Data Evaluation Report on the Acute Toxicity of GF-443 to Rainbow Trout (*Oncorhynchus mykiss*)
PMRA Submission Number {.....} EPA MRID Number 45831011

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D288160
OECD Data Point	
EPA MRID	45831011
EPA Guideline	§72-1c

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

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Company Code:
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Date Evaluation Completed:

CITATION: Marino, T.A., *et al.* 2002. GF-443: An Acute Toxicity Study with the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Laboratory Study No. 021024. Study submitted by Dow AgroSciences LLC, Indianapolis, IN. Study initiated April 1, 2002 and completed July 29, 2002.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, juvenile Rainbow trout (*Oncorhynchus mykiss*) were exposed under static conditions to GF-443 [an end-use product containing 22% XDE-638 (penoxsulam)] at nominal concentrations of 0 (negative control), 59.3, 98.8, 165, 274, 457, and 762 ppm. The equivalent nominal concentrations of active ingredient (XDE-638) were 0, 13.0, 21.6, 36.0, 60.1, 100, and 167 ppm a.i. Mean-measured concentrations were <5.91 (LOQ, negative control), 13.3, 20.8, 37.1, 57.0, 91.4, and 147 ppm a.i.

After 96 hours of exposure, 10% mortality was observed in the 91.4 ppm a.i. treatment group. No other mortalities or sub-lethal effects were observed. The LC_{50} was >147 ppm a.i., which categorizes GF-443 [an end-use product containing 22% XDE-638 (penoxsulam)] as practically nontoxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The NOAEC (for mortality) was 91.4 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 fish (72-1c) using an end-use product.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	Juvenile: 0.607 ± 0.085 g and 43 ± 2 mm (mean of all definitive test fish at test termination)
Test Type (Flow-through, Static, Static Renewal):	Static

96-Hour
LC_{50}: >147 ppm a.i.
NOAEC: 91.4 ppm a.i. (based on mortality)
LOAEC: 147 ppm a.i.
Endpoints affected: Mortality

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in the OECD Guidelines for Testing of Chemicals, No. 203 (1992); and the EEC Method C.1, Acute Toxicity for Fish (1992). Deviations from U.S. EPA guideline §72-1 included:

- The initial weight and length of the fish were not specified.
- The storage conditions of test chemical was not reported.

- The size of the test vessels (12 L with a fill volume of 10 L) was less than required (fill volume of 15-30 L).
- Mild aeration was provided during the study.
- The water hardness (63-67 mg $CaCO_3$/L) was slightly greater than recommended (40-48 mg $CaCO_3$/L).
- The pH range (6.9-7.3) slightly exceeded recommendations (7.2-7.6).

These deviations do 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. 26). Overall mean recoveries were 102% at Day 0 and 90.4% at Day 4. Slight declines in concentrations were observed at the ≥274 ppm (nominal) test levels. 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: Rainbow trout (*Oncorhynchus mykiss* Walbaum)

Age at test initiation: Juvenile

Weight at test initiation: Not provided; 0.607 ± 0.085 g (average weight of all test and control fish at test termination)

Length at test initiation: Not provided; 43 ± 2 mm (average length of all test and control fish at test termination)

Source: Thomas Fish Company, Anderson, CA.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A 96-hour static range-finding study was conducted with five Rainbow trout per single replicate and GF-443 at nominal concentrations of 0 (negative control), 505, and 1000 ppm (equivalent to 0, 110, and 219 ppm XDE-638, respectively, p. 15). No mortalities or sub-lethal effects were observed at the control or any test level.

A 96-hour static definitive study was attempted with five trout per replicate, and six replicates per test level (p. 15). Nominal GF-443 concentrations were 0 (negative control), and 457 ppm (equivalent to 100 ppm XDE-638). However, 27% mortality was observed after 24 hours of exposure and was 43% following 96 hours of exposure in the test solution.

