EPA
United States
Environmental Protection
Agency

June 15, 2004

SUBJECT: PXTS Ecotoxicity Study Submitted in Support of Wood Preservative Use

DP Barcode: 302772
PC Code: 006929

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The RASSB has reviewed ecotoxicity study submitted in support of PXTS registration as a wood preservative. An aquatic animal toxicity test was submitted and reviewed for the active ingredient PXTS. See the "Status/Results of Submitted PXTS Ecological Effects Study - 06/15/04" below:

Status/Results of Submitted PXTS Ecological Effects Study

Study	Species	MRID	Status	Results
Eastern Oyster	*Crassostrea*	462115	Core	EC_{50} = 64 ug/L, NOEC= 31 ug/L

DP Barcode: D302772 **MRID No: 462115-01**

DATA EVALUATION RECORD
OYSTER ACUTE TOXICITY TEST (SHELL DEPOSITION)
OPPTS GUIDELINE 850.1025

1. **CHEMICAL:** Polyxylenol tetrasulfide (PXTS) **PC Code No.:** 006929

2. **TEST MATERIAL:** Polyxylenol tetrasulfide - PXTS **Purity:** 100%
Batch No: 1685-23
EPA File Symbol: 75799-R

3. **CITATION**

Authors:	Susan J. Palmer (Study Director) Raymond L. Van Hoven Henry O. Krueger
Title:	PXTS: A 96-hour Shell Deposition Test with the Eastern Oyster, *Crassostrea virginica.*
Study Completion Date:	January 19, 2004
Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Sponsor:	Akzo Nobel Functional Chemicals LLC 5 Livingstone Avenue Dobbs Ferry, New York 10522
Laboratory Report ID:	Wildlife International, Ltd. Project No. 497A-112A
MRID No.:	462115-01

4. **REVIEWED BY:** Srinivas Gowda, Biologist
US EPA/OPP/AD/RASSB/Team 1

Signature: Srinivas Gowda **Date:** 6/15/04

5. **APPROVED BY:** Norm Cook, Chief
US EPA/OPP/AD/RASSB

Signature: **Date:** 4/14/04

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Crassostrea virginica* (Eastern Oyster)
Valve Height: Mean: 45.0 mm (39.4 - 49.4 mm)
Definitive Test Duration: 96 hours

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Study Method: Flow-through
Type of Concentrations: Nominal and Mean measured.

7. CONCLUSIONS

Results Synopsis (Based on nominal concentrations):

96 hr

EC_{50} (µg/L): 64 µg/L
95% CI: 40 - 105 µg/L
NOEC (µg/L): 31 µg/L
No Mortality Concentration: 125 µg/L

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: Study not discounted for minor guideline deviations discussed in Section 9.

C. Repairability: Not applicable.

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1025:

- The study report did not provide information on whether oysters were in a pre-spawn condition of gonadal development prior to and during the test.
- Natural seawater was filtered to limit exposure to oyster diseases and diluted with well water. After adjusting the salinity to 20‰, the water was filtered to remove microorganisms and fine particles prior to test.
- No information was provided for the range-finding test.
- The dilution factor between concentrations used in this study (approx. 2.0) was slightly higher than the recommended dilution factor of 1.8.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • Eastern oyster - *Crassostrea virginica*	• Eastern oyster - *Crassostrea virginica* (p.8).
Life Stage/Size • 30-50 mm in valve height • As similar in age and/or size as possible to reduce variability • Standard deviation of valve height < 20% of the mean • Should be in pre-spawn condition of gonadal development prior to and during test as determined by direct or histological observation of the gonadal tissue for presence of gametes	• Mean oyster length: 45.0 mm (p.8). • Yes, oyster length range: 39.4 - 49.4 mm (p.8). • Yes, standard deviation: 3.2 mm (p.11). • No information provided regarding whether oysters were in a pre-spawn condition of gonadal development prior to and during test.
Acquisition • Oysters may be cultured in laboratory, purchased from culture facilities or commercial harvesters, or collected from a natural population in an unpolluted area free from epizootic disease	• Oysters obtained from a culture facility (Taylor Shellfish Farms) (p.10).
Acclimation • Upon receipt, oysters brushed clean of fouling organisms and gradual transfer of oysters to holding water • Oysters held for at least 12-15 days before testing • Oyster held in dilution water at test temperature for at least 2 days before used • During holding, dissolved oxygen > 60% saturation and temperature of holding water should be same as testing water • Batch of oysters acceptable for testing if mortality over 7-day period prior to testing is <5%; if mortality between 5 and 10%, acclimation should continue for 7 additional days; if mortality >10%, entire batch should be rejected • Oysters which appear diseased, have cracked, chipped, bored or gaping shells, or are infested with mudworms or boring sponges should not be used	• No information provided on whether oysters were cleaned upon arrival and gradually transferred to holding tank. • Oysters held in filtered saltwater (same source as used for the test) for 12 days prior to study initiation (p.10). • Holding tank water temperatures ranged from 20.5 to 22.7 °C (Test temperature: 20 ± 2°C) (p.11). • Dissolved oxygen concentrations in holding tank ranged from 6.3 to 7.1 mg/L (≥79%) (p.11). • Oysters showed no signs of disease or stress during the 12-day holding period (p.11).

