

US EPA ARCHIVE DOCUMENT

**DATA EVALUATION RECORD
FRESHWATER FISH EARLY LIFE-STAGE TEST
GUIDELINE 72-4(A)**

1. **CHEMICAL**: Molinate

Shaughnessey #: 041402

2. **TEST MATERIAL**: ¹⁴C-Ordram

Purity: "Technical"

3. **CITATION**:

Authors: William McAllister

Title: Early Life Stage Toxicity of ¹⁴C-Ordram to Rainbow Trout (Salmo gairdneri) in a Flow-through System

Study Completion Date: June 19, 1987

Laboratory: Analytical Bio-Chemistry Laboratories, Inc.

Laboratory Report ID: 35223

Sponsor: ICI Americas

MRID No.: 406578-01

4. **REVIEWED BY**:

F. Nicholas Mastrota, Wildlife Biologist
ERB II, EFED, USEPA

Signature:

Date:

5. **PEER REVIEW BY**:

Andrew C. Bryceland, Fisheries Biologist
ERB II, EFED, USEPA

Signature:

Date:

6. **CONCLUSIONS**: This study is scientifically sound, but cannot be used to fulfill the test requirement for an early life-stage study with molinate because of significant guideline deviation, and because raw data and pertinent information on test conditions were not provided. According to this study, the NOAEL and LOAEL for effects of molinate on the early life stage of the trout are 0.39 and 0.83 mg ai/L, respectively.

7. **ADEQUACY OF THE STUDY**:

A. Classification: Supplemental

B. Rationale: The dilution water had excessive hardness and pH, the time to hatch was not measured, the raw data were not provided, and incomplete information was not

provided on test methodology.

C. Reparability: Not repairable.

8. MAJOR GUIDELINE DEVIATIONS:

1. The study report did not include raw data, i.e., counts and means for each replicate. This precluded the statistical results from being verified. Furthermore, raw data on the morphological and behavioral observations were not provided.
2. The purity of the test material was not reported. The test material was labeled as "Technical Ordram", which, according to REFS, has a purity of 96%.
3. The age of the trout embryos at the beginning of the study was not reported.
4. The study report did not state how often the fish were fed, or if feeding ceased 24 hours prior to termination of the study.
5. Counts of surviving fish were made at day 36 and 60 posthatch, whereas the Standard Evaluation Procedure (SEP) states that, at a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.
6. The dilution water exceeded SEP standards for hardness and pH. The hardness was 316 mg/L as CaCO₃, whereas the SEP standard is 40-48 mg/L as CaCO₃. The pH was 7.8-8.3, whereas the SEP standard is 7.2-7.6.
7. Test temperatures were not consistent. Readings were generally between 11 and 13 °C, but dropped to 10 °C on Day 63 and to 8 °C on Day 73.
8. DO and pH were measured in the control and only two test concentrations, whereas the SEP states that measurements should be made at each concentration. Also, the freshwater parameters of alkalinity and hardness, which should be measured weekly, were only measured on Day 30.
9. Two required endpoints, time to hatch and time to swim-up, were not measured.
10. Embryo cups were made of polyethylene tubing. Polyethylene is an inappropriate material for a test vessel since it is known to absorb residues of some pesticides.

9. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: Any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See SEP for complete listing.	Rainbow trout (<i>Onchorhynchus mykiss</i>)
Source	Mt. Lassen Trout Farm, Red Bluff, Cal.
Age at beginning of test: Embryos 2 to 24 hours old.	Embryos (age not reported)
Replicates: Minimum of 20 embryos per replicate cup, 4 replicates per concentration. Minimum of 30 fish per treatment for post-hatch exposure.	20 embryos per replicate, with 4 replicates per concentration. All hatched fry were exposed during the post-hatch period.
Post Hatch: % of embryos that produce live fry must be $\geq 50\%$ in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.	100% of eggs in control cups hatched.
Feeding: Fish should be fed at least twice daily. Fish should not be fed for at least 24 hr prior to termination on day 32.	Fish were fed beginning 11 days post-hatch.
Counts: At a minimum, live fish should be counted 11, 18, 25, and 32 days after hatching.	Counts of surviving fish were made at day 36 and 60 posthatch.
Controls: Avg. survival at end of test must be $\geq 80\%$. Survival in any control chamber must not be $< 70\%$.	Overall survival in the control was 90%. Survival was not reported for individual chambers.
Controls: Negative control and carrier control (when applicable) are required.	Negative control and solvent control

Comments:

B. Physical System:

Guideline Criteria	Reported Information

<p>Test Water: 1) May be natural or reconstituted; 2) Natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants. 3) Hardness of 40 to 48 mg/L as CaCO₃ and pH of 7.2 to 7.6 is recommended.</p>	<p>Aerated well water was used. Analysis indicated that pesticides and other contaminants were below detection limits. Hardness: 316 mg/L as CaCO₃ pH: 8.0-8.1</p>
<p>Test Temperature: Depends upon test species; should not deviate by more than 2°C from appropriate temperature.</p>	<p>Temperatures readings generally ranged from 11 to 13 °C, but was 10 °C on Day 63 and was 8 °C on Day 73.</p>
<p>Photoperiod: Recommend 16L/8D.</p>	<p>16L/8D</p>
<p>Dosing Apparatus: Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.</p>	<p>The study used an intermittent flow proportional diluter with a Hamilton® Model 420 syringe dispenser. The measured test concentrations were 0.049, 0.098, 0.20, 0.39, and 0.83 mg/l (approximately a 0.5 dilution factor).</p>
<p>Toxicant Mixing: 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%.</p>	<p>Mixing chambers without aeration were used. Flow-splitting accuracy was not reported.</p>
<p>Test Vessels: All glass or glass with stainless steel frame.</p>	<p>The study used glass vessels with stainless steel drains.</p>
<p>Embryo Cups: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</p>	<p>Embryo cups were made of 7-cm polyethylene tubing with 16 mesh stainless steel screening fused to the bottom.</p>
<p>Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours. Flow rate must maintain DO at above 75% of saturation and maintain the toxicant level.</p>	<p>The flow rate was approximately 60 ml/minute/replicate, enough to replace a replicate volume 7.4 times in a 24 hr period. DO ranged from 62 to 90% of saturation.</p>
<p>Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.</p>	<p>The dilution water was aerated.</p>

Comments: The study report states that diluter malfunctioned near the end of the study, sometime late on Day 72 or early on Day 73. The authors claim that this caused the refrigerator system to over cool the water in the test chambers, resulting in the low temperature of 8°C

recorded on Day 73.

C. Chemical System:

Guideline Criteria	Reported Information
<p>Concentrations: Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</p> <ul style="list-style-type: none"> - Toxicant conc. must be measured in one tank at each toxicant level every week. - One concentration must adversely affect a life stage and one concentration must not affect any life stage. 	<p>There were 5 test concentrations, a control, and a solvent control, each with 4 replicates. Concentrations were measured at each test level. The test concentrations yielded both a LOAEL and an NOAEL.</p>
<p>Other Variables:</p> <ol style="list-style-type: none"> 1) DO must be measured at each conc. at least once a week; 2) Freshwater parameters in a control and one concentration must be analyzed once a week. 	<p>DO, pH, and conductivity were measured on Day 0 and Day 1, and weekly thereafter. Measurements were made in the control, at a medium concentration, and at the highest concentration with surviving eggs and fry. The only freshwater parameter that was measured weekly was conductivity. Hardness and alkalinity were measured only on Day 30.</p>
<p>Solvents: Should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p>	<p>Acetone was used as a solvent. The highest solvent concentration was 27 ppm (0.027 ml/L).</p>

Comments:

10. REPORTED RESULTS:

Guideline Criteria	Reported Information
<p>Data Endpoints must include:</p> <ul style="list-style-type: none"> - Number of embryos hatched; - Time to hatch; - Mortality of embryos, larvae, and juveniles; - Time to swim-up (if appropriate); - Measurement of growth; - Incidence of pathological or histological effects; - Observations of other effects or clinical signs. 	<p>Data Endpoints included:</p> <ul style="list-style-type: none"> - Number of embryos hatched; - Mortality of embryos, larvae, and juveniles; - Measurements of growth (length and wet weight); - Observations of morphological and behavioral effects.
<p>Raw data included? (Y/N)</p>	<p>No. Only summary data was provided; data on individual replicates were absent.</p>

Effects Data:

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Toxicant Conc. ($\mu\text{g/L}$)		Percent Hatch	Percent Survival (36 days)	60-Day Total Length (mm)	60-Day Wet weight (mg)
Nom.	Meas.				
Ctrl	--	100	90	38	860
Solv	--	100	91	38	870
0.50	0.049	100	96	37	830
0.10	0.098	100	98	37	870
0.20	0.20	100	80	38	940
0.40	0.39	100	91	38	900
0.80	0.83	100	29*	43	1300

* Significantly different from the control ($P < 0.05$).

Toxicity Observations: "Morphological and behavioral abnormalities such as curved spine, fish resting on the bottom of the test chamber, loss of equilibrium, and quiescence were minimal and were scattered throughout the controls and test level. For most part, effects were transient and did not appear to be part of a dose response pattern."

Statistical Results:

Statistical Method: ANOVA/Tukey's test for mean comparisons

NOAEL: 0.39 mg/L

LOEAL: 0.83 mg/L

MATC: 0.57 mg/L

Most sensitive endpoint: Survival

Comments: Growth measurements at the highest test level were not included in the ANOVA analysis because there was significant variability in response between replicates at this level. The apparent increase in growth at this level was attributed to the decreased competition for food caused by the decreased survival.

11. Reviewer's Statistical Results:

Comments: Statistical results could not be verified because the study report did not include data on individual replicates. However, visual inspection of the treatment level means indicate that the NOAEL and LOAEL are 0.39 and 0.57 mg/L, respectively.