DATA EVALUATION RECORD
ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK
SHELL DEPOSITION STUDY
§72-3(B)

1. **CHEMICAL:** Penoxsulam PC Code No.: 119031

2. **TEST MATERIAL:** XDE-638 Purity: 97.5%

3. **CITATION:**

Author: Boeri, R.L., *et al.*

Title: XDE-638: Flow-Through Mollusc Shell Deposition Test

Study Completion Date: January 4, 2000

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

Sponsor: Dow AgroSciences LLC
Indianapolis, Indiana

Laboratory Report ID: 1847-DO/Dow Study No. 990126

MRID No.: 45831023

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

5. **APPROVED BY:** James J. Goodyear, Ph.D., Biologist, OPP/EFED/ERB - III

Signature: JGoodyear **Date:**

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Crassostrea virginica*

Age or Size of Test Organism: Valve height: 31-48 mm

Definitive Test Duration: 96 hours

Study Method: Flow-through

Type of Concentrations: Mean -measured

7. CONCLUSIONS:

In this 96-hour, flow-through acute EC_{50} test with an estuarine/marine mollusk, the Eastern oyster (*Crassostrea virginica*) was exposed to XDE-638 (penoxsulam) at nominal concentrations of 0 (negative control), 16, 26, 43, 73, and 120 ppm. Mean-measured concentrations of were ≤0.00673 (LOQ, control), 15.8, 26.4, 45.4, 73.6, and 127 ppm a.i. (99-106% of nominal values).

After 96 hours of exposure, there was one mortality in the control and no mortalities in the treatment groups. No significant reductions were shell deposition were observed at any test level. Mean shell growth was 2.4 mm for the negative control, and 2.4, 2.4, 2.6, 2.6, and 2.1 mm for the 15.8, 26.4, 45.4, 73.6, and 127 mg/L groups, respectively. The **EC_{50} is >127 ppm a.i.,** which categorizes XDE-638 (penoxsulam) as **practically non-toxic** to the Eastern oyster (*Crassostrea virginica*) on an acute toxicity basis. The **NOEC was 127 ppm a.i.**

This study is scientifically valid and fulfills the requirements of an acute toxicity test with an estuarine/marine mollusk (§72-3b). This study is classified as **CORE**.

Results Synopsis

EC_{50}: >127 ppm a.i.
NOEC: 127 ppm a.i.
LOEC: >127 ppm a.i.

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 DEVIATIONS:

I. Pretest mortality was not reported.

II. The study author failed to describe the positioning of the oysters within the test vessels during the loading procedure.

III. DO levels were not provided in terms of percent saturation.

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

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster (*Crassostrea gigas*) and the Eastern oyster (*Crassostrea virginica*)	*Crassostrea virginica*
Mean valve height 25 - 50 mm along the long axis	31-48 mm

Guideline Criteria	Reported Information
Supplier	P. Cummins Oyster Company, Baltimore, Maryland
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	21 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Amount of peripheral shell growth removed prior to testing	3-5 mm
Feeding during the acclimation Must be fed to avoid stress.	Live marine phytoplankton provided as supplement to existing food in natural, unfiltered seawater.
Pretest Mortality <3% mortality 48 hours prior to testing	Not reported

C. Test System

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural, unfiltered 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) salinity, weekly range: <6 ‰	34-35‰
Water Temperature 15-30°C, consistent in all	20.6-21.9°C (mean = 21.2°C)
pH	7.7-8.0
Dissolved Oxygen ≥60% throughout	6.6-8.3 mg O_2/L (mean = 7.6 mg/L)
Total Organic Carbon	1.2 mg/L
Test Aquaria Should be constructed of glass or stainless steel.	Glass; 20 L (15 L fill volume, 19-cm depth)
Type of Dilution System Must provide reproducible supply of toxicant	Intermittent-flow proportional diluter
Flow rate Consistent flow rate	8.1 volume additions/day, or 0.50 L/oyster/hr.
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Not reported; study authors reported that oysters were randomly distributed among two replicates of each treatment.
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark (15 minute transition period)

Guideline Criteria	Reported Information
Solvents Not to exceed 0.5 mL/L	N/A

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 or more oysters, then no definitive test is required.	A 96-hour range-finding study was performed under static renewal conditions with a negative control, and nominal test concentrations of 1.0, 10, 50, and 100 ppm. No mortality was observed. Shell growth averaged 2.9 mm in the negative control, 3.3 mm at 1.0 ppm, 3.5 at 10 ppm, 4.6 at 50 ppm, and 3.3 mm at 100 ppm (p. 12).
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; conc. should be in a geo-	0 (negative control), 16, 26, 43, 73, and 120 ppm
Number of Test Organisms Minimum 20 individual per test level and in each control	20 oysters/level, divided into two replicates of 10 oysters each
Test organisms randomly or impartially assigned to test	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	1. Measured daily in each aquarium and continuously in one test vessel. 2. Measured daily in each aquarium.

