

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD
ACUTE LC₅₀ TEST WITH AN ESTUARINE/MARINE ORGANISM
§72-3(C) - SHRIMP

1. **CHEMICAL**: Propazine PC Code No.: 080808

2. **TEST MATERIAL**: Propazine Purity: 98%

3. **CITATION**:

Author: Boeri, R. L., P. L. Kowalski and T. J. Ward

Title: Static Acute Toxicity of Propazine To The Mysid
(*Mysidopsis bahia*)

Study Completion Date: July 12, 1995

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

Sponsor: Griffin Corporation
P.O. Box 1847
Rocky Ford Road
Valdosta, Georgia 31603-1847

Laboratory Report ID: 571-AB

MRID No.: 441848-01

DP Barcode: D237791

4. **REVIEWED BY**: John Marton, Staff Scientist, Dynamac Corporation

Signature: **Date:** 8/29/2005

APPROVED BY: Teri S. Myers, Staff Scientist, Dynamac Corporation

Signature: **Date:** 8/30/2005

5. **APPROVED BY**: Anita Pease, OPP/EFED/ERB-III

Signature: **Date:**

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Mysidopsis bahia*

Age or Size of Test Organism: <24 hours

Definitive Test Duration: 96 hours

Study Method: Static

Type of Concentration: Mean-measured

7. CONCLUSIONS:

The 96-hour acute toxicity of Propazine to the saltwater mysid, *Mysidopsis bahia*, was studied under static conditions. Mysids (<24 hours old) were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 0.65, 1.1, 1.8, 3.0 and 5.0 ppm a.i.; mean measured concentrations were <0.167 (<LOQ; controls), 0.586, 1.04, 1.71, 2.98 and 4.77 ppm a.i., and recoveries were 90-99% of nominal. During the 96-hour test, mortality was 0% in both the negative and solvent controls and in the mean-measured 0.586 ppm a.i. treatment level. Mortality was 15, 30, 30 and 55% in the mean-measured 1.04, 1.71, 2.98 and 4.77 ppm a.i. treatment levels, respectively, by 96 hours. The **96-hour LC₅₀ value was 4.20 ppm a.i.**, which categorizes Propazine as **moderately toxic** to the saltwater mysid, *Mysidopsis bahia*, on an acute toxicity basis. From 24- to 72-hours, mysids in the mean-measured 1.71-4.77 ppm a.i. treatment levels were observed swimming erratically, however by 96-hours, all surviving mysids appeared to be swimming normally. At test termination, 100% of the surviving mysids in the mean-measured 4.77 ppm a.i. treatment level were visually smaller than the control organisms; no other sub-lethal effects were observed at 96-hours. Based on mortality and sub-lethal effects, the **NOEC and LOEC values were 0.586 and 1.04 ppm a.i.**, respectively.

This study is scientifically sound and fulfills the requirements of an acute LC₅₀ test with an estuarine/marine organism (Subdivision E, §72-3(C) [mysid]) and is classified as **Acceptable**. The study does provide information that may be useful for future risk-assessment purposes.

Results Synopsis

96-Hour:

LC₅₀: 4.20 ppm a.i. 95% C.I.: 2.94-9.11 ppm a.i.

Probit slope: 2.0 95% C.I.: 1.0-3.0 ppm a.i.

NOEC: 0.586 ppm a.i.

LOEC: 1.04 ppm a.i.

Endpoints affected: Mortality and sub-lethal effects

8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable

B. Rationale: This study is scientifically sound and fulfills the requirements of an acute LC₅₀ test with an estuarine/marine organism (Subdivision E, §72-3(C) [mysid]).

C. Repairability: N/A

9. BACKGROUND:

10. GUIDELINE DEVIATIONS:

1. It was not reported if all test mysids were from the same year class.
2. The reported pH (7.7-8.1) ranged slightly higher than recommended (7.7-8.0).
3. The percent saturation of the dissolved oxygen concentration in the dilution water during the definitive test was not reported.
4. The TOC in the dilution water was not reported.
5. The size and fill volume of the test chambers (2 L and 1 L, respectively) were smaller than recommended for the test organism (3.9 L and 2-3 L, respectively).

