

US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the Acute Toxicity of XDE-638 to Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....}

EPA MRID Number 45834804

Data Requirement:	PMRA DATA CODE	
	EPA DP Barcode	D288160
	OECD Data Point	
	EPA MRID	45834804
	EPA Guideline	§72-1c

Test material: XDE-638 **Purity:** 97.5%
Common name: Penoxsulam
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

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Reference/Submission No.:

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Active Code:
EPA PC Code: ~~109031~~ 119031

Date Evaluation Completed:

CITATION: Marino, T.A., *et al.* 2000. XDE-638: 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 Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI.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. 991214. Study submitted by Dow AgroSciences, Indianapolis, IN. Study initiated October 18, 1999 and submitted April 3, 2000. (*study # 991214*)

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Staff Scientist, Dynamac Corporation **Date:** 10/17/03

Primary Reviewer: Richard Felthousen &
James J. Goodyear, Ph.D., Biologists, ERB 3,
Environmental Fate and Effects Division **Date:**
Office of Pesticide Programs, US EPA

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, juvenile Rainbow trout (*Oncorhynchus mykiss*) were exposed under static conditions to XDE-638 (penoxsulam) at nominal concentrations of 0 (negative and solvent controls) and 100 ppm (limit test). Mean-measured concentrations were <12 (LOQ, controls) and 102 ppm a.i.

After 96 hours of exposure, no mortality or sub-lethal effects were observed in any control or test group. The 96-hour LC₅₀ was >102 ppm a.i., which categorizes XDE-638 as practically nontoxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The NOAEC and LOAEC were 102 and >102 ppm a.i., respectively.

Since the mean fish weight of 0.287 g, determined from pooling data from all definitive test fish at test termination, was less than the required initial weight range of 0.5 to 5 g, this study does not fulfill guideline requirements for an acute toxicity study with the Rainbow trout (§72-1c) and is classified SUPPLEMENTAL, but it need not be repeated.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): At least 14 days old: 0.287 ± 0.066 g and 29 ± 2 mm (mean of all definitive test fish at test termination)

Test Type (Flow-through, Static, Static Renewal): Static

96-Hour

LC₅₀: >102 ppm a.i.

NOAEC: 102 ppm a.i.

LOAEC: >102 ppm a.i.

Endpoints affected: None

I. MATERIALS AND METHODS**GUIDELINE FOLLOWED:**

The study protocol was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, Series §72-1 (1986), and the U.S. EPA Standard Evaluation Procedure (1985). Deviations from guideline §72-1c include:

- The initial age, weight, and length of the fish were not specified.
- Mean fish weight (0.287 ± 0.066 g) was determined from pooling data from all control and test fish at study termination, and was less than the recommended initial range of 0.5-5g.
- The storage conditions of test chemical was not reported.
- The size of the test vessels (4 L with a fill volume of 3.5 L) was significantly less than required (fill volume of 15-30 L).
- The water hardness (60 mg CaCO₃/L) was greater than recommended (40-48 mg CaCO₃/L).
- The pH range (6.6-7.6) exceeded recommendations (7.2-7.6).

These deviations do not affect the validity of the study. However, this study does not fulfill guideline requirements. It need not be repeated.

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

A. MATERIALS:

1. Test Material XDE-638 (penoxsulam)

Description: Pink powder

Lot No./Batch No. : ND05167938

Purity: 97.5%

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. Results are presented in Table 3, p. 22.

Storage conditions of test chemicals: Not specified

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*)

Age at test initiation: Juvenile, at least 14 days old (not further specified)

Weight at test initiation: Not provided; 0.287 ± 0.066 g (average weight of all test and control fish at test termination)

Length at test initiation: Not provided; 29 ± 2 mm (average length of all test and control fish at test termination)

Source: Eyed embryos were obtained from Mt. Lassen Trout Farms, Red Bluff, 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 XDE-638 at nominal concentrations of 0 (negative and 0.5 mL DMF/L controls), 2.50, 25.0, and 100 ppm (p. 13). Dissolution of test substance in the 100 ppm treatment group was not complete on Day 0 and test material was observed on bottom of test vessel on Day 3; it was believed that the solubility limit of the material was exceeded. The 100 ppm group was terminated after 72 hours. No mortality was observed at any control or test level.

