

US EPA ARCHIVE DOCUMENT

6-16-92

DATA EVALUATION RECORD

- 1. CHEMICAL: Mancozeb.  
Shaughnessey No. 014504.
- 2. TEST MATERIAL: Dithane® M-45 Fungicide; Lot No. 76777;  
82.4% active ingredient; a yellow powder.
- 3. STUDY TYPE: Estuarine Shrimp Flow-Through Acute Toxicity  
Test. Species Tested: Mysid (*Mysidopsis bahia*).
- 4. CITATION: Ward, G.S. and T. Leak. 1990. Acute Toxicity of  
Dithane® M-45 Fungicide to Mysids (*Mysidopsis bahia*) Under  
Flow-Through Conditions. Laboratory Project ID. 89328-0500-  
2130. Rohm and Haas Report No. 88RC-0069. Prepared by  
Hunter/ESE, Inc., Gainesville, FL. Submitted by Rohm and  
Haas Company, Spring House, PA. MRID No. 418229-01.

5. REVIEWED BY:

Louis M. Rifici, M.S.  
Associate Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *Louis M. Rifici*  
Date: *4/17/92*

6. APPROVED BY:

Pim Kosalwat, Ph.D.  
Senior Scientist  
KBN Engineering and  
Applied Sciences, Inc.

Signature: *P. Kosalwat*  
Date: *4/17/92*

Henry T. Craven, M.S.  
Supervisor, EEB/EFED  
USEPA

Signature: *Henry T. Craven*  
Date: *6/16/92*  
*Disruptive 6/16/92*

7. CONCLUSIONS: This study is scientifically sound ~~but does~~  
*and* ~~not~~ meet the guideline requirements for an acute estuarine  
shrimp toxicity study. ~~The mysids used were older than~~  
~~recommended.~~ Under the conditions of the test, the 96-hour  
LC<sub>50</sub> of 10.5 µg a.i./l mean measured concentration  
classifies Dithane M-45 as very highly toxic to mysid  
shrimp. The NOEC was estimated to be 3.4 µg a.i./l mean  
measured concentration.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

**11. MATERIALS AND METHODS:**

- A. **Test Animals:** Mysids (*Mysidopsis bahia*) were obtained from in-house cultures. The mysids were held for three days after being released from the adults in natural seawater (21-23 parts per thousand [ppt]) at a temperature of  $22 \pm 1^\circ\text{C}$ . During holding, they were fed live brine shrimp nauplii at least once daily. Mortality during holding was  $\leq 2\%$ .
- B. **Test System:** The test was conducted using a diluter system with a dilution factor of 60%. The diluter was calibrated volumetrically and equilibrated for four days prior to test initiation. The test chambers were glass aquaria (25.5 x 27.5 x 15 cm) designed to maintain a volume of 6 l. The mysids were housed in retention chambers constructed of petri dishes (1.5 x 9 cm) with nitex screen collars (report page 89). Flow rate to each aquarium was sufficient to provide approximately 11.8 volume replacements per day. Water temperature was maintained at  $22 \pm 1^\circ\text{C}$ . The photoperiod was 16-hour light/8-hour dark with 30-minute dawn and dusk simulations. Mysids were fed brine shrimp during the study (report page 112).

Natural filtered ( $5 \mu\text{m}$ ) seawater (collected from the Atlantic Ocean near Marineland, FL) was adjusted to a salinity of 20 ppt with well water (obtained on-site) before use as test dilution water.

Stock solutions ( $550 \mu\text{g a.i./l}$ ) were prepared using an automatic continuous stock dispensing system. The solutions were mixed for one hour before being delivered to the diluter stock supply. During each cycle, 1100 ml of dilution water were mixed with 87 ml of stock from the dispensing chamber to prepare the final stock. The test solutions were prepared by additions of the final stock solution to dilution water by the diluter system.

- C. **Dosage:** Ninety-six-hour flow-through test. Based on the results of a preliminary test, six nominal concentrations (3.0, 5.0, 9.0, 14.0, 24.0, and  $40.0 \mu\text{g a.i./l}$ ) and a dilution water control were selected.
- D. **Design:** Twenty mysids were placed in each test chamber (one chamber per concentration). Observations of mortality and sublethal responses were made every 24 hours. Salinity and temperature were measured in the seawater control once daily. Temperature was monitored

continuously with a recording device. The dissolved oxygen and pH of the test solutions were measured in each chamber daily.

Water samples from each aquarium were collected at test initiation and on days 2 and 4. The samples were sent by overnight courier to ABC Laboratories in Columbia, MO, for quantitative analysis of Dithane M-45 in solution using gas chromatography.

- 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 (1977).

12. **REPORTED RESULTS:** The test material was detected in the control at test initiation and after 48 hours (Table 3-2, attached). The mean measured concentrations were 1.1, 3.4, 3.4, 4.4, 6.9, 12.0, and 17.0  $\mu\text{g a.i./l}$ . These values were 43-113% of nominal concentrations.

