DATA EVALUATION RECORD

§ 72-1 -- ACUTE LC₅₀ TEST WITH A COLDWATER FISH

1. CHEMICAL: Pirate (AC 303,630)  PC Code No.: 129093
2. TEST MATERIAL: CL 357,806
   (A photolytic degrade of AC 303,630)
   Purity: 97%
3. CITATION:
   Authors: J.W. Davis, M.R. Dunham, and J.D. Wisk
   Title: Acute Toxicity of CL 357,806 to the Rainbow Trout (Oncorhynchus mykiss) Under Static Test Conditions
   Study Completion Date: December 14, 1995
   Laboratory: Toxikon Environmental Sciences, Jupiter, FL
   Sponsor: American Cyanamid Company, Princeton, NJ
   Laboratory Report ID: J9504005a
   MRID No.: 438870-08
   DP Barcode: D210808 and D222690
4. REVIEWED BY: William Evans, Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division
   Signature: William Evans  Date: 10/9/96
5. APPROVED BY: Ann Stavola, Section Chief, Review Section 5
   Ecological Effects Branch
   Environmental Fate and Effects Division
   Signature: Ann Stavola  Date: 11/5/96
6. STUDY PARAMETERS:
   Age or Size of Test Organism: 0.20-0.53 g
   Definitive Test Duration: 96 hours
   Study Method: Static
   Type of Concentrations: Nominal

7. CONCLUSIONS: This study is not scientifically sound and does not meet the guideline requirements for an acute freshwater fish toxicity test. Results of toxicity values are not recorded for invalid studies.

Results Synopsis

LC₅₀:
NOEC:

95% C.I.:
Probit Slope: N/A
8. ADEQUACY OF THE STUDY:

A. Classification: Invalid.

B. Rationale: The exposure concentrations are unknown.

C. Repairability: No

9. GUIDELINE DEVIATIONS:

1. The test solutions were aerated during the test and exposure concentrations were not measured.

2. The dissolved oxygen concentration in the test solutions (≥44%) was lower than recommended (≥60%).

3. The test temperature ranged from 11.3 to 14.5°C. The test temperature should not vary >1°C.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

<table>
<thead>
<tr>
<th>Guideline Criteria</th>
<th>Reported Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
<td>Oncorhynchus mykiss</td>
</tr>
<tr>
<td>Preferred species is the rainbow trout</td>
<td></td>
</tr>
<tr>
<td><em>(Oncorhynchus mykiss)</em></td>
<td></td>
</tr>
<tr>
<td><strong>Mean Weight</strong></td>
<td>0.36 (0.20-0.53) g</td>
</tr>
<tr>
<td>0.5-5 g</td>
<td></td>
</tr>
<tr>
<td><strong>Mean Standard Length</strong></td>
<td>3.13 (2.7-3.7) cm</td>
</tr>
<tr>
<td>Longest not &gt; 2x shortest</td>
<td></td>
</tr>
<tr>
<td><strong>Supplier</strong></td>
<td>Mount Lassen Trout Farm, Red Bluff, CA</td>
</tr>
<tr>
<td><strong>All fish from same source?</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>All fish from the same year class?</strong></td>
<td>Yes</td>
</tr>
</tbody>
</table>

B. Source/Acclimation

<table>
<thead>
<tr>
<th>Guideline Criteria</th>
<th>Reported Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acclimation Period</td>
<td>14 days</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Minimum 14 days</td>
<td></td>
</tr>
<tr>
<td>Wild caught organisms were</td>
<td>N/A</td>
</tr>
<tr>
<td>quarantined for 7 days?</td>
<td></td>
</tr>
<tr>
<td>Were there signs of disease or</td>
<td>No</td>
</tr>
<tr>
<td>injury?</td>
<td></td>
</tr>
<tr>
<td>If treated for disease, was there</td>
<td>N/A</td>
</tr>
<tr>
<td>no sign of the disease remaining</td>
<td></td>
</tr>
<tr>
<td>during the 48 hours prior to testing?</td>
<td></td>
</tr>
<tr>
<td>Feeding</td>
<td></td>
</tr>
<tr>
<td>No feeding during the study</td>
<td></td>
</tr>
<tr>
<td>Pretest Mortality</td>
<td></td>
</tr>
<tr>
<td>&lt; 3% mortality 48 hours prior to testing</td>
<td>No mortality in the 48 hours prior to testing.</td>
</tr>
</tbody>
</table>

