DP Barcode: Not provided **MRID No:** 248883

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 (ADL ref. #82167) **Purity:** Not provided

3. **CITATION**

Author:	Sousa, J.V. and LeBlanc, G.A.
Title:	Acute Toxicity of Milidin X-2 to Bluegill (*Lepomis macrochirus*)
Laboratory:	EG&G, Bionomics Aquatic Toxicology Laboratory, Wareham, MA
Date:	March 30- April 3, 1982
Sponsor:	Not reported
Study report ID:	Not provided
Laboratory report ID:	BW-82-4-1144

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: *Lepomis macrochirus*
Age of Test Organism: Juvenile
Definitive Test Duration: 96-hours
Study Method: Static
Type of Concentrations: Nominal

7. **CONCLUSIONS:**

Results Synopsis:
Statistical Method: Moving average angle
96-hr LC_{50}: 39 mg/L
95% C.I.: 31-51 mg/L

NOEC: < 13

Verified Results Synopsis: same as reported

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.
- Physical condition and signs of disease in the fish were not provided
- 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.
- The temperature in the holding tank during the 48 hours prior to testing was 20 °C; protocol suggests
  22± 1 °C.
- Saturation was not maintained at >60% throughout the whole test.
- Pretest mortality rates were only given for the 48-hours prior to testing, not for the entire 14-day acclimation period,
- 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 determine appropriate 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 (time 0 hrs, 48 hrs, 96 hrs).
- Mortality observations were not recorded at 6-hours of exposure 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
Species ▪ Preferred freshwater species: bluegill sunfish (*Lepomis macrochirus*) or rainbow trout (*Oncorhynchus mykiss*) ▪ Preferred saltwater species: Atlantic silverside (*Menidia menidia*) or Sheepshead minnow (*Cyprinodon variegatus*)	▪ Bluegill sunfish (*Lepomis macrochirus*)
Weight ▪ Juvenile fish < 3.0 g	▪ 0.34 (0.20-0.52) grams wet weight
Length ▪ Longest not > 2x shortest	▪ 34 (30-38) mm
Supplier	▪ Commercial fish supplier in Connecticut (Bionomics lot #81A27)
All fish from same source and population?	▪ Same supplier as noted above
Fish used in previous tests?	▪ Not specified
If wild fish used, quarantined 7 days before acclimation?	▪ Not applicable
Signs of stress or injury?	▪ Not specified

B. Acclimation

Guideline Criteria	Reported Information
Acclimation Period ▪ Minimum 12 days (14 days recommended) ▪ Minimum 7 days in test dilution water	▪ 14 day acclimation period
Holding Water ▪ Same source as test dilution water (if not, acclimation to dilution water done gradually over 48 hr period)	▪ Source of water was a well that flowed through tank at a rate of 9-10 tank volume replacements/day. ▪ Hardness: 30-34 mg/L $CaCO_3$ ▪ Alkalinity: 26-29 mg/L $CaCO_3$ ▪ Specific conductance: 105-120 µmhos/cm ▪ pH: 6.8-7.0 ▪ DO: >100% saturation at 20°C

Guideline Criteria	Reported Information
Disease Treatment ▪ No treatments within 48 hrs of test initiation or during test	▪ Not specified
Feeding ▪ No feeding within 48 hrs of test initiation. Feed daily prior to this period.	▪ Fed dry pellet food daily , *ad libitum* ▪ Not fed 48 hours prior to testing.
Pretest Mortality ▪ < 5% during acclimation; reject entire batch if > 10%.	▪ 1.0% mortality in test fish population during 48-hours prior to testing
Water Temperature ▪ Temperature changes should not exceed 3°C per day ▪ Hold fish minimum 7 days at test temperature prior to testing	▪ 20-21°C during whole acclimation period ▪ Measured with a Brooklyn alcohol thermometer ▪ Test temperature and acclimation tank temperature were the same.
Background ▪ During final 48 hrs, colors and light intensities similar to testing area	▪ Photoperiod of 16 hours of light and 16 hours of dark ▪ Same as test conditions