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Guideline Criteria	Reported Information
Preparation for Definitive Test • Oysters which have been acclimated and meet condition criteria should have 3-5 mm of the shell periphery, at the rounded end, ground away with a small electric disc grinder or other appropriate device to produce a smooth, rounded, blunt profile • Oyster's valves should be held together tightly during grinding to avoid vibrating shell and injuring abductor muscle • If an opening into the shell cavity is visible, oyster should not be used	• Prior to test initiation, recently deposited shell was removed from all oysters by grinding the periphery of the oyster with an electric grinder (p.11). • Condition of oysters during grinding not reported.

B. Test System

Guideline Criteria	Reported Information
Test Chamber • Tanks should be made of chemically inert material	• Yes. Test chambers made of glass (p.12).
Temperature • Test temperature should be 20°C • Temporary fluctuations (less than 8 hr) within ± 5°C permissible • Should be recorded continuously	• Test temperature ranged from approximately 19.5 to 22.0°C (p.18 and 22). • Temperature measured manually at test initiation and termination using a liquid-in-glass thermometer. Temperature was also measured continuously in the negative test chamber (p.16).
Salinity • Dilution water – salinity in excess of 12 ppt • Natural seawater – weekly range of <10 ppt • Artificial seawater – should not vary by more than 2 ppt	• Salinity of dilution water at test initiation and termination ranged from 20 to 21‰ (p.18 and 22).

Guideline Criteria	Reported Information
Dissolved Oxygen • Dissolved oxygen concentrations should be at least 60% during and at the end of the test. • Measurements should be made daily from the beginning to the end of the test in each chamber	• Dissolved oxygen concentration remained ≥5.7 mg/L (71%) saturation throughout the test (p.18 and 22). • Daily measurements of dissolved oxygen concentration (p.22).
Photoperiod • No recommendations provided in guidelines	• 16-hour light/8-hr dark with 30-min transition period (p.16).
pH • Measured in each test chamber at the beginning and end of test. • Dilution water should have a monthly range of <0.8 unit • Artificial seawater pH should not vary by more than 0.5 unit • Test should be carried out without adjustment of pH unless there is evidence of marked change, in which case the guidelines advise that test be repeated with pH adjustment to dilution water	• pH measured in each test chamber at 0, 48 and 96 hours (p.16). • pH of dilution water ranged from 8.1 to 8.2 (p.18 and 22).
Feeding • Cultured algae may be added to dilution water sparingly as needed	• Suspension of marine microalgae was delivered to test chambers at a rate of 5.8×10^9 cells/oyster/day (p.11).

Guideline Criteria	Reported Information
Dilution Water • Constant supply of good quality unfiltered seawater should be available throughout holding, acclimation, and testing periods • Natural seawater recommended, but artificial seawater with food added can be used • Should be delivered at a flow rate of at least 1 and preferably 5 L/hr/oyster • Flowrate should be ±10% of nominal flow • Dilution water is acceptable if oysters survive and grow normally for 14 days without exhibiting signs of stress	• Natural seawater was filtered to limit exposure to oyster diseases and diluted with well water. After adjusting the salinity to 20‰, it was filtered to remove microorganisms and fine particles prior to test (p.12). • Flow rate: at least 1 L/oyster/hour (p.12). • Flow of dilution water was controlled by rotameters, which were calibrated prior to the test (p.12), but no information regarding deviations from nominal flow was given. • Oyster held in filtered water during the 12-day holding period showed no signs of disease or stress (p.11).
Carriers • Stock solutions of test substances of low aqueous solubility may be prepared by ultrasonic dispersion or by use of organic solvents, emulsifiers, or dispersants of low toxicity to oysters • When used, control oysters should be exposed to same concentration of carrier as that used in highest concentration treatment • Concentration of carriers should not exceed 0.1 mL/L	• Stock solutions prepared in acetone (p.13). • Concentration of acetone in the solvent control and all PXTS treatment groups was 0.1 mL/L (p.13).

C. **Test Design**

Guideline Criteria	Reported Information
Range-Finding Test • Should be conducted to establish test chemical concentrations for the definitive test • Test should be conducted in same way as definitive test except a widely spaced concentration series (i.e., log-interval) is used	• No information provided on range-finding test.

