DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE ORGANISM
§72-3(C) - MYSID

1. CHEMICAL: Penoxsulam PC Code No.: 119031

2. TEST MATERIAL: XDE-638 Purity: 97.7%

3. CITATION:

Author: Ward, T.J., *et al.*

Title: XDE-638: Acute Toxicity to the Mysid, *Americamysis bahia*

Study Completion Date: October 9, 2000

Laboratory: T.R. Wilbury Laboratories, Inc.
40 Doaks Lane
Marblehead, Massachusetts 01945

Sponsor: The Dow Chemical Company
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for
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Indianapolis, Indiana 46268-1054

Laboratory Report ID: 1998-DO/Dow Study No. 000052

MRID No.: 45831024

DP Barcode: D288160

4. REVIEWED BY: Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: **Date:** 10/17/03

APPROVED BY: Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: **1Date:** 10/17/03

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

JGoodyear

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Americamysis bahia*

Age or Size of Test Organism: <24 hours old

Definitive Test Duration: 96 hours

Study Method: Static

Type of Concentration: Mean-measured

7. CONCLUSIONS:

The 96-hour acute toxicity of XDE-638 (penoxsulam) to the saltwater mysid, *Americamysis bahia*, was studied under static conditions. Mysids were exposed to the test material at nominal concentrations of 0 (negative control), 16, 26, 43, 72, and 120 ppm. Mean-measured concentrations were <0.0270 (LOQ; control), 14.4, 25.6, 42.8, 71.1, and 114 ppm a.i. (90-100% of nominal values).

After 96 hours, mortality was 5% in the 114 ppm a.i. test level. No other mortality or sub-lethal effects were observed in any control or test level. The 96-hour LC_{50} value was >114 ppm a.i., which categorizes XDE-638 (penoxsulam) as practically nontoxic to the saltwater mysid, *Americamysis bahia*, on an acute toxicity basis. Based on mortality and sub-lethal effects, the NOAEC value was 114 ppm a.i., the highest concentration tested.

This study is scientifically valid and fulfills the requirements of an acute LC_{50} test with an estuarine/marine organism (Subdivision E, §72-3(C)). This study is classified as CORE.

Results Synopsis
96-Hour:
LC_{50}: >114 ppm a.i.
NOAEC: 114 ppm a.i.
LOAEC: >114 ppm a.i.
Endpoints affected: None

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: The guideline deviations were considered to be minor and did not impact the acceptability or validity of the study. Missing information should be provided to U.S. EPA.

C. Repairability: N/A

9. BACKGROUND:

10. GUIDELINE DEVIATION:

1. The salinity of 15-17‰ was less than recommended (30-34‰) for marine (stenohaline) mysid.
2. The pH levels (7.6-8.0) were less than recommended (8.0-8.3) for marine (stenohaline) mysid.
3. DO levels were not provided in terms of percent saturation.
4. The fill volume of the test vessels (1 L) was smaller than recommended (2-3 L).

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of XDE-638 (penoxsulam) to mysids for the purpose of chemical registration.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Americamysis bahia*, *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.*	*Americamysis bahia*
Age Juvenile (≤ 24 hours old) mysids should be used	<24 hours old

Guideline Criteria	Reported Information
Supplier	Juveniles were collected from in-house laboratory cultures. The original brood stock was obtained from Aquatic BioSystems, Inc., Ft. Collins, Colorado (January 26, 1999).
All mysids are from same source?	Yes
All mysids are from the same year class?	Not reported.

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	Continuous
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No apparent disease, injuries or abnormalities at beginning of test.
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study and no feeding for 24 hours before the beginning of the test if organisms are over 0.5 g each. Mysids should be fed throughout the study.	Fed live brine shrimp, *Artemia salina*, during acclimation and testing.
Pretest Mortality <3% mortality 48 hours prior to testing	<3% mortality 48 hours prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Carbon filtered, natural seawater collected directly from the Atlantic Ocean at T.R. Wilbury Laboratories in Marblehead, MA.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) for marine (stenohaline) mysids and 10-17 ‰ for estuarine (euryhaline) mysids, weekly range <6 ‰	15-17‰ (mean = 16‰)
Water Temperature Approx. 22 ± 1 °C	21.0-22.1°C (mean = 21.6°C)
pH 8.0-8.3 for marine (stenohaline) mysids, 7.7-8.0 for estuarine (euryhaline) mysids, monthly range < 0.8	7.6-8.0
Dissolved Oxygen Between 60 and 105% saturation. If needed, aerate prior to introduction of chemical.	7.2-7.9 mg/L (mean = 7.6 mg/L)
Total Organic Carbon Should be <5 mg/L in reconstituted seawater	2.4 mg/L
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown	1. Glass culture dishes 2. 2.0 L

