

US EPA ARCHIVE DOCUMENT





**11. MATERIALS AND METHODS:**

- A. **Test Animals:** Juvenile sheepshead minnows (*Cyprinodon variegatus*) were obtained from laboratory cultures and maintained for 17 days in natural seawater with a salinity of 20-22 parts per thousand (ppt) and a temperature of 20°-25°C. The fish were fed live brine shrimp nauplii at least once daily. Less than one percent mortality was observed during the 48-hour period prior to test initiation. Test fish were 5-8 mm long and averaged 0.0038 g wet weight.
- B. **Test System:** The proportional diluter system, similar to that of Mount and Brungs (1967), consisted of seven glass aquaria. Each aquarium contained approximately 9 L of test solution or dilution water. The number of diluter cycles was sufficient to provide 4.7 daily volume turnovers. Stock solutions were prepared in the dilution water using an automatic stock dispensing system. The stock was delivered to the toxicant mixing cells with a peristaltic pump.

Filtered (5  $\mu$ m) natural seawater, with the characteristics listed in Appendix A (attached) was diluted to 20 ppt with well water and used as dilution water in the test. Water temperature was regulated to maintain temperature at 22° $\pm$ 1°C. The laboratory containing the diluter was maintained on a 16-hour light/8-hour dark photoperiod. The test was initiated after allowing the diluter to run for 96 hours.

- C. **Dosage:** Ninety-six-hour flow-through test. Based on a preliminary test, six nominal concentrations (0.6, 1.0, 1.7, 2.8, 4.6, and 7.7 mg a.i./l) and a dilution water control were used.
- D. **Design:** Twenty sheepshead minnows were used per concentration. Loading was approximately 0.002 g/l/day.

Observations of mortality were made every 24 hours. The dissolved oxygen (D.O.) and pH were measured in all test vessels every 24 hours. The salinity and temperature in the control vessel were measured every 24 hours. The temperature was also monitored continuously using a continuous recording device.

Samples of each test solution were taken on days 0, 2, and 4 to determine the actual exposure concentrations.

The samples were analyzed using gas chromatography by Analytical Bio-Chemistry Laboratories in Columbia, MO.

- E. **Statistics:** The median lethal concentration ( $LC_{50}$ ) and associated 95% confidence interval (C.I.) for each 24-hour interval were calculated using a computer program developed by Stephan et al. (1977).
12. **REPORTED RESULTS:** The mean measured concentrations are given in Table 3-2 (attached). Measured concentrations were fairly consistent between sampling days and averaged 43-50% of nominal concentrations.

The responses of sheepshead minnows to Dithane M-45 Fungicide are given in Table 3-3 (attached). The 96-hour  $LC_{50}$ , based on mean measured concentrations, was 1.7 mg a.i./l (95% C.I. = 1.2-2.3 mg a.i./l) using the binomial method. The NOEC was 0.82 mg a.i./l.

The D.O. was 7.0-7.8 during the test. The pH values ranged from 8.1 to 8.2. The temperature and salinity were 21°-22°C and 19-22 ppt, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors presented no conclusions.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards, with the exception of 40 CFR Part 160.113.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

- A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated from the SEP as follows:

The average weight of the fish used in the test (0.0038 g wet weight) was much lower than recommended (0.1-5 g) and seems unrealistically low.

The report did not state if the fish were randomly placed in the test chambers.

The system used to maintain the temperature during the test was not described.

The salinity of the test dilution water was approximately 20 ppt. The recommended salinity for tests involving euryhaline fish is 10-17 ppt.

The use of 15- to 30-minute dawn and dusk simulations was not described in the report.

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the LC<sub>50</sub> value (see attachment).
- C. **Discussion/Results:** This study is scientifically sound but does not satisfy the guideline requirement for a marine/estuarine fish acute toxicity test with technical material. The 96-hour LC<sub>50</sub> value of 1.6 mg a.i./l mean measured concentration classifies technical mancozeb as moderately toxic to sheepshead minnows. The NOEC is 0.8 mg a.i./l mean measured concentration.
- D. **Adequacy of the Study:**
- (1) **Classification:** Supplemental.
  - (2) **Rationale:** The weight of the test fish is in doubt.
  - (3) **Repairability:** Yes, if the weight of test fish is clarified.
15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 12/09/94.

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Mancozeb

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Pages 6 through 7 are not included.

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The material not included contains the following type of information:

- Identity of product inert ingredients.
  - Identity of product impurities.
  - Description of the product manufacturing process.
  - Description of quality control procedures.
  - Identity of the source of product ingredients.
  - Sales or other commercial/financial information.
  - A draft product label.
  - The product confidential statement of formula.
  - Information about a pending registration action.
  - FIFRA registration data.
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MANCOZEB ACUTE FISH TEST

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3.7	20	20	100	9.536742E-05
2.3	20	20	100	9.536742E-05
1.2	20	0	0	9.536742E-05
.8	20	0	0	9.536742E-05
.48	20	0	0	9.536742E-05
.28	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.2 AND 2.3 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 1.661325

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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