6-3-04

DP Barcode: 299969 MRID No: 460626-28

DATA EVALUATION RECORD
MYSID ACUTE TOXICITY TEST
GUIDELINE OPPTS 850.1035/OPP§72-3

1. **CHEMICAL:** PXTS **PC Code No.:** 006929

2. **TEST MATERIAL:** PXTS TECHNICAL **Purity:** 100%
Batch No. 1685-23, Bottle #2
EPA File Symbol 75799-R

3. **CITATION**

Author: Susan Palmer, Raymond Van Hoven, Henry Krueger
Title: PXTS: A 96-Hour Flow-Through Acute Toxicity Test With the Saltwater Mysid, *Mysidopsis bahia*
Study Completion Date: January 31, 2003
Laboratory: Wildlife International, LTD.
8598 Commerce Drive
Easton, MD 21601
Sponsor: Akzo Nobel Functional Chemicals LLC
5 Livingstone Ave.
Dobbs Ferry, NY 10522
Laboratory Report ID: 497A-110A
MRID No.: 460626-28

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

Signature: Srinivas Gowda **Date:** 05-13-04

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

Signature: [signature] **Date:** 6/3/04

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Mysidopsis bahia*
Age of Test Organism: < 24 hours
Definitive Test Duration: 96 hours
Study Method: Flow-through
Type of Concentrations: Nominal and Mean measured

7. **CONCLUSIONS:**

Results Synopsis:

	Reported (Nominal)	Verified (Nominal)	Verified (Mean Measured)
96-hour			
LC_{50} (µg ai/L):	>125	>125	>52.3
95% CI:	NA	NA	NA
NOEC (µg ai/L):	125	125	52.3

The submitted flow-through acute saltwater Mycid toxicity study is scientifically sound and provides useful information for risk assessment. Based on nominal concentrations, the 96-hour LC_{50} was >125 µg ai/L. NOEC was 125 µg ai/L. The study can be classified as supplemental for a technical grade active ingredient because it failed to establish a valid LC_{50} value for *Mysidopsis bahia.* The study could be upgraded to core category if the study is repeated and established a valid LC_{50} value for *Mysidopsis bahia.*

8. **ADEQUACY OF THE STUDY**

A. Classification: Supplemental.

B. Rationale: This study did not determine an LC_{50} value. A range finding test was not conducted to establish test solution concentrations for the definitive test.

C. Repairability: This study may be upgraded to core if the registrant submits a valid range finding study for *Mysidopsis bahia* and provides additional description of good faith efforts taken to solubilize PXTS.

9. **GUIDELINE DEVIATIONS:**

The following guideline deviations were based on EPA OPPTS Guideline 850.1035:

- During the holding period and the exposure period, the study used a photoperiod of 16 hours light and 8 hours dark instead of the guideline recommended photoperiod of 14 hours light and 10 hours dark.
- Preliminary testing was not reported in the Study Report.
- Concentration-response curves were not provided in the Study Report.
- The LC50 and NOEC values provided in the Study Report are based on nominal concentrations, not the mean measured concentrations.
- The flow rate was not reported.

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species • Mysids (*Mysidopsis bahia*; now *Americamysis*)	• *Mysidopsis bahia* (p.10)
Life Stage/Size • Mysids used in a particular test should be of similar age and be of normal size and appearance for their age. • The definitive test should be conducted on the mysid life stage (juveniles or young adults) which is most sensitive to the test substance being evaluated.	• Mysids used in test were juveniles (<24 hours old) (p.10)
Acquisition • Mysids should originate from laboratory cultures in order to ensure the individuals are of similar age and experimental history. • Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. • Taxonomic verification should be obtained from the commercial supplier by experienced laboratory personnel or by an outside expert.	• Mysid cultures maintained by Wildlife International Ltd. (p.10) • Supplier not reported • Identification of species verified by supplier of original culture stock (p.10)
Acclimation • Any change in the temperature and chemistry of the dilution water used for holding or culturing the test organisms to those of the test should be gradual. • Within a 24–h period, changes in water temperature should not exceed 1°C, while salinity changes should not exceed 5 percent. • During acclimation mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas.	• Mysids held in water from same source and at approximately same temperature as during test (p.10) • During holding period prior to test, temperature ranged from 24.9-25.8°C • Salinity measured 20-22‰ (p.11)