A second definitive study was attempted with five trout per replicate, two replicates per test level, and nominal concentrations of 59.3, 98.8, 165, 274, 457, and 762 ppm GF-443 (p. 15). This test was terminated following 24 hour due to one vessel in each of the two lowest test levels exhibiting 100% mortality (other replicate vessel/level showed no adverse effects). In addition, no adverse effects were observed in the 165, 274, and 457 ppm test levels. Mortality was 20% in the 762 ppm level and was all from one replicate. It was concluded that the high variability in the dose response from this test and from the previous definitive test attempt was due to vessel contamination from the screened inserts that were used to aid in the observation of the fish. The screens were necessary due to the highly turbid nature of the treated solutions, which made observations difficult. It was found that the inserts were contaminated with bleach, and prior to repeated the test, all glass inserts were re-screened with new mesh and all glassware re-cleaned.

b) Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	At least 14 days prior to testing. Same as test Standard diet once per day except during the 48 hours prior to testing. <5% mortality during the last 48 hours of acclimation.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Duration of the test	96 hours	EPA/OECD requires: 96 hours
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static	 Static N/A N/A	*EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	Due to the excess turbidity of the test solutions at the higher dose levels, mild aeration (~100 bubbles/minute per vessel) was provided to each vessel during the study.	Aeration is not recommended. *EPA requires: no aeration; OECD permits aeration*
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	 Glass beakers 12 L 10 L	The test vessel size and fill volume were less than required. To assist in observations, each replicate contained a 20 x 29-cm glass insert with a

Parameter	Details	Remarks Criteria
		nylon screen (~1000 µm pore size), which was used for moving organisms near surface of the solutions. *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
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 prior to use.	*EPA 1975; Soft reconstituted water or water from a natural source,* ***not*** *dechlorinated tap water;* *OECD permits dechlorinated tap water.*
<u>Water parameters</u>: Hardness pH Dissolved oxygen Total Organic Carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 63-67 mg $CaCO_3$/L 6.9-7.3 9.5-10.5 mg/L (≥92%) <1000 µg/mL 4,000 µg/mL See Table 1, p. 24 Not detected (Table 2, p. 25). <20 µg/L 12.9-13.5°C N/A DO, pH, and temperature were determined daily. Temperature was continuously recorded from	The water hardness was slightly greater than recommended. The pH range exceeded recommendations. Results from inorganic and organic analysis of the dilution water are provided in Tables 1 and 2, pp. 24-25. ***Hardness and pH*** *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *<u>Renewal</u>: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *<u>Flow-through</u>: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.*

Parameter	Details	Remarks
		Criteria
	one test vessel.	***Salinity*** *30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰* ***EPA water quality*** *measured at beginning of test and every 48 hours*
Concentration of test material: nominal:	0 (negative control), 59.3, 98.8, 165, 274, 457, and 762 ppm GF-443	Mean-measured concentrations are provided in Table 3, p. 26.
		EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series
XDE-638 equivalent concentrations:	0, 13.0, 21.6, 36.0, 60.1, 100, and 167 ppm	
measured:	<5.91 (LOQ, control), 13.3, 20.8, 37.1, 57.0, 91.4, and 147 ppm a.i.	
Solvent (type, percentage, if used)	N/A	
		EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.
Number of fish/replicates:		
negative control:	10 fish, divided into 2 replicates containing 5 fish each	*EPA: ≥10/concentration; OECD requires at least 7 fish/concentration*
solvent control:	10 fish, divided into 2 replicates containing 5 fish each	
treated:	10 fish, divided into 2 replicates containing 5 fish each	
Biomass loading rate	0.304 g fish/L (p. 19).	
		Static: ≤0.8 g/L at ≤17°C, ≤0.5 g/L at >17°C; flow-through: ≤1 g/L/day; OECD requires maximum of 1 g fish/L

Parameter	Details	Remarks
		Criteria
		for static and semi-static with higher rates accepted for flow-through
Lighting	16-hours light/8-hours dark.	
		EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.
Feeding	Animals were not fed during testing.	
		EPA/OECD requires: No feeding during the study
Recovery of chemical Level of Quantization Level of Detection	71.9-105% of nominal 5.91 ppm a.i. Not reported.	Recoveries are based on test solutions analyzed on Days 0 and 4 (Table 3, p. 26).
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 sublethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	Every 24 hours.	
		EPA/OECD requires: minimally every 24 hours
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 96 hours of exposure, 10% mortality was observed in the 147 ppm a.i. test level (mean-measured equivalent XDE-638 of 762 ppm GF-443; Table 6, p. 29). No other mortalities were observed.