A 96-hour static definitive study was attempted with five trout per replicate, and two replicates per test level (p. 13). Nominal XDE-638 concentrations were 0 (negative and 0.1 mL DMF/L controls), 7.78, 13.0, 21.6, 36.0, 60.0, and 100 ppm. Test solutions were sonicated for 30 minutes, however, undissolved test material was still observed at ≥ 21.6 ppm levels. No mortality was observed at any control or test level during the study. Samples collected for analytical verification of the test concentrations indicated that XDE-638 increased from approximately 74% of nominal concentrations on Day 0 to 95% on Day 4.

A subsequent solubility study was conducted, and it was observed that the test material would completely dissolve in the test solutions following a minimum of 1.5 hours of sonication.

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	At least 14 days prior to testing.	
Conditions: (same as test or not)	Same as test	
Feeding:	Standard diet once per day except during the 48 hours prior to testing.	<i>EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.</i>
Health: (any mortality observed)	<5% mortality during the last 48 hours of acclimation.	
Duration of the test	96 hours	
		EPA/OECD requires: 96 hours
<u>Test conditions:</u> static/flow through	Static	
Type of dilution system- for flow through method.	N/A	<i>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</i>
Renewal rate for static renewal	N/A	
Aeration, if any	No aeration during the study.	
		<i>EPA requires: no aeration; OECD permits aeration</i>
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Glass beakers 4 L 3.5 L	Vessels were loosely covered. The size of the test vessels were significantly less than required. <i>EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm Fill volume: 15-30 L of solution</i>
Source of dilution water	The dilution water was pumped to the laboratory from the upper Saginaw Bay of Lake Huron. The	

Parameter	Details	Remarks	
		Criteria	
		<i>EPA 1975; Soft reconstituted water or water from a natural source, not dechlorinated tap water; OECD permits dechlorinated tap water.</i>	
<u>Water parameters:</u> Hardness	60 mg CaCO ₃ /L	The water hardness was slightly greater than recommended.	
pH	6.6-7.6	The pH range exceeded recommendations.	
Dissolved oxygen	7.8-10.4 mg/L (≥76% saturation)	Results from inorganic and organic analysis of the dilution water are provided in Tables 1 and 2, pp. 20-21.	
Total Organic Carbon	3,845 ng/mL		
Particulate Matter	<LOD (<1000 ng/mL, total suspended solids)		
Metals	See Table 1, p. 20	<p>Hardness and pH EPA requires hardness of 40-48 mg/L as CaCO₃ 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 CaCO₃ and pH between 6 and 8.5.</p> <p>Dissolved Oxygen <i>Renewal:</i> ≥60% during 1st 48 hrs and ≥40% during 2nd 48 hrs <i>Flow-through:</i> ≥60% through out test. OECD requires at least 80% saturation value.</p> <p>Temperature EPA requires 12°C for coldwater species and 17-22°C for warmwater species. OECD requires range of 21 - 25°C for bluegill and 13-17°C for rainbow trout.</p> <p>Salinity 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰</p> <p>EPA water quality measured at beginning of test and every 48 hours</p>	
Pesticides	<LOD (Table 2, p.21)		
Chlorine	Not reported		
Temperature	12.2-12.9°C		
{Salinity for marine or estuarine species}	N/A		
Intervals of water quality measurement	DO, pH, and temperature were determined daily. Temperature was continuously recorded from one test vessel.		
<u>Concentration of test material:</u> nominal:	0 (negative and 0.1 mL DMF/L controls) and 100 ppm		This study was conducted as a limit test, demonstrating that the acute toxicity to the Rainbow trout

