockville, Maryland.
Incubation conditions: 25 ± 2 °C, continuous lighting with pH 7.5-8.5
Acclimation period: 8 weeks

C. STUDY DESIGN:

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

1. Aquatic Vascular Plant Growth Medium:

Table 1: Composition of a standard medium for aquatic vascular plants.*

Parameter		Details
Standard Growth Medium*		Modified (20x) US EPA Algal Assay Medium (AAM) (Duckweed)
Chelator		not used
Carbon source		$NaHCO_3$
Water source and purity		sterile deionized water
Method of sterilization		not stated
pH	Prior to sterilization	
	After sterilization	7.5-8.5
pH adjustments		

* If a standardized growth medium is not used or has been modified, fill the nutrient composition into the following table.

Table 1b: Chemical composition of a non-standard growth medium for aquatic vascular plants. The stock solutions listed below were diluted with deionized water to make up the final assay medium (60 mL of each stock solution in 3 L of sterile deionized water)

Solution Number	Chemical	Concentration of stock solution	Solution Number	Chemical	Concentration of stock solution
Macro-nutrients	$NaNO_3$	25.5 g/L	Micro nutrients	H_3BO_3	1.86 g/L
	$NaHCO_3$	15 g/L		$MnCl_2•4H_2O$	4.16 g/L
	K_2HPO_4	1.044 g/L		$ZnCl_2$	0.0327 g/L
	$MgSO_47H2O$	14.7 g/L		$CoCl_2•6H_20$	2.86 g/L
	$MgCl_2•6H_2O$	12.16 g/L		$CuCl_2•2H_2O$	0.022 g/L
	$CaCl_2•2H_2O$	4.4 g/L		$Na_2MoO_4•2H_2O$	0.0726 g/L
				$FeCl_3•6H_20$	0.16 g/L
				$Na_2EDTA•2H_2O$	

3. Experimental conditions:

Table 3: Experimental design.

Experimental Design Parameters			**Details**
Storage conditions of freshwater growth medium			stored
Volume of freshwater growth medium			100 mL medium/replicate
Controls	Negative		dilution assay medium
	Solvent control		dimethyl formamide
Test organisms	Age of plant culture		originally received in 1987
	Inoculum frond count at Day 0		4 plants per replicate, 4 fronds per plant
Test concentrations (nominal)* [µg a.i./L]			0.15, 0.314, 0.628, 1.26, 2.50, and 4.99
Pesticide addition method			stock solution prepared, compound dissolved in medium.
Method of analytical verification			HPLC/UV
Number of replicates		Control	3
		Treatments	3
Test conditions	Test duration		14 days
	Test vessel		250 mL, borosilicate Erlenmeyer flask with Shimadzu covers
	Incubation facility		growth chamber
	Aeration or agitation		not specified
	Static, static-renewal or flow-through test system		static
	Temperature (°C) (mean ± s.d.)		23.8 ± 0.25
	Photoperiod		continuous light
	Light fluence rate**		5382 ± 1076
	Light wavelengths		not provided
	Light source		not provided

*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}.

4. Observations:

Table 4: Observations

Observation Parameters		**Details**
Test dates	Initiation	34799
	Termination	34813
Observation or sampling intervals		Daily: light, temperature every 3 days: pH (in flasks containing no plants), growth in each concentration and control. pH in test flasks containing plants was checked on day 0 and 14. XDE-570 concentrations were measured in the bulk dosing solutions on day 0 and from each test flask on day 14.
Measurement endpoint parameter(s)		frond count
Measurement technique		-

No additional observations were made.

5. Description of analytical procedures:

Extraction: The samples were acidified, concentrated using liquid/liquid extraction with methylene chloride, the extract was blown down then reconstituted with DMF/water.

Identification and quantification of parent compound: High Performance Liquid Chromatography with UV detection (HPLC/UV, 254 nm) using a MetaChem Abzelute ODS-DB, 3 mm x 150 mm, 5 µm column.

Detection limits (LOD, LOQ): 5 µg/L diluent

6. Statistical Analysis: The results expressed in terms of plant growth were reported as EC50 and EC25 values, (with 95% confidence intervals) with fronds as the end point for each term. The NOEC was also calculated using ANOVA with Dunnett's test comparing each treatment group to the control. The EC50 and EC25 values were determined by the least squares linear regression of the log of the concentration against the day 14 frond counts.

