

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

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

2. **TEST MATERIAL:** Milidin X-2 **Purity:** Not provided

3. **CITATION**

Author: Sousa, J.V. and LeBlanc, G.A.

Title: Acute Toxicity of Milidin X-2 to Rainbow Trout (*Salmo gairdneri*)

Laboratory: EG&G, Bionomics Aquatic Toxicology Laboratory, Wareham, MA

Date: April, 1982

Sponsor: Not reported

Study report ID: Not provided

Laboratory report ID: BW-82-4-1145

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: *Salmo gairdneri*

Age of Test Organism: Juvenile

Definitive Test Duration: 96 hours

Study Method: Static, Acute

Type of Concentrations: Nominal

7. **CONCLUSIONS:**

Results Synopsis:

96-hr LC₅₀: 71 mg/L

95% C.I.: 46-110 mg/L

NOEC: 17 mg/L



8. **ADEQUACY OF THE STUDY**

Classification: Supplemental

9. **GUIDELINE DEVIATIONS:**

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

- Purity and physiochemical characteristics of the test material were not provided.
- Study report identification number not reported.
- No chemical analysis of water used in test dilution was reported.
- Holding water and test water dilution came from two different sources and fish were not held in the test water dilution for a minimum of 7 days during the acclimation period.
- No discussion of pretest mortality rates for the 14-day acclimation period, only for the 48-hours prior to testing.
- No recording of the photoperiod, background colors, and light intensities during the acclimation and testing periods.
- Fish supplier, source population, breeding, and previous testing of test animals not reported.
- Biomass loading rate not supplied.
- Water salinity not specified.
- 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
<p>Species</p> <ul style="list-style-type: none"> ▪ Preferred freshwater species: bluegill sunfish (<i>Lepomis macrochirus</i>) or rainbow trout (<i>Oncorhynchus mykiss</i>) ▪ Preferred saltwater species: Atlantic silverside (<i>Menidia menidia</i>) or Sheepshead minnow (<i>Cyprinodon variegatus</i>) 	<ul style="list-style-type: none"> ▪ Rainbow Trout, <i>Oncorhynchus mykiss</i> (formerly <i>Salmo gairdneri</i>)
<p>Weight</p> <ul style="list-style-type: none"> ▪ Juvenile fish < 3.0 g 	<ul style="list-style-type: none"> ▪ 0.28 (0.18-0.42) grams wet weight
<p>Length</p> <ul style="list-style-type: none"> ▪ Longest not > 2x shortest 	<ul style="list-style-type: none"> ▪ 35(30-38) mm
<p>Supplier</p>	<ul style="list-style-type: none"> ▪ Information not provided
<p>All fish from same source and population?</p>	<ul style="list-style-type: none"> ▪ Information not provided
<p>Fish used in previous tests?</p>	<ul style="list-style-type: none"> ▪ Information not provided
<p>If wild fish used, quarantined 7 days before acclimation?</p>	<ul style="list-style-type: none"> ▪ Information not provided
<p>Signs of stress or injury?</p>	<ul style="list-style-type: none"> ▪ Information not provided

B. Acclimation

Guideline Criteria	Reported Information
<p>Acclimation Period</p> <ul style="list-style-type: none"> ▪ Minimum 12 days (14 days recommended) ▪ Minimum 7 days in test dilution water 	<ul style="list-style-type: none"> ▪ 14 day holding period prior to study
<p>Holding Water</p> <ul style="list-style-type: none"> ▪ Same source as test dilution water (if not, acclimation to dilution water done gradually over 48 hr period) 	<ul style="list-style-type: none"> ▪ The source of the holding water was well water, with a flow-through rate of 13-tank volume replacements/day. ▪ Total hardness range: 30-34 mg/L CaCO₃ ▪ Alkalinity range: 26-29 mg/L CaCO₃, ▪ Specific conductance range: 105-120 μhos/cm ▪ pH range: 7.1-7.2 ▪ DO range: 98-100% saturation at 12°C

Guideline Criteria	Reported Information
<p><u>Disease Treatment</u></p> <ul style="list-style-type: none"> No treatments within 48 hrs of test initiation or during test 	<ul style="list-style-type: none"> No data available
<p><u>Feeding</u></p> <ul style="list-style-type: none"> No feeding within 48 hrs of test initiation. Feed daily prior to this period. 	<ul style="list-style-type: none"> Fed dry pellets of food daily, <i>ad libitum</i> Not fed 48 hours prior to testing
<p><u>Pretest Mortality</u></p> <ul style="list-style-type: none"> < 5% during acclimation; reject entire batch if > 10%. 	<ul style="list-style-type: none"> 0.3% mortality rate in the test population during 48-hour period prior to testing
<p><u>Water Temperature</u></p> <ul style="list-style-type: none"> Temperature changes should not exceed 3°C per day Hold fish minimum 7 days at test temperature prior to testing 	<ul style="list-style-type: none"> 11-12°C in holding tank. Acclimation tank temperature and test temperature were the same.
<p><u>Background</u></p> <ul style="list-style-type: none"> During final 48 hrs, colors and light intensities similar to testing area 	<ul style="list-style-type: none"> No information available