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of Propazine to mysids for the purpose of chemical registration.

12. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
<p><u>Species</u> Preferred species are <i>Americamysis bahia</i>, <i>Penaeus setiferus</i>, <i>P. duorarun</i>, <i>P. aztecus</i> and <i>Palaemonetes sp.</i></p>	<p><i>Mysidopsis bahia</i> (lot #1205941)</p>
<p><u>Age</u> Juvenile (\leq 24 hours old) mysids should be used</p>	<p><24 hours (mean wet weight- 0.37 mg; mean length-2.6 mm, based on control mysids at test termination)</p>
<p><u>Supplier</u></p>	<p>Juveniles were collected from in-house laboratory cultures. Original culture was obtained from Aquatic Biosystems Inc., Fort Collins, Colorado</p>
<p>All shrimp are from same</p>	<p>Yes</p>
<p>All shrimp are from the same year class?</p>	<p>Not reported</p>

B. Source/Acclimation

Guideline Criteria	Reported Information
<p><u>Acclimation Period</u> Minimum 10 days</p>	<p>Continuous</p>
<p>Wild caught organisms were quarantined for 7 days?</p>	<p>N/A</p>
<p>Were there signs of disease or injury?</p>	<p>During the holding period, the adults showed no signs of sickness, injuries or abnormalitites.</p>
<p>If treated for disease, was there no sign of the disease remaining during the 48</p>	<p>N/A</p>

Guideline Criteria	Reported Information
hours prior to testing?	
<p>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.</p>	Live brine shrimp (<i>Artemia salina</i> nauplii; lot BS02) were provided daily during acclimation and testing to prevent cannibalism.
<p>Pretest Mortality <3% mortality 48 hours prior to testing</p>	0%

C. Test System

Guideline Criteria	Reported Information
<p>Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water</p>	The dilution water was natural filtered seawater collected from T. R. Wilbury Laboratories, Marblehead, Massachusetts. Dilution water was stored in polyethylene tanks where it was aerated and recirculated through particle filters, activated carbon and an ultraviolet sterilizer.
<p>Does water support test animals without observable signs of stress?</p>	Yes
<p>Salinity 30-34 ‰ (parts per thousand) for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range <6 ‰</p>	16‰
<p>Water Temperature Approx. 22 ± 1 °C</p>	21.5-22.9°C

Guideline Criteria	Reported Information
<p>pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8</p>	7.7-8.1
<p><u>Dissolved Oxygen</u> Between 60 and 105% saturation. If needed, aerate prior to introduction of chemical.</p>	6.3-7.8 mg/L (% saturation was not reported)
<p><u>Total Organic Carbon</u> Should be <5 mg/L in reconstituted seawater</p>	Not reported
<p><u>Test Aquaria</u> 1. <u>Material</u>: Glass or stainless steel 2. <u>Size</u>: 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. <u>Fill volume</u>: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.</p>	Test chambers were 2 L glass aquaria filled with approximately 1 L of test solution (depth: ~4 cm). Test vessels were randomly arranged within a water bath during the definitive exposure period.
<p><u>Type of Dilution System</u> Must provide reproducible supply of toxicant</p>	N/A; Test was conducted under static conditions
<p><u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period</p>	N/A; Test was conducted under static conditions
<p><u>Biomass Loading Rate</u> 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)</p>	0.004 g/L

Guideline Criteria	Reported Information
<p><u>Photoperiod</u> 16 hours light, 8 hours dark</p>	16 hours light, 8 hours dark, with a 15-minute transition period. Light intensity was 40 footcandles.
<p><u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests</p>	Dimethylformamide, 0.1 mL/L

