DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4(B)

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

2. TEST MATERIAL: Propazine PC Code No.: 080808
Purity: 98%

3. CITATION

Authors: H. R. Murrell; J. L. Veltri
Title: Chronic toxicity of propazine to *Daphnia magna* under flow-through conditions
Study Completion Date: 7/3/97
Laboratory: ABC Laboratories, Inc.
Sponsor: Griffin Corporation
Laboratory Report ID: ABC Labs #41958
MRID No.: 443276-02
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: Nicholas E. Federoff, Wildlife Biolgist, EFED, ERB IV, U.S. EPA

Signature: Ann Stavola Date: 10/15/98

6. STUDY PARAMETERS

Scientific Name of Test Organism: *Dahnia magna*
Age of Test Organism: first instar (< 24-hrs old)
Definitive Test Duration: 21 days
Study Method: Flow-through
Type of Concentrations: Mean measured

7. CONCLUSIONS: This study is scientifically sound and fulfills the 72-4(B) guideline requirements for a 21-day aquatic invertebrate life cycle test. Dissolved oxygen levels were negatively correlated with dose. The NOEC and LOEC were 0.047 ppm a.i. and 0.091 ppm a.i., respectively, based on growth measured in terms of length (mm) and weight (g).

Results Synopsis

NOEC: 0.047 mg ai/L LOEC: 0.091 mg ai/L*

LOEC's for specific effects

Growth (Length): 0.091 mg a.i./L*
Growth (Weight): 0.091 mg a.i./L*
Egg Production: 0.37 mg ai/L

Larvae Survival: 0.37 mg ai/L

*See reviewer's comments

8. **ADEQUACY OF THE STUDY**

A. **Classification:** Core

B. **Rationale:**

C. **Repairability:**

9. **GUIDELINE DEVIATIONS**

1. DO not measured in all treatment groups.

2. (etc.)

10 **SUBMISSION PURPOSE:** A flow-through toxicity study to determine the maximum acceptable toxicant concentration (MATC) limits.

11. **MATERIALS AND METHODS**

A. **Test Organisms/Acclimation**

| Guideline Criteria | Reported Information |
|---|---|
| **Species**<br>*Daphnia magna* | Daphnia magna |
| **Source** | ABC Laboratories' in-house culture |
| **Parental Acclimation Conditions**<br>Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. | Culturing and testing environment the same. |
| **Parental Acclimation Period**<br>At least 21 days. | No acclimation necessary since the culturing and testing environment are the same. |

| Guideline Criteria | Reported Information |
|---|---|
| **Age of Parental Stock**<br>At least 10-12 days old at the beginning of the acclimation period. | 16 - 24 days old |
| **Food**<br>Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae. | Algae (*Selenastrum capricornutum* / *Ankistrodesmus falcatus*); trout chow (Zeigler Bros.); Fleismann's active dry yeast (*Saccharomyces* sp.) |
| **Food Concentration**<br>5 mg/L (dry wt.) of synthetic food or $10^8$ cells/L of algae is recommended. | Algal suspension: 4 x 108 cells/mL test solution; supplemented with o.5 mL of 5.0 mg/mL suspension of trout chow and yeast. |
| **Were daphnids in good health during acclimation period?** | Not reported |

**B. Test System**

| Guideline Criteria | Reported Information |
|---|---|
| **Test Water**<br>Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details). | ABC hard-blended dilution water |
| **Water Temperature**<br>20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours. | Target: 20 ± 2°C<br>Range: 19.3 to 20.4°C |
| **pH**<br>7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours. | 7.8 to 8.3 |

| Guideline Criteria | Reported Information |
|---|---|
| **Total Hardness**<br>160 to 180 mg/L as $CaCO_3$ is recommended. | 156 mg/L as $CaCO_3$ |
| **Dissolved Oxygen**<br>Renewal: must not drop below 50% for more than 48 hours.<br>Flow-through: ≥ 60% throughout test. | 60 to 97% |
| **Test Vessels or Compartments**<br>1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics<br>2. Size: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable. | 1-liter borosilicate glass beakers. Given a loading rate of 1 daphnid/100 mL and a total of 40 daphnids per 4 replicate, the text implies that the fill volume is a full liter |
| **Covers**<br>Renewal: Test vessels should be covered with a glass plate.<br>Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen. | Covered with 50-mesh stainless steel screens |
| **Type of Dilution System**<br>Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used. | Half-liter proportional diluter system with a Hamilton MicroLab 420 syringe dispenser |
| **Flow Rate**<br>Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period. | 3.6 mL/min; sufficient to replace 1 L test volume 5.1 times in a 24-hr period. |
| **Aeration**<br>Dilution water should be vigorously aerated, but the test tanks should not be aerated. | Aerated hard blended dilution water; test solutions were not aerated during the study. |

| Guideline Criteria | Reported Information |
|---|---|
| **Photoperiod**<br>16 hours light, 8 hours dark. | 16-hr light, 8-hr dark |
| **Solvents**<br>Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol. | Solvent: dimethylformamide (DMF)<br>Maximum conc.: 0.05 ml/L. |