C. Test System

Guideline Criteria	Reported Information
<p><u>Dilution Water</u></p> <ul style="list-style-type: none"> Reconstituted water or water from natural source preferred. If dechlorinated tap water, daily chlorine analysis performed. Chemical analysis performed and maximum concentrations not exceeded (see guideline) 	<ul style="list-style-type: none"> Soft water reconstituted from deionized water, using recommended EPA recommended procedure. Data from chemical analysis was not reported.
<p><u>Solutions</u></p> <ul style="list-style-type: none"> Distilled water used to make stock solutions of test substances. If stock volume > 10% of test solution volume, dilution water used. 	<ul style="list-style-type: none"> Milidin X-2 working stock solution of 15 mg/ml was prepared by adding 7.5 grams of Milidin X-2 to distilled water and then diluting to calculated volume in a 500 ml volumetric flask (p 5). 19.6 L glass jars, each with 15 L of test solution.

Guideline Criteria	Reported Information
<p>Water Temperature</p> <ul style="list-style-type: none"> ▪ 10 or 12 ± 2°C for cold water species (see guideline) ▪ 22 or 23 ± 2°C for warm water species (see guideline) ▪ Vary no more than 1°C in any 24-hr period ▪ Record in all replicates at beginning of test and every 24 hrs; record hourly in one replicate. 	<ul style="list-style-type: none"> ▪ Temperature was maintained at 12 ±1°C for the controls and test water ▪ Recorded temperatures ranged from 11-12 °C. ▪ Hourly record of temperature for one replicate not provided.
<p>pH</p> <ul style="list-style-type: none"> ▪ > 6.0 and < 8.0 for freshwater testing ▪ > 7.5 and < 8.5 for marine testing ▪ Measured in each replicate at beginning of test and every 24 hrs 	<ul style="list-style-type: none"> ▪ pH values were recorded at 0, 24, 48, and 96 hours in the control and high, middle, and low test concentrations. ▪ pH values ranged from 7.0-9.4 ▪ Measured pH values exceeded range for freshwater fish testing.
<p>Dissolved Oxygen</p> <ul style="list-style-type: none"> ▪ Static: > 60% saturation at all times ▪ Flow-through: > 75% saturation at all times ▪ Measured in each replicate at beginning of test and every 24 hrs 	<ul style="list-style-type: none"> ▪ The DO content was recorded at 0, 24, 48, and 96 hours in the control and high, middle, and low-test concentrations. ▪ In test and control conditions pH ranged from 8.6-11.6 mg/L (76 to >100 % saturation).
<p>Total Hardness</p> <ul style="list-style-type: none"> ▪ 40 to 180 mg/L as CaCO₃ (freshwater species) ▪ Measured at beginning of each test 	<ul style="list-style-type: none"> ▪ Dilution water used had a total hardness as CaCO₃ of 46 mg/L ▪ Measured at the beginning of the definitive toxicity test. ▪ Dilution water has a specific conductance of 140 μhos /cm.
<p>Salinity</p> <ul style="list-style-type: none"> ▪ 20 ± 5ppt (estuarine species) ▪ Measured at beginning of each test and, for flow-through tests, on day 4, and if extended days 7 and 14 	<ul style="list-style-type: none"> ▪ Not specified
<p>Test Aquaria/Equipment</p> <ul style="list-style-type: none"> ▪ Material: Glass, stainless steel, nylon screen or perfluorocarbon plastic (e.g., Teflon®) ▪ Test chambers loosely covered 	<ul style="list-style-type: none"> ▪ 19.6 L glass jars
<p>Aeration</p> <ul style="list-style-type: none"> ▪ Static systems only if < 60% saturation; if aeration used test concentrations measured. ▪ No aeration in flow-through tests 	<ul style="list-style-type: none"> ▪ Test solutions were not aerated ▪ 60% saturation at all times

