

4/23/96

DATA EVALUATION RECORD  
AQUATIC INVERTEBRATE LIFE CYCLE TEST  
GUIDELINE 72-4(B)

1. **CHEMICAL:** Molinate PC Code No.: 041402  
~~108401~~
2. **TEST MATERIAL:** Technical grade molinate  
**Purity:** Not reported
3. **CITATION**  
**Authors:** Bailey, Howard C.  
**Title:** Acute and chronic toxicity of the rice herbicides thiobencarb and molinate to Opposum shrimp (*Neomysis mercedis*)  
**Publication Date:** 1993  
**Laboratory:** SRI International  
**Sponsor:** California State Water Resources Control Board  
**MRID No.:** 43976801
4. **REVIEWED BY:** F. Nicholas Mastrotta, Biologist, ERCB  
**Signature:** *F. Nicholas Mastrotta* **Date:** 4/15/96
5. **PEER REVIEW BY:** Robert K. Hitch, Ecologist, ERCB  
**Signature:** *Robert K. Hitch* **Date:** 4/23/96
6. **STUDY PARAMETERS**  
**Age of Test Organism:** Not reported  
**Definitive Test Duration:** 56 days  
**Study Method:** Flow-through  
**Type of Concentrations:** Mean measured
7. **CONCLUSIONS:** This published paper provides supplemental information on the chronic toxicity of molinate to saltwater shrimp and shrimp-like species. It is best described as a shrimp early life-stage test. This test is scientifically sound, but does not satisfy the guideline requirements for a aquatic invertebrate life-cycle test since effects on reproduction were not evaluated. The results show that chronic exposure to molinate at concentrations greater or equal to 45.2 µg/L can significantly reduce the survival of young opossum shrimp.

**Results Synopsis**

NOEC: 25.6 µg ai/L      LOEC: 45.2 µg ai/L  
MATC: 34.0 µg ai/L

LOEC's for specific effects



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Larvae Survival: 25.6 µg ai/L  
Growth (Length): ≥ 25.6 µg ai/L

**8. ADEQUACY OF THE STUDY**

**A. Classification:** Supplemental.

**B. Rationale:** The test was not a life-cycle test and have several grave deviations from the guidelines for a life-cycle aquatic invertebrate test (GLN 72-4b). The experiment design was similar to that used for a fish early life-stage test (GLN 72-4a), but it cannot be used to fulfill this guideline since the test species was not a fish. Also, the published paper was lacking some information required for analyzing this study.

**C. Repairability:** None.

**8. MAJOR GUIDELINE DEVIATIONS:**

1) The experimental design was not a life-cycle test. Adult shrimp were not paired. Instead, gravid shrimp were selected from the parental stock and placed into test solutions to provide offspring. The young were then maintained in the test solutions until the termination of the test, at which time their survival and length were recorded. Thus, this study examines developmental effects rather than reproductive effects. Although the experimental design is scientifically sound, it is not acceptable for fulfilling the data requirement for the life-cycle aquatic invertebrate test with an aquatic invertebrate (guideline 72-4b).

2) The endpoints measured differed from those required for the life-cycle aquatic invertebrate test. Survival data was recorded on the young rather than the adult organisms, and survival of males and females were not recorded separately. Although the number of young produced was measured, this measure does not reflect effects on reproduction since the females were already gravid when they were first exposed. The dry weights were not measured for either the first or second generation. The lengths of the young alive at the end of the study were measured, but not separately for males and females. Also, results on length were only reported for concentrations that did not have a significant decrease in survival. A reduction in survival should not preclude the analysis of effects on length.

3) The duration of the acclimation period was not reported.

- 4) Therefore, there was an insufficient number of test organisms per treatment level. The ASTM guidelines for a mysid life-cycle toxicity test (E 1191-90) states that at least two compartments should be placed in each two replicate chambers at each treatment level, and at least 15 organisms should be placed in each of these compartments. This means that there should be at least 60 organisms per treatment level. In the present test, two replicate test chambers were used without compartments, and 15-20 shrimp were placed in each chamber. Therefore, only 30-40 organisms were used per treatment level.
- 5) Summary statistics were only reported on a per treatment level basis. Summary statistics for each replicate is needed to confirm statistical analysis.
- 6) The dilution water was dechlorinated tap water, which is not recommended since dechlorination is usually not complete.
- 7) A serial diluter system was not used to deliver test concentrations. Instead, separate solutions were prepared for each of five test concentrations which were delivered to test chambers from Mariotte bottles.
- 8) Crystallizing dishes were used for test vessels. These dishes are not designed for this purpose and may not be appropriate. The volume of these dishes was not reported.
- 9) The flow rate was approximately 2 volumes/24 h, whereas it should have been 5 to 10 volumes/24 h.
- 10) Quality assurance and GLP compliance statements were not provided.
- 11) Observations for clinical signs of toxicity were not reported.

