# Data Evaluation Report on the Toxicity of Propazine to Sheepshead Minnow, Early Life Cycle

PMRA Submission Number {.....} EPA MRID Number 441848-02

**Data Requirement:**

| | |
|---|---|
| PMRA Data Code | {.....} |
| EPA DP Barcode | D237791 |
| OECD Data Point | {.....} |
| EPA MRID | 441848-02 |
| EPA Guideline | 72-4(a) |

**Test Material:** Propazine Technical **Purity (%):** 98%
Common name Propazine
Chemical name: IUPAC: Not reported
CAS name: 2-Chloro-4,6-bis(isopropylamino)-s-triazine
CAS No.: 139-40-2
Synonyms: None reported

**Primary Reviewer:** Christie E. Padova **Signature:** Christie E. Padova
**Staff Scientist, Dynamac Corporation** **Date:** 2/22/06

**Secondary Reviewer:** Teri S. Myers **Signature:** Teri S Myers
**Staff Scientist, Cambridge Environmental** **Date:** 2/26/06

**Primary Reviewer:** Anita Pease **Date:** {.....}
**EPA/OPP/EFED/ERB – II**

**Secondary Reviewer(s):** {.....} **Date:** {.....}
**{EPA/OECD/PMRA}**

**Reference/Submission No.:** {.....}

| | | |
|---|---|---|
| **Company Code** | {.....} | [For PMRA] |
| **Active Code** | {.....} | [For PMRA] |
| **Use Site Category** | {.....} | [For PMRA] |
| **EPA PC Code** | 080808 | |

**Date Evaluation Completed:** {dd-mm-yyyy}

**CITATION:** Boeri, R.L., P.L. Kowalski, and T.J. Ward. 1995. Early Life-Stage Toxicity of Propazine to the Sheepshead Minnow, *Cyprinodon variegates.* Unpublished study performed by T.R. Wilbury Laboratories, Inc., Marblehead, MA and ABC Laboratories, Inc., Columbia, MO. Study submitted by Griffin Corporation, Valdosta, GA. Study initiated September 16, 1994 and completed November 21, 1995.

**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the toxicity of a pesticide to fish, early life cycle. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

**EXECUTIVE SUMMARY:**

The 36-day chronic toxicity of propazine to the early life stage of sheepshead minnow (*Cyprinodon variegates*) was studied under flow-through conditions. Fertilized eggs/embryos (80/level, <24 hours old) of sheepshead minnow were exposed to 0 (negative and solvent control), 0.30, 0.65, 1.3, 2.5, and 5.0 mg ai/L. Mean-measured concentrations were <0.0562 (<LOD, controls), 0.259, 0.668, 1.34, 2.59, and 5.25 mg ai/L, respectively. The test system was maintained at 29.0-30.8 °C and a pH of 7.5-8.3. The 36-day NOAEC and LOAEC values were 1.34 and 2.59 mg ai/L, respectively, based on a treatment-related delay in hatching (i.e., hatching success), the most sensitive endpoint. Embryo survival was 0% at the highest test level. At the 2.59 mg ai/L, hatching was complete by Day 5, whereas hatching was complete at all lower treatment levels and controls by Day 4. No other treatment-related effects were observed (normal versus abnormal fish, post-hatch survival, and terminal growth).

This study is scientifically sound and {does or does not} satisfy the guideline requirement for an early life toxicity study with sheepshead minnow.

**Results Synopsis**

Test Organism Size/Age(mean Weight or Length): Embryos, <24 hours old
Test Type (Flow-through, Static, Static Renewal): Flow-through

LOAEC: 2.59 mg ai/L

NOAEC: 1.34 mg ai/L

Endpoint(s) Affected: Embryo survival and hatching success
Most Sensitive Endpoint(s): Hatching success

## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** The study protocol was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, Subdivision E, §72-4 (1982); the U.S. EPA Standard Evaluation Procedure EPA 540/9-86-138 (1986); and the ASTM Standard Guide for Conducting Early Life-Stage Toxicity Test with Fishes (1992). Deviations from §72-4a included:

1. Only two replicate aquaria/test level were maintained in this study, whereas four replicates/level are required.

2. Aeration was initiated after 24 hours of exposure to maintain the DO concentration above acceptable levels. Although not recommended, aeration did not adversely affect exposure concentrations.

