

DP Barcode: Not provided

MRID No: 247712

**DATA EVALUATION RECORD  
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE  
GUIDELINE OPPTS 850.1075**

1. **CHEMICAL:** Grotan **PC Code No.:** 083301

2. **TEST MATERIAL:** Triadine™ 10 **Purity:** 65.9% ai  
(Industrial Microbiostat, lot # OD-9211)

3. **CITATION**

Author: Sousa, J.V. and LeBlanc, G.A.  
Title: Acute Toxicity of Triadine 10 to Rainbow Trout (*Salmo gairdneri*)  
Laboratory: EG&G, Bionomics, Aquatic Toxicology Laboratory, Wareham, MA  
Date: February 12-16, 1982  
Sponsor: Not provided  
Study Report ID: Not provided  
Laboratory Report ID: BW-82-2-1117

4. **REVIEWED BY:** W. Erickson, Biologist

Signature:

Date:

5. **APPROVED BY:** N. Cook, Branch Chief

Signature:

Date:

6. **STUDY PARAMETERS**

**Scientific Name of Test Organism:** *Oncorhynchus mykiss* (formerly *Salmo gairdneri*)  
**Age of Test Organism:** Juvenile  
**Definitive Test Duration:** 96-hours  
**Study Method:** Static, Acute  
**Type of Concentrations:** Nominal Concentrations



7. **CONCLUSIONS:**

**Results Synopsis:**

**Statistical Method:** Moving average angle

96-h LC<sub>50</sub>: 42 mg/L

95% C.I.: 34-53 mg/L

NOEC: < 7.8 mg/L

8. **ADEQUACY OF THE STUDY**

**Classification:** Supplemental

9. **GUIDELINE DEVIATIONS:**

The following guideline deviations were based on EPA OPPTS Guideline 850.1075:

- The purity of the chemical was not specified.
- No chemical analysis of water used in test dilution was reported.
- Holding water and test water dilution came from two different sources and Rainbow Trout were not held in the test water dilution for a minimum of 7 days during the acclimation period.
- Pretest mortality was only provided for the 48 hours prior to testing and not for the entire acclimation period.
- Salinity of water not specified.
- No mention of health of fish prior to test initiation
- Use and type of cover for test vessels was not reported.
- DO data was not recorded at the 72-hours exposure condition; guidelines require DO values to be collected at every 24-hour interval.
- No evidence of range-finding test, to provide a basis for definitive test concentrations.
- The number of replicates for each concentration was not explicitly stated, reviewer made assumption based on note that N=30.
- Some pH measurements reported were outside the range for freshwater species.
- Recorded hourly temperatures in one replicate throughout the study were not provided.
- No analysis of the stability of the test chemical was reported therefore, it could not be determined if measured concentrations are also required.
- Test chemical concentrations were not monitored and recorded throughout the experiment (guidelines require at time 0 hrs, 48 hrs, and 96- hours).
- Mortality observations were not recorded at 6- hours for each test.
- No graph of the concentration-mortality curve was provided.
- Quality assurance and GLP compliance statements were not provided.

10. **SUBMISSION PURPOSE:** Reregistration

11. **MATERIALS AND METHODS**

A. **Test Organisms**

Guideline Criteria	Reported Information
<b>Species</b> <ul style="list-style-type: none"> <li>▪ Preferred freshwater species: bluegill sunfish (<i>Lepomis macrochirus</i>) or rainbow trout (<i>Oncorhynchus mykiss</i>)</li> <li>▪ Preferred saltwater species: Atlantic silverside (<i>Menidia menidia</i>) or Sheepshead minnow (<i>Cyprinodon variegatus</i>)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Rainbow Trout (<i>Oncorhynchus mykiss</i>)</li> <li>▪ Formerly <i>Salmo gairdneri</i></li> </ul>
<b>Weight</b> <ul style="list-style-type: none"> <li>▪ Juvenile fish &lt; 3.0 g</li> </ul>	<ul style="list-style-type: none"> <li>▪ 0.63 (0.41-0.94) g wet weight</li> </ul>
<b>Length</b> <ul style="list-style-type: none"> <li>▪ Longest not &gt; 2x shortest</li> </ul>	<ul style="list-style-type: none"> <li>▪ 43 (39-48) mm</li> </ul>
<b>Supplier</b>	<ul style="list-style-type: none"> <li>▪ Commercial fish supplier in Montana (Bionomics lot #81A33)</li> </ul>
<b>All fish from same source and population?</b>	<ul style="list-style-type: none"> <li>▪ All from above supplier</li> </ul>
<b>Fish used in previous tests?</b>	<ul style="list-style-type: none"> <li>▪ Not specified</li> </ul>
<b>If wild fish used, quarantined 7 days before acclimation?</b>	<ul style="list-style-type: none"> <li>▪ Not applicable</li> </ul>
<b>Signs of stress or injury?</b>	<ul style="list-style-type: none"> <li>▪ Not specified</li> </ul>