10. MATERIALS AND METHODS:A. Biological System:

Guideline Criteria	Reported Information
<b>Species:</b> An estuarine shrimp species, preferably <i>Americamysis bahia</i> .	Opossum shrimp ( <i>Neomysis mercedis</i> )
<b>Duration</b> 28 days/one generation	56 days
<b>Source</b> (or supplier)	Montezuma Lough, Sacramento-San Joaquin Delta, CA
<b>Parental Acclimation</b> 1) Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids should be in good health.	Parental stock was maintained in dilution water and under test conditions.
<b>Parental Acclimation Period</b> At least 14 days	Not reported
<b>Chamber Location:</b> Treatments should be randomly assigned to test chamber locations.	Adult gravid shrimp were randomly assigned to treatments. Young produced within each treatment level were pooled and then randomly distributed between the two replicate test chambers.
<b>Duration of the Test:</b> A mysid test must not be terminated before 7 days past the median time of 1 <sup>st</sup> brood release in the control treatment.	Test was terminated 56 days after gravid adults were placed into treatment solutions.
<b>Brood Stock:</b> Test started with mysids: 1) from only one brood stock or 2) from brood stock which has not obtain sexual maturity or had been maintained for > 14 days in a laboratory with same food, water, temperature, and salinity used in the test.	The brood stock was a wild population from the Sacramento-San Joaquin Delta. The duration of acclimation was not reported.

Guideline Criteria	Reported Information
<p><b>Distribution:</b>  <b>No. of mysids before pairing:</b>  Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/level.  <b>No. of mysids after pairing:</b>  <math>\geq 20</math> randomly selected pairs/treatment (excess males should be held in separate compartment to replace paired males).</p>	<p>There were two replicate chambers per treatment level. Each chamber held 3 adult gravid shrimp during the first 14 days, and 15-20 young thereafter. During the first 14 days, water from each chamber drained into a second tier chamber that was used to hold the young produced by the gravid females. There were a total of 30-40 shrimp per level (excluding adults). No mysids were paired.</p>
<p><b>Pairing:</b>  1) Should be conducted when most of the mysids are sexually mature (usu. 10-14 days after test initiation).  2) Should be paired on the same day</p>	<p>Shrimp were not paired. Instead, gravid shrimp were selected from the parental stock and placed into test solutions to provide offspring.</p>
<p><b>Feeding:</b>  1) Mysids should be fed live brine shrimp nauplii at least once daily.  2) 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.</p>	<p>Shrimp were fed <i>Artemia</i> nauplii once daily. Algal and vitamin supplements were also provided three times per week.</p>
<p><b>Counts:</b>  Live adult mysids should be counted  1) at initiation,  2) at pairing,  3) and daily after pairing.  4) Live young must be counted and removed daily.  5) Missing or impinged animals should be recorded.</p>	<p>Counts of adult shrimp were not recorded. Young produced were counted and removed to a separate chamber daily for the first 14 days. Thereafter, the number of young surviving were counted daily.</p>

Guideline Criteria	Reported Information
<b>Controls:</b> Survival in any control chamber (between pairing and test termination) must not be less than 70%.	Survival was not reported per chamber. Overall, the control chambers had 78% survival.
<b>Controls:</b> Negative control and carrier control (when applicable) are required.	The test included a negative control. (No carrier was used.)

Comments: *Neomysis mercedis* is a small, primarily estuarine, crustacean found along the Pacific Coast of North America.

**B. Physical System:**

Guideline Criteria	Reported Information
<b>Test Water:</b> 1) May be natural (sterilized and filtered) or a commercial mixture; 2) During the test, difference between highest and lowest measured salinities must be less than 10 ‰ (parts per thousand). Should be measured daily. 3) Salinity should be between 15 and 30 ‰. 4) Measured pH should be between 7.6 and 8.2. Must not deviate by more than one unit for more than 48 hours. Should be measured at the beginning, end of test and weekly. 5) Water must be free of pollutants. 6) DO must be measured @ each conc. @ least once a wk. (see details in ASTM)	The test water was artificial sea water prepared from tap water dechlorinated with activated carbon filters.  Salinity: Not reported Conductance: 3500 µmhos pH: 6.9 - 7.9 DO: 7.9 - 9.4 mg/L

Guideline Criteria	Reported Information
<p><b>Test Temperature:</b></p> <ol style="list-style-type: none"> <li>1) Mean measured temperature for each chamber at test termination should be within 1pC of selected test temperature.</li> <li>2) Each individual measured temperature must be within 3pC of the mean of the time-weighted averages.</li> <li>3) For mysid shrimp, 27°C is recommended.</li> <li>4) Whenever temp. is measured concurrently in more than one test chamber the highest &amp; lowest temp. must not differ by more than 2°C.</li> </ol>	<p>Test temperature ranged from 17.5 to 18.5 °C</p>
<p><b>Photoperiod:</b> Recommend 16L/8D.</p>	<p>16 h light / 8 h dark</p>
<p><b>Dosing Apparatus:</b></p> <ol style="list-style-type: none"> <li>1) Intermittent flow proportional diluters or continuous flow serial diluters should be used.</li> <li>2) A minimum of 5 toxicant concentrations</li> <li>3) with a dilution factor not greater than 0.5 and controls should be used.</li> </ol>	<p>Diluters were not used. Separate solutions were prepared for each of five test concentrations. Test solutions were delivered to test chambers from Mariotte bottles.</p>
<p><b>Toxicant Mixing:</b></p> <ol style="list-style-type: none"> <li>1) Mixing chamber is recommended but not required;</li> <li>2) Aeration should not be used for mixing;</li> <li>3) It must be demonstrated that the test solution is completely mixed before intro. into the test system;</li> <li>4) Flow splitting accuracy must be within 10%.</li> </ol>	<p>Since a diluter system was not used, there were no mixing chambers.</p>