B. Test System

Guideline Criteria	Reported Information
Test Chamber • Materials and equipment that contact test solutions should be chosen to minimize sorption of test chemicals from dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect test results. • Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.	• Test chambers were 25 L Teflon®-lined stainless steel aquaria filled with approximately 22 L of test water (p.12) • Nylon screens were attached to holes on opposite sides of test compartments to allow for flow of water through the compartments (p.12)
Temperature • The test temperature should be 25°C. • Excursions from the test temperature should be not greater than –2°C.	• Temperature measurements taken manually ranged from 24.5 to 24.6°C (p.20). • Temperature measured continuously throughout study ranged from 24.0 to 25°C (p.20)
Salinity • Salinity of 20 ± 3 ppt.	• Average salinity measurement: 20‰ (p.20)
Dissolved Oxygen • Dissolved oxygen concentration between 60 and 105% saturation. • Aeration, if needed to achieve this level, should be done before the addition of the test substance. All treatment and control chambers should be given the same aeration treatment.	• Dissolved oxygen remained above 75% saturation throughout the test (p.20) • Range: 6.6 to 7.4 mg/L (p.20)
Photoperiod • Photoperiod of 14 hours light and 10 hours dark, with a 15 to 30 min transition period.	• 16 hour light/8 hours darkness with a 30 minute transition period of low light intensity (p.15)
pH • Measured in each test chamber at the beginning and end of test.	• The pH was measured in alternating test chambers of each treatment and control group at the beginning and end of test and at 24 hour intervals. (p.15) • Average measurement: 8.2 (8.1-8.2) (p.23)

Guideline Criteria	Reported Information
Feeding • Mysids should be fed daily during testing. Any food utilized should support survival, growth, and reproduction of the mysids. A recommended food is live Artemia spp. (48–h–old nauplii).	• Fed live brine shrimp (*Artemia* sp.) nauplii daily occasionally enriched with ALGAMAC-2000 to prevent cannibalism. (p.11)
Dilution Water • Natural seawater or artificial seawater is acceptable as dilution water if mysids will survive and successfully reproduce in it for the duration of the holding, acclimating, and testing periods without showing signs of stress. • Mysids should be cultured and tested in dilution water from the same origin. • Natural seawater should be filtered through a filter with a pore size of <20 mm prior to use in a test. • Artificial seawater can be prepared by adding commercially available formulations or specific amounts of reagent-grade chemicals to deionized water.	• The dilution water was natural seawater collected at Indian River Inlet, Delaware that was filtered and diluted to salinity of 20‰ with well water. (p.11) • The seawater was passed through sand filter to remove particles >25 μm. (p.11) • The 20 ‰ water was filtered to 0.45 μm to remove microorganisms and fine particles prior to use in the test. (p.11)
Carriers • Use of carriers should be avoided, if possible, as they may serve as a carbon source for bacteria. • If solvents, solubilizing agents, or emulsifiers have to be used, they should be commonly used carriers and should not possess a synergistic or antagonistic effect on the toxicity of the test substance. Preferred carriers are dimethyl formamide, triethylene glycol, acetone, or ethanol. • Concentration of carriers should not exceed 0.1 mL/L	• Stock solution and test solutions prepared in acetone at a concentration of 0.1 mL/L. (p.12)

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • Range finding test should be conducted to determine (1) which life stage (juvenile or young adult) is to be utilized in the definite test; and (2) the test solution concentrations for the definite test. • The mysids should be exposed to a series of widely spaced concentrations of test substance (e.g. 1, 10, 100 mg/L, etc.), usually under static conditions. • A minimum of 10 mysids for each age class (juvenile or young adult) should be exposed to each concentration of test substance for up to 96 hours. The age class which is most sensitive to the test substance in the range-finding test should be utilized in the definitive test.	• Range finding test not mentioned
Dose Range • Dilution factor between concentration should be chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g. 2, 4, 8, 16, 32, and 64 mg/L).	• Dilution factor ratio ~2 (p.14)
Doses • At least 5 test concentrations should be used.	• Five concentrations chosen: 7.8, 16, 31, 63, and 125 µg/L (nominal concentrations) (p.20)
Controls • Every test should include controls consisting of the same dilution water, conditions, and procedures, and mysids from the same population or culture container, except that none of the test chemical is added.	• Solvent and negative controls included (p.12)
Replicates Per Dose • An equal number of mysids are introduced into the test and control chambers by stratified random assignment and should be placed in two or more replicates.	• Mysids collected from culture and placed in transfer chambers one or two at a time for a total of 10 mysids per container (p.11) • Transfer chambers then placed into test chambers indiscriminately (p.11) • Two replicates per test concentrations for total of 20 mysids per dose (p.20).

