

DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4(A)

1. CHEMICAL: 2-chloro-4,6-bis(isopropylamino)-s-triazine
Shaughnessey, #: 080808

2. TEST MATERIAL: Propazine Purity: 98.0%

3. CITATION:

Authors: J. E. Rhodes

Title: Early life-stage toxicity of propazine to
the fathead minnow (*Pimephales promelas*)
under flow-through conditions

Study Completion Date: 08/10/95

Laboratory: ABC Laboratories, Inc.

Laboratory Report ID: ABC Laboratories #41957

Sponsor: Griffin Corporation

MRID No.: 442873-07

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. CONCLUSIONS: This study is scientifically sound but does not fulfill the 72-4 (A) guideline requirements for freshwater fish early life-stage tests. 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. Under the conditions tested, Propazine at concentrations of 2.44 and 4.64 mg a.i./L resulted in significant ($P < 0.05$) reduction in length of fathead minnows. At concentrations of 1.14, 2.44, and 4.64 mg a.i./L, reductions in blotted wet weight were observed. The MATC was calculated at 0.938 mg a.i./L; thus, propazine is mildly toxic to fathead minnows.

Results Synopsis

NOEL: 0.772 mg a.i./L

LOEL: 1.14 mg a.i./L

7. ADEQUACY OF THE STUDY:



2041122

A. Classification: Supplemental

B. Rationale: 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. Although vehicle (DMF) blank contaminated with test compound, it was not significantly different ($P < 0.05$) from control parameters. Interpretation of the results do not appear to have been effected.

C. Reparability: Upgradeable if registrant can demonstrate that neither pH nor water hardness affect the solubility or toxicity of propazine.

8. **MAJOR GUIDELINE DEVIATIONS:** 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.

9. **MATERIALS AND METHODS:**

A. **Biological System:**

Guideline Criteria	Reported Information
<p>Species: Any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See SEP for complete listing.</p>	<p><i>Pimephales promelas</i></p>
<p>Source</p>	<p>ABC Laboratories, Inc., in-house culture</p>
<p>Age at beginning of test: Embryos 2 to 24 hours old.</p>	<p>< 24 hrs post-fertilization</p>
<p>Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for post-hatch exposure.</p>	<p>25 newly fertilized eggs (< 24 hrs post-fertilization) per replicate cup, 4 replicates per concentration.</p>

Guideline Criteria	Reported Information
<p>Post Hatch: % of embryos that produce live fry must be $\geq 50\%$ in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.</p>	<p>90.5 to 100% hatch in control and solvent control.</p>
<p>Feeding: Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32.</p>	<p>Fry fed rotifers (<i>Branchionus</i> sp.) and brine shrimp (<i>Artemia</i>) nauplii <i>ad libitum</i> three times a day at approximately 4-hr intervals on weekdays at 2 times a day on weekends.</p>
<p>Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.</p>	<p>Counts of surviving fry were made periodically throughout the study and also at termination; frequency of counts not reported.</p>
<p>Controls: Avg. survival at end of test must be $\geq 80\%$. Survival in any control chamber must not be $< 70\%$.</p>	<p>100% 30-day post-hatch survival in control and solvent control.</p>
<p>Controls: Negative control and carrier control (when applicable) are required.</p>	<p>Dilution water control and DMF-vehicle blank</p>

Comments:

B. Physical System:

Guideline Criteria	Reported Information
<p>Post Hatch: % of embryos that produce live fry must be $\geq 50\%$ in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.</p>	<p>90.5 to 100% hatch in control and solvent control.</p>
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<p>Controls: Negative control and carrier control (when applicable) are required.</p>	<p>Dilution water control and DMF-vehicle blank</p>

Comments:

B. Physical System:

Guideline Criteria	Reported Information
<p>Test Water:</p> <p>1) May be natural or reconstituted;</p> <p>2) Natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants.</p> <p>3) Hardness of 40 to 48 mg/L as CaCO₃ and pH of 7.2 to 7.6 is recommended.</p>	<p>1) Dilution water from deep well; portion passed thorough reverse osmosis</p> <p>2) Dilution water heated, passed through polypropylene cartridge filter and sterilized by exposure to UV; water screened for contaminants.</p> <p>3) hardness ranged 138 to 152 mg/L and pH ranged from 8.01 to 8.31.</p>
<p>Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature.</p>	<p>Mean water temperature across all treatments: 24.5 - 24.8°C</p>
<p>Photoperiod: Recommend 16L/8D.</p>	<p>Developing embryos kept in dark until hatch nearly complete; afterwards 16 hr light/8 hr dark.</p>
<p>Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters should be used: A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.</p>	<p>2-L proportional diluter system. Toxicant concentrations included: 0.33, 0.65, 1.3, 2.5, and 5.0 mg a.i./L. Control and vehicle blank used.</p>

DP Barcode D237791 Guideline Criteria	MRID No.:442873-0 Reported Information
<p>Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.</p>	<p>1) Flow splitting and mixing cells (4-L volume) used. 2) To facilitate test solution circulation, cups oscillated vertically by rocker-arm apparatus. 3) Accuracy of split of test solutions verified by volumetric calibration before test initiation and on day 31 of test. 4) Measured concentrations ranged from 88 to 119% of the nominal test concentrations.</p>
<p>Test Vessels: All glass or glass with stainless steel frame.</p>	<p>18 X 20.5 cm with a water depth of 25.5 cm (9.4 L)</p>
<p>Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</p>	<p>9-cm diameter glint glass jars with 40-mesh Nytex screen.</p>
<p>Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain DO at above 75% of saturation and maintain the toxicant level.</p>	<p>9.4-L chamber volume replaced 7.3 times in 24-hr period. During last 10 days 9.4 volume replacements in 24-hr period.</p>
<p>Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.</p>	<p>Mean dissolved oxygen in control, vehicle blank, and levels 1 - 5 ranged from 6.7 to 7.7 mg/L, representing 85-97% saturation at 25°C.</p>

Comments: No comments.

C. Chemical System:

Guideline Criteria	Reported Information
<p>Concentrations: Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</p> <ul style="list-style-type: none"> - Toxicant conc. must be measured in one tank at each toxicant level every week. - One concentration must adversely affect a life stage and one concentration must not affect any life stage. 	<p>-- Control, vehicle control, 0.33, 0.65, 1.3, 2.5, and 5.0 mg a. i./L.</p> <p>-- Toxicant concentration test on days -3, 0, 7, 14, 21, 28, and 35.</p> <p>--NOEL (0.772 mg a.i./L) reported; LOEL reported (1.14 mg a.i./L); MATC (0.938 mg a.i./L) reported.</p>
<p>Other Variables:</p> <ol style="list-style-type: none"> 1) DO must be measured at each conc. at least once a week; 2) Freshwater parameters in a control and one concentration must be analyzed once a week. 	<ol style="list-style-type: none"> 1) DO measured at each concentration daily; 2) water quality measurements of temperature, DO, conductivity, p H, hardness, and alkalinity were measured on days 0, 1, 7, 14, 21, 28, and 35.
<p>Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p>	<p>Text implies 0.4 ml DMF/4 L diluant; thus, 0.1 ml/L</p>

Comments:

10. REPORTED RESULTS:

Guideline Criteria	Reported Information
<p>Data Endpoints must include:</p> <ul style="list-style-type: none"> - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if approp.); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs. 	<p>-63 to 92 embryos hatched (Table VII)</p> <p>- 3 - 6 days to hatch</p> <p>-85.1 to 96.5% survival embryos, 96.7 to 100% survival of 30 day post-hatch (Table VII)</p> <p>-30 day post-hatch actual length (mm) and blotted wet weight (g) (Table VIII)</p> <p>-no significant physical or behavioral abnormalities</p>
Raw data included? (Y/N)	Yes