C. Test System

Guideline Criteria	Reported Information
Dilution Water ▪ 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)	▪ Dilution water used was soft water reconstituted from deionized water, using EPA recommended procedure ▪ No chemical analysis data provided.
Solutions ▪ Distilled water used to make stock solutions of test substances. If stock volume > 10% of test solution volume, dilution water used.	▪ 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 diluting to calculated volume in a 500 ml volumetric flask ▪ Definitive test was conducted in 19.6 L glass jars, which contained 15 L of test solution.

Guideline Criteria	Reported Information
Water Temperature ▪ 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.	▪ Recorded temperatures range from 21-22°C
pH ▪ > 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	▪ Recorded pH values ranged from 6.8-9.4. ▪ Measured pH values exceeded range for freshwater fish testing. ▪ Recorded at 0, 24, 48, and 96 hours in the control and high, middle, and low test concentrations.
Dissolved Oxygen ▪ 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	▪ DO values: 1.2-11.4 mg/L or 14% saturation to > 100% saturation ▪ Recorded at 0, 24, 48, and 96 hours in the control and high, middle, and low test concentrations.
Total Hardness ▪ 40 to 180 mg/L as $CaCO_3$ (freshwater species) ▪ Measured at beginning of each test	▪ Total hardness: 46 mg/L $CaCO_3$ ▪ Total hardness was measured at the beginning of the definitive toxicity test. ▪ Alkalinity: 31 mg/L $CaCO_3$ ▪ Specific conductance: 140 µmhos/cm ▪ Specific conductance was only measured once, at the beginning of the definitive toxicity test.
Salinity ▪ 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	▪ Not provided

Guideline Criteria	Reported Information
Test Aquaria/Equipment ▪ Material: Glass, stainless steel, nylon screen or perfluorocarbon plastic (e.g., Teflon®) ▪ Test chambers loosely covered	▪ 19.6 L glass jars were used as test vessels. ▪ No discussion of vessel coverings
Aeration ▪ Static systems only if < 60% saturation; if aeration used test concentrations measured. ▪ No aeration in flow-through tests	▪ Test solutions were not aeration even though DO was below 60% saturation.
Type of Dilution System ▪ Must provide reproducible supply of toxicant	▪ Static
Flow Rate ▪ 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	▪ No flow
Biomass Loading Rate ▪ Static/Static-renewal: ≤ 0.8 g FWF/L ▪ Flow-through: ≤ 0.5 g FWF/L	▪ Not specified
Photoperiod ▪ Range from 12D/12N to 16D/8N, with 15 min transition period ▪ Intensity 30 to 100 lm at water surface	▪ Photoperiod of 16 hours of light and 16 hours of dark ▪ Same as acclimation period
Solvents ▪ 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	▪ Not specified

D. Test Design

Guideline Criteria	Reported Information
Range-Finding Test ▪ If LC_{50} > 100 mg/L with 30 fish, then no definitive test required	▪ No evidence of range- finding test

Guideline Criteria	Reported Information
Test Concentrations ▪ Minimum of control and 5 concentrations in geometic 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	▪ Five concentrations and a control were analyzed (13, 22, 36, 60, and 100 mg/L) during the definitive toxicity test. ▪ Concentrations were used to calculate LC_{50} values and NOEC. ▪ No information provided on stability of chemical in test solution, thus cannot determine if measured concentrations are required.
Concentration Analysis ▪ 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	▪ Not specified
Controls ▪ 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.	▪ The negative control contained the same dilution water as used in the exposure jars (p16). ▪ Control data indicates that maximum allowable mortality was not exceeded.
Replicates ▪ Two per test concentration ▪ Equal volume test solution and number test fish	▪ 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

