DATA EVALUATION RECORD
§ 72-2 -- ACUTE LC_{50} TEST WITH A FRESHWATER INVERTEBRATE

1. **CHEMICAL:** 2-chloro-4,6 bis (isopropylamino)-s-trizine
PC Code No.: 080808

2. **TEST MATERIAL:** Propazine Purity: 98.0%

3. **CITATION**

Authors: H. R. Murrell; J. L. Veltri
Title: Acute toxicity of propazine to *Daphnia magna*
Study Completion Date: 11/28/94
Laboratory: ABC Laboratories
Sponsor: Griffin Corporation
Laboratory Report ID: 41954
MRID No.: 442873-05
DP Barcode: D237791

4. **REVIEWED BY:** Thomas M. Steeger, Ph.D., Fishery Biologist, EFED, ERB IV, U.S. EPA

Signature: Thomas M Steeger **Date:** 10/2/97

5. **APPROVED BY:** Ann Stavola, Aquatic Biologist, EFED, ERB IV, U.S. EPA

Signature: Ann Stavola **Date:** 10/15/98

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Daphnia magna*
Age of Test Organism: first instar daphnids (<24 hr)
Definitive Test Duration: 48 hours
Study Method: Static
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study was scientifically sound; however, it did not fulfill the 72-2 guideline requirements for an acute LC_{50} test with freshwater invertebrates. The 96-hr LC_{50} for Propazine was estimated to be greater than the highest dose tested, i.e., 5.32 mg a.i./L, under the conditions tested, and the no-observed effect concentration was >5.32 mg a.i./L. Both pH and hardness exceeded the recommended guidelines. Water solubilities and the adsorption process to organic matter can be affected by pH, thus it is important to adhere to the recommended guidelines regarding the ranges for these values. This study is classified as supplemental;

based on the results of this study, propazine is classified as moderately toxic to freshwater invertebrates.

Results Synopsis

LC_{50}: >5.32 ppm ai 95% C.I.: ____-____ ppm ai
NOEL:>5.32 ppm ai Probit Slope: ____

8. **ADEQUACY OF THE STUDY**

A. Classification: supplemental

B. Rationale: pH and hardness exceeded guideline requirements

C. Repairability: Upgradeable to core provided the registrant demonstrates that both hardness and pH have no effect on the toxicity of Propazine to Daphnids. Additionally, registrant must provide an explanation as to why higher concentrations of propazine were not tested.

9. **Guideline Deviations**

1. pH (8.1 - 8.4) exceeded recommended range 7.2 - 7.6
2. Hardness (150 mg/L as $CaCO_3$) exceeded recommended range of 40 - 48.

10. **SUBMISSION PURPOSE:** Determine the acute toxicity of Propazine to *Daphnia magna*

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is *Daphnia magna*	*Daphnia magna*
All organisms are approximately the same size and weight?	Yes

Guideline Criteria	Reported Information
Life Stage Daphnids: 1st instar (<24 h). Amphipods, stoneflies, and mayflies: 2nd instar. Midges: 2nd & 3th instar.	1st instar
Supplier	in-house (ABC Laboratories) culture
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	Since the culturing and testing parmeters of temperature, dilution water, and lighting were the same, no acclimation period was necessary.
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
Feeding No feeding during the study.	Not fed during 48-hr study
Pretest Mortality No more than 3% mortality 48 hours prior to testing.	0% mortality 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.	Blended water representing a combination of well water and reverse osmosis water to achieve final hardness 130-160 mg/L.
Does water support test animals without observable signs of stress?	Yes
Water Temperature Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20 ± 1 °C
pH Prefer 7.2 to 7.6.	8.1 - 8.4
Dissolved Oxygen Static: ≥ 60% during 1st 48 h and ≥ 40% during 2nd 48 h, flow-through: ≥ 60%.	7.8 mg/L (91% saturation at 20°C) on Day 0)
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$.	150 mg/L as $CaCO_3$
Test Aquaria 1. Material: Glass or stainless steel. 2. Size: 250 ml (daphnids and midges) or 3.9 L (1 gal). 3. Fill volume: 200 ml (daphnids and midges) or 2-3 L.	1. Glass 2. 250 ml 3. 200 ml
Type of Dilution System Must provide reproducible supply of toxicant.	N/A

Guideline Criteria	Reported Information
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.	1 daphnid/25 ml
Photoperiod 16 hours light, 8 hours dark.	16 hours light
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	Dimethylformamide 0.1 ml/L

D. **Test Design:**

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L, then no definitive test is required.	Yes (0.01, 0.1, 1.0, and 5.0 mg/L)
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	Control, vehicle control, 0.33, 0.65, 1.3, 2.5, and 5.0 mg/L
Number of Test Organisms Minimum 20/level, may be divided among containers.	(10/replicate)(2 replicates/treatment) = 20 organisms/treatment
Test organisms randomly or impartially assigned to test vessels?	Yes

Water Parameter Measurements 1. Temperature Measured continuously or, if water baths are used, every 6 h, 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. Temperature in water bath measured and recorded continuously. 2. DO and pH measured at 0 and 48 hrs.
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 system was used	yes

12. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Static: ≤10% Flow-through: ≤10%	0%
Percent Recovery of Chemical	102 ± 2.5%
Raw data included?	Yes

Mortality

Concentration (ppm)		Number of Organ-isms	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	--	20	0	0	--	--

Concentration (ppm)		Number of Organ-isms	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Solvent Control	--	20	0	0	--	--
0.33	0.325	20	0	0	--	--
0.65	0.556	20	0	0	--	--
1.3	1.07	20	0	0	--	--
2.5	2.15	20	0	0	--	--
5.0	5.32	20	0	0	--	--

Other Significant Results:

B. Statistical Results

Method: No statistics run

48-hr LC_{50}: >5.32 ppm ai 95% C.I.: ____-____ ppm ai

Probit Slope: ____ NOEC: 5.32 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	NA____ (____-____) ppm ai
Moving Average Angle LC_{50} (95% C.I.)	NA____ (____-____) ppm ai
Probit LC_{50} (95% C.I.)	NA____ (____-____) ppm ai
Probit Slope	NA
NOEC	>5.32 ppm ai

14. REVIEWER'S COMMENTS:

The study failed to establish an LC50. Preliminary tests revealed no adverse effects up to 5 mg/L; however, the registrant only tested up to 5 mg/L instead of 100 mg/l. If no adverse effects were noted at 100 mg/L, the study would have established that propazine was practically nontoxic to daphnids. At present,

propazine could be moderately toxic to daphnids.

Hardness 150 mg/L exceeded the preferred range (40 - 48 mg/L); pH (8.1 - 8.4) exceeded the preferred range (7.2 - 7.6). In Methods for Acute Toxicity test with Fish, Macroinvertebrates and Amphibians (EPA 1975) it states that whenever possible the soft reconstituted fresh water should be used for test with freshwater animals. The guideline defines hardness of 40 to 48 ppm and a pH range of 7.2 to 7.6. The ASTM (1980) states that soft reconstituted fresh water should be used whenever possible when testing freshwater animals.

Water solubilities are determined by the pH level, with triazines being more soluble at low pH levels. Adsorption of triazines through an exchange process to organic matter is also dependent on pH (Menzer 1991). Thus, the recommended guidelines for both hardness and pH should be followed to facilitate comparison with previous studies.

References

ASTM 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. ASTM Committee on Standards, Philadelphia, E 729-80.

EPA 1975. Methods for acute toxicity test with fish, macroinvertebrates and amphibians. Committee on methods for toxicity tests with aquatic organisms. Ecol. Res. Series, EPA 660/375-009.