B. **Acclimation**

Guideline Criteria	Reported Information
<b>Acclimation Period</b> <ul style="list-style-type: none"> <li>▪ Minimum 12 days (14 days recommended)</li> <li>▪ Minimum 7 days in test dilution water</li> </ul>	<ul style="list-style-type: none"> <li>▪ 14 day acclimation period</li> </ul>

Guideline Criteria	Reported Information
<p><b><u>Holding Water</u></b></p> <ul style="list-style-type: none"> <li>Same source as test dilution water (if not, acclimation to dilution water done gradually over 48 hr period)</li> </ul>	<ul style="list-style-type: none"> <li>Source of water in holding tank was from a well that flowed- through tank at a rate of 13-15 tank volume replacements/day</li> <li>Total hardness: 30 mg/L CaCO<sub>3</sub></li> <li>Alkalinity: 24-30 mg/L CaCO<sub>3</sub></li> <li>Specific conductance: 100 µhos/cm</li> <li>pH range: 6.7-6.9</li> <li>DO range of 83-94% of saturation</li> </ul>
<p><b><u>Disease Treatment</u></b></p> <ul style="list-style-type: none"> <li>No treatments within 48 hrs of test initiation or during test</li> </ul>	<ul style="list-style-type: none"> <li>Not Specified</li> </ul>
<p><b><u>Feeding</u></b></p> <ul style="list-style-type: none"> <li>No feeding within 48 hrs of test initiation. Feed daily prior to this period.</li> </ul>	<ul style="list-style-type: none"> <li>Fed dry pellet food daily, <i>ad libitum</i></li> <li>Food withheld 48-hours prior to testing.</li> </ul>
<p><b><u>Pretest Mortality</u></b></p> <ul style="list-style-type: none"> <li>&lt; 5% during acclimation; reject entire batch if &gt; 10%.</li> </ul>	<ul style="list-style-type: none"> <li>0.2% mortality (48 hours prior to testing)</li> </ul>
<p><b><u>Water Temperature</u></b></p> <ul style="list-style-type: none"> <li>Temperature changes should not exceed 3°C per day</li> <li>Hold fish minimum 7 days at test temperature prior to testing</li> </ul>	<ul style="list-style-type: none"> <li>11-12 °C in holding tank</li> <li>Testing and acclimation temperature were the same</li> </ul>
<p><b><u>Background</u></b></p> <ul style="list-style-type: none"> <li>During final 48 hrs, colors and light intensities similar to testing area</li> </ul>	<ul style="list-style-type: none"> <li>16 hours light/8 hours dark</li> <li>Same as definitive test</li> </ul>

**C. Test System**

Guideline Criteria	Reported Information
<p><b><u>Dilution Water</u></b></p> <ul style="list-style-type: none"> <li>Reconstituted water or water from natural source preferred. If dechlorinated tap water, daily chlorine analysis performed.</li> <li>Chemical analysis performed and maximum concentrations not exceeded (see guideline)</li> </ul>	<ul style="list-style-type: none"> <li>The test dilution water used was soft water reconstituted from deionized water, using EPA recommended procedures</li> <li>Chemical analysis data was not provided.</li> </ul>
<p><b><u>Solutions</u></b></p> <ul style="list-style-type: none"> <li>Distilled water used to make stock solutions of test substances. If stock volume &gt; 10% of test solution volume, dilution water used.</li> </ul>	<ul style="list-style-type: none"> <li>Stock solution of Triadine 10 (15 mg/L) was prepared by adding distilled water to 7.5 grams of Triadine 10 in a 500 mL volumetric flask, until an appropriate volume had been reached.</li> <li>19.6 L glass jars with 15 L of test solution.</li> </ul>

