

00073588

DP Barcode: Not provided

MRID No: ~~240113106~~

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: G. Raasina
Title: Four-Day Static Fish Toxicity Studies with Milidin X-2 in Rainbow Trout and Bluegills
Report Date: July 23, 1973
Laboratory: Industrial Bio-test Laboratories, Inc.
Sponsor: DeMille Chemical Corporation
Laboratory Report ID: IBT No. 665-03509
MRID No.: Corrected MRID is 00073588 B. Montague

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

Signature: **Date:**

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

Signature: **Date:**

- 6. **STUDY PARAMETERS:**

Scientific Name of Test Organisms: *Oncorhynchus mykiss* (formerly *Salmo gairdneri*) and *Lepomis macrochirus*
Age of Test Organism: Juvenile
Definitive Test Duration: 96 hours
Study Method: Static
Type of Concentrations: Nominal: 18, 32, 56, 78, and 100 mg/L

- 7. **CONCLUSIONS:**

Results Synopsis: Rainbow Trout: 96-hr LC₅₀: 74 mg/L



	95% C.I.: 61 – 89 mg/L
	NOEC: 18 mg/L
Bluegill:	96-hr LC ₅₀ : 40 mg/L
	95% C.I.: 33 – 49 mg/L
	NOEC: 18 mg/L
Verified Results Synopsis:	
Rainbow Trout	96-hr LC ₅₀ : 73.99 mg/L
	95% C.I.: 64.2 – 83.4 mg/L
	NOEC: Not available through TOXANAL program
Bluegills	96-hr LC ₅₀ : 39.84 mg/L
	95% C.I.: 32.2 – 48.3 mg/L
	NOEC: Not available through TOXANAL program

8. ADEQUACY OF THE STUDY:

Classification: Supplemental

9. GUIDELINE DEVIATIONS:

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

- Length of longest fish is > 2x the shortest fish
- Fish were fed until 72 hours prior to test initiation rather than the recommended 48 hours
- pH and dissolved oxygen (DO) were only measured daily for the control and if mortality occurred rather than daily for the control and all test groups
- pH and DO were outside of the recommended ranges for a static test
- One replicate per group was tested
- Not provided:
 - LC50 and 95% confidence limits for 24, 48, and 72 hours
 - Range-finding study data
 - Data on test material (purity, physiochemical characteristics, etc.)
 - Temperature of water during test period
 - Water hardness and salinity
 - Methods and data from water sample analysis verifying concentration and impurities
 - Data on measured concentrations
 - Pretest mortality and disease treatment
 - Description of acclimation and test facilities
 - Weight, supplier of fish, and whether fish were used in other tests prior to this study
 - Data on fish placement in test chamber and whether distributed randomly
 - Loading information (<0.5 FWF per L of test material)

- Construction materials used for the test tanks, covers over tanks
- Calibration or aeration of test system and flow rate
- Photoperiod and light intensity
- Method of stock preparation
- Test date and personnel
- List of any protocol deviations occurring during test dates
- Quality assurance and GLP compliance statements
- Concentration-response and concentration-mortality curves

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>Salmo gairdnerii</i>) and bluegill sunfish (<i>Lepomis macrochirus</i>)
<p>Weight</p> <ul style="list-style-type: none"> ▪ Juvenile fish < 3.0 g 	<ul style="list-style-type: none"> ▪ Not provided
<p>Length</p> <ul style="list-style-type: none"> ▪ Longest not > 2x shortest 	<ul style="list-style-type: none"> ▪ Length: 35 to 75 mm ▪ Longest is > 2x the shortest
<p>Supplier</p>	<ul style="list-style-type: none"> ▪ Not provided
<p>All fish from same source and population?</p>	<ul style="list-style-type: none"> ▪ Not provided
<p>Fish used in previous tests?</p>	<ul style="list-style-type: none"> ▪ Not provided
<p>If wild fish used, quarantined 7 days before acclimation?</p>	<ul style="list-style-type: none"> ▪ Not provided
<p>Signs of stress or injury?</p>	<ul style="list-style-type: none"> ▪ No

B. Acclimation

Guideline Criteria	Reported Information
<p><u>Acclimation Period</u></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"> ▪ All fish were observed for general health and suitability as test animals for a period of not less than 14 days prior to experimental use in preliminary screening studies ▪ 24-hour acclimation period before definitive study
<p><u>Holding Water</u></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"> ▪ Reconstituted deionized water supplemented, per liter, with 30 mg calcium sulfate, 30 mg magnesium sulfate, 48 mg sodium bicarbonate, and 2 mg potassium chloride
<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"> ▪ Not provided
<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 frozen brine shrimp or Purina Trout Chow #2 until 72 hours prior to test initiation
<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"> ▪ Not provided
<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"> ▪ Rainbow trout held at 12°C for a minimum of 14 days prior to testing ▪ Bluegills held at 18°C for a minimum of 14 days prior to testing
<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"> ▪ Not provided