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

D. Test Design

Guideline Criteria	Reported Information
Range-Finding Test <ul style="list-style-type: none"> If $LC_{50} > 100$ mg/L with 30 fish, then no definitive test required 	<ul style="list-style-type: none"> No evidence of range finding test
Test Concentrations <ul style="list-style-type: none"> Minimum of control and 5 concentrations in geometric series Concentrations 50 to 120% greater than next lowest concentration No more than 25% variation between test concentrations within same treatment Concentrations selected to produce NOEC and, preferably, at least 2 partial mortalities ($>$ and $<$ 50%) after 96 hrs Measured concentrations required if test chemical unstable or flow-through system, and must remain at least 80% of nominal concentrations 	<ul style="list-style-type: none"> In the definitive test, 5 concentrations (17, 28, 46, 48, and 110 mg/l) were analyzed for test organism effects. All concentrations were 50-120% greater than next lowest concentration. Data from range-finding test was not provided therefore appropriate concentrations could not be selected. One test concentration (17 mg/l) did produce NOEC. No evidence reported to indicate the stability of the test chemical and therefore it cannot be determined if measured concentrations are required.

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

12. REPORTED RESULTS

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements included in the report?</p>	<ul style="list-style-type: none"> ▪ No
<p>Name of test facilities, test dates and personnel reported?</p>	<ul style="list-style-type: none"> ▪ Yes, test facilities and dates reported on page 2 and personnel on page 11.

Guideline Criteria	Reported Information
Identification of test substance (including physicochemical characteristics) and purity provided?	<ul style="list-style-type: none"> No
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"> Yes, page 5 Accuracy of method not described.
LC ₅₀ concentration-response curves, LC ₅₀ values, and associated 95% C.I. determined for 24, 48, 72, and 96 hrs? NOEL also reported?	<ul style="list-style-type: none"> Yes, page 8 No LC₅₀ concentration-response curves
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"> No
Any protocol deviations which may have influenced final results of test reported?	<ul style="list-style-type: none"> No
Raw data included?	<ul style="list-style-type: none"> Yes, pages 9 and 10
Signs of abnormal behavior by test fish (if any) described?	<ul style="list-style-type: none"> Yes, page 10
Statistical methods reported?	<ul style="list-style-type: none"> Yes, page 6

Dose Response

Definitive Test

Nominal Concentration (mg ai/L)	Cumulative % Mortality			
	24 hour	48 hour	72 hour	96 hour
Control	0	0	0	0
17	0	0	0	0
28	0	0	0 ^d	0 ^d
46	0 ^d	0 ^d	0 ^d	0 ^e
68	0 ^d	0 ^{a,b}	0 ^{a,b}	20 ^{a,b}
110	100 ^{a,b,c}	100	100	100

- a- Some fish were swimming at the surface of the test solution
- b- Some fish exhibited darkened pigmentation.
- c- One fish exhibited a partial loss of equilibrium.
- d- One fish exhibited darkened pigmentation.
- e- Fish were respiring rapidly.

Other Effects Observed: Darkened pigmentation was observed in the latter half of the study in the 28 mg/L and 46 mg/L dose groups, as well as all timeframes of the 68 mg/L test group and at the 24 hour mark in the 110 mg/L test group. Fish were found swimming at the surface of the test solution during the 24-hour mark for the 110 mg/L test group, as well as at the 48, 72, and 96-hour test marks for the 69 mg/L test group. Fish were observed respiring rapidly at the 96-hour mark for the 46 mg/L test group.

Statistical Results: The dose related mortality data from the definitive toxicity test was used to estimate the 24-, 48, 72, and 96-hour median lethal concentrations (LC₅₀) and 95% confidence intervals. A computer program (Stephan, 1978) was used to perform the statistical analysis. The 24-hour value was empirically based and the rest of the values were estimated using the binomial probability method.

Results Synopsis:

Duration	LC ₅₀ (mg a.i./L)	95% Upper CI	95% Lower CI
24-hr ^a	>110		
48-hr	86	110	68
72-hr	86	110	68
96-hr	71	110	46

NOEC through 96 hours = 17

^a Empirically estimated

^b Estimated by the binomial probability method

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13. VERIFICATION OF STATISTICAL RESULTS

Toxanal was used to estimate the LC₅₀ values using the moving average angle analysis and the probit method.

```

C:\WINDOWS\system32\cmd.exe
EXPOSED      DEAD      DEATHS      PROB. <PERCENT >
110          10        100         9.765625E-02
68           10        20          5.46875
46           10        0           9.765625E-02
28           10        0           9.765625E-02
17           10        0           9.765625E-02

THE BINOMIAL TEST SHOWS THAT 46 AND 110 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 79.11428

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.

*****
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
?

```

Statistical Method: Binomial probability test

Results Verification Synopsis: 96-hr LC₅₀: 79.11 mg/L 95% C.I: 46 and 110 mg/L
NOEC: 17 mg/L

14. REVIEWER'S COMMENTS: Many deficiencies were noted in this study. Areas of major concern include 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.

References:

Stephan, Charles. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication (secondary reference as cited in study report).

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