3. The pH range (7.5-8.3) was higher than recommended (7.2-7.6).

4. An adequate discussion regarding sub-lethal effects observed at the 2.59 mg ai/L level (i.e., hatching delay and smaller appearance) was not provided. Based upon the reported NOAEC and LOAEC, the study authors apparently dismissed these effects as being significant to treatment.

These deviations do not affect the validity of the study.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided. The study followed the U.S. EPA (40 CFR, Part 160) GLP standards with the following exceptions: survival data collected on day 3 from the 2-2 replicate test vessel with a mean-measured concentration of 0.668 mg ai/L, and from the 4-1 replicate test vessel with a mean-measured concentration of 2.59 mg ai/L could not be verified from the raw data; and the stability of the test substance under exposure conditions was assumed by not verified.

### A. MATERIALS:

**1. Test Material** Propazine Technical

**Description:** White powder

**Lot No./Batch No. :** 309027

**Purity:** 98%

**Stability of compound under test conditions:** Stable, as indicated by relatively constant (within 20% of mean) measured concentrations determined on days 0, 7, 14, 21, 28, 35, and 36 in all aquaria containing live organisms.

**Storage conditions of test chemicals:** Room temperature in the dark

**Physicochemical properties of Propazine.**

| Parameter | Values | Comments |
|---|---|---|
| **Water solubility at 20EC** | 8.6 mg/L | |
| **Vapor pressure** | Not reported | |
| **UV absorption** | Not reported | |
| **pKa** | Not reported | |
| **Kow** | Not reported | |

*(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)*

**2. Test organism**:

**Species:** Sheepshead minnow (*Cyprinodon variegates*) [*EPA recommends any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See Standard Evaluation Procedure for listing of recommended species. OECD recommends rainbow trout, fathead minnows, zebra fish, and ricefish but does not exclude the use of other species.*]

**Age /embryonic stage at test initiation:** Embryos, <24 hours old [*EPA recommends fish embryos 2 to 24 hours old.*]

**Method of collection of the fertilized eggs:** Collected during natural spawning of 84 female and 28 male conditioned adult fish, which were introduced into spawning tanks approximately 24 hours prior to the test initiation.

**Source:** Aquatic BioSystems, Inc., Fort Collins, CO

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a. Range-finding study: A 7-day static renewal screening study was conducted with sheepshead minnow embryos (<24 hours old) and nominal concentrations of 0.010, 0.10, 0.50, 1.0, and 5.0 mg/L. After 7 days of exposure, there was >50% survival at 0.10, 0.10, and 0.50 mg/L, 40% survival at 1.0 mg/L, and 35% survival at 5.0 mg/L.

b. Definitive study

**Table 1: Experimental Parameters**

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| Parental acclimation, if any<br><br>Period:<br><br>Conditions (same as test or not):<br><br>Feeding (type, source, amount given, frequency):<br><br>Health: (any mortality observed) | Acclimation of the brood stock fish was not described. | |
| Number of fertilized eggs/embryos in each treatment at test initiation | 80 embryos/treatment level, divided into 20 embryos/cage, 2 cages/aquarium, and 2 replicate aquaria/treatment. | Fish were thinned to 30 level (15 per replicate) following hatch.<br>*Each treatment should include a minimum of 20 embryos per replicate cup and a minimum of 30 fish per treatment for post-hatch exposure (OECD recommends at least 60 eggs, divided between at least 2 replicates)* |
| Concentration of test material<br>nominal:<br><br>measured: | 0 (negative and solvent controls), 0.30, 0.65, 1.3, 2.5, and 5.0 mg ai/L<br><br><0.0562 (<LOD, controls), 0.259, 0.668, 1.34, 2.59, and 5.25 mg ai/L, respectively | Test substance concentrations were determined at study initiation, weekly thereafter, and at study termination in all aquaria containing live organisms. All measured concentrations were within 20% of mean values.<br>*A minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate should be used.*<br>*- Toxicant concentration should be measured in one tank at each toxicant level every week.*<br>*- One concentration should adversely affect a life stage and one concentration should not affect any life stage.*<br>*OECD recommends that 5 concentrations be spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution should be within ∀20% of the mean measured values.* |

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| Solvent (type, percentage, if used) | Dimethylformamide, 0.1 ml/L | *The solvent should not exceed 0.1 ml/L in a flow-through system. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, ethanol.*<br>*OECD recommends that the solvent not have an effect on survival nor produce any other adverse effects;concentration should not be greater than 0.1 ml/L.* |
| <u>Number of replicates</u><br><br>control:<br>solvent control:<br>treated ones: | <br><br>2<br>2<br>2/level | *Number of replicates should be 4 per concentration.*<br>*A solvent control should be used in conjunction with a solubilizing agent.* |
| <u>Test condition</u><br><br>static renewal/flow-through:<br><br>type of dilution system for flow through method:<br><br>flow rate:<br><br>renewal rate for static renewal: | <br><br>Flow-through<br><br>Intermittent-flow proportional diluter<br><br>7.9 volume additions/day<br><br>N/A | The diluter was calibrated before and after the test and observed for normal operation twice daily during the test.<br>*Intermittent flow proportional diluters or continuous flow serial diluters should be used. EPA recommends that flow rate to larval cups should provide 90% replacement in 8 to 12 hours (OECD recommends 5 test chamber volumes/24 hours). For static-renewal, OECD recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or reatain organisms in vessels and change at least 2/3 test water. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.*<br>*Toxicant Mixing:*<br>*1) Mixing chamber is preferred;*<br>*2) Aeration should not be used for mixing;*<br>*3) The test solution should be completely mixed before introduction into the test system;*<br>*4) Flow splitting accuracy should be within 10%.* |

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| Aeration, if any | Initiated after 24 hours of exposure to maintain the DO concentration above acceptable levels. | Aeration did not adversely affect exposure concentrations.<br>*Dilution water should be aerated to ensure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.* |
| Duration of the test | 36 days (32 days post-hatch) | *Recommended test duration is 32 days for EPA. OECD recommendations for test duration are species specific and range from 28-60 days.* |
| <u>Embryo cups, if used</u><br>type/material (glass/stainless steel):<br>size:<br>fill volume: | <br>Glass cylinders closed at one end with Nitex screen<br>Not reported<br>Not reported | The embryo cages were oscillated slowly to assure an adequate flow of media around the embryos.<br>*Recommended embryo cups are 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.* |
| <u>Test vessel</u><br>type/material: (glass/stainless steel)<br>size:<br>fill volume: | <br>Glass<br>20 L<br>15 L | *Recommended test vessel is all glass or glass with stainless steel frame.* |
| Source of dilution water | Natural seawater collected in Marblehead, MA was adjusted to a salinity of 16-17 ppt with de-chlorinated tap water and stored in polyethylene tanks where it was aerated and continuously passed through a particle filter, UV sterilizer, and activated carbon. | Results of chemical analysis of a representative sample of dilution water were provided. Results indicated: pH of 8.3, salinity of 17 ppt, TOC of 6 mg/L, and boron concentration of 3.3 mg/L. Other metals, pesticides, and PCBs were below limits of detection.<br>*Source of dilution water should be natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants.*<br>*OECD accepts any water in which the test species show control survival at least as good as presented in SEP.* |

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| <u>Water parameters</u><br><br>hardness:<br><br>pH:<br><br>dissolved oxygen:<br><br>temperature (s) (record all the temperatures used for different life stages):<br><br>photoperiod:<br><br>salinity (for marine or estuarine species):<br><br>other measurements:<br><br>interval of water quality measurements: | <br><br>N/A<br><br>7.5-8.3<br><br>4.8-7.7 mg/L<br><br>29.0-30.8°C (constant throughout study)<br><br>16 hours light/8 hours dark, with 15-minute transition periods<br><br>16-17 ppt<br><br>None<br><br>DO, pH, salinity, and temperature were recorded daily in each test chamber that contained live organisms. The temperature was also continuously monitored in one control vessel. | Dissolved oxygen was not provided in terms of % saturation. As aeration was initiated 24 hours following test initiation, DO levels likely remained in the acceptable range.<br><br>Light intensity averaged 36 footcandles.<br><br>*Recommended hardness: 40-48 mg/L as $CaCO_3$;*<br>*Recommended pH:* 7.2 to 7.6<br>*Dissolved Oxygen (DO) should be measured at each concentration at least once a week;*<br>*Freshwater parameters in a control and one concentration should be analyzed once a week.*<br>*Temperature depends upon test species and should not deviate by more than 2EC from appropriate temperature.*<br>*OECD recommends that DO concentration be between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously.* |
| <u>Post-hatch details</u><br><br>when the post-hatch period began:<br><br>number of hatched eggs (alevins)/ treatment released to the test chamber:<br><br>on what day, the alevins were released from the incubation cups to the test chamber: | <br><br>Day 4, when hatching was at least 90% complete in the control exposure.<br><br>Newly hatched larvae were thinned to 30 organisms per treatment level (15/replicate) on Day 4.<br><br>Day 4 | Survival was at least 97.5% in all negative and solvent control replicates.<br><br>*Percentage of embryos that produce live fry should be <u>≥</u> 50% in each control; percentage of hatch in any control embryo cup should not be more than 1.6 times that in another control cup.* |

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| <u>Post-hatch Feeding</u><br>start date:<br><br>type/source of feed:<br><br>amount given:<br><br>frequency of feeding: | <br>Day 4<br><br>Newly-hatched *Artemia salina* nauplii (lot number BS03)<br><br>*Ad libitum*<br><br>Twice daily except during the final 24 hours of the test | Fish were fed to excess as observed by live *Artemia salina* present approximately 10 minutes after feeding. Exposure vessels were cleaned daily beginning on day 8 of the exposure. |
| Stability of chemical in the test system | Stable, as indicated by relatively constant (within 20% of mean) measured concentrations. | |
| Recovery of chemical:<br><br>Frequency of measurement:<br><br>LOD:<br>LOQ: | 99.2-105% of nominal<br><br>Days 0, 7, 14, 21, 28, 35, and 36<br><br>0.0562 mg ai/L<br>Not reported | Reviewer-calculated, based on fortified and concurrently-analyzed QC samples (at 0.30, 1.3, and 5.0 mg/L). |
| Positive control {if used, indicate the chemical and concentrations} | N/A | |
| <u>Fertilization success study, if any</u><br><br>number of eggs used:<br><br>on what day the eggs were removed to check the embryonic development: | N/A | |
| Other parameters, if any | N/A | |

**2. Observations:**

**Table 2: Observations**

| Parameters | Details | Remarks<br>*Criteria* |
|---|---|---|
| Parameters measured including the sublethal effects/toxicity symptoms | - Number of embryos hatched (normal and abnormal)<br>- Larval survival (normal and abnormal)<br>- Measurement of growth (length and wet weight)<br>- Clinical signs of toxicity or abnormal behavior | *Recommended parameters measured include:*<br>*- Number of embryos hatched;*<br>*- Time to hatch;*<br>*- Mortality of embryos, larvae, and Juveniles:*<br>*- Time to swim-up (if appropriate);*<br>*- Measurement of growth;*<br>*- Incidence of pathological or Histological effects;*<br>*- Observations of other effects or clinical signs.* |
| Observation intervals/dates for:<br><br>egg mortality:<br>no. of eggs hatched:<br>mortality of fry (e.g.,alevins):<br>swim-up behavior:<br>growth measurements:<br>embryonic development:<br>other sublethal effects | <br><br>Daily<br>Daily<br>Daily<br>N/A<br>Day 36<br>Not determined<br>Daily | |
| Water quality was acceptable (Yes/No) | Yes | |
| Were raw data included? | Yes | |
| Other observations, if any | N/A | |

## II. RESULTS AND DISCUSSION

### A. MORTALITY:

Following 48 hours of exposure, the percent survival of embryos averaged 97.5 and 100% for the negative and solvent control groups, respectively, and 98.8, 97.5, 98.8, 87.5, and 37.5% for the mean-measured 0.259, 0.668, 1.34, 2.59, and 5.25 mg ai/L levels, respectively. No statistically-significant differences were reported. On Day 4, percent survival at hatch averaged 97.5 and 100% for the negative and solvent control groups, respectively, and 98.8, 97.5, 91.3, 65.0, and 0% for the mean-measured 0.259, 0.668, 1.34, 2.59, and 5.25 mg ai/L levels, respectively. The highest tested concentration caused complete mortality, was assumed to be different than the control, and was not included in statistical analyses. Post-hatch survival was assessed on days 7, 14, 21, 28, and 32 post-hatch. After 32 days post-hatch, survival averaged 100% for both control groups, compared to 96.7, 100, 90.0, and 83.3% for the mean-measured 0.259, 0.668, 1.34, and 2.59 mg ai/L treatment groups, respectively, with no statistically-significant differences reported. The subsequent NOAEC, LOAEC, and MATC for survival (all assessments) were 2.59, 5.25, and 3.69 mg ai/L, respectively.

**Table 3: Effect of Propazine Technical on egg hatching and survival at different life stage of fish**.

| Treatment (mg ai/L) Measured (and nominal) concentrations | Egg hatched/embryo viability | | | Time to hatch[1] | | | Juvenile-survival on day 32 after hatch | |
|---|---|---|---|---|---|---|---|---|
| | No. of eggs at study initiation | hatch/embryo viability | | day x1 | day x2 | day xn | No. dead | % mortality |
| | | No. | % | | | | | |
| Control (dilution water only) | 80 | 78 | 97.5 | ND | | | 0 | 0.0 |
| Solvent control | 80 | 80 | 100.0 | ND | | | 0 | 0.0 |
| 0.259 (0.30) | 80 | 79 | 98.8 | ND | | | 1 | 3.30 |
| 0.668 (0.65) | 80 | 78 | 97.5 | ND | | | 0 | 0.0 |
| 1.34 (1.3) | 80 | 73 | 91.3 | ND | | | 3 | 10.0 |
| 2.59 (2.5) | 80 | 47 | 65.0 | ND | | | 5 | 16.7 |
| 5.25 (5.0) | 80 | 0 | 0.0* | ND | | | --- | --- |
| NOAEC | | 2.59 mg ai/L | | ND | | | 2.59 mg ai/L | |
| $EC_{50}$ | | ND | | ND | | | ND | |
| Positive control, if used<br><br>mortality:<br>$EC_{50}$:<br>NOAEC | N/A | | | | | | | |

ND – Not determined

[1] It was reported that the time to fist hatch was not statistically analyzed because first hatch occurred on the same day (Day 2) for all concentrations producing live fish.

* The highest tested concentration caused complete mortality and was assumed to be different than the control and not included in statistical analyses.

**Table 4: Effect of Propazine Technical on Growth of Juvenile Fish.**

| Treatment (mg ai/L) Measured (and nominal) concentrations | Swim-up[1] | | | Growth -length (cm) | Growth-wet weight (g) |
|---|---|---|---|---|---|
| | day x1 | day x2 | day xn | | |
| Control (dilution water only) | N/A | | | 24 | 248 |
| Solvent control | N/A | | | 24 | 268 |
| 0.259 (0.30) | N/A | | | 24 | 265 |
| 0.668 (0.65) | N/A | | | 24 | 263 |
| 1.34 (1.3) | N/A | | | 24 | 253 |
| 2.59 (2.5) | N/A | | | 24 | 271 |
| 5.25 (5.0) | N/A | | | --- | --- |
| NOAEC | N/A | | | 2.59 mg ai/L | 2.59 mg ai/L |
| LOAEC | N/A | | | 5.25 mg ai/L | 5.25 mg ai/L |
| $EC_{50}$ | N/A | | | ND | ND |
| Positive control, if used<br><br>mortality:<br>$EC_{50}$:<br>NOAEC | N/A | | | | |

[1] Swim-up is not applicable for this species.
ND – Not determined

**B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:**

The time to first hatch occurred on the same day (Day 2) for all concentrations producing live fish. However, it was reported that hatching was complete on Day 5 of the exposure at the 2.59 mg ai/L level (compared to Day 4 for all lower treatment levels). All hatched fish fed when first presented with food. In addition to survival, normal versus abnormal survivors were assessed at each interval (48 hours, at hatch, and days 7, 14, 21, 28, and 32 post-hatch). Results were identical for percent survival data and percent normal at all intervals. However, it was reported that several fish exposed to 2.59 mg ai/L appeared visually smaller than controls during the test (not further described quantitatively) and that no other sub-lethal effects were observed. At study termination, total lengths and wet weights averaged 24 mm and 248-271 mg for all treatment and control groups, with no statistically-significant differences observed. The reported NOAEC, LOAEC, and MATC for sub-lethal endpoints were 2.59, 5.25, and 3.69 mg ai/L, respectively.

**Table 5: Sub-lethal Effect of Propazine Technical on Sheepshead Minnow.**

| Treatment (mg ai/L) Measured (and nominal) concentrations | % Deformed larvae | Behavioral effects (specify) | Behavioral effects (specify) | Smaller Appearance[1] | Toxicity symptoms (specify) |
|---|---|---|---|---|---|
| Control (dilution water only) | None | None | None | ---- | None |
| Solvent control | None | None | None | ---- | None |
| 0.259 (0.30) | None | None | None | None | None |
| 0.668 (0.65) | None | None | None | None | None |
| 1.34 (1.3) | None | None | None | None | None |
| 2.59 (2.5) | None | None | None | Several | None |
| 5.25 (5.0) | None | None | None | N/A | None |
| NOAEC | N/A | N/A | N/A | 2.59 mg ai/L | N/A |
| LOAEC | N/A | N/A | N/A | 5.25 mg ai/L | N/A |
| Positive control, if used % sublethal effect: NOAEC: | N/A | N/A | N/A | | N/A |

[1] No discussion was provided regarding this effect and associated NOAEC and LOAEC levels. Based upon provided values, this effect was apparently dismissed as being treatment-related.

**C. REPORTED STATISTICS:**

Data that were statistically analyzed included 1) the percent of healthy embryos after 48 hours, 2) the percent of healthy and unhealthy embryos hatched, 3) mortality of embryos, larvae, and juveniles 7, 14, 21, 28, and 32 days post-hatch, 4) the mean total length of surviving fish at study termination, and 5) the mean wet weight of surviving fish at study termination. The time to first feeding was not statistically analyzed because all fish fed when first presented with food. The time to first hatch was not statistically analyzed because first hatch occurred on the same day (Day 2) for all concentrations producing live fish.

A parametric "t" test determined that control and solvent control growth data were not significantly different (at the $\alpha = 0.05$ level) and therefore the means were pooled for subsequent comparisons for length and weight. The data were then checked for normality using Shapiro-Wilks' Test and for homogeneity of variance using Bartlett's Test. A parametric one-way analysis of variance (ANOVA) and Tukey's method of multiple comparisons were used to compare treatment and pooled control means. For survival and percent normal data, the Shapiro Wilks test was performed to determine normality; homogeneity of variances, however, could not be determined due to zero variance in at least one group. Kruskal and Wallis's test was then used to compare treatment and control data.

The NOAEC and LOAEC were based on significance data. The MATC was calculated as the geometric mean of the NOAEC and LOAEC. A computer program (TOXSTAT; Gulley *et al.,* 1990) was used to perform the statistical analyses, and mean-measured values were used in all estimations.

**D. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Survival at 48 hours and after 32 days hatch and wet weight data were analyzed. None of these data satisfied the assumptions of normality or homogeneity of variances, so the NOAEC and LOAEC values were determined using the non-parametric Kruskal-Wallis test via TOXSTAT statistical software. Data for length and time to first hatch could be verified visually.

$EC_{50}$: >2.59 mg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 1.34 mg ai/L
LOAEC: 2.59 mg ai/L

**E. STUDY DEFICIENCIES:**

There were no study deficiencies.

**F. REVIEWER=S COMMENTS:**

The reviewer's statistical results were similar to the study authors'; however, the conclusions regarding the NOAEC and LOAEC differed. No discussion was provided by the study authors to support the dismissal of sub-lethal effects observed at the 2.59 mg ai/L test level. Specifically, the completion of hatch was delayed at this level until Day 5, whereas the hatch time for all remaining groups with live young was complete by Day 4. Furthermore, visual inspection of fish (not further described) indicated "several" hatched fish from the 2.59 mg ai/L level appeared smaller than controls. No quantitative data were provided aside from terminal growth measurements, at which time no statistically-significant differences in length were observed. Based on the reported NOAEC and LOAEC levels, these sub-lethal effects were apparently dismissed by the study authors as being significant to treatment. The reviewer disagrees with this conclusion. As growth measurements were not determined at intervals during the study, no conclusions may be made regarding the size of the fish. However, it is obvious from the data provided that a treatment-related effect on hatching occurred at the 2.59 mg ai/L level. On Day 4, only 47/80 embryos hatched at this level, compared to 78/80 and 80/80 at the negative and solvent control levels, respectively. The NOAEC and LOAEC levels reported in the Executive Summary and Conclusions sections are modified from the study author's conclusions to reflect these treatment-related effects.

Insoluble test material, observed as a slight turbidity, was noted in test vessels with a mean-measured concentration of 5.25 mg ai/L on Days 0-5 of the test. Complete mortality occurred at this concentration level on Day 4 and observations of insolubility were discontinued after day 5 (at this level). Insoluble material was not observed at any other level tested.

The maximum loading rate during the study was approximately 0.25 g/L at any one time and 0.03 g/L/24 hours.

Experimental test dates were December 30, 1994 to February 4, 1995.

**G. CONCLUSIONS:**

This study is scientifically sound and is thus acceptable or unacceptable. Based upon a treatment-related effect upon the time of hatch at the 2.59 mg ai/L level (the most sensitive endpoint), the NOAEC and LOAEC are 1.34 and 2.59 mg ai/L, respectively. In addition, a treatment-related effect on survival was observed at the 5.25 mg ai/L, whereas hatching success was 0% at this test level.

LOAEC: 2.59 mg ai/L

NOAEC: 1.34 mg ai/L

Endpoint(s) Affected: Embryo survival and hatching success
Most Sensitive Endpoint(s): Hatching success

## III. REFERENCES:

Gulley, D.D., A.M. Boelter, and H.L. Bergman. 1990. TOXSTAT Version 3.3. Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie, WY.

Steel, R.G.D., and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill. New York.

U.S. EPA. 1985. Hazard Evaluation Division Standard Evaluation Procedure – Fish Early Life Stage. Hazard Evaluation Division, Office of Pesticide Programs, Washington, D.C.

U.S. EPA. 1988. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. EPA/600/4-87/028.

U.S. EPA. 1993, 40 CFR Part 160. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards. Final Rules.

## APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

survival at 48 h
File: 4802s Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | RANK SUM |
|---|---|---|---|---|
| 1 | solvent control | 100.000 | 100.000 | 25.000 |
| 2 | neg control | 97.500 | 97.500 | 14.000 |
| 3 | 0.259 | 98.750 | 98.750 | 19.500 |
| 4 | 0.668 | 97.500 | 97.500 | 14.000 |
| 5 | 1.34 | 98.750 | 98.750 | 19.500 |
| 6 | 2.59 | 87.500 | 87.500 | 10.000 |
| 7 | 5.25 | 37.500 | 37.500 | 3.000 |

Calculated H Value = 10.410 Critical H Value Table = 12.590
**Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.**

survival at 48 h
File: 4802s Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | ORIGINAL MEAN | GROUP 7 | 6 | 2 | 4 | 3 | 5 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 5.25 | 37.500 | 37.500 | \ | | | | | | |
| 6 | 2.59 | 87.500 | 87.500 | . | \ | | | | | |
| 2 | neg control | 97.500 | 97.500 | . | . | \ | | | | |
| 4 | 0.668 | 97.500 | 97.500 | . | . | . | \ | | | |
| 3 | 0.259 | 98.750 | 98.750 | . | . | . | . | \ | | |
| 5 | 1.34 | 98.750 | 98.750 | . | . | . | . | . | \ | |
| 1 | solvent control | 100.000 | 100.000 | . | . | . | . | . | . | \ |

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 3.868

survival d32
File: 4802s2 Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | RANK SUM |
|---|---|---|---|---|
| 1 | solvent control | 100.000 | 100.000 | 21.000 |

| | | | | |
|---|---|---|---|---|
| 2 | neg control | 100.000 | 100.000 | 21.000 |
| 3 | 0.259 | 96.650 | 96.650 | 16.500 |
| 4 | 0.668 | 100.000 | 100.000 | 21.000 |
| 5 | 1.34 | 90.000 | 90.000 | 14.000 |
| 6 | 2.59 | 83.350 | 83.350 | 8.500 |
| 7 | 5.25 | 0.000 | 0.000 | 3.000 |

Calculated H Value = 10.481 Critical H Value Table = 12.590
**Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.**

survival d32
File: 4802s2 Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | ORIGINAL MEAN | GROUP 0 0 0 0 0 0 0 7 6 5 3 2 1 4 |
|---|---|---|---|---|
| 7 | 5.25 | 0.000 | 0.000 | \ |
| 6 | 2.59 | 83.350 | 83.350 | . \ |
| 5 | 1.34 | 90.000 | 90.000 | . . \ |
| 3 | 0.259 | 96.650 | 96.650 | . . . \ |
| 2 | neg control | 100.000 | 100.000 | . . . . \ |
| 1 | solvent control | 100.000 | 100.000 | . . . . . \ |
| 4 | 0.668 | 100.000 | 100.000 | . . . . . \ |

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 3.767

wet weight
File: 4802w Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | RANK SUM |
|---|---|---|---|---|
| 1 | GRPS 1&2 POOLED | 257.750 | 257.750 | 33.500 |
| 2 | 0.259 | 265.000 | 265.000 | 19.500 |
| 3 | 0.668 | 261.000 | 261.000 | 16.000 |
| 4 | 1.34 | 253.000 | 253.000 | 9.000 |
| 5 | 2.59 | 271.000 | 271.000 | 24.000 |
| 6 | 5.25 | 0.000 | 0.000 | 3.000 |

Calculated H Value = 8.276 Critical H Value Table = 11.070
**Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.**

wet weight
File: 4802w Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | ORIGINAL MEAN | GROUP 0 6 | 0 4 | 0 1 | 0 3 | 0 2 | 0 5 |
|---|---|---|---|---|---|---|---|---|---|
| 6 | 5.25 | 0.000 | 0.000 | \ | | | | | |
| 4 | 1.34 | 253.000 | 253.000 | . | \ | | | | |
| 1 | GRPS 1&2 POOLED | 257.750 | 257.750 | . | . | \ | | | |
| 3 | 0.668 | 261.000 | 261.000 | . | . | . | \ | | |
| 2 | 0.259 | 265.000 | 265.000 | . | . | . | . | \ | |
| 5 | 2.59 | 271.000 | 271.000 | . | . | . | . | . | \ |

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values