The responses of mysid shrimp are presented in Table 3-3 (attached). The 96-hour  $LC_{50}$  based on mean measured concentrations was 10.5  $\mu\text{g a.i./l}$  (95% C.I. = 8.6-14.0  $\mu\text{g a.i./l}$ ). Sublethal (gyrating movement or lethargy) and lethal effects occurred at concentrations  $\geq 4.4 \mu\text{g a.i./l}$ . One mortality occurred at the lowest concentration, 3.4  $\mu\text{g a.i./l}$ . The no-observed-effect concentration (NOEC) was judged to be 3.4  $\mu\text{g a.i./l}$ .

Dissolved oxygen ranged from 6.9 to 7.3 mg/l or  $\geq 92\%$  of saturation. The pH values ranged from 8.1 to 8.3. The temperature was 22-23°C and the salinity was 20-23 ppt.

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

Quality assurance and good laboratory practice statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards set forth in FIFRA 40 CFR Part 160. Audit and review dates were also presented.

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

- A. **Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following:

The control solutions appeared to be contaminated with the test material at test initiation and after 48 hours

(Table 3-2, attached). However, a letter (report page 79, attached) from G. Scott Ward to Doris Milligan/Rohm and Haas Company indicated that this test would be acceptable by EPA since no mortality occurred in the control group.

The test mysids were at least three days old at test initiation. An amendment to the SEP states that mysids  $\leq 24$  hours old must be used to initiate tests.

Methods used to select and load the mysids into the test chambers were not described. Mysids must be randomly distributed to the test aquaria.

The system used to maintain test solution temperature was not described.

The salinity of the dilution water in the study was 20-23 ppt with a pH of 8.1-8.3. The recommended salinity and pH for estuarine shrimp tests are 10-17 ppt and 7.7-8.0, respectively.

The number of volume replacements used (11.8 replacements/day) was higher than recommended (5-10 replacements/day).

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal program and reviewer calculated mean measured concentrations (Table 3-2, attached) to calculate the  $LC_{50}$  value and obtained similar results (see attached printout). The slope of the probit line was 3.0.
- C. **Discussion/Results:** This study is scientifically sound but does not meet the guideline requirements for a flow-through acute saltwater shrimp toxicity study. The mysids used were older than recommended. Under the conditions of the test, the 96-hour  $LC_{50}$  of 10.5  $\mu\text{g}$  a.i./l mean measured concentration classifies Dithane M-45 as very highly toxic to mysid shrimp. The NOEC was estimated to be 3.4  $\mu\text{g}$  a.i./l mean measured concentration.
- D. **Adequacy of the Study:**
- (1) **Classification:** Supplemental.
  - (2) **Rationale:** The mysids used were older than recommended and were not fed during the test. The control solution appeared contaminated with the test material.

(3) Repairability: No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 04-14-92.

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Mancozeb

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Page \_\_\_\_\_ is not included in this copy.

Pages 6 through 8 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.
  - The document is a duplicate of page(s) \_\_\_\_\_.
  - The document is not responsive to the request.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
17.3	20	16	80	.5908966
12.1	20	8	40	25.17223
6.9	20	8	40	25.17223
4.4	20	4	20	.5908966
3.5	20	0	0	9.536742E-05
3.4	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 4.4 AND 17.3 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 13.18146

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.1012125	10.2658	8.435874	13.45672

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1141502	1	.1493678

SLOPE = 3.039973  
 95 PERCENT CONFIDENCE LIMITS = 2.012884 AND 4.067062

LC50 = 10.65272  
 95 PERCENT CONFIDENCE LIMITS = 8.663474 AND 14.22127

LC10 = 4.070928  
 95 PERCENT CONFIDENCE LIMITS = 2.702935 AND 5.195109

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Shaughnessey # 014504 Chemical Name Alumex 206 Chemical Class \_\_\_\_\_ Page 1 of 1

Study/Species/Lab/Chemical MRID # \_\_\_\_\_ % a.i. \_\_\_\_\_ Results \_\_\_\_\_ Reviewer/Validation Date \_\_\_\_\_ Status \_\_\_\_\_

48-Hour EC<sub>50</sub> \_\_\_\_\_ EC<sub>50</sub> - \_\_\_\_\_ pp ( \_\_\_\_\_ ) 95% C.L. \_\_\_\_\_ Control Mortality (%) - \_\_\_\_\_  
Slope - \_\_\_\_\_ # Animals/Level - \_\_\_\_\_ Solvent Control Mortality (%) - \_\_\_\_\_  
Temperature - \_\_\_\_\_

Lab: \_\_\_\_\_  
MRID # \_\_\_\_\_ 48-Hour Dose Level pp / (% Effect) \_\_\_\_\_  
( \_\_\_\_\_ ), ( \_\_\_\_\_ ), ( \_\_\_\_\_ ), ( \_\_\_\_\_ )

Comments:

96-Hour LC<sub>50</sub> 82.4 LC<sub>50</sub> - 11.5 \* 95% C.L. Flubot pp<sub>10</sub> ( 86-14.1 ) Control Mortality (%) - 0  
Slope - N/A # Animals/Level - 20 Solvent Control Mortality (%) - N/A  
Temperature - 22°C

Species: Hyndopsis balnea Supplemental

Lab: Hunter/ESL, Inc. LMR  
7/17/82

MRID # 418279-01 96-Hour Dose Level pp<sub>10</sub> / (% Mortality) 3.4 (5), 3.1 (0), 4.4 (20), 6.9 (40), 12.0 (40), 17.0 (80)

Comments:

\* from standard concentration