Data Evaluation Report on the Acute Toxicity of GF-443 to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number{.....} EPA MRID Number 45831020

Data Requirement:	PMRA DATA CODE	
	EPA DP Barcode	D288160
	OECD Data Point	
	EPA MRID	45831020
	EPA Guideline	§72-2

Test material: GF-443 (a SC end-use product) **Purity:** 21.9% w:w a.i.
Common name: Penoxsulam (a.i.)
Chemical name: IUPAC: Not reported
CAS name: 2-(2,2-Difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-C]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide
CAS No.: Not reported
Synonyms: None reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature: Rebecca Bryan
Date: 10/17/03

QC Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation
Signature: C E Padova
Date: 10/17/03

Primary Reviewer: ~~William Erickson~~ Richard Fehrsen - Biologist
OPP/EFED/ERB - III
Date: 3/8/04

Secondary Reviewer(s):
{EPA/OECD/PMRA}
Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: ~~119031~~ 119031

Date Evaluation Completed:

CITATION: Marino, T.A., *et al.* 2002. GF-443: An Acute Toxicity Study with the Daphnid, *Daphnia magna* Straus. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Laboratory Study No. 021025. Study submitted by Dow AgroSciences, Indianapolis, IN. Study initiated February 26, 2002 and completed July 29, 2002.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of GF-443 [an end-use product containing 22% XDE-638 (penoxsulam)] to *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at mean-measured concentrations were <0.6 (LOQ, negative control), 7.92, 13.3, 22.2, 36.5, 58.0, and 90.1 ppm a.i. The water hardness was higher than recommended

No mortality was observed during the study, and incidental immobilization was observed at 5, 10, 10, and 5% in the 13.3, 22.2, 36.5, and 58.0 ppm a.i. test levels. No immobilization was observed in the negative control group, the 7.92 ppm a.i. test group, or in the highest level tested, 90.1 ppm a.i. The 48-hour LC/EC_{50} was >90.1 ppm a.i., which categorizes GF-443 as slightly toxic to *Daphnia magna* on an acute toxicity basis. The 48-hour NOAEC level, based on mortality/immobilization, was 90.1 ppm a.i.

This study is classified as SUPPLEMENTAL, but it need not be repeated. It is scientifically sound and fulfills the guideline requirements for an acute toxicity test with freshwater invertebrate (72-2) using an end-use product.

Results Synopsis

Test Organism Age (e.g., 1st instar): <24 hours old
Test Type (Flow-through, Static, Static Renewal): Static

48-Hour
LC/EC_{50}: >90.1 ppm a.i.
NOAEC: 90.1 ppm a.i. (based on mortality/immobilization)
LOAEC: >90.1 ppm a.i.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the OECD Guidelines for Testing of Chemicals, No. 202; the EC Directive 91/414 Annex I 8.2.5; and the EEC Method C.1, Acute Toxicity for *Daphnia* (1992). Deviations from U.S. EPA guideline §72-2 included:

1. The storage conditions of the test material were not reported.

2. Pre-test health (including mortality) of the laboratory culture and/or brood was not described.

3. The hardness (172 mg/L as $CaCO_3$) was significantly higher than recommended (40-48 mg/L as $CaCO_3$).

4. The loading rate was not specified.

5. Sub-lethal effects were not monitored.

These deviations did not affect the validity or acceptability of the study.

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

A. MATERIALS:

1. Test Material GF-443 [a suspension concentrate (SC) herbicide formulation]

Description: Cream to light tan liquid

Lot No./Batch No. : E-828-59

Purity: 21.9% XDE-638 (w/w)

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was verified by analytical determination at 0 and 96 hours (Table 3, p. 25). Overall mean recoveries were 101% at Day 0 and 97.4% at Day 4. A slight decline in concentration was observed at the 457 ppm (nominal) test level (101% on Day 0 to 79.2% on Day 2). Similar declines were not observed at the lower test levels.

Storage conditions of test chemical: Not reported.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

2. Test organism:

Species: *Daphnia magna* Straus

Age at test initiation: <24 hours old

Source: In-house laboratory cultures (initially obtained from Yale University, New Haven, Connecticut).

