Data Evaluation Report on the Acute Toxicity of XDE-638 to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number{.....} EPA MRID Number 45831012

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D288160
OECD Data Point	
EPA MRID	45831012
EPA Guideline	§72-2

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
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Signature: Rebecca Bryan
Date: 10/17/03

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

Reference/Submission No.:

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

Date Evaluation Completed:

CITATION: Marino, T.A., *et al.* 2000. XDE-638: An Acute Toxicity Study with the Daphnia, *Daphnia magna* Straus. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Laboratory Study No. 991215. Study submitted by Dow AgroSciences, Indianapolis, IN. Study initiated October 26, 1999 and completed April 17, 2000.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of XDE-638 (penoxsulam) to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at a single, nominal concentration of 100 ppm (limit test), with negative and solvent controls. Mean-measured concentrations were <12 (LOQ, negative and solvent controls) and 98.3 ppm a.i.

After 48 hours, no immobilization was observed in the controls or 98.3 ppm a.i. treatment group. The 48-hour LC/EC_{50} was >98.3 ppm a.i., which categorizes XDE-638 (penoxsulam) as slightly toxic to the water flea (*Daphnia magna*) on an acute toxicity basis. The 48-hour NOAEC level, based on mortality/immobilization, was 98.3 ppm a.i., the only concentration tested. The water hardness was four times the recommended level.

This study is scientifically sound, but, because of the high water hardness it does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2). This study is classified as SUPPLEMENTAL, but it need not be repeated..

Results Synopsis

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

48-Hour
LC/EC_{50}: >98.3 ppm a.i.
NOAEC: 98.3 ppm a.i. (based on mortality/immobilization)
LOAEC: >98.3 ppm a.i.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, Series §72-2, and the U.S. EPA Standard Evaluation Procedure. Deviations from guideline §72-2 include:

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 water hardness (176 mg/L as $CaCO_3$) was significantly higher than recommended (40-48 mg/L as $CaCO_3$).

4. The pH (7.4-7.8) was slightly higher than recommended (7.2-7.6).

5. The loading rate was not specified.

6. Sub-lethal effects were not monitored.

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

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

A. MATERIALS:

1. Test Material XDE-638 (penoxsulam)

Description: Pink powder

Lot No./Batch No. : TSN101773

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 (97.6% of nominal) and 48 hours (99.0% of nominal, Table 3, p. 22).

Storage conditions of test chemicals: 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.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A 48-hour static range-finding study was conducted with 10 daphnid per single replicate and XDE-638 at nominal concentrations of 0 (negative and 0.1 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 1; it was believed that the solubility limit of the material was exceeded. No mortality was observed at any control or test level.

A 48-hour static definitive study was attempted with 10 daphnid 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 from an acute study with the Rainbow trout (MRID 45834804) conducted concurrently with this study indicated that concentrations of 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:	Continuous laboratory cultures were maintained.	*EPA requires 7 day minimum acclimation period.*
Conditions: (same as test or not)	Same as test	
Feeding:	*Daphnia* cultures were	

Parameter	Details	Remarks *Criteria*
Health: (any mortality observed)	fed 4 times/week with mixed diet of *Ankistrodesmus convolutus* (algae) and YCT trout chow (yeast-ceraphyll trout). Not specified	
Duration of the test	48 hours	*EPA requires 48 hours*
Test condition - static/flow through	Static	
Type of dilution system (for flow through method)	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*
Renewal rate (for static renewal)	N/A	
Aeration, if any	No aeration during the study.	
Test vessel		Test vessels were covered to prevent evaporation.
Material: *(glass/stainless steel)* Size: Fill volume:	Glass jars 250 mL 200 mL	*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	 176 mg/L as $CaCO_3$ 7.4-7.8 8.9-9.1 mg/L (averaged	The hardness was higher than recommended. The pH was slightly greater than recommended.