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Effects Data:

Toxicant Conc. ($\mu\text{g/L}$) ¹		Per cent Hatch				Time to Hatch			
Nom.	Meas.	A	B	C	D	A	B	C	D
Ctrl	--	95.7	90.5	100	100	5	5	5	5
Solv*	--	90.9	100	90.5	95.7	5	4	5	5
0.33	0.343	86.4	95.7	85.0	90.5	4	5	5	4-5
0.65	0.772	75.0	95.0	84.2	84.2	5	4-5	4-5	4
1.3	1.14	90.9	95.5	100	90.5	4	4	4	4
2.5	2.44	95.5	87.0	100	83.3	4	4	4	4
5.0	4.64	92.0	100	100	88.0	4	4	4	4

Toxicant Conc. ($\mu\text{g/L}$) ¹		Survival (37 days)				Total Length (mm)			
Nom.	Meas.	A	B	C	D	A	B	C	D
Ctrl	--	100	100	100	100	23.3	23.8	23.5	24.0
Solv*	--	100	100	100	100	23.8	23.4	23.3	23.2
0.33	0.343	100	100	93.8	100	24.3	22.9	24.2	23.6
0.65	0.772	91.7	94.7	100	100	23.6	23.1	23.6	24.0
1.3	1.14	100	100	95.2	100	23.5	22.8	23.3	22.9
2.5	2.44	100	100	96.0	100	22.2	23.0	22.3	22.7
5.0	4.64	91.3	100	100	95.5	20.6	21.4	21.3	21.1

Toxicant Conc. ($\mu\text{g/L}$) ¹		Wet weight (gm)			
Nom.	Meas.	A	B	C	D
Ctrl	--	0.229	0.255	0.244	0.254
Solv*	--	0.251	0.223	0.241	0.219
0.33	0.343	0.247	0.211	0.258	0.244
0.65	0.772	0.242	0.224	0.242	0.241
1.3	1.14	0.226	0.208	0.221	0.213
2.5	2.44	0.198	0.216	0.200	0.210
5.0	4.64	0.162	0.172	0.177	0.169

Toxicity Observations:

Statistical:

Discrete data (egg hatchability and survival) analyzed using contingency table methods. Sample sizes for some contingency table cells too small (<10); pairwise comparisons performed using one-tailed Fisher exact test.

Continuous scale data (length, weight) analyzed by ANOVA; one-tailed Dunnett's multiple means comparison used to assess

differences between treatments.

Statistical Method:

NOEL: 0.772 mg/L LEL: 1.14 mg/L

MATC: 0.938 mg/L

Most sensitive endpoint: growth (mm)

Comments: None

11. Reviewer's Statistical Results:

Statistical Method:

NOEL: 0.772 mg/L LEL: 1.14 mg/L MATC: 0.938 mg/L

Most sensitive endpoint: weight (g)

Comments: *Propazine contamination (0.0784 - 0.231 mg a.i./L) discovered on days -3 and 0 in the vehicle blank test; vehicle blank stock solution contained 317 mg/L. Thus, a vehicle blank was not run.

Evidence of fungal infection was observed in all treatments during the incubation; all affected embryos were removed; the initial number of eggs in each replicate was adjusted to account for fungal mortality.

Considerable variation, RSE = 5.35%, in level 2 (0.772 mg/L) treatment (84.6 ± 4.53 mg/L) (mean ± standard error). Table VII does not report mean hatch nor survival but rather represents a summary of simple quotients. It is not very meaningful to compare treatment groups using Table VII as the overall values do not represent means.

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. Thus, this study is classified as supplemental; it is upgradeable to core if the registrant can demonstrate that neither pH nor water hardness affect the solubility or toxicity of propazine.

12. COMPLETION OF ONE-LINER FOR STUDY: