

DATA EVALUATION RECORD

§ 72-1(A) -- ACUTE LC₅₀ TEST WITH A WARMWATER FISH

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

2. TEST MATERIAL: Propazine Purity: 98.2%

3. CITATION

Authors: J. Bowman; J. Veltri
Title: Acute toxicity of Propazine to bluegill
Study Completion Date: 6/26/95
Laboratory: ABC Laboratories, Inc.
Sponsor: Griffin Corporation
Laboratory Report ID: ABC Labs # 41953
MRID No.: 442873-04
DP Barcode: D237791

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

Signature: *Thomas M Steeger*

Date: 10/7/97

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: *Lepomis macrochirus*
Age or Size of Test Organism: Young of year
Definitive Test Duration: 96 hrs
Study Method: Static
Type of Concentrations: Mean measured

7. CONCLUSIONS: This study was not scientifically sound and did not fulfill the 72-1(A) guideline requirements for an acute LC₅₀ test in warmwater fish. Although the 96-hr LC₅₀ was estimated to have exceeded the maximum dose (4.5 mg a.i./L), precipitate in aquaria receiving higher doses raises the question of solubility and whether Propazine was completely dissolved at the higher doses tested. Erratic swimming behavior in fish treated with 1.0 and 1.8 mg/L may represent a compound-related affect in treatments where Propazine was freely soluble. Thus, the reported NOEL of > 4.5 mg a.i./L may greatly overestimate this value; behavioral effects may dictate that the NOEL be adjusted downward to 0.065 mg a.i./L.



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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.

Results SynopsisLC₅₀: >4.5 ppm ai

95% C.I.: _____ - _____ ppm ai

NOEL: >4.5 ppm ai

Probit Slope: _____

8. ADEQUACY OF THE STUDY**A. Classification: INVALID****B. Rationale:**

Precipitate in higher treatment groups suggests that the compound was not in solution. Water analysis revealed that mean measured concentrations were similar to nominal levels; however the analysis relied on extraction of Propazine with toluene. The limited solubility of the compound as evidenced through precipitate in the higher treatment levels raises a question of actual exposure, i.e., bioavailability. Conventional centrifugation is required for all test media where precipitate is observed in the test chambers. Thus it is unknown whether the fish were exposed to the nominal and/or mean-measured concentrations.

Hardness and pH exceeded the guideline ranges. 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 acceptable ranges for these values.

C. Repairability: none**9. GUIDELINE DEVIATIONS**

1. Total hardness 130 - 160 mg/L (desired: 40 - 48 mg/L)
2. Temperature dropped to 19°C
3. pH (8.2 - 8.5) exceeded recommended range (7.2 - 7.6)

10. SUBMISSION PURPOSE:**11. MATERIALS AND METHODS****A. Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is the bluegill sunfish (<i>Lepomis macrochirus</i>)	<i>Lepomis macrochirus</i>
<u>Mean Weight</u> 0.5-5 g	0.14 ± 0.06
<u>Mean Standard Length</u> Longest not > 2x shortest	Mean: 19 ± 2 mm Range:
<u>Supplier</u>	Osage Catfisheries, Inc.
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 14 days	14 days (temperature acclimation 3 days)
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Feeding</u> No feeding during the study	cessation 72 hrs prior to test
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing	0% mortality prior to testing.

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water	blended naturally hard well water with well water demineralized through reverse osmosis (see note)
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> 17°C or 22°C	22°C (see note)
<u>pH</u> Prefer 7.2 to 7.6	8.2 - 8.5
<u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 st 48 hrs and $\geq 40\%$ during 2 nd 48 hrs, flow-through: $\geq 60\%$	85% at 96 hr in 1.8 mg/L treatment group
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO ₃	130 -160 mg/L as CaCO ₃
<u>Test Aquaria</u> 1. <u>Material</u> : Glass or stainless steel 2. <u>Size</u> : Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume</u> : 15-30 L of solution	1. Glass 2. 19 L 3. 15 L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	N/A
<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	static volume of 15 L/24 hours
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^\circ\text{C}$, ≤ 0.5 g/L at $> 17^\circ\text{C}$; flow-through: ≤ 1 g/L/day	0.1 g/L (or g/L/day)

Guideline Criteria	Reported Information
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hr light; 8 hr dark
<u>Solvents</u> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: dimethylformamide Maximum conc.: 0.5 ml DMF/L.

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	1.0, 5.0, 20.0 mg/L
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	0, 0.65, 1.1, 1.8, 3.0, 5.0 mg ai/L
<u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers	10 per treatment level
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
<p>Water Parameter Measurements</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. 0, 48, 96 hrs (continuous recording of water bath temperatures)</p> <p>2. 0, 48, 96 hrs</p>
<p>Chemical Analysis</p> <p>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>Measured concentrations of Propazine determined from the five test levels, control, vehicle blank, and propazine-fortified quality control samples at 0 and 96 hours of definitive test.</p>

12. REPORTED RESULTSA. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	98 ± 3.1%
<p><u>Control Mortality</u></p> <p>Not more than 10% control organisms may die or show abnormal behavior.</p>	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes (see note)

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	0	10	0	0	0	0
Solvent Control	0	10	0	0	0	0
0.65	0.67	10	0	0	0	0
1.1	1.0	10	0	0	0	0
1.8	1.8	10	0	0	0	0
3.0	2.7	10	0	0	0	0
5.0	4.5	10	0	0	0	0

Other Significant Results:

B. Statistical Results

Method:

96-hr LC₅₀: >4.5 ppm ai 95% C.I.: _____ - _____ ppm ai

Probit Slope: _____ NOEC: 4.5 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	(_____ - _____) ppm ai
Moving Average Angle LC ₅₀ (95% C.I.)	(_____ - _____) ppm ai
Probit LC ₅₀ (95% C.I.)	(_____ - _____) ppm ai
Probit Slope	
NOEC	ppm ai

14. REVIEWER'S COMMENTS:

Abnormal effects of erratic swimming were observed in the 1.0 and 1.8 mg/L solutions at 48 and 72 hours of the test. The

researchers concluded that since the effects were observed in only a couple fish in either concentration, the effects were not believed to be compound related.

Compound was tested at two levels (3.0 and 5.0 mg/L) that approached the solubility limit (3.0 mg/L) and there was a precipitate at both test concentrations. The precipitate formed in spite of the presence of DMF (0.5m ml/L) to facilitate solubility. It is noteworthy that the only aberrant behavior was observed in treatments where precipitate was not present and it is likely that the compound was completely solubilized. This is despite analysis indicating that mean measured concentrations approached nominal concentrations. Analysis relied on toluene extracts of Propazine. The conclusion that the NOEL and LC₅₀ exceed 4.5 mg/L may not be warranted given the limited solubility of Propazine. The limited solubility of the compound as evidenced through precipitate in the higher treatment levels raises a question of actual exposure, i.e., bioavailability. Conventional centrifugation is required for all test media where precipitate is observed in the test chambers (EPA 1994). Thus it is unknown whether the fish were exposed to the nominal and/or mean-measured concentrations.

Total hardness (130-160 mg/L) exceeded the recommended range (40-48 mg/L); p H (8.2 - 8.5) exceeded the recommended 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 p H 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

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EPA 1994. Pesticide reregistration rejection rate analysis: ecological effects. Office of Prevention, Pesticides, and Toxic Substances Publication EPA 738-R-94-035, Washington D. C.

Menzer, R. E. 1991. Water and soil pollutants. In M. O. Amdur, J. Doull and C. D. Klaassen, editors. Casarett and Doull's Toxicology. Pergamon Press, New York: 872 - 902.