C. Test Design

| Guideline Criteria | Reported Information |
|---|---|
| **Duration**<br>21 days | 21 days |
| **Nominal Concentrations**<br>Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. | 0.025, 0.050, 0.10, 0.20, and 0.40 mg/L |
| **Number of Test Organisms**<br>22 daphnids/level;<br>7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each. | 10 daphnids/replicate<br>4 replicates per treatment level; thus, 40 daphnids per level. |
| **Test organisms randomly or impartially assigned to test vessels?** | Yes |
| **Renewal**<br>Parent daphnids in all beakers must be transferred to containers with fresh test solution (< 4 hours old) three times each week (e.g. every Monday, Wednesday and Friday). | Changeovers (removal of neonates) performed on Monday, Wednesday, and Friday after first brood release in the control. |

| Guideline Criteria | Reported Information |
|---|---|
| Water Parameter Measurements<br>1. Dissolved oxygen must be measured at each concentration at least once a week.<br>2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control.<br>3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers. | 1. DO measured in control and level 1, 3, and 5 of test concentrations at 0,4, 7, 14, and 21 days.<br><br>2. pH measured in control and levels 1, 3, and 5 at 0, 4, 7, 14, and 21 days. Alkalinity, hardness and conductance measured on the dilution water daily.<br><br>3. Temperature measured in control and levels 1, 3, and 5 at 0, 4, 7, 14, and 21 days. |
| Chemical Analysis<br>Needed 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. | Measured concentrations of propazine in test dilution water determined at -8, 0, 7, 14, and 21 days of the toxicity test. |

12. REPORTED RESULTS

A. General Results

| Guideline Criteria | Reported Information |
|---|---|
| Quality assurance and GLP compliance statements were included in the report? | Yes |
| Control Mortality<br>≤ 30% | <10% |
| Did daphnids in each control produce at least 40 young after 21 days? | 47, 40, 39, and 10 young produced by each control during 21-day study. |
| Were no ephippia produced in any of the controls? | Not reported |

| Guideline Criteria | Reported Information |
|---|---|
| **Data Endpoints**<br>- Survival of first-generation daphnids,<br>- Number of young produced per female,<br>- Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test,<br>- Observations of other effects or clinical signs. | -Survival data<br>-Days to first brood<br>-Young/adult reproduction days<br>-length<br>-weights<br>-$EC_{50}$ and MATC |
| **Raw data included?** | SAS outputs; no raw data |

Effects Data

| Toxicant Concentration (mg[or µg]/L) | | No. (%) Dead or Immobile (21 Days) | Young per Female per Repro. Day | Total Length (mm) | Dry Weight (mg) |
|---|---|---|---|---|---|
| Nominal | Measured | | | | |
| Control | -- | 2 (5%) | 10.42 | 4.37 | 0.88 |
| Solvent Control | -- | 1 (2.5%) | 10.30 | 4.46 | 1.02 |
| 0.025 | 0.020 | 1 (2.5%) | 11.33 | 4.47 | 0.995 |
| 0.050 | 0.047 | 0 (0%) | 10.69 | 4.39 | 0.900 |
| 0.10 | 0.091 | 0 (0%) | 9.76 | 4.21 | 0.720 |
| 0.20 | 0.17 | 3 (7.5%) | 11.26 | 4.33 | 0.858 |
| 0.40 | 0.37 | 6 (15%) | 8.15 | 4.13 | 0.705 |

Toxicity Observations:

**B. Statistical Results**

Most sensitive endpoint: 21-day survival

| Endpoint | Method | NOEC | LOEC | MATC |
|---|---|---|---|---|
| Survival | 21 day | 0.17 | 0.37 | 0.25 |
| Reproduction | time to 1st brood; | ns | ns | -- |
| | young/adult reproduction days (YAD) | 0.17 | ns | -- |
| Weight | | 0.17 | * | -- |
| Length | | 0.17 | * | -- |

13. **VERIFICATION OF STATISTICAL RESULTS**

Most sensitive endpoint: growth (weight and length)

| Endpoint | Method | NOEC | LOEC | MATC |
|---|---|---|---|---|
| Survival | 21-day chronic | 0.17 | 0.37 | 0.25 |
| Reproduction | Young/Adult | 0.17 | 0.37 | 0.25 |
| Weight | | 0.047 | 0.091 | 0.065 |
| Length | | 0.047 | 0.091 | 0.065 |

14. **REVIEWER'S COMMENTS:** Dissolved oxygen concentrations were negatively correlated with the treatment level:

control 92 - 97% level 1 82 - 93% level 5 60- 75%
vehicle 91 - 94% level 3 63 - 85%

Dissolved oxygen levels were roughly 20% lower in level 5 compared to controls; pH was also negatively correlated with treatment level.

Dissolved oxygen was not measured at each test concentration but rather only at level 1, 3, and 5.

*The NOEC level for weight and length was reported at 0.17 mg/L; however, both of these parameters were significantly different from controls at the 0.091 mg/L treatment level. The authors concluded that the 0.091 mg/L test level was aberrant since the data did not follow a normal dose-response pattern.

Without knowing DO for level 4, it is difficult to conclude that the treatment effect observed in level 3 was not a result of diminished oxygen. Oxygen levels in level 3 and level 5 were roughly similar; a combination of lower DO and propazine may have had a synergistic affect that confounded the "traditional" dose response.

This synopsis reports the LOEC for growth, i.e., length and weight, as 0.091 mg/L and is based on an empirical interpretation of the data present in Table VI.