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A 48-hour range-finding study was conducted with ten daphnia per single replicate at nominal test concentrations of 0 (negative control), 45.7, 229, and 457 ppm (equivalent to 0, 10.0, 50.0, and 100 ppm XDE-638, respectively, p. 15). No treatment-related effects were observed during the study, with the exception of a single daphnid at the 229 ppm level, which was believed to be incidental.

b) Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Continuous laboratory cultures were maintained. Same as test *Daphnia* cultures were fed 5 times/week with mixed diet of *Selenastrum capricornutum* (algae) and YCT trout chow (yeast-ceraphyll trout). Not specified	*EPA requires 7 day minimum acclimation period.*
Duration of the test	48 hours	*EPA requires 48 hours*
Test condition - static/flow through Type of dilution system (for flow through method)	Static N/A	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*

Parameter	Details	Remarks *Criteria*
Renewal rate (for static renewal)	N/A	
Aeration, if any	No aeration during testing.	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass jars with 6 x 8 cm glass inserts with NITEX® screening (425 µm). 250 mL 200 mL	Vessels were covered to reduce evaporation. To assist in observations, each replicate contained a glass insert with a nylon screen, which was used for moving organisms near surface of the solutions. *EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	The dilution water was pumped to the laboratory from the upper Saginaw Bay of Lake Huron. The water was filtered (sand and carbon), pH-adjusted, and UV-irradiated. The hardness was adjusted to approximately 170 mg/L as $CaCO_3$, then the water was autoclaved for 30 minutes and aerated for 24 hours prior to use.	*EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 172 mg/L as $CaCO_3$ 7.0-7.6 8.2-10.0 mg/L (≥92% saturation) 20.2-20.6°C <1000 µg/mL (<LOD) 1000 µg/mL (total suspended solids) See Table 1, p. 23 <LOD (Table 2, p. 24) <1 ppb	The hardness was higher than recommended. Results from inorganic and organic analysis of the dilution water are provided in Tables 1 and 2, pp. 23-24. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 24 hr and ≥ 40% during 2nd 24 hr* *Flow-through: ≥60%*

Parameter	Details	Remarks Criteria
Number of replicates Solvent control: Negative control: Treatments:	 N/A 2 2	
Number of organisms per replicate Solvent control: Negative control: Treatments:	 N/A 10 10	The biomass loading rate was not specified. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17 °C, ≤ 0.5 g/L at > 17 °C; flow-through: ≤ 1 g/L/day.*
Concentration of test material: nominal: XDE-638 equivalent concentrations:	 0 (negative control), 35.5, 59.2, 98.7, 165, 274, and 457 ppm GF-443 0, 7.78, 13.0, 21.6, 36.0, 60.0, and 100 ppm a.i.	Mean-measured concentrations are provided in Table 3, p. 26. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	N/A	 *EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light/8 hours dark	Light intensity ranged from 1265-1756 lux. *EPA requires 16 hours light, 8 hours dark.*
Feeding	Animals were not fed during testing.	 *EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Verified. The measured test concentrations were all 101% of nominal on Day 0 and 79.2-104% of nominal on Day 2 (Table 3, p. 25).	
Recovery of chemical Level of Quantitation Level of Detection	79.2-104% of nominal 0.6 ppm a.i. Not reported	Based on mean-measured concentrations (Table 3, p. 25).

Parameter	Details	Remarks *Criteria*
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sub-lethal effects	Mortality/immobility	
Observation intervals	After 24 and 48 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed during the study. After 48 hours, immobilization was observed at 5, 10, 10, and 5% in the 13.3, 22.2, 36.5, and 58.0 ppm a.i. test levels (mean-measured XDE-638). No immobilization was observed in the negative control group, the 7.92 ppm a.i. test group, or the highest level tested, 90.1 ppm a.i., and the study authors believed the immobilization observed at the other levels to be incidental to treatment with GF-443 (p. 18).

Table 3: Effect of GF-443 on mortality/immobilization of *Daphnia magna*.

Treatment			No. of organisms	Observation Period			
				24 Hours		48 Hours	
Nominal GF-443, ppm	Nominal XDE-638, ppm	Measured XDE-638, ppm a.i.		No.	%	No.	%
Negative control	Negative control	<0.6	20	0	0	0	0
35.5	7.78	7.92	20	0	0	0	0
59.2	13.0	13.3	20	0	0	1	5
98.7	21.6	22.2	20	0	0	2	10
165	36.0	36.5	20	0	0	2	10
274	60.0	58.0	20	0	0	1	5
457	100	90.1	20	0	0	0	0
NOAEC (mortality)			90.1 ppm a.i.				
LC_{50} (95% C.I.)			>90.1 ppm a.i.				
Positive control, if used mortality: LC_{50}:			N/A	N/A	N/A	N/A	N/A

B. SUB-LETHAL TOXICITY ENDPOINTS:

Not observed.

C. REPORTED STATISTICS:

The 48-hour LC/EC_{50} value was determined visually. The results were based on mean-measured concentrations of active ingredient.

D. VERIFICATION OF STATISTICAL RESULTS:

The LC/EC_{50} could be determined visually because immobility did not exceed 50% in this study. The NOAEC was determined using Fisher's Exact Test (to compare the control to the treatment levels with immobility) via TOXSTAT statistical software. The results were based on mean-measured concentrations of active ingredient.

48-Hour
LC/EC_{50}: >90.1 ppm a.i.
NOAEC: 90.1 ppm a.i. (based on mortality/immobilization)
LOAEC: >90.1 ppm a.i.

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-2 that affected the acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors.

GF-443 is a suspension concentrate (SC) herbicide formulation containing 22% active ingredient, XDE-638 (penoxsulam). The results of this study are provided as mean-measured concentrations of active ingredient in the test solutions (ppm a.i.).

It was reported that test solutions were cloudy white/turbid following preparation (p. 15).

G. CONCLUSIONS:

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) using an end-use product containing 22% (w:w) XDE-638. Because of the higher than recommended water hardness, this study is classified as SUPPLEMENTAL, but it need not be repeated. The 48-hour LC/EC_{50} was >90.1 ppm a.i., the highest concentration tested, which categorizes GF-443 [an end-use product containing 22% XDE-638 (penoxsulam)] as slightly toxic to the water flea, *Daphnia magna*, on an acute toxicity basis. The NOAEC (for mortality/immobilization) was 90.1 ppm a.i.

48-Hour
LC/EC_{50}: >90.1 ppm a.i.
NOAEC: 90.1 ppm a.i. (based on mortality/immobilization)
LOAEC: >90.1 ppm a.i.

III. REFERENCES:

Organisation for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals. Method 202, Daphnia sp., Acute Immobilization Test, Part 1. ISBN 92-64-12221-4.

European Community (EC) Directive 91/414 Annex I 8.2.5.

Official Journal of the European Communities. (EEC) Method C.1. Acute Toxicity Test for *Daphnia*. ISSN 0378-6978. 29 December 1992.

EPA-FIFRA. Environmental Protection Agency. Hazard Evaluation Division, Standard Evaluation Procedure: Acute Toxicity Test for Freshwater Invertebrates. EPA-540/9-85-005.

Environmental Protection Agency. Office of Pesticide and Toxic Substances. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Guideline 72-2, Acute Toxicity Test For Freshwater Aquatic Invertebrates. EPA-540/09-87-198.

OECD Series on Principles on Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

Dow AgroSciences LLC Test Substance Distribution Certificate. TSN102739, 02 October 2001.

Nelson, R.M. Certificate of Analysis for Test/Reference/Control Substances: FA&PC Number 013276, Dow AgroSciences LLC, Indianapolis, Indiana. 27 September 2001.

Product Technology Information Platform (PTIP) Database. Dow AgroSciences LLC, Indianapolis, Indiana.

Dow AgroSciences Test Substance Assay. TSN101773, Dow AgroSciences LLC, Indianapolis, Indiana. 20 May 1999.

Toxicology & Environmental Research and Consulting Pharmacy Inventory Database, Test Material Information for TSN101773. The Dow Chemical Company, Midland, MI.

Smith A.J. Dow AgroSciences Certificate of Analysis for Test/Reference/Control Substances: FA & PC 993090, Dow AgroSciences LLC, Indianapolis, Indiana. 20 May 1999.

McClymont, L.G., *et al.* Analytical Data for XDE-638: 21 Day Chronic Toxicity Test with the Daphnia, *Daphnia magna,* Straus, Study # 001018, 28 August 2002.

Marino, T.A. et al. XDE-638: An Acute Toxicity Study with the Daphnia, *Daphnia magna* Straus. Study ID# 991215. Unpublished report of The Dow Chemical Company, Midland, Michigan. 17 April 2000.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	59.2	20	1	
2	98.7	20	2	
3	165	20	2	
4	274	20	1	