

US EPA ARCHIVE DOCUMENT



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

LC<sub>50</sub>: 12 ppm

95% C.I.: 9.2-16 ppm

NOEC: 5.3 ppm

Probit Slope: N/A

8. **ADEQUACY OF THE STUDY:**

A. **Classification:** Core

B. **Rationale:** N/A

C. **Repairability:** N/A

9. **GUIDELINE DEVIATIONS:** No significant deviations. ;

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. **Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species are the sheepshead minnow ( <i>Cyprinodon variegatus</i> ) or the Silverside ( <i>Menidia sp.</i> ).	<i>Cyprinodon variegatus</i>
<b><u>Mean Weight</u></b> 0.5-5 g	0.31 g
<b><u>Mean Standard Length</u></b> Longest not > 2x shortest	Mean: 21 mm Range: 18-26 mm
<b><u>Supplier</u></b>	In-house cultures
<b>All fish from same source?</b>	Yes
<b>All fish from the same year class?</b>	Yes

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<b>Acclimation Period</b> Minimum 14 days	Cultures maintained under conditions similar to testing; test fish acclimated to test conditions for 3 days.
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	No feeding during acclimation and test periods.
<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	Seawater collected from Indian River Inlet, DE, was adjusted to a salinity of 20 ‰ with well water. The water was aerated and filtered.
Does water support test animals without observable signs of stress?	Yes
<b>Salinity</b> 30-34 ‰ salinity, weekly range <6 ‰	20 ‰
<b>Water Temperature</b> 22 ± 1 °C	22.0-22.9 °C

Guideline Criteria	Reported Information
<p><b><u>pH</u></b> 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range &lt; 0.8</p>	8.3-8.4
<p><b><u>Dissolved Oxygen</u></b> Static: <math>\geq 60\%</math> during 1<sup>st</sup> 48 hrs and <math>\geq 40\%</math> during 2<sup>nd</sup> 48 hrs, flow-through: <math>\geq 60\%</math></p>	$\geq 64\%$ saturation during the test
<p><b><u>Test Aquaria</u></b> 1. <b><u>Material:</u></b> Glass or stainless steel 2. <b><u>Size:</u></b> Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. <b><u>Fill volume:</u></b> 15-30 L of solution</p>	Teflon®-lined polyethylene  25-L  15 L
<p><b><u>Type of Dilution System</u></b> Must provide reproducible supply of toxicant</p>	Continuous-flow diluter
<p><b><u>Flow Rate</u></b> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period</p>	6 volume replacements/day, meter systems calibrated before the test and checked twice daily during the test.
<p><b><u>Biomass Loading Rate</u></b> Static: <math>\leq 0.8</math> g/L at <math>\leq 17^\circ\text{C}</math>, <math>\leq 0.5</math> g/L at <math>&gt; 17^\circ\text{C}</math>; flow-through: <math>\leq 1</math> g/L/day</p>	0.034 g/L/day
<p><b><u>Photoperiod</u></b> 16 hours light, 8 hours dark</p>	16 h light, 8 h dark
<p><b><u>Solvents</u></b> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests</p>	Solvent: DMF Maximum conc.: 0.1 ml/L

**D. Test Design**

Guideline Criteria	Reported Information
<p><b><u>Range Finding Test</u></b> If <math>LC_{50} &gt; 100</math> mg/L with 30 fish, then no definitive test is required.</p>	Range-finding test was conducted but results were not reported.
<p><b><u>Nominal Concentrations of Definitive Test</u></b> Control &amp; 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series</p>	Negative control, solvent control; and 3.2, 5.4, 9.0, 15, and 25 mg/L, not corrected for percentage active ingredient
<p><b><u>Number of Test Organisms</u></b> Minimum 10/level, may be divided among containers</p>	20 fish per treatment level and control, 10 per replicate.
<p><b>Test organisms randomly or impartially assigned to test vessels?</b></p>	Yes
<p><b>Biological observations made every 24 hours?</b></p>	Yes
<p><b><u>Water Parameter Measurements</u></b> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary <math>&gt; 1^{\circ}C</math> 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control</p>	<p>Temperature measured in each test chamber at test initiation and termination, and monitored continuously in one negative control chamber.</p> <p>DO and pH measured daily in alternate replicate chambers.</p>
<p><b><u>Chemical Analysis</u></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</p>	Solutions from alternate replicates were collected and analyzed at 0, 48, and 96 hours using GC.

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

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	98-108% of nominal
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured*		Hour of Study			
			24	48	72	96
Control	<0.25	20	0	0	0	0
Solv. Cont.	<0.25	20	0	0	0	0
3.2	3.4	20	0	0	0	0
5.4	5.3	20	0	0	0	0
9.0	9.2	20	0	0	0	0
15	16	20	0	9	19	20
25	27	20	5	20	20	20

\* Measured as total dimethenamid

Other Significant Results: Signs of toxicity observed at the three highest test levels included erratic swimming, discoloration, lying on bottom with little motion, and lethargy.

**B. Statistical Results**

Method: Binomial method

96-hr LC<sub>50</sub>: 12 ppm

95% C.I.: 9.2-16 ppm

Probit Slope: N/A

NOEC: 5.3 ppm

**13. VERIFICATION OF STATISTICAL RESULTS:**

Parameter	Result
Binomial Test LC <sub>50</sub> (95% C.I.)	12 ppm (9.2-16 ppm)
Moving Average Angle LC <sub>50</sub> (95% C.I.)	N/A
Probit LC <sub>50</sub> (95% C.I.)	N/A
Probit Slope	N/A
NOEC	5.3 ppm

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and can be classified as **Core**. Based on mean measured concentrations as total dimethenamid, the 96-hour LC<sub>50</sub> for sheepshead minnow was determined to be 12 ppm, which classifies the test material as slightly toxic to the sheepshead minnow. The NOEC was 5.3 ppm.

KOSALWAT SAN 1289H TECHNICAL CYPRINODON VARIEGATUS 10-23-97

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
27	20	20	100	9.536742E-05
16	20	20	100	9.536742E-05
9.2	20	0	0	9.536742E-05
5.3	20	0	0	9.536742E-05
3.4	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 9.2 AND 16 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 12.1326

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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