Guideline Criteria	Reported Information
Number and Placement of Organisms: • A minimum of 20 mysids per concentration. • Impartially distributed among test chambers. • Test chambers within the testing area should be positioned in a random manner. • An equal number of mysids should be introduced into the test and control chambers.	• Two replicate chambers per dose and 10 mysids per replicate for a total of 20 mysids per dose (p.20)
Duration of Test • 96 hours	• 96 hours (p.8)
Endpoints • mortality	• Mortality (p.8)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	• Yes (p.3 and 4)
The nature of the test, laboratory, name of the investigator, test substance, and dates of test reported?	• Yes (cover page)
Control • **Mortality should not exceed 10% at end of test**	• Yes–no mortality in controls (p.21)
Detailed description of the test substances (e.g. the source, lot number, composition, physical and chemical properties, shelf life and storage conditions, and any carrier or additives used)?	• Yes (p.10)
Detailed information about the shrimp (e.g. common and scientific names, source of supply, age, history, weight, acclimation procedure, and feeding history)?	• Yes (p.10)
A description of the experimental design including the number of test solution concentrations, number of replicates, and number of shrimp per replicate?	• Yes (p.11, 12, 21)

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Since none of the test concentrations resulted in mortality, the LC50 and NOEC values were empirically estimated to be greater than the highest test concentration.

Time	LC_{50} (µg/L) Nominal concentration	LC_{50} (µg/L) Mean Measured concentration	95% Confidence Interval	NOEC (µg/L) Nominal concentration	NOEC (µg/L) Mean Measured concentration
24-hour	>125	>52.3	NA	125	52.3
48-hour	>125	>52.3	NA	125	52.3
72-hour	>125	>52.3	NA	125	52.3
96-hour	>125	>52.3	NA	125	52.3

[1] Values based on the mean of the three replicates at nominal 125 µg/L. One replicate was reported to be <LOD; therefore, ½ the LOD value was used to calculate this value.

14. REVIEWER'S COMMENTS:

- Guideline deviations are presented in Section 9.

Guideline Criteria	Reported Information
The source of the dilution water, its chemical characteristics (e.g. salinity), and a description of any pretreatment?	• Yes (p.11 and 24)
A description of the test chambers, the depth and volume of solution in the chamber, the number of organisms per treatment, the number of replicates, the loading, the lighting, the test substance delivery system and flow rate expressed as volume additions per 24 h?	• Yes (p.11-12) • Flow rate not provided
The concentration of the test substance in each test chamber before the start of the test and at the end?	• Yes (p.19)
Number of dead shrimp and measurements of water temperature, salinity, and dissolved oxygen concentration in each test chamber recorded at the protocol-designated times?	• Yes (p.20-23)
Methods and data records of all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks?	• Yes (p.13-15)
Recorded data for the holding and acclimation period (temperature, salinity, etc.)?	• Yes (p.10-11)
Concentration-response curves should be fitted to mortality data collected at 24, 48, 72, and 96 h. A statistical test of goodness-of-fit performed?	• No
For each set of mortality data, the 48– and 96–h LC_{50} and 95 percent confidence limits calculated on the basis of the average measured concentration of the test substance. When data permits, LC_{50} values with 95 percent confidence limits should be computed for 24– and 72–h observations. The NOEC and slope of the dose-response curves calculated?	• 24, 48, 72, and 96 hour LC50 provided (no confidence limits) (p.22) • Based on nominal concentrations
The methods used in calculating the concentration-response curves and the LC_{50} values should be fully described?	• Yes (p.16)

Dose Response

Nominal Concentration (µg/L)	24-hour Cumulative Mortality (%)		48-hour Cumulative Mortality (%)		72-hour Cumulative Mortality (%)		96-hour Cumulative Mortality (%)	
	Replicate A	Replicate B	Replicate A	Replicate B	Replicate A	Replicate B	Replicate A	Replicate B
Control	0	0	0	0	0	0	0	0
Solvent Control	0	0	0	0	0	0	0	0
7.8	0	0	10	0	10	0	10	0
16	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0
125	0	0	0	0	0	0	0	0

Statistical Results

Statistical Method: The Study Report states that the absence of mortality precluded the statistical calculation of an LC50. Therefore, the LC50 values reported were estimated to be greater than the highest test concentration. The LC50s and the NOEC were determined by visual interpretation of the mortality and observation data.

Time	LC_{50} (µg/L)[a]	95% Confidence Interval	NOEC (µg/L)
24-hour	>125	NA	125
48-hour	>125	NA	125
72-hour	>125	NA	125
96-hour	>125	NA	125

a) LC_{50} value was empirically estimated to be greater than the highest mean measured concentration tested.
NA: Confidence intervals could not be calculated from data