Guideline Criteria	Reported Information
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	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
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	One mortality (5%) occurred in the controls (Table 3, p. 17).
Control Shell Deposition Must be at least 2 mm.	2.4 mm (Table 4, p. 18)
Recovery of Chemical	103% of nominal, based on matrix spikes run concurrently with the test solution samples (Table B.1., p. 25).
Raw data included?	Yes
Signs of toxicity (if any) were described?	None detected.

Shell Growth

Concentration (mg/L) Nominal	Mean Measured	Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent of Control
Negative Control	≤LOQ	20	1	2.4 ± 0.8	---
16	15.8	20	0	2.4 ± 0.9	100
26	26.4	20	0	2.4 ± 1.1	100
43	45.4	20	0	2.6 ± 1.1	108
73	73.6	20	0	2.6 ± 0.8	108
120	127	20	0	2.1 ± 1.0	88

Limit of quantitation = 0.00673 ppm a.i.

B. Statistical Results

The EC_{50} could not be calculated because there was no effect on treatment shell growth compared to the control. The NOEC was calculated using TOXSTAT 3.3. Normal distribution was determined by a Chi-square test and homogeneity of variances was determined by the Bartlett's test. ANOVA and Bonferroni's test were used to compare the shell deposition data for the treatments to the control. The results are based on mean-measured concentrations.

EC_{50}: >127 ppm a.i.
NOEC: 127 ppm a.i.
LOEC: >127 ppm a.i.

14. VERIFICATION OF STATISTICAL RESULTS:.

The EC_{50} could be determined visually because reductions in shell deposition did not exceed 50% in this study. Shell deposition data were confirmed to be normally distributed and the variances were homogeneous. The NOEC was determined using ANOVA via TOXSTAT statistical software. The results are based on mean-measured concentrations.

EC_{50}: >127 ppm a.i.
NOEC: 127 ppm a.i.
LOEC: >127 ppm a.i.

15. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors; XDE-638 (penoxsulam) is categorized as practically non-toxic to the Eastern oyster 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.

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.

Bruce, R.D. and J.D. Versteeg. 1992. A Statistical Procedure for Modeling Continuous Toxicity Data. Environ. Toxicol. and Chem. Vol. 11. No. 10, pp. 1485-1494.

Gulley, D.D., *et al.* 1990. TOXSTAT Version 3.3. Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie, Wyoming.

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

OECD. 1997. The OECD Principles of Good Laboratory Practice. [C(97)186/Final].

Smith, R.I. 1964. Keys to the Marine Invertebrates of the Woods Hole Region. Woods Hole Marine Biological Laboratory Contribution Number 11.

U.S. EPA. 1985. Standard Evaluation Procedure. Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 96-hour Flow-Through Shell Deposition Study). Hazard Evaluation Division, Office of Pesticide Programs, Washington, DC, EPA-540/9-85-011.

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. 72-3(b).

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

17. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

shell deposition
File: 1023sd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3.602	0.720	0.753
Within (Error)	113	108.037	0.956	
Total	118	111.639		

Critical F value = 2.37 (0.05,5,60)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

shell deposition
File: 1023sd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	2.389	2.389		
2	15.8	2.410	2.410	-0.066	
3	26.4	2.445	2.445	-0.177	
4	45.4	2.580	2.580	-0.608	
5	73.6	2.630	2.630	-0.768	
6	127	2.090	2.090	0.956	

Bonferroni T table value = 2.36 (1 Tailed Value, P=0.05, df=110,5)

shell deposition
File: 1023sd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	19			
2	15.8	20	0.740	31.0	-0.021
3	26.4	20	0.740	31.0	-0.056
4	45.4	20	0.740	31.0	-0.191
5	73.6	20	0.740	31.0	-0.241

6	127	20	0.740	31.0	0.299

shell deposition
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	19	2.389	2.389	2.492
2	15.8	20	2.410	2.410	2.492
3	26.4	20	2.445	2.445	2.492
4	45.4	20	2.580	2.580	2.492
5	73.6	20	2.630	2.630	2.492
6	127	20	2.090	2.090	2.090

shell deposition
File: 1023sd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	2.492				
15.8	2.492	0.327		1.67	k= 1, v=113
26.4	2.492	0.327		1.75	k= 2, v=113
45.4	2.492	0.327		1.77	k= 3, v=113
73.6	2.492	0.327		1.78	k= 4, v=113
127	2.090	0.956		1.79	k= 5, v=113

s = 0.978
Note: df used for table values are approximate when v > 20.