Table 3: Effect of GF-443 on mortality of Rainbow trout (*Oncorhynchus mykiss*).

Treatment			No. of fish at start of study	Observation Period					
				0-24 Hours		48-72 Hours		96 Hours	
Nominal GF-443, ppm	Nominal XDE-638, ppm	Measured XDE-638, ppm a.i.		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	Negative control	<5.91	10	0	0	0	0	0	0
59.3	13.0	13.3	10	0	0	0	0	0	0
98.8	21.6	20.8	10	0	0	0	0	0	0
165	36.0	37.1	10	0	0	0	0	0	0
274	60.1	57.0	10	0	0	0	0	0	0
457	100	91.4	10	0	0	0	0	0	0
762	167	147	10	0	0	0	0	1	10
NOAEC (mortality)			91.4 ppm a.i.						
LC_{50} (95% C.I.)			>147 ppm a.i.						
Positive control, if used mortality: LC_{50}:			N/A	N/A	N/A	N/A	N/A	N/A	N/A

B. NONLETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed in the control or any treatment group during the study (Table 6, p. 29).

C. REPORTED STATISTICS:

The 96-hour LC_{50} value was estimated because there was only one fish mortality during the study. The NOAEC was determined based on mortality data. The results were based on mean-measured concentrations of active ingredient.

LC_{50}: >147 ppm a.i.
NOAEC: 91.4 ppm a.i. (based on mortality)
LOAEC: 147 ppm a.i.
Endpoints affected: Mortality

D. VERIFICATION OF STATISTICAL RESULTS:

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

LC_{50}: >147 ppm a.i.
NOAEC: 91.4 ppm a.i. (based on mortality)
LOAEC: 147 ppm a.i.
Endpoints affected: Mortality

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-1c 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 the mean-measured concentrations of active ingredient in the test solutions (ppm a.i.).

The test solutions were turbid and white in color for all treatment groups and fish visibility

was impaired in the 91.4 and 147 ppm a.i. treatment groups (p. 16). Therefore, they aerated. Turbidity is a sign of incomplete solution of the chemical being studied. Both the aeration and the turbidity decreased the acceptability of this study. By day 4, turbidity decrease in the solutions, but fish visibility was still impaired in the 91.4 and 147 ppm a.i. treatment groups.

[Although most of the deviations from guideline were minor, aerating due to excess turbidity should not have been conducted. The study should only be classified as "Supplemental." RF 2/4-4: I concur. jjg 8-11-4]

G. CONCLUSIONS:

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish (§72-1) using an end-use product containing 22% (w:w) XDE-638. This study is classified as SUPPLEMENTAL, but it need not be repeated. The LC_{50} was >147 ppm a.i., the highest concentration tested, which categorizes GF-443 [an end-use product containing 22% XDE-638 (penoxsulam)] as practically nontoxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The NOAEC (for mortality) was 91.4 ppm a.i. No sub-lethal effects were observed.

96-Hour
LC_{50}: >147 ppm a.i.
NOAEC: 91.4 ppm a.i. (based on mortality)
LOAEC: 147 ppm a.i.
Endpoints affected: Mortality

III. REFERENCES:

Organisation for Economic Cooperation and Development (OECD) (1992). OECD Guidelines for Testing of Chemicals. Method 203, "Fish, Acute Toxicity Test", ISBN 92-64-12221-4. Adopted July 1992.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	0	
1	762/167	10	1	