D. Test Design

Guideline Criteria	Reported Information
<p><u>Range Finding Test</u> If LC₅₀ >100 mg/L with 30 shrimp, then no definitive test is required.</p>	<p>Nominal concentrations were based on the 96-hour survival from a static renewal screening test which was performed for 18-days from November 14 to December 2, 1994 which was conducted to determine the approximate chronic toxicity levels. Nominal concentrations were 0 (negative and solvent controls), 0.01, 0.1, 0.5, 1 and 5 ppm a.i. After 96-hours, survival was at least 95% in the controls and 0.0-0.5 ppm a.i. treatment levels, 70% survival at the 1 ppm a.i. treatment level and 15% survival at the 5 ppm a.i. treatment level. One of the fourteen surviving mysids at the 1 ppm a.i. treatment level and all surviving mysids at the 5 ppm a.i. treatment level exhibited erratic swimming after 96-hours of exposure.</p>
<p><u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.</p>	0 (negative and solvent controls), 0.65, 1.1, 1.8, 3.0 and 5.0 ppm a.i.
<p><u>Number of Test Organisms</u></p>	

Guideline Criteria	Reported Information
Minimum 20/level, may be divided among containers	20 mysids/level, divided into two replicates of 10 mysids each for both controls and all treatment levels.
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
<p><u>Water Parameter Measurements</u></p> <p>1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary >1°C</p> <p>2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control</p>	<p>1. Measured at test initiation every 24-hours thereafter in every test vessel that contained live mysids. Temperature was also recorded in one test vessel at least every 6 hours.</p> <p>2. DO, pH and salinity were measured daily in each test vessel containing live mysids.</p>
<p><u>Chemical Analysis</u> 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 system was used</p>	<p>Samples were collected from each test vessel at 0- and 96-hours. Samples were collected in duplicate, then pooled prior to shipping for analysis. Each batch of samples was accompanied by fortified QC samples at 0.65, 1.8 and 5.0 ppm a.i. as well as a sample of the stock solution (50,000 ppm a.i.). Samples were shipped to ABC Laboratories, Columbia, Missouri for analysis.</p>

13. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements were included in the report?</p>	<p>Yes</p>
<p><u>Recovery of Chemical</u></p>	<p>Percent recovery of the mean-measured concentrations ranged from 90-99% of nominal. The primary stock solution (50,000 ppm a.i.) analyzed from Day 0 had a recovery of 96%; QC fortifications (0.65, 1.8 and 5.0 ppm a.i.) had recoveries of 99-105%; and the laboratory spikes (0.625 and 5.63 ppm a.i.) had recoveries of 101%.</p>
<p><u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.</p>	<p>Mortality was 0% for both the negative and solvent controls after 96-hours.</p>
<p>Raw data included?</p>	<p>Yes</p>
<p>Signs of toxicity (if any) were described?</p>	<p>Yes</p>

Mortality

Concentration (ppm a.i.)		Number of Shrimp	Mean Cumulative Mortality (%)			
Nominal	Mean Measured		Hours of Study			
			24	48	72	96
Negative Control	<LOQ	20	0	0	0	0
Solvent Control	<LOQ	20	0	0	0	0
0.65	0.586	20	0	0	0	0
1.1	1.04	20	5	5	10	15
1.8	1.71	20	10	20	30	30
3.0	2.98	20	5	25	25	30
5.0	4.77	20	30	50	50	55

LOQ = 0.167 ppm a.i.

During the 96-hour test, mortality was 0% in both the negative and solvent controls and in the mean-measured 0.586 ppm a.i. treatment level. Mortality was 15, 30, 30 and 55% in the mean-measured 1.04, 1.71, 2.98 and 4.77 ppm a.i. treatment levels, respectively, by 96 hours.

Sub-lethal Effects:

Treatment, ppm a.i., 96 Hour Mean- Measured and (Nominal Conc.)	Number of Shrimp	Observation Period			
		Endpoint at 24- Hours	Endpoint at 48- Hours	Endpoint at 72- Hours	Endpoint at 96- Hours
		% Affected ^a	% Affected	% Affected	% Affected
Negative Control	20	AN ^b	AN	AN	AN
Solvent Control	20	AN	AN	AN	AN
0.586 (0.65)	20	AN	AN	AN	AN
1.04 (1.1)	20	AN	AN	AN	AN
1.71 (1.8)	20	11%-erratic swimming	13%-erratic swimming	AN	AN
2.98 (3.0)	20	16%-erratic swimming	20%-erratic swimming	20%-erratic swimming	AN
4.77 (5.0)	20	50%-erratic swimming	20%-erratic swimming	10%-erratic swimming	100%-visually smaller than control mysids

^a % Affected = (Number of Affected Mysids/Number of Surviving Mysids) x 100.

^b AN = Appears Normal.

B. Statistical Results

Statistical Method(s): The 96-hour LC₅₀ was determined using the probit method via the computer program of C.E. Stephan (1983). The NOEC and LOEC values were determined by visual interpretation of the mortality and observation data. All toxicity values were determined using the 0-96 hour mean-measured concentrations.

96-Hour:

LC₅₀: 4.20 ppm a.i. 95% C.I.: 2.94->4.77 ppm a.i.

Slope: 2.0

NOEC: 0.586 ppm a.i.

LOEC: 1.04 ppm a.i.

Endpoints affected: Mortality and sub-lethal effects

14. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Negative and solvent control data were pooled for all statistical analyses since mortality (0%) was identical in both control groups. The 96-hour LC₅₀ and respective 95% confidence interval based on mortality were determined using the Probit method via Toxanal statistical software. The NOEC and LOEC based on mortality and sub-lethal effects were determined by visual interpretation. All toxicity values were determined in terms of the 0-96 hour mean-measured concentrations.

96-Hour:

LC₅₀: 4.20 ppm a.i. 95% C.I.: 2.94-9.11 ppm a.i.

Probit slope: 2.0 95% C.I.: 1.0-3.0 ppm a.i.

NOEC: 0.586 ppm a.i.

LOEC: 1.04 ppm a.i.

Endpoints affected: Mortality and sub-lethal effects

15. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study authors, except the reviewer was able to provide 95% confidence intervals associated with the probit slope. Consequently, the study authors' reported LC₅₀ value and associated 95% confidence interval is reported in the Conclusion section of this DER.

The reported solubility of the test material was approximately 8.6 ppm a.i.

The study was conducted using estuarine salinity (16 ‰). If salinity were to be found to

affect the activity of Propazine, a study reflecting marine salinity (30-35 ‰) would be necessary to address the salinity difference between marine and estuarine habitats. The periodic screening analysis of the dilution water showed that contaminants were detected at levels that prove to negatively affect mysids in acute exposure.

Routine biannual dilution water analyses for pesticides, PCBs and metals were conducted according to standard EPA procedures by Pace, Incorporated, Hampton, New Hampshire. These analyses were not conducted strictly according to GLPs as no distinct protocol or study director was identified. The stability of the test substance under exposure conditions was assumed but not verified.

Based on the LC₅₀ value (4.20 ppm a.i.), Propazine is categorized as moderately toxic to juvenile (<24 hours old) saltwater mysids (*Mysidopsis bahia*) on an acute toxicity basis.

This study was conducted in accordance with USEPA (40 CFR Part 160) Good Laboratory Practice Regulations. Quality Assurance and No Data Confidentiality Statements were included.

16. REFERENCES

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

U.S. EPA. 1985. *Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Estuarine and Marine Organisms (Shrimp 96-Hour Acute Toxicity Test)*. Hazard Evaluation Division. Office of Pesticide Programs, EPA-540/9-85-010.

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

U.S. EPA. 1992. 40 CFR Part 160. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards. Final Rule.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
4.77	20	11	55	41.19014
2.98	20	6	30	5.765915
1.71	20	6	30	5.765915
1.04	20	3	15	.1288414
.586	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.04 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.349579

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
1	1.581135	4.349579	0 +INFINITY

RESULTS CALCULATED USING THE **PROBIT METHOD**

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2452205	1	.4581812

SLOPE = 2.012094
95 PERCENT CONFIDENCE LIMITS = 1.01571 AND 3.008478

LC50 = 4.197979
95 PERCENT CONFIDENCE LIMITS = 2.935083 AND 9.110914

LC10 = .9814252
95 PERCENT CONFIDENCE LIMITS = .3922337 AND 1.449241