Guideline Criteria	Reported Information
<p><b>Water Temperature</b></p> <ul style="list-style-type: none"> <li>▪ 10 or 12 ± 2°C for cold water species (see guideline)</li> <li>▪ 22 or 23 ± 2°C for warm water species (see guideline)</li> <li>▪ Vary no more than 1°C in any 24-hr period</li> <li>▪ Record in all replicates at beginning of test and every 24 hrs; record hourly in one replicate.</li> </ul>	<ul style="list-style-type: none"> <li>▪ All test solution temperatures were maintained at 12 ± 1 °C upon the addition of Rainbow Trout,</li> <li>▪ Temperature was only recorded once for each exposure time during the definitive test.</li> </ul>
<p><b>pH</b></p> <ul style="list-style-type: none"> <li>▪ &gt; 6.0 and &lt; 8.0 for freshwater testing</li> <li>▪ &gt; 7.5 and &lt; 8.5 for marine testing</li> <li>▪ Measured in each replicate at beginning of test and every 24 hrs</li> </ul>	<ul style="list-style-type: none"> <li>▪ pH values ranged from 6.8-9.1.</li> <li>▪ pH values were measured at 0, 24, 48, and 96-hours in the control, high, middle, and low-test concentrations</li> </ul>
<p><b>Dissolved Oxygen</b></p> <ul style="list-style-type: none"> <li>▪ Static: &gt; 60% saturation at all times</li> <li>▪ Flow-through: &gt; 75% saturation at all times</li> <li>▪ Measured in each replicate at beginning of test and every 24 hrs</li> </ul>	<ul style="list-style-type: none"> <li>▪ DO values ranged from 6.9- 10.6 mg/L or 64-98%.</li> <li>▪ DO values were measured at 0, 24, 48, and 96-hours in the control, high, middle, and low test concentrations</li> </ul>
<p><b>Total Hardness</b></p> <ul style="list-style-type: none"> <li>▪ 40 to 180 mg/L as CaCO<sub>3</sub> (freshwater species)</li> <li>▪ Measured at beginning of each test</li> </ul>	<ul style="list-style-type: none"> <li>▪ The test dilution water had a total hardness as CaCO<sub>3</sub> of 42 mg/L</li> <li>▪ Total hardness was reported once during the definitive test.</li> <li>▪ The test dilution water had a specific conductance of 140 μhos/cm (p 16).</li> <li>▪ Specific conductance was reported once during the definitive test.</li> </ul>
<p><b>Salinity</b></p> <ul style="list-style-type: none"> <li>▪ 20 ± 5ppt (estuarine species)</li> <li>▪ Measured at beginning of each test and, for flow-through tests, on day 4, and if extended days 7 and 14</li> </ul>	<ul style="list-style-type: none"> <li>▪ Not specified</li> </ul>
<p><b>Test Aquaria/Equipment</b></p> <ul style="list-style-type: none"> <li>▪ Material: Glass, stainless steel, nylon screen or perfluorocarbon plastic (e.g., Teflon®)</li> <li>▪ Test chambers loosely covered</li> </ul>	<ul style="list-style-type: none"> <li>▪ 19.6 L glass jars</li> <li>▪ The acclimation holding tank was made of fiber glass</li> </ul>
<p><b>Aeration</b></p> <ul style="list-style-type: none"> <li>▪ Static systems only if &lt; 60% saturation; if aeration used test concentrations measured.</li> <li>▪ No aeration in flow-through tests</li> </ul>	<ul style="list-style-type: none"> <li>▪ The test solutions were not aerated</li> </ul>

Guideline Criteria	Reported Information
<b>Type of Dilution System</b> <ul style="list-style-type: none"> <li>Must provide reproducible supply of toxicant</li> </ul>	<ul style="list-style-type: none"> <li>Static</li> </ul>
<b>Flow Rate</b> <ul style="list-style-type: none"> <li>Consistent flow rate of 6-10 vol/24 hours</li> <li>Measured at beginning and end of each test</li> <li>No more than a factor of 10 variation between replicates</li> </ul>	<ul style="list-style-type: none"> <li>No flow</li> </ul>
<b>Biomass Loading Rate</b> <ul style="list-style-type: none"> <li>Static/Static-renewal: <math>\leq 0.8</math> g FWF/L</li> <li>Flow-through: <math>\leq 0.5</math> g FWF/L</li> </ul>	<ul style="list-style-type: none"> <li>Not specified</li> </ul>
<b>Photoperiod</b> <ul style="list-style-type: none"> <li>Range from 12D/12N to 16D/8N, with 15 min transition period</li> <li>Intensity 30 to 100 lm at water surface</li> </ul>	<ul style="list-style-type: none"> <li>16 hours light/8 hours dark</li> <li>Same as acclimation period</li> </ul>
<b>Solvents</b> <ul style="list-style-type: none"> <li>Not to exceed 0.5 ml/L for static or static-renewal tests or 0.1 ml/L for flow-through tests</li> <li>Preferred solvents dimethyl formamide, triethylene glycol, methanol, acetone, or ethanol</li> </ul>	<ul style="list-style-type: none"> <li>Not Specified</li> </ul>