Parameter	Details	Remarks Criteria
Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	100% saturation) 19.6-19.9°C 3,611 ng/mL <1000 ng/mL (<LOD, total suspended solids) See Table 1, p. 20 <LOD (Table 2, p.21) <20 ng/mL (<LOD, residual)	Results from inorganic and organic analysis of the dilution water are provided in Tables 1 and 2, pp. 20-21. *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%*
Number of replicates Solvent control: Negative control: Treatments:	 3 3 3	
Number of organisms per replicate Solvent control: Negative control: Treatments:	 10 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.*
Treatment concentrations nominal: measured:	 0 (negative and 0.1 mL DMF/L controls) and 100 ppm <12 (<LOQ, controls) and 98.3 ppm a.i.	This study was conducted as a limit test, demonstrating that the acute toxicity to the daphnia magna exceeds 100 ppm. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	Dimethyl formamide (DMF), 0.1 mL/L	 *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 was 2143 lux at test termination. *EPA requires 16 hours light, 8 hours dark.*
Feeding	Animals were not fed during testing.	 *EPA/OECD requires: No feeding during*

Parameter	Details	Remarks Criteria the study
Stability of chemical in the test system	Verified. Analyzed concentrations were 97.6% of nominal concentrations for Day 0 samples and 99.0% for Day 4 samples. The mean-measured concentration was 98.3% of nominal.	
Recovery of chemical Level of Quantitation Level of Detection	97.6-99.6% of nominal 12 ppm a.i. 0.03 ppm a.i.	
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	Mortality/immobility	
Observation intervals	After 6, 24, and 48 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY

After 48 hours, no mortality/immobilization was observed in the controls or 98.3 ppm a.i. test group (p. 16).

Table 3: Effect of XDE-638 on mortality/immobilization of *Daphnia magna*.

		Observation period	

Treatment, ppm a.i. Measured and (nominal) concn.	No. of organisms	24 Hours		48 Hours	
		No.	%	No	%
Negative Control	30	0	0	0	0
Solvent Control	30	0	0	0	0
98.3 (100)	30	0	0	0	0
NOAEC, ppm a.i.		Not determined			
LC/EC_{50} (95% C.I.), ppm a.i.		>98.3			

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.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical analyses were not required, as there was no immobility in this study. The LC_{50} and NOAEC (for mortality/immobilization) could be visually determined.

48-Hour
LC/EC_{50}: >98.3 ppm a.i.
NOAEC: 98.3 ppm a.i. (based on mortality/immobilization)
LOAEC: >98.3 ppm a.i.

E. STUDY DEFICIENCIES:

There were significant deviations from U.S. EPA guideline §72-2 that affected the acceptability of this study.

F. REVIEWER'S COMMENTS:

The water hardness was four times the recommended level, otherwise, the reviewer's conclusions were identical to the study authors.

G. CONCLUSIONS:

[Because water concentration was significantly higher than specified in guidelines, study can only be classified as "Supplemental." rf, 2-11-4] This study is scientifically sound, fulfills U.S. EPA guideline §72-2, and is classified as SUPPLEMENTAL, but it need not be redone.. The 48-hour LC/EC_{50} was >98.3 ppm a.i., the only

concentration tested. Based on the results of this study, XDE-638 (penoxsulam) is categorized as slightly toxic to the water flea, *Daphnia magna*, on an acute toxicity basis.

48-Hour
LC/EC_{50}: >98.3 ppm a.i.
NOAEC: 98.3 ppm a.i. (based on mortality/immobilization)
LOAEC: >98.3 ppm a.i.

III. REFERENCES:

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.

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

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.

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

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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. November, 1993.

Certificate of Analysis for XDE-638 TGAI-TOX. The Dow Chemical Company, 20 May 1999.

K.E. Engle, Analytical Report AL #99-148, XDE-638 RLCAT #2, August 27, 1999.

Kirk, H.D., *et al.* XDE-638: Twenty-one Day Chronic Toxicity Study with the Daphnia, *Daphnia magna* Straus". Toxicology & Environmental Research and Consulting laboratory, The Dow Chemical Company, Study ID 001018.

Marino, T.A., *et al.* "XDE-638: An Acute Toxicity Study with the Rainbow Trout, *Oncorrhynchus mykiss* Walbaum". The Dow Chemical Company R & D Unpublished Report, Study ID# 991214.