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"> ▪ Reconstituted deionized water supplemented, per liter, with 30 mg calcium sulfate, 30 mg magnesium sulfate, 48 mg sodium bicarbonate, and 2 mg potassium chloride ▪ Chemical analysis not provided
<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"> ▪ Stock solution prepared within one hour of dosing ▪ Stock solution dispensed as a 10% (w/v) aqueous solution
<p><u>Water Temperature</u></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"> ▪ Not measured during the test ▪ Water temperature in the holding tanks was 12°C for rainbow trout and 18°C for bluegills
<p><u>pH</u></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 measured for the groups which experienced mortality and daily for the untreated control group ▪ For the test groups measured for rainbow trout, the pH ranged from 7.8 to 9.4 ▪ For the test groups measured for bluegills, the pH ranged from 8.7 to 9.3 ▪ The control pH ranged from 7.1 to 7.3 for rainbow trout and 6.9 to 7.1 for bluegills

Guideline Criteria	Reported Information
<p><u>Dissolved Oxygen</u></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"> ▪ Dissolved oxygen values were measured for the groups which experienced mortality and daily for the untreated control group ▪ For the test groups measured for rainbow trout, the DO ranged from 7.7 to 9.3 ppm (61.6 to 74.4%) ▪ For the test groups measured for bluegills, the DO ranged from 6.5 to 8.5 ppm (52 to 68%) ▪ The control DO ranged from 6.6 to 8.0 ppm (52.8 to 64%) for rainbow trout and 7.4 to 7.9 ppm (59.2 to 63.2%) for bluegills
<p><u>Total Hardness</u></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"> ▪ Not provided
<p><u>Salinity</u></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 provided
<p><u>Test Aquaria/Equipment</u></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"> ▪ Vessel lined with polyethylene bags
<p><u>Aeration</u></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"> ▪ Not provided
<p><u>Type of Dilution System</u></p> <ul style="list-style-type: none"> ▪ Must provide reproducible supply of toxicant 	<ul style="list-style-type: none"> ▪ Static
<p><u>Flow Rate</u></p> <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"> ▪ Not provided

Guideline Criteria	Reported Information
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"> Mass of fish not provided so loading rate can not be calculated
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"> Not provided
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"> Solvent not used in this study

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"> A preliminary screening test was performed; however the methods and results were not provided
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"> Test concentrations: 18, 32, 56, 78, and 100 mg/L Concentrations within the 50 to 120% greater than next lowest concentration range

Guideline Criteria	Reported Information
<p><u>Concentration Analysis</u></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><u>Controls</u></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"> ▪ Untreated control consisted of dilution water and was treated similarly to the dosed groups ▪ A 'quality check' using toxaphene was also used and dispensed in a 0.01 % (w/v) solution in acetone ▪ The untreated controls for both rainbow trout and bluegills had zero mortality
<p><u>Replicates</u></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"> ▪ One per test concentration
<p><u>Test Organisms</u></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 fish per test concentration per test vessel ▪ Not fed during treatment ▪ Methods for placement of fish into vessels were not provided ▪ Observations made at 1-6 hours, 24, 48, 72, and 96 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 and No, page 2 (test dates and all personnel not reported)
Identification of test substance (including physicochemical characteristics) and purity provided?	▪ Yes and No, page 1 (only description and source were reported)
Methods used in preparation of stock solutions and analysis of test concentrations described? Accuracy of method (i.e., detection limit and quantification limit) reported?	▪ No
LC₅₀ concentration-response curves, LC₅₀ values, and associated 95% C.I. determined for 24, 48, 72, and 96 hrs? NOEL also reported?	▪ Yes and No (only LC values at 96 hours were reported on pages 2, 6 & 8 and NOEL on pages 11 & 12)
Graph of concentration-mortality curve at test termination and any control mortality observed during acclimation or study period provided?	▪ No and Yes (pages 7 & 8 for study period mortality)
Any protocol deviations which may have influenced final results of test reported?	▪ No
Raw data included?	▪ Yes, pages 6 to 18
Signs of abnormal behavior by test fish (if any) described?	▪ Yes, pages 11, 12, 17 & 18
Statistical methods reported?	▪ Litchfield and Wilcoxon tests used to determine the LC ₅₀ when possible

Dose Response

Rainbow Trout:

Nominal Concentration (mg/L)	Percent Survival	Number of Surviving Fish (10 fish per group)				
		1 - 6 hour	24 hour	48 hour	72 hour	96 hour
Control	100	10	10	10	10	10
18	100	10	10	10	10	10
32	100	10	10	10	10	10
56	90	10	10	10	10	9
78	50	10	10	7	5	5
100	0	10	8	0	0	0

Bluegills:

Nominal Concentration (mg/L)	Percent Survival	Number of Surviving Fish (10 fish per group)				
		1 - 6 hour	24 hour	48 hour	72 hour	96 hour
Control	100	10	10	10	10	10
18	100	10	10	10	10	10
32	80	10	10	8	8	8
56	10	10	7	4	3	1
78	0	10	3	1	0	0
100	0	10	3	0	0	0

Statistical Results: Litchfield and Wilcoxon tests used to determine the LC₅₀ when possible.