II. RESULTS AND DISCUSSION:

The test conditions outlined in the study protocol were maintained throughout the study. The two lowest test concentrations resulted in a slight stimulation of frond growth in *Lemna gibba* (up to 10.7%). The 14 day NOEC was 0.616 µg/L (with 5.2% inhibition of growth) and the 14 day EC50 and EC25 values (with 95% CI) were 1.18 (0.39-3.53) and 0.57 (0-1.86) µg/L, respectively. These values were based on the mean measured day 0 concentrations. The LOEC was 1.35 µg/L; this concentration caused 61.3% inhibition in frond growth compared to controls. The highest test concentration of 4.59 µg/L caused 93.3% inhibition in growth. The EC25 value

was lower than the NOEC value, likely due to high variability and thus low power of the ANOVA.

A. RESIDUE ANALYSIS:

Table 5: Concentrations of XDE-570 used in the acute aquatic vascular plant toxicity test.

Treatment	Nominal Concentration (µg a.i./L)	Actual Concentration (µg a.i./L) (Validated by chemical analyses)*		
		Initial	Day 14 **	Mean
Negative control	0	0	0	0
Solvent control	0	0	0	0
Treatment 1	0.157	0.137	-	-
Treatment 2	0.314	0.306	-	-
Treatment 3	0.628	0.616	-	-
Treatment 4	1.26	1.35	-	-
Treatment 5	2.5	2.3	-	-
Treatment 6	4.99	4.59	-	-

* Validated by HPLC/UV analysis.** The day 14 samples were not usable for analysis due to high levels of interference in chromatography.

B. INHIBITORY EFFECTS: XDE-570 significantly reduced frond production (growth) in *Lemna gibba* at concentrations of 1.35 µg/L and higher. The 14 day NOEC was 0.616 µg/L (with 5.2% inhibition of growth) and the 14 day EC50 and EC25 values (with 95% CI) were 1.18 (0.39-3.53) and 0.57 (0-1.86) µg/L, respectively. These values were based on the mean measured day 0 concentrations and the effects were compared to those of the solvent (dimethyl formamide) controls.

Table 7: Effect of XDE-570 on aquatic vascular plant growth.

Treatment (measured concentration) (µg a.i./L medium)	**Response**	
	Day 14	
	Frond Number	**% Inhibition**
Solvent control	460	0
0.137	485	-5.4
0.306	509	-10.7
0.616	436	5.2
1.35	178	61.3
2.3	55	88
4.59	31	93.3

Table 8: Statistical endpoint values. *[for Tier II studies]**

Statistical Endpoint	**Value for frond counts (day 14)**
NOEC (µg a.i./L)	0.62
LOEC (µg a.i./L)	1.35
EC_{50} (µg a.i./L) (95% C.I.)	1.18
EC_{25} (µg a.i./L) (95% C.I.)	0.57

* Do not use this table, if the study was deemed unacceptable.

C. **OTHER EFFECTS**: There was not a major change in pH during the study. A recovery period was not included. Stimulation was observed at 0.137 and 0.306 µg/L.

III. STUDY DEFICIENCIES AND COMMENTS: Exposure to the test chemical was through water (dissolved), however, this route of exposure does not address foliar absorption and potential differences in toxicity between the two routes. *Lemna* are floating aquatic plants and are most likely to be exposed in the environment through foliar deposition. Furthermore, the toxicity of herbicides to *Lemna* varies depending on the route of exposure, and in some cases may be more inhibitory via surface deposition. The concentrations of XDE-570 in test vessels containing *Lemna* were measured on the first and last days of the experiment, however, chromatography was poor during the analysis of the 14 day samples and these data were not used. Consequently, exposure concentrations were not true mean exposures, rather, day 0

concentrations. These are considered to be a minor deficiency in light of the stability of the test chemical in water (e.g. during range finding study).

V. CONCLUSIONS: The current study indicates that XDE-570 is highly toxic to the floating vascular plant *Lemna gibba* at very low concentrations. A significant adverse effect on frond counts was observed at a test concentration of 1.35 µg/L. The agricultural EEC of XDE-570 in surface water is 2.5 µg/L (based on a single application at 7.5 g a.i./ha) and exceeds the NOEC (0.62 µg/L) as well as the EC50 value of 1.18 µg/L.

VI. REFERENCES: No references were cited.

Template dated: *April 8, 1999*
Template name: *9_8_5_D_Aquatic_vascular_plant.wpd*

Study review filename: *X:\EDO\CRO\OECD\Review Exchange\MISC REVIEWS\Florasulam for EPA by DOW Request\Environment\9.8.5b Florasulam 14 day Lemna.wpd*