



**Results Synopsis:** Results are based on mean measured concentrations as total dimethenamid.

LC<sub>50</sub>: 3.2 ppm  
 NOEL: 1.2 ppm

95% C.I.: 2.7-3.9 ppm  
 Probit Slope: 4.1

**8. ADEQUACY OF THE STUDY:**

- A. **Classification:** Supplemental.
- B. **Rationale:** The age of test mysids was not reported.
- C. **Repairability:** Yes, if the test mysids were ≤24 hours old at test initiation.

**9. BACKGROUND:**

**10. GUIDELINE DEVIATIONS:** The age of the test mysids was not reported; guidelines require ≤24 hour-old mysids.

**11. SUBMISSION PURPOSE:**

**12. MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species are <i>Mysidopsis bahia</i> , <i>Penaeus setiferus</i> , <i>P. duorarum</i> , <i>P. aztecus</i> and <i>Palaemonetes sp.</i>	<i>Mysidopsis bahia</i>
<b><u>Age</u></b> Juvenile, mysids should be ≤ 24 hours old	Juveniles; age of the test organisms was not reported.
<b><u>Supplier</u></b>	In-house cultures
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<b>Acclimation Period</b> minimum 10 days	Mysid cultures were maintained under environmental conditions similar to those for testing.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<b>Feeding</b> No feeding during the study and no feeding for 24 hours before the beginning of the test if organisms are over 0.5 g each.	Mysids were fed live brine shrimp nauplii daily during the study.
<b>Pretest Mortality</b> <3% mortality 48 hours prior to testing	Not reported.

**C. Test System**

Guideline Criteria	Reported Information
<b>Source of dilution water</b> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Natural filtered seawater (collected from Indian River Inlet, Delaware) diluted with well water, filtered, and aerated.
Does water support test animals without observable signs of stress?	Yes
<b>Salinity</b> 30-34 % for marine (stenohaline) shrimp and 10-17 % for estuarine (euryhaline) shrimp, weekly range < 6 %	20-21%

Guideline Criteria	Reported Information
<u>Water Temperature</u> Approx. $22 \pm 1$ °C	24.5 to 25.5°C
<u>pH</u> 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	8.3
<u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 <sup>st</sup> 48 hrs and $\geq 40\%$ during 2 <sup>nd</sup> 48 hrs, Flow-through: $\geq 60\%$	$\geq 69\%$ saturation throughout the test.
<u>Total Organic Carbon</u>	1.4 mg/L
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel 2. <u>Size:</u> 19.6 L is acceptable for organisms $\geq 0.5$ g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. <u>Fill volume:</u> 15 L is acceptable for organisms $\geq 0.5$ g, 2-3 L is acceptable for smaller organisms.	Each test chamber was a 500-ml glass beaker, with nylon mesh screen covered on both ends. The chamber was suspended in an 8-L Teflon®-lined polyethylene aquarium filled with 6.5 L of test solution.
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Continuous-flow diluter
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	14 volume replacements/day; meter systems calibrated before the test and checked twice daily during the test.
<u>Biomass Loading Rate</u> Static: $\leq 0.8$ g/L at $\leq 17^\circ\text{C}$ , $\leq 0.5$ g/L at $> 17^\circ\text{C}$ ; flow-through: $\leq 1$ g/L/day	Not reported.

Guideline Criteria	Reported Information
<b>Photoperiod</b> 16 hours light, 8 hours dark	16 h light, 8 h dark.
<b>Solvents</b> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: DMF Maximum conc.: 0.1 ml/L

**D. Test Design**

Guideline Criteria	Reported Information
<b>Range Finding Test</b> If $LC_{50} > 100$ mg/L with 30 shrimp, then no definitive test is required.	A range-finding test was conducted but results were not reported.
<b>Nominal Concentrations of Definitive Test</b> Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	Negative control, solvent control; and 1.0, 1.7, 2.9, 4.8, and 8.0 mg/L, not corrected for percentage active ingredient.
<b>Number of Test Organisms</b> Minimum 20/level, may be divided among containers	20 mysids/level, 10 per replicate chamber.
<b>Test organisms randomly or impartially assigned to test vessels?</b>	Yes
<b>Biological observations made every 24 hours?</b>	Yes
<b>Water Parameter Measurements</b> 1. <b>Temperature</b> Measured constantly or, if water baths are used, every 6 hrs, may not vary $> 1^{\circ}C$ 2. <b>DO and pH</b> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Temperature measured in each test chamber at test initiation and termination, and monitored continuously in a negative control chamber.  2. DO and pH measured daily in alternate replicate chambers of each level.



Guideline Criteria	Reported Information
<b>Chemical Analysis</b> needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Solutions from alternate replicates were collected and analyzed at 0, 48, and 96 hours using GC.

**13. REPORTED RESULTS:****A. General Results**

Guideline Criteria	Reported Information
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Yes
<b>Recovery of Chemical</b>	103-120% of nominal
<b>Control Mortality</b> Not more than 10% of control organisms may die or show abnormal behavior.	5% mortality in negative control and no mortality in solvent control.
<b>Raw data included?</b>	Yes
<b>Signs of toxicity (if any) were described?</b>	Yes

**Mortality**

Concentration (ppm)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured*		Hour of Study			
			24	48	72	96
Control	<0.25	20	1	1	1	1
Solv. Cont.	<0.25	20	0	0	0	0
1.0	1.2	20	0	0	0	0
1.7	1.8	20	0	1	3	4
2.9	3.0	20	0	0	5	9
4.8	5.5	20	0	0	16	17

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
9.2	20	19	95	2.002716E-03
5.5	20	17	85	.1288414
3	20	9	45	41.19014
1.8	20	4	20	.5908966
1.2	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.8 AND 5.5 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.217475

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	6.572957E-02	3.240558	2.715184 3.882731

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.0945431	1	.6719156

SLOPE = 4.124771  
 95 PERCENT CONFIDENCE LIMITS = 2.856492 AND 5.39305

LC50 = 3.225196  
 95 PERCENT CONFIDENCE LIMITS = 2.679909 AND 3.911673

LC10 = 1.587305  
 95 PERCENT CONFIDENCE LIMITS = 1.100596 AND 1.989598

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Concentration (ppm)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured*		Hour of Study			
			24	48	72	96
8.0	9.2	20	3	6	19	19

\* Measured as total dimethenamid.

**Other Significant Results:** Signs of toxicity observed at the four highest treatment levels included erratic swimming and lethargy.

**B. Statistical Results:** Results are based on mean measured concentrations as total dimethenamid.

Method: Probit analysis

96-hr LC<sub>50</sub>: 3.2 ppm

95% C.I.: 2.7-3.9 ppm

Probit Slope: 4.1

NOEC: 1.2 ppm

#### 14. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test LC <sub>50</sub> (95% C.I.)	3.2 (1.8-5.5) ppm
Moving Average Angle LC <sub>50</sub> (95% C.I.)	3.2 (2.7-3.9) ppm
Probit LC <sub>50</sub> (95% C.I.)	3.2 (2.7-3.9) ppm
Probit Slope	4.1
NOEC	1.2 ppm

**15. REVIEWER'S COMMENTS:** This study is scientifically sound but does not fulfill the guideline requirements. The exact age of the test organisms was not reported. The authors only reported that "juvenile" mysids were used. The use of mysids <24 hours old is required.

Based on mean measured concentrations as total dimethenamid, the 96-hour LC<sub>50</sub> for juvenile mysids was 3.2 ppm which classifies this compound as moderately toxic to *Mysidopsis bahia*. The NOEC was 1.2 ppm. This study is classified as Supplemental.