3-19-90

Accession No. 408851-02

72-3

DATA EVALUATION RECORD

1. CHEMICAL: Dithane M-45.
Shaughnessey No. 014504.

2. TEST MATERIAL: Dithane M-45, TD #87-139, Lot #76777, a yellow powder, 82.4 percent active ingredient.

16 AI

3. STUDY TYPE: Mollusc Flow-Through Shell Deposition Study.
Species Tested: Eastern Oyster (Crassostrea virginica).

4. CITATION: Manning, C.S. 1988. Dithane M-45: Acute Toxicity on Shell Growth of the Eastern Oyster (Crassostrea virginica). Conducted by Environmental Science and Engineering, Inc., Gainesville, FL. ESE No. 89328-0200-2130. Submitted by Rohm and Haas Company, Spring House, PA. EPA Accession No. 408851-02.

5. REVIEWED BY:

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: January 20, 1989

6. APPROVED BY:

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Isabel C. Johnson
Date: January 30, 1989

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

Signature: C.E.L. 3-7-90 Henry T. Craven
Date: 3/19/90

7. CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for a mollusc flow-through shell deposition test. With a 96-hour EC50 of 1.60 mg a.i./L mean measured concentration, Dithane M-45 is considered moderately toxic to eastern oyster (Crassostrea virginica). The NOEC could not be determined due to reduction in shell growth observed at all test concentrations.

8. RECOMMENDATIONS: N/A.

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9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Oysters (Crassostrea virginica) obtained from Ocean Pond Corporation, Fishers Island, NY, were maintained for 7 days in a cage submerged in water at Marineland, FL. They were then transferred to Environmental Science and Engineering, Inc. (ESE) and maintained at 26 ± 1°C for an additional 3 days in unfiltered seawater. Their length was approximately 27 to 50 mm from umbo to distal valve edge.

Just prior to test initiation, oysters which demonstrated shell growth during the holding period were carefully ground by hand with a fine-grit grinding wheel. Approximately 2-5 millimeters of shell were removed from margins of each oyster to provide a smooth flattened edge.

B. **Test System:** The test system consisted of 15.6-L glass aquaria, each containing approximately 8.3 L of test solution or control seawater at a depth of 8.0 cm. Each aquarium received a continuous flow of natural, unfiltered seawater at a combined rate of 21 L/hour through calibrated standpipes located in control and chemical headboxes. The test was conducted at 26 ± 1°C under fluorescent lighting on a 16-hour light and 8-hour dark photoperiod.

The water used for holding and testing was natural unfiltered seawater, collected from Marineland, Florida. The water temperature was adjusted to 26°C prior to entry into the test system. The stock solution was prepared every 6 hours by the addition of 3.15 g of Dithane M-45 into a 400-L fiberglass reservoir containing 315 L of unfiltered seawater. This stock was then stirred for 1 hour prior to its use in the diluter system. The diluter consisted of two 122 x 20 x 20 cm headboxes with calibrated standpipes used for the distribution of appropriate flows of stock to dilution water to provide a series of concentrations, each being 60% of the next higher concentration.

C. Dosage: Ninety-six-hour EC50 shell deposition test.

D. **Design:** Based on a range-finding study, five nominal concentrations (i.e., 1.3, 2.2, 3.6, 6.0, and 10.0 mg/L as whole material) were selected for the definitive test. A total of 120 oysters were indiscriminately distributed to five treatment groups and one seawater control group (20 per group). The oysters were evenly spaced in each test chamber and oriented to the incoming flow of water.

During the test, salinity was measured in the seawater control test container daily. Temperature was recorded in the control test container hourly. Dissolved oxygen concentrations and pH were measured daily in all treatments. Concentrations of Dithane M-45 in the test solutions and control were analyzed on Days 0, 1, 2, 3, and 4 of the test.

The oysters were removed from the test containers after 96 hours of continuous exposure and new shell growth of each oyster was measured to the nearest 0.1 mm. Percentage shell growth of oysters exposed to Dithane M-45 was calculated by the following equation:

$$\text{Percentage Reduction} = \frac{\text{Mean shell growth of exposed oysters} - \text{Mean Shell growth of control oysters}}{\text{Mean shell growth of control oysters}} \times 100$$

Following measurement of new shell growth, the control oysters were measured for total length, then shucked and wet tissue weights determined.

E. **Statistics:** Differences in shell growth between the control and Dithane M-45 oysters were determined by analysis of variance (ANOVA) followed by Williams' multiple comparison test to identify those concentrations producing effects statistically greater than the control at a confidence level of 95 percent.

A probit transformation was applied to the response variable and then regressed, using least square regression, with the log concentration. An F test for linearity was conducted to determine whether the regression technique adequately described the experimental data. From these data, a concentration-response curve was drawn and an EC50 value and 95% confidence limits were determined from the curve.

12. **REPORTED RESULTS:** The diluter functioned properly during the

96-hour exposure. Mean measured concentrations of Dithane M-45 were presented in Table 3.1 (attached).

Table 3.2 (attached) presents mean shell deposition and percentage change after 96 hours of exposure. Reductions in growth were statistically significant in test concentrations ≥1.4 mg a.i./L mean measured concentration. The 96-hour EC50 value was 2.01 mg a.i./L mean measured concentration, with 95% confidence limits of 0.33 to 12.1 mg a.i./L.

At test termination, control oysters ranged from 27 to 50 mm in total valve height (excluding the new shell growth) and averaged 0.91 g/oyster on a shucked wet weight basis. Loading was calculated to be 0.036 g/L of test solution or dilution water passing through the test aquaria in 24 hours.

During the test, temperature was maintained at 26 ± 1°C and the salinity of the seawater ranged from 30 to 35 parts per thousand. Dissolved oxygen concentrations remained ≥ 6.5 mg/L (≥98% of saturation) and pH ranged from 8.1 to 8.2 in all test aquaria throughout the test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author. A statement was included by the Study Director, indicating that the study was conducted and complied with published Good Laboratory Practices (GLP) for tests of substances regulated under the Toxic Substances Control Act and the Federal Insecticide, Fungicide, and Rodenticide Act. The test data were reviewed by ESE's Quality Assurance Unit to assure that standard operating procedures and protocol used in the conductance of this test were followed.

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

A. **Test Procedure:** The test procedures generally followed the SEP guidelines, except that there was no 15- to 30-minute transition period between light and dark photoperiod.

B. **Statistical Analysis:** The reviewer reanalyzed shell growth data using analysis of variance and multiple comparisons with 3 tests (i.e., Tukey's, Bonferroni's, and Dunnett's) and obtained slightly different results from those performed by the author. The author found no significant difference in shell growth between the control group and the 0.71-mg/L group. Multiple comparison tests performed by the reviewer showed reduction in shell growth