#### D. Test Design

Guideline Criteria	Reported Information
<b>Range-Finding Test</b> <ul style="list-style-type: none"> <li>If <math>LC_{50} &gt; 100</math> mg/L with 30 fish, then no definitive test required</li> </ul>	<ul style="list-style-type: none"> <li>No evidence of range-finding test</li> </ul>
<b>Test Concentrations</b> <ul style="list-style-type: none"> <li>Minimum of control and 5 concentrations in geometric series</li> <li>Concentrations 50 to 120% greater than next lowest concentration</li> <li>No more than 25% variation between test concentrations within same treatment</li> <li>Concentrations selected to produce NOEC and, preferably, at least 2 partial mortalities (<math>&gt;</math> and <math>&lt;</math> 50%) after 96 hrs</li> <li>Measured concentrations required if test chemical unstable or flow-through system, and must remain at least 80% of nominal concentrations</li> </ul>	<ul style="list-style-type: none"> <li>Five test concentrations (7.8, 13, 22, 36, 60 mg/L) and a control were analyzed during the definitive test.</li> <li>One test concentration (7.8 mg/L) did produce NOEC.</li> <li>No information provided about the stability of the test chemical, cannot determine if measured concentrations are required.</li> </ul>

Guideline Criteria	Reported Information
<p><b>Concentration Analysis</b></p> <ul style="list-style-type: none"> <li>▪ Performed at test initiation and every 48 hrs</li> <li>▪ Static: each replicate, minimally at test initiation (before organisms added), at 48 hrs and at end of test</li> <li>▪ Static-renewal: each replicate, at test initiation and end, and just before and after each renewal</li> <li>▪ Flow-through: each replicate at 0, 48, and 96 hrs, and every 96 hrs thereafter</li> </ul>	<ul style="list-style-type: none"> <li>▪ Not specified</li> </ul>
<p><b>Controls</b></p> <ul style="list-style-type: none"> <li>▪ Consist of same dilution water, conditions, procedures and test population</li> <li>▪ Negative and/or solvent</li> <li>▪ Maximum allowable mortality 10% (or 1 mortality if 7 to 10 fish used) for 96 hr period; 10% additional past 96 hrs.</li> </ul>	<ul style="list-style-type: none"> <li>▪ The negative control jar contained the same dilution water as in the exposure jars.</li> <li>▪ Control data indicates that maximum allowable mortality was not exceeded.</li> </ul>
<p><b>Replicates</b></p> <ul style="list-style-type: none"> <li>▪ Two per test concentration</li> <li>▪ Equal volume test solution and number test fish</li> </ul>	<ul style="list-style-type: none"> <li>▪ Number of replicates was not explicitly stated.</li> <li>▪ When quantifying the weight and length range of fish, study noted that N=30. Reviewer assumed this meant that there were three replicates for each treatment concentration.</li> </ul>
<p><b>Test Organisms</b></p> <ul style="list-style-type: none"> <li>▪ Minimum 7/replicate (10 preferred)</li> <li>▪ Equal number per test chamber</li> <li>▪ Not fed during treatment period</li> <li>▪ Randomly or impartially assigned to test vessels within 30 min of addition of test substance</li> <li>▪ Biological observations made at 6 hrs and every 24 hours</li> </ul>	<ul style="list-style-type: none"> <li>▪ Ten test organisms per replicate were used</li> <li>▪ The Rainbow Trout were distributed randomly to each test jar within 10 minutes after the preparation of the test solution.</li> <li>▪ Biological observations made every 24 hours.</li> </ul>

**12. REPORTED RESULTS**

Guideline Criteria	Reported Information
<p><b>Quality assurance and GLP compliance statements included in the report?</b></p>	<ul style="list-style-type: none"> <li>▪ No</li> </ul>
<p><b>Name of test facilities, test dates and personnel reported?</b></p>	<ul style="list-style-type: none"> <li>▪ Yes, pages 13 &amp; 23</li> </ul>