Guideline Criteria	Reported Information
Test Organisms ▪ 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	▪ 10 Bluegill per replicate ▪ The Bluegill were randomly distributed to each test jar within 30 minutes of addition of the test substance ▪ Biological observations made every 24 hours.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in the report?	▪ No
Name of test facilities, test dates and personnel reported?	▪ Yes, page 13
Identification of test substance (including physicochemical characteristics) and purity provided?	▪ Yes, page 14
Methods used in preparation of stock solutions and analysis of test concentrations described? Accuracy of method (i.e., detection limit and quantification limit) reported?	▪ Yes, page 16
LC_{50} concentration-response curves, LC_{50} values, and associated 95% C.I. determined for 24, 48, 72, and 96 hrs? NOEL also reported?	▪ Yes, page 20 ▪ No LC_{50} concentration-response curves
Graph of concentration-mortality curve at test termination and any control mortality observed during acclimation or study period provided?	▪ No
Any protocol deviations which may have influenced final results of test reported?	▪ Yes, page 18
Raw data included?	▪ Yes, pages 21 and 22
Signs of abnormal behavior by test fish (if any) described?	▪ Yes, page 22
Statistical methods reported?	▪ Yes

Dose Response

Nominal Concentration (mg ai/L)	Cumulative % Mortality			
	24 hour	48 hour	72 hour	96 hour
Control	0	0	0	0
13	0	0	0[c]	0[c,f,g]
22	0	0	0[c,d]	10[c,f]
36	10	10	20	30[c,f]
60	40[a]	80[b]	80[b]	90[d,e]
100	100	100	100	100

[a] Six fish were lethargic
[b] Two fish were lethargic
[c] Test solutions became cloudy
[d] One fish was lethargic
[e] Fish was swimming erratically
[f] Fish were respiring rapidly
[g] Two fish had darkened pigmentation

Other Effects Observed: Sublethal effects were seen in all test groups, except the high dose group where the effects were 100% mortality. At the 72- and 96-hour marks for the 13 and 22 mg/L dose groups, cloudy test solutions, lethargy, rapid respiration, erratic swimming, and darkened pigmentation were observed. Cloudiness and rapid respiration were also observed at the 96-hour mark in the 36 mg/L dose group. Six fish were lethargic at 24 hours in the 60 mg/L dose group, with two still lethargic at the 48- and 72-hour marks. Lethargy and erratic swimming were also seen at 96 hours in the 60 mg/L dose 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 (LC_{50}) and 95% confidence intervals. A computer program (Stephan, 1978) estimated LC_{50} values using the moving average angle method.

Results Synopsis:

Duration	LC_{50} (mg a.i./L)	95% Upper CI	95% Lower CI
24-hr	57	71	47
48-hr	49	60	40
72-hr	47	57	38
96-hr	39	50	31

NOEC through 96 hours = < 13

VERIFICATION OF STATISTICAL RESULTS: Toxanal was used to estimate the LC_{50} values using the moving average angle analysis and the probit method.

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AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 42.22463


RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
 SPAN          G              LC50          95 PERCENT CONFIDENCE LIMITS
 4              .1145314       39.84167       38.88863       50.39293


RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS     G              H              GOODNESS OF FIT PROBABILITY
 5              .2436731       1              .8164625

SLOPE     =      6.276357
95 PERCENT CONFIDENCE LIMITS   = 3.178144      AND     9.374571

LC50 =           39.52614
95 PERCENT CONFIDENCE LIMITS = 31.50432 AND  49.73477

LC10 =           24.80492
95 PERCENT CONFIDENCE LIMITS = 14.6508 AND  31.19545
**********************************************************************
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
?
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Statistical Method: Moving Average
Results Verification Synopsis: 96-hr LC_{50}: 39.04 mg/L 95% C.I: 30.89- 50.39 mg/L
NOEC: Not available

Statistical Method: Probit Method
Results Verification Synopsis: 96-hr LC_{50}: 39.53 mg/L 95% C.I: 31.50-49.73 mg/L
NOEC: Not available

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.

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