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Guideline Criteria	Reported Information
Dose Range • Dilution factor between concentrations should not exceed 1.8	• Dilution factor between concentrations was approx. 2.0 (p.13).
Doses • At least 5 test concentrations should be used • Test chemical concentrations should be documented	• Five test concentrations used: 7.8 µg/L, 16 µg/L, 31 µg/L, 63 µg/L and 125 µg/L (p. 8).
Controls • No recommendations provided in guidelines	• Two controls used (p.8): – Negative control – Solvent control
Replicates Per Dose • No recommendations provided in guidelines.	• One test chamber for each treatment and control group (p.9).
Number and Placement of Organisms: • 20 oysters per test chamber. • Impartially distributed among test chambers • Spread out equidistantly from one another with left (cupped) valve down and open, unhinged ends all oriented in same direction facing the incoming flow of test solution	• 20 oysters per test chamber (p.9) • Yes (p.11). • No information provided on how the oysters were positioned in the test chamber.
Duration of Test • 96 hours	• 96 hours (p.8).
Endpoints • Shell growth	• Shell growth (p.9).
Shell Growth Measurements • Oysters should be inspected at least after 24, 48, 72, and 96 hours • Dead oysters should be removed • Shell growth increments measured after 96 hours • Record the length of the longest "finger" of new shell growth to the nearest 0.1 mm	• Daily observations for mortality and signs of toxicity (p.18 and 23). • No mortality among oysters during test (p.23). • Oyster shell growth measured at test termination (p.18, 24 and 42). • Longest finger of new shell growth measured to the nearest 0.1 mm (p.16).

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12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes (p.3 and 4).
Name of test and investigator, name and location of laboratory, and start/end dates of test reported?	Yes (cover page).
Control • Mortality should not exceed 10% at end of test • Minimum of 2 mm of new shell growth should be observed	• No mortality in controls (p18 and 23). • Mean shell deposition in the negative control and solvent control was 3.43 and 3.64 mm, respectively (p.18 and 24).
Information on test chemical (e.g., water solubility, vapor pressure, purity, stability in water and light, n-octanol/water partition coefficient, and pKa values)?	• No information provided.
Source of dilution water, the mean, standard deviation and range of salinity, pH, temperature, and dissolved oxygen during test period?	• Yes (p.11 and 22).
Description of test procedures used (e.g., flow-through system, test chambers, chemical delivery system, aeration, etc.)?	• Yes (p.12).
Detailed information on oysters used, including the age and/or size, source, history, method of confirmation of pre-spawn condition, acclimation procedures, and food used?	• Yes (p.10), but no information was provided regarding whether oysters were in a pre-spawn condition prior to and during test.
Number of organisms tested, loading rate, and flow rate?	• Yes (p.11and 12).

Guideline Criteria	Reported Information
Methods of preparation of stock and test solutions, and test chemical concentrations used?	• Yes (p.13).
Number of dead and live test organisms, the percentage of organisms that died, and the number that showed any abnormal effects in the control and in each test chamber at each observation period?	• Yes (p.23).
96 hr shell growth measurements of each oyster, the mean, standard deviation and range of measured growth at 96 hr of oysters in each concentration of test substance and control?	• Yes (p.24 and 42).
Calculated 96 hr EC50 and its 95% confidence limits and statistical methods used to calculate values?	• Yes (p.8 and 17).
Graph of concentration-response curve based on 96 hr chemical concentration and shell growth measurements upon which EC50 calculated?	• No.
When observed, the 96 hr NOEC?	• Yes (p.8).
Raw data included?	• Yes (p.42).
Methods and data records reported?	• Yes (p.13-16, 21 and 25-41).
Statistical methods reported?	• Yes (p.17).

Dose Response

Nominal Concentration (µg/L)	Mean Measured Concentration (µg/L)	Mean Shell Deposition (mm)
Control	N/A	3.43
Solvent Control	N/A	3.64
Pooled Control	N/A	3.53
7.8	ND	3.32
16	ND	2.90
31	ND	2.59
63	< 50	1.78
125	89	1.14

*N/A = Not applicable.
ND = Not determined. Due to the limits of the analytical method, concentrations ≤31 µg/L could not be analyzed.

Statistical Results

Statistical Method:
Statistical analyses were conducted using the TOXSTAT computer program. No significant differences were found between the negative control and solvent control data when compared using an appropriate t-test. Thus, control data were pooled for comparison with treatment groups.

NOEC

ANOVA and Bonferroni's t-test were conducted to compare shell deposition data for the treatment groups to the pooled control data. The data were first evaluated for normality using the Chi-Square Test and for homogeneity of variance using Bartlett's Test. The NOEC was determined from the statistical analysis of the data and an assessment of the concentration-response pattern.

EC_{50}

The EC_{50} value, the concentration of test substance that would reduce shell deposition by 50% relative to the pooled control, and 95% confidence limits were calculated using linear interpolation.

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Results Synopsis:

96 hr

EC_{50} (µg/L):	64 µg/L
95% CI:	40 - 105 µg/L
NOEC (µg/L):	31 µg/L
No Mortality Concentration:	125 µg/L

13. REVIEWER'S COMMENTS:

Guideline deviations are presented in Section 9.