Guideline Criteria	Reported Information
Identification of test substance (including physicochemical characteristics) and purity provided?	<ul style="list-style-type: none"> <li>Yes, page 14</li> </ul>
Methods used in preparation of stock solutions and analysis of test concentrations described? Accuracy of method (i.e., detection limit and quantification limit) reported?	<ul style="list-style-type: none"> <li>Yes, page 16</li> <li>No accuracy of method described.</li> </ul>
LC <sub>50</sub> concentration-response curves, LC <sub>50</sub> values, and associated 95% C.I. determined for 24, 48, 72, and 96 hrs? NOEL also reported?	<ul style="list-style-type: none"> <li>Yes, page 20</li> <li>No LC<sub>50</sub> concentration-response curves provided</li> </ul>
Graph of concentration-mortality curve at test termination and any control mortality observed during acclimation or study period provided?	<ul style="list-style-type: none"> <li>No</li> </ul>
Any protocol deviations which may have influenced final results of test reported?	<ul style="list-style-type: none"> <li>No</li> </ul>
Raw data included?	<ul style="list-style-type: none"> <li>Yes, pages 21 and 22</li> </ul>
Signs of abnormal behavior by test fish (if any) described?	<ul style="list-style-type: none"> <li>Yes, page 22</li> </ul>
Statistical methods reported?	<ul style="list-style-type: none"> <li>Yes</li> </ul>

### Dose Response

Nominal Concentration (mg ai/L)	Cumulative % Mortality			
	24 hour	48 hour	72 hour	96 hour
Control	0	0	0	0
7.8	0	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>a,b</sup>
13	0	0	0 <sup>b</sup>	0 <sup>a,b</sup>
22	0	0 <sup>a</sup>	0 <sup>a,b</sup>	0 <sup>a,b</sup>
36	100	40 <sup>a</sup>	40 <sup>a,b</sup>	40 <sup>a,b</sup>
60	30	70 <sup>a,b</sup>	70 <sup>a,c</sup>	80 <sup>a,c</sup>

- a- All of the fish were respiring rapidly  
b- Some of the fish were dark colored  
c- All of the fish were dark colored



**Other Observed Effects:** Sublethal effects were recorded in survivors. Darkened pigment was noted at all concentrations and at all exposure times except 24 hours. Fish were respiring rapidly over all concentrations after 96-h exposure, and at concentrations greater than 22 mg ai/L after 72 and 48-h exposure.

**Statistical Results:** The dose related mortality data from the definitive toxicity test was used to estimate the 24-, 48-, 72-, and 96-h median lethal concentrations (LC<sub>50</sub>) and 95% confidence intervals. Toxanol was used to perform the statistical analysis.

Statistical Method: Moving average angle

96-hr LC<sub>50</sub>: 42 mg/L

95% C.I.: 34-53 mg/L

NOEC: < 7.8 mg/L

**Results Synopsis:**

Duration	LC <sub>50</sub> (mg a.i./L)	95% Upper CI	95% Lower CI
24-hr	> 60		
48-hr	44	59	35
72-hr	44	59	35
96-hr	42	53	34

NOEC through 96 hours = < 7.8

**13. VERIFICATION OF STATISTICAL RESULTS**

```

C:\WINDOWS\system32\cmd.exe
AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 40.6999

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
SPAN          G          LC50          95 PERCENT CONFIDENCE LIMITS
2             .2148829       41.719       34.04308      55.26382

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS    G          H          GOODNESS OF FIT PROBABILITY
6             .3312454       1           .8641912

SLOPE = 6.605585
95 PERCENT CONFIDENCE LIMITS = 2.799797 AND 10.41137

LC50 = 42.54592
95 PERCENT CONFIDENCE LIMITS = 34.04161 AND 55.62246

LC10 = 27.32727
95 PERCENT CONFIDENCE LIMITS = 14.74104 AND 34.13073
*****
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
?
    
```

Statistical Method: Moving Average

Results Verification Synopsis: 96-hr LC<sub>50</sub>: 41.7 mg/L

95% C.I.: 34.0-55.3 mg/L

14. **REVIEWER'S COMMENTS:** This study was completed before EPA OPPTS Guidelines were established. Many deficiencies were noted, including failure to explicitly state the number of replicates per treatment concentration, the absence of data to indicate a range-finding test was completed, failure to report proper concentration analysis throughout the definitive test to ensure accuracy of nominal values, absence of chemical analysis of water, and the absence of a concentration-mortality curve. In addition, the condition of the fish prior to testing was not noted. Based on these deficiencies, this study is considered to be Supplemental.

Sign-off Date : 01/02/08  
DP Barcode No. : D346246