Guideline Criteria	Reported Information
<b>Test Vessels:</b> 1) Material: all glass, No. 316 stainless steel, or perfluorocarbon plastic 2) Size: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume acceptable 3) 90 or 140 mm inside dia. glass Petri dish bottoms with collars made of 200 - 250 um mesh screen.	Crystallizing dishes, 150 mm x 75 mm, were used for test vessels. The depth or volume was not reported.
<b>Covers</b> 1) Renewal: Test vessels should be covered with a glass plate. 2) Flow-through: Openings in the test compartments should be covered with nylon mesh or stainless steel screen.	Not reported.
<b>Flow Rate:</b> 1) Flow rates should provide 5 to 10 volume additions per 24 hr. 2) Flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level. 3) Meter systems calibrated before study and checked twice daily during test period 4) Renewal must not drop below 50% for more than 48 hours.	The flow rate was approximately 2 volumes/24 h
<b>Aeration:</b> 1) Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2) Test tanks may be aerated.	Aeration was not mentioned, but the DO levels were adequate (86-100%).



**C. Chemical System:**

Guideline Criteria	Reported Information
<b>Concentrations:</b> 1) Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate. 2) Toxicant conc. must be measured in one tank at each toxicant level every week. 3) One concentration must adversely affect a life stage and one concentration must not affect any life stage. 4) The measured conc. of the test material of any treatment should be at least 50% of the time-weighted average measured conc. for >10% of the duration of the test. 5) The measured conc. for any treatment level should not be more than 30% higher than the time-weighted average measured conc. for more than 5% of the duration of the test.	<u>Test concentrations:</u> 0, 15.1, 25.6, 45.2, 89.6, and 173.7 µg/L  Test solutions were sampled weekly. The concentrations of test material was measured using gas chromatography with TSD detection. The CV for measured concentrations was 15.2.
<b>Solvents:</b> 1) Should not exceed 0.1 ml/L in a flow-through system. 2) Following solvents are acceptable: triethylene glycol, methanol, acetone, ethanol.	Aqueous solutions without solvents were used.

**11. REPORTED RESULTS:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	No

Guideline Criteria	Reported Information
1) At least 75% of the paired 1 <sup>st</sup> generation females in the control produced young or 2) the average number of young produced by the 1 <sup>st</sup> generation females in the control(s) was more than 3.	Not applicable.
<b>Data Endpoints</b> must include: 1) Survival of first-generation mysids Female Male 2) Number of live young produced per female 3) Dry weight of each first-generation mysid alive at the end of the test Female Male 4) Length of each 1 <sup>st</sup> generation mysid alive at the end of the study Female Male 5) Incidence of pathological or histological effects; 6) Observations of other effects or clinical signs.	<b>Data Endpoints</b> were:  1) Number of young produced per gravid female  2) Mortality of young over 42 days following the 14-day hatching period  3) Mean survival times of young  4) Length of survivors
<b>Raw data included?</b> (Y/N)	No

Effects Data:

Measured Toxicant Conc. ( $\mu\text{g/L}$ )	45-Day Percent Mortality	Average Survival Time for Young (days)	Mean Total Length (mm)
Control	78	30	8.7 (SD = 1.9)
15.1	68	29	7.5 (SD = 1.5)
25.6	70	31	8.2 (SD = 2.3)
45.2	0	10	
89.6	45	17	
173.7	14	6	

**Toxicity Observations:** Not reported.

**Statistical Results:**

Endpoint	Method	NOEC	LOEC
Average survival time for young	Mantel-Cox test	25.6 $\mu\text{g/L}$	45.2 $\mu\text{g/L}$
Number of young produced	Dunnett's test	$\geq 173.7 \mu\text{g/L}$	Not determined
Length	Dunnett's test	$\geq 25.6 \mu\text{g/L}$	Not determined

**Comments:**

Significant difference in length, compared to the control, was only tested at the 15.1 and 25.6  $\mu\text{g/L}$  test levels. There was no significant effect at these levels. Although the NOEC and LOEC for effect on length can not be determined, it is clear that this endpoint is not more sensitive than survival time.

**12. Reviewer's Statistical Results:**

**Comments:** Statistical results could not be verified since this was a published paper that only provided summary data.