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

1. CHEMICAL: Thiobencarb PC Code No.: 108401

2. TEST MATERIAL: Technical grade thiobencarb
Purity: Not reported

3. CITATION
Authors:Bailey, Howard C.
Title:Acute and chronic toxicity of the rice herbicides thiobencarb and molinate to Oppossum shrimp (*Neomysis mercedis*)
Publication Date: 1993
Laboratory: SRI International
Sponsor:California State Water Resources Control Board
MRID No.:43976801

4. REVIEWED BY: F. Nicholas Mastrota, Biologist, ERCB

Signature: **Date:** 4/15/96

5. PEER REVIEW BY: Robert K. Hitch, Ecologist, ERCB

Signature: **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 thiobencarb 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 thiobencarb at concentrations greater or equal to 6.2 mg/L can significantly reduce the survival of young opossum shrimp.

Results Synopsis
NOEC: 3.2 mg ai/L LOEC: 6.2 mg ai/L
MATC: 4.5 mg ai/L

LOEC's for specific effects
Larvae Survival: 6.2 mg ai/L
Growth (Length): Not determined

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
Species: An estuarine shrimp species, preferably *Americamysis bahia*.	Opossum shrimp (*Neomysis mercedis*)
Duration 28 days/one generation	56 days
Source (or supplier)	Montezuma Lough, Sacramento-San Joaquin Delta, CA
Parental Acclimation 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.
Parental Acclimation Period At least 14 days	Not reported
Chamber Location: 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.
Duration of the Test: A mysid test must not be terminated before 7 days past the median time of 1st brood release in the control treatment.	Test was terminated 56 days after gravid adults were placed into treatment solutions.
Brood Stock: 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	The brood stock was a wild population from the Sacramento-San Joaquin Delta. The duration of acclimation was not reported.

Guideline Criteria	Reported Information
salinity used in the test.	
Distribution: **No. of mysids before pairing**: Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/level. **No. of mysids after pairing**: ≥ 20 randomly selected pairs/treatment (excess males should be held in separate compartment to replace paired males).	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.
Pairing: 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	Shrimp were not paired. Instead, gravid shrimp were selected from the parental stock and placed into test solutions to provide offspring.
Feeding: 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.	Shrimp were fed *Artemia* nauplii once daily. Algal and vitamin supplements were also provided three times per week.
Counts: 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.	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.
Controls: Survival in any control chamber (between pairing and test	Survival was not reported per chamber. Overall, the

Guideline Criteria	Reported Information
termination) must not be less than 70%.	control chambers had 80% survival.
Controls: 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
Test Water: 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 $^{0}/_{00}$ (parts per thousand). Should be measured daily. 3) Salinity should be between 15 and 30 $^{0}/_{00}$. 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 mmhos pH: 6.9 - 7.9 DO: 7.9 - 9.4 mg/L
Test Temperature: 1) Mean measured temperature for each chamber at test termination should be within 1°C of selected test temperature.	Test temperature ranged from 17.5 to 18.5 °C

Guideline Criteria	Reported Information
2) Each individual measured temperature must be within 3°C of the mean of the time-weighted averages. 3) For mysid shrimp, 27°C is recommended. 4) Whenever temp. is measured concurrently in more than one test chamber the highest & lowest temp. must not differ by more than 2°C.	
Photoperiod: Recommend 16L/8D.	16 h light / 8 h dark
Dosing Apparatus: 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations 3) with a dilution factor not greater than 0.5 and controls should be used.	Diluters were not used. Separate solutions were prepared for each of five test concentrations. Test solutions were delivered to test chambers from Mariotte bottles.
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%.	Since a diluter system was not used, there were no mixing chambers.
Test Vessels: 1) Material: all glass, No. 316 stainless steel, or perflorocarbon 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	Crystallizing dishes, 150 mm x 75 mm, were used for test vessels. The depth or volume was not reported.

Guideline Criteria	Reported Information
collars made of 200 - 250 um mesh screen.	
Covers 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.
Flow Rate: 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
Aeration: 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
Concentrations: 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.	Test concentrations: 0, 3.2, 6.2, 12.8, 23.5, and 53.4 mg/L Test solutions were sampled weekly. The concentrations of test material was measured using gas chromotagraphy with TSD detection. The CV for measured concentrations was 9.6.
Solvents: 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
1) At least 75% of the paired 1st generation females in the	Not applicable.

Guideline Criteria	Reported Information
control produced young or 2) the average number of young produced by the 1st generation females in the control(s) was more than 3.	
Data Endpoints 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 1st 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.	Data Endpoints 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
Raw data included? (Y/N)	No

Effects Data:

Measured Toxicant Conc. (mg/L)	45-Day Percent Mortality	Average Survival Time for Young (days)	Mean Total Length (mm)
Control	80	31	8.4 (SD = 1.8)
3.2	75	29	7.4 (SD = 1.5)
6.2	60	25	
12.8	40	20	
23.5	0	2	
53.4	8	8	

Toxicity Observations: Not reported.

Statistical Results:

Endpoint	Method	NOEC	LOEC
Average survival time for young	Mantel-Cox test	3.2 mg/L	6.2 mg/L
Number of young produced	Dunnett's test	$\geq$ 53.4 mg/L	Not determined
Length	Dunnett's test	$\geq$ 3.2 mg/L	Not determined

Comments:
1) The average survival time at the 6.2 mg/L was marginally significant (P=0.06). Considering the concentration response shown, this was considered a significant treatment-related response.
2) Significant difference in length, compared to the control, was only tested at the 3.2 mg/L test level. There was no significant effect at this level. 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.