Results Synopsis:

Rainbow Trout:

Duration	LC ₅₀ (mg a.i./L)	95% Upper CI	95% Lower CI
24-hr	Not provided	Not provided	Not provided
48-hr	Not provided	Not provided	Not provided
72-hr	Not provided	Not provided	Not provided
96-hr	74 mg/L	89 mg/L	61 mg/L

NOEC through 96 hours = 18 mg/L

Bluegills:

Duration	LC ₅₀ (mg a.i./L)	95% Upper CI	95% Lower CI
24-hr	Not provided	Not provided	Not provided
48-hr	Not provided	Not provided	Not provided
72-hr	Not provided	Not provided	Not provided
96-hr	40 mg/L	49 mg/L	33 mg/L

NOEC through 96 hours = 18 mg/L

Other Effects Observed: The following tables exhibit other toxic effects observed in rainbow trout and bluegills during the study.

Rainbow Trout:

Concentration (mg/L)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
Control	None	-	-	-
18	No observable difference from control	-	-	-
32	Dark discoloration	24	No recovery	-
	Quiescent	24	No recovery	
56	Dark discoloration	1-6	No recovery	72-96
	Quiescent	1-6	No recovery	
78	Dark discoloration	1-6	No recovery	24-72
	Quiescent	1-6	No recovery	
	Surfacing	24	No recovery	
100	Dark discoloration	1-6	Until death	6-48
	Quiescent	1-6	Until death	
	Surfacing	24	Until death	

Bluegills:

Concentration (mg/L)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
Control	None	-	-	-
18	No observable difference from control	-	-	-
32	Dark discoloration	24	No recovery	24-48
	Quiescent	24	No recovery	

56	Dark discoloration	24	No recovery	6-96
	Quiescent	24	No recovery	
	Surfacing	24	No recovery	
78	Dark discoloration	24	Until death	6-72
	Quiescent	24	Until death	
	Surfacing	24	Until death	
100	Dark discoloration	24	Until death	6-48
	Quiescent	24	Until death	
	Surfacing	24	Until death	

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Toxanal was used to verify results. The screen caps from the results of the program appear below.

Rainbow Trout

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RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
SPAN      G      LC50      95 PERCENT CONFIDENCE LIMITS
4          9.429869E-02      67.45261      58.14929
80.87186

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS  G      H      GOODNESS OF FIT PROBABILITY
15          .3084593      1      .2298245

SLOPE = 12.69295
95 PERCENT CONFIDENCE LIMITS = 5.643842 AND 19.74405

LC50 = 73.99808
95 PERCENT CONFIDENCE LIMITS = 64.21668 AND 83.48776

LC10 = 58.76612
95 PERCENT CONFIDENCE LIMITS = 41.17338 AND 66.81251
    
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Bluegills

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RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
SPAN      G      LC50      95 PERCENT CONFIDENCE LIMITS
3          .1051672      38.54733      31.61095      46.12952

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS  G      H      GOODNESS OF FIT PROBABILITY
6          .2549674      1      .9954882

SLOPE = 9.122963
95 PERCENT CONFIDENCE LIMITS = 4.1682 AND 14.07773

LC50 = 39.64321
95 PERCENT CONFIDENCE LIMITS = 32.18533 AND 48.33411

LC10 = 28.91646
95 PERCENT CONFIDENCE LIMITS = 17.92572 AND 34.95697
    
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Verified Results Synopsis:

Rainbow Trout 96-hr LC₅₀: 73.99 mg/L
 95% C.I.: 64.2 – 83.4 mg/L

Bluegill 96-hr LC₅₀: 39.84 mg/L
95% C.I.: 32.2 – 48.3 mg/L

14. REVIEWER'S COMMENTS: Verification statistics indicate that the rainbow trout LC₅₀ for the 96-hour study is 73.99 mg/L, which is similar to what was calculated by the study's author (LC₅₀ = 74 mg/L). Verification statistics also indicate that the bluegill LC₅₀ for the 96-hour study is 39.84 mg/L, which is similar to what was calculated by the study's author (LC₅₀ = 40 mg/L).

Deviations from the protocol do not likely impact the main conclusions of the study. However, since the study did not provide information concerning fish weight, chemical analyses on dilution water, or concentration analysis, and because temperature, pH, and dissolved oxygen levels were reported only for selective groups rather than daily for each group, this study is considered to be Supplemental.

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