Parameter	Details	Remarks
		Criteria
measured:	<12 (<LOQ, controls) and 102 ppm a.i.	exceeds 100 ppm. Mean-measured concentrations are provided in Table 3, p. 22. <i>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.</i>
Solvent (type, percentage, if used)	Dimethylformamide (DMF), 0.1 mL/L	<i>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.</i>
<u>Number of fish/replicates:</u> negative control: solvent control: treated:	30 fish, divided into 6 replicates containing 5 fish each 30 fish, divided into 6 replicates containing 5 fish each 30 fish, divided into 6 replicates containing 5 fish each	<i>EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration</i>
Biomass loading rate	0.410 g fish/L (p. 16).	<i>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 for static and semi-static with higher rates accepted for flow-through</i>
Lighting	16-hours light/8-hours dark.	<i>EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.</i>
Feeding	Animals were not fed during testing.	<i>EPA/OECD requires: No feeding during the study</i>
Recovery of chemical	97.6-105% of nominal	Recoveries are based on test solutions analyzed on Days 0 and 4 (Table 3, p. 22).
Level of Quantitation	12 ppm a.i.	
Level of Detection	0.04 ppm a.i.	

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 sublethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	6, 24, 48, 72 and 96 hours of exposure	<i>EPA/OECD requires: minimally every 24 hours</i>
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:**A. MORTALITY:**

After 96 hours of exposure, no mortalities were observed in the controls or 102 ppm a.i. treatment group.

Table 3: Effect of XDE-638 on mortality of Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, ppm a.i. measured and (nominal conc.)	No. of fish at start of study	Observation Period					
		0-24 Hours		48-72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	30	0	0	0	0	0	0
Solvent control	30	0	0	0	0	0	0
102 (100)	30	0	0	0	0	0	0
NOAEC (mortality)	102 ppm a.i.						
LC ₅₀ (95% C.I.)	>102 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. NON-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed during the study in the controls or 102 ppm a.i. treatment groups.

C. REPORTED STATISTICS:

The 96-hour LC₅₀, NOAEC, and LOAEC values were visually determined, based on observed mortality data.

96-Hour

LC₅₀: >102 ppm a.i.

NOAEC: 102 ppm a.i.

LOAEC: >102 ppm a.i.

Endpoints affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC₅₀, NOAEC, and LOAEC for mortality and sub-lethal effects could be determined visually.

96-Hour

LC₅₀: >102 ppm a.i.

NOAEC: 102 ppm a.i.

LOAEC: >102 ppm a.i.

Endpoints affected: None

E. STUDY DEFICIENCIES:

This study is scientifically valid. However, the mean fish weight of 0.287 g was determined from all fish at study termination and was less than the required initial weight range of 0.5-5 g. As a result, this study does not fulfill guideline requirements for an acute toxicity study with the Rainbow trout (§72-1c) and is classified SUPPLEMENTAL, but it need not be repeated.

F. REVIEWER'S COMMENTS:

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

The aqueous stability of XDE-638 was determined in a 14-day static daphnid study conducted at nominal concentrations of 0.1 and 10 ppm (p. 10). The 14-day recoveries were 98-104% of nominal.

G. CONCLUSIONS:

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with freshwater fish (§72-1) because the mean weight of the fish at study termination was 0.287 g, which is less than the required initial weight range of 0.5 to 5 g. This study provides useful information, and is classified SUPPLEMENTAL, but it need not be repeated. The 96-hour LC₅₀ of XDE-638 was >102 ppm a.i., which classifies XDE-638 (penoxsulam) as practically nontoxic to juvenile Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The 96-hour NOAEC was 102 ppm a.i.

96-Hour

LC₅₀: >102 ppm a.i.

NOAEC: 102 ppm a.i.

LOAEC: >102 ppm a.i.

Endpoints affected: None

III. REFERENCES:

- U.S. Environmental Protection Agency. Office of Pesticide and Toxic Substances. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Guideline 72-1, Acute Toxicity Test For Freshwater Fish. EPA-540/09-87-198. December 1986.
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- EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).
- The Dow Chemical Company, Research Sample Safety Data Sheet. 2 February, 1999.
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