C. Test System

<table>
<thead>
<tr>
<th>Guideline Criteria</th>
<th>Reported Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source of dilution water</strong></td>
<td>Town of Jupiter water which was carbon-treated, aerated, and filtered prior to use.</td>
</tr>
<tr>
<td>Soft reconstituted water or water from a natural source,</td>
<td></td>
</tr>
<tr>
<td>not dechlorinated tap water</td>
<td></td>
</tr>
<tr>
<td><strong>Does water support test animals without observable signs of stress?</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Water Temperature</strong></td>
<td>11.3-14.5°C</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>6.5-7.4</td>
</tr>
<tr>
<td><strong>Dissolved Oxygen</strong></td>
<td>≥44% saturation during the first 24 hours. Aeration was initiated when the DO fell</td>
</tr>
<tr>
<td>Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs,</td>
<td>below 40% of saturation at 31 hours. Except for the highest test concentration, DO was</td>
</tr>
<tr>
<td>flow-through: ≥ 60%</td>
<td>≥87% of saturation during the 2nd half the test.</td>
</tr>
<tr>
<td><strong>Total Hardness</strong></td>
<td>70 mg/L as CaCO₃</td>
</tr>
<tr>
<td><strong>Test Aquaria</strong></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td><strong>1. Material:</strong></td>
<td>1. Glass</td>
</tr>
<tr>
<td>Glass or stainless steel</td>
<td></td>
</tr>
<tr>
<td><strong>2. Size:</strong></td>
<td>2. 10 liters (22-cm diameter, 30-cm height)</td>
</tr>
<tr>
<td>Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm</td>
<td></td>
</tr>
<tr>
<td><strong>3. Fill volume:</strong></td>
<td>3. 9 liters</td>
</tr>
<tr>
<td>15-30 L of solution</td>
<td></td>
</tr>
<tr>
<td><strong>Type of Dilution System</strong></td>
<td>Static system</td>
</tr>
<tr>
<td><strong>Must provide reproducible supply of toxicant</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Flow Rate</strong></td>
<td>N/A</td>
</tr>
<tr>
<td>Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period</td>
<td></td>
</tr>
<tr>
<td><strong>Biomass Loading Rate</strong></td>
<td>0.4 g/L</td>
</tr>
<tr>
<td>Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at &gt; 17°C; flow-through: ≤ 1 g/L/day</td>
<td></td>
</tr>
<tr>
<td><strong>Photoperiod</strong></td>
<td>16 hours light, 8 hours dark</td>
</tr>
<tr>
<td>16 hours light, 8 hours dark</td>
<td></td>
</tr>
<tr>
<td><strong>Solvents</strong></td>
<td>Solvent: DMF</td>
</tr>
<tr>
<td>Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests</td>
<td>Maximum conc.: 0.1 mL/L</td>
</tr>
</tbody>
</table>
### D. Test Design

<table>
<thead>
<tr>
<th>Guideline Criteria</th>
<th>Reported Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range Finding Test</strong>&lt;br&gt;If LC₅₀ &gt;100 mg/L with 30 fish, then no definitive test is required.</td>
<td>A range-finding test showed 0% mortality at 1.0 µg ai/L and 100% mortality at concentrations ≥10 µg ai/L.</td>
</tr>
<tr>
<td><strong>Nominal Concentrations of Definitive Test</strong>&lt;br&gt;Control &amp; 5 treatment levels; dosage should be 50% of the next highest concentration; concentrations should be in a geometric series</td>
<td>Five nominal test concentrations (0.63, 1.3, 2.5, 5, and 10 µg/L), a dilution water control and a solvent control were used.</td>
</tr>
<tr>
<td><strong>Number of Test Organisms</strong>&lt;br&gt;Minimum 10/level, may be divided among containers</td>
<td>10 fish/replicate, 2 replicates/treatment</td>
</tr>
<tr>
<td><strong>Test organisms randomly or impartially assigned to test vessels?</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Biological observations made every 24 hours?</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Water Parameter Measurements</strong>&lt;br&gt;1. Temperature&lt;br&gt;Measured constantly or, if water baths are used, every 6 hrs, may not vary &gt; 1°C&lt;br&gt;2. DO and pH&lt;br&gt;Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control</td>
<td>1. Temperature was measured hourly in the dilution water control and continuously in the water bath. &lt;br&gt;2. DO and pH were measured daily in each test vessel.</td>
</tr>
<tr>
<td><strong>Chemical Analysis</strong>&lt;br&gt;Necessary 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</td>
<td>No chemical analysis of test solutions was conducted.</td>
</tr>
</tbody>
</table>
12. REPORTED RESULTS:

A. General Results

<table>
<thead>
<tr>
<th>Guideline Criteria</th>
<th>Reported Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality assurance and GLP compliance statements were included in the report?</td>
<td>Yes</td>
</tr>
<tr>
<td>Recovery of Chemical</td>
<td>N/A</td>
</tr>
<tr>
<td>Control Mortality</td>
<td>5% mortality in each control</td>
</tr>
<tr>
<td>Not more than 10% control organisms may die or show abnormal behavior.</td>
<td></td>
</tr>
<tr>
<td>Raw data included?</td>
<td>Yes</td>
</tr>
<tr>
<td>Signs of toxicity (if any) were described?</td>
<td>Yes; signs of toxicity were observed in fish exposed to test concentrations ≥1.3 ppb, which included dark coloration and loss of equilibrium.</td>
</tr>
</tbody>
</table>

### Mortality

<table>
<thead>
<tr>
<th>Concentration (ppb)</th>
<th>Number of Fish</th>
<th>Cumulative Number Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Nominal (ppb)</td>
<td>Mean Measured (ppb)</td>
<td>Hour of Study</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Control</td>
<td>N/A</td>
<td>20</td>
</tr>
<tr>
<td>Solvent Control</td>
<td>N/A</td>
<td>20</td>
</tr>
<tr>
<td>0.63</td>
<td>N/A</td>
<td>20</td>
</tr>
<tr>
<td>1.3</td>
<td>N/A</td>
<td>20</td>
</tr>
<tr>
<td>2.5</td>
<td>N/A</td>
<td>20</td>
</tr>
<tr>
<td>5.0</td>
<td>N/A</td>
<td>20</td>
</tr>
<tr>
<td>10.0</td>
<td>N/A</td>
<td>20</td>
</tr>
</tbody>
</table>
Other Significant Results: Dissolved oxygen concentrations decreased from 100-102% of saturation at test initiation to 44-64% of saturation at 24 hours. Aeration of the test solutions began at 31 hours and continued until test termination. DO levels were ≥87% of saturation for the remainder of the test in all test solutions, except the highest test concentration.

B. Statistical Results - based on nominal concentrations.

Method: Moving Average

96-hr LC₅₀: 2.6 ppb 95% C.I.: 2.1-3.4 ppb

Probit Slope: N/A NOEC: 0.63 ppb

13. VERIFICATION OF STATISTICAL RESULTS:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binomial Test LC₅₀ (C.I.)</td>
<td>3.2 (2.5-5.0) ppb</td>
</tr>
<tr>
<td>Moving Average Angle LC₅₀ (95% C.I.)</td>
<td>N/A</td>
</tr>
<tr>
<td>Probit LC₅₀ (95% C.I.)</td>
<td>N/A</td>
</tr>
<tr>
<td>Probit Slope</td>
<td>N/A</td>
</tr>
<tr>
<td>NOEC</td>
<td>0.63 ppb</td>
</tr>
</tbody>
</table>

14. REVIEWER'S COMMENTS: Although the test solutions were aerated at approximately 31 hours after test initiation and continued until test termination, chemical analysis of the test solutions was not performed. Therefore, the actual concentrations to which the fish were exposed are unknown. This study is not scientifically sound and is classified as Invalid. The toxicity values are not recorded for studies classified as invalid.
NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT’S CORRECTION.

RGM O. mykiss CL 357-806

<table>
<thead>
<tr>
<th>CONC.</th>
<th>NUMBER EXPOSED</th>
<th>NUMBER DEAD</th>
<th>PERCENT DEAD</th>
<th>BINOMIAL PROB. (PERCENT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>19</td>
<td>19</td>
<td>100</td>
<td>1.907348E-04</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>19</td>
<td>100</td>
<td>1.907348E-04</td>
</tr>
<tr>
<td>2.5</td>
<td>19</td>
<td>3</td>
<td>15.7895</td>
<td>.2212524</td>
</tr>
<tr>
<td>1.3</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>1.907348E-04</td>
</tr>
<tr>
<td>.63</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>1.907348E-04</td>
</tr>
</tbody>
</table>

THE BINOMIAL TEST SHOWS THAT 2.5 AND 5 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.175985

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.