Guideline Criteria	Reported Information
shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	3. 1 L (3.5-cm depth)
Type of Dilution System Must provide reproducible supply of toxicant	N/A; static conditions
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day (N/A for mysids)	N/A
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark, with a 15-minute transition period.
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	N/A

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 mysids, then no definitive test is required.	A 96-hour static range finding study was performed at nominal test concentrations of 0 (negative control), 1.2, 6.0, 12, 60, and 120 ppm. After 96 hours, no mortality or sub-lethal effects were observed in any control or test group.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	0 (negative control), 16, 26, 43, 72, and 120 ppm
Number of Test Organisms Minimum 20/level, may be divided among containers	20 mysids/level, divided into two replicates of 10 mysids each.
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary >1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Measured daily in each aquarium and continuously in one control vessel. 2. Measured daily in each aquarium.

Guideline Criteria	Reported Information
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through	Analytical determination of test substance was performed on samples collected from each test vessel at the beginning and end of the test.

13. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	91% of nominal, based on matrix spikes run concurrently with the test solution samples (Table 2, p. 16).
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	No control mortality was observed.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (mg/L)		Number of Mysids	Mean cumulative mortality (%)			
			Hours of Study			
Nominal	Mean Measured		24	48	72	96
Negative Control	≤LOQ	20	0	0	0	0
16	14.4	20	0	0	0	0
26	25.6	20	0	0	0	0
43	42.8	20	0	0	0	0
72	71.1	20	0	0	0	0
120	114	20	0	0	0	5

Limit of quantitation = 0.0270 ppm a.i.

After 96 hours of exposure, mortality was 5% in the 114 ppm a.i. test group; no other mortality was observed in any control or test group (Table 3, p. 17). In addition, no sub-lethal effects were observed during the study (Table 4, p. 18).

B. Statistical Results

The LC_{50} value could not be calculated because of <50% mortality in all treatment groups. The 96-hour NOAEC was estimated by visual interpretation of the mortality and clinical observation data. The results are based on mean-measured concentrations.

96-Hour:
LC_{50}: >114 ppm a.i.
NOAEC: 114 ppm a.i.
LOAEC: >114 ppm a.i.
Endpoints affected: None

14. 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 level) via TOXSTAT statistical software. The results are based on mean-measured

concentrations.

96-Hour:

LC_{50}: >114 ppm a.i.
NOAEC: 114 ppm a.i.
LOAEC: >114 ppm a.i.
Endpoints affected: None

15. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors; XDE-638 (penoxsulam) is categorized as practically nontoxic to the mysid mysids on an acute toxicity basis.

The guideline deviations were considered to be minor and did not impact the acceptability or validity of the study. Missing information should be provided to U.S. EPA.

Insoluble test material was observed on the surface of all test level vessels at 0 hours, and on the surface of the ≥42.8 ppm a.i. vessels throughout the study (p. 15).

This study was conducted in accordance with USEPA Good Laboratory Practice Regulations. A Quality Assurance Statement was included.

16. REFERENCES

ASTM. 1986. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E-729-80. In Annual Book of Standards.

Japan MAFF. 1984. Good Laboratory Practice Standard. 59 NohSan No. 3850.

OECD. 1997. OECD Guidelines for Testing of Chemicals. Annex 2. OECD Principles of Good Laboratory Practice. [C(97)186/Final].

Stephan, C.E. 1983. Computer Methods for the Calculation of LC50 Values. Personal Communication. U.S. EPA. Duluth, MN.

U.S. EPA. 1985. Standard Evaluation Procedure, Acute Toxicity Test for Estuarine and Marine Organisms (Shrimp 96-hour Acute Toxicity Test). Hazard Evaluation Division, Office of Pesticide Programs, Washington, D.C.

U.S. EPA. 1988. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Ecological Effects Branch, Hazard Evaluation Division, Office of Pesticide Programs, Washington, D.C. Draft, March 1988.

U.S. EPA. 1993. 40 CFR Part 160. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards. Final Rule.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER	NUMBER EXPOSED	SIG DEAD	(P=.05)
	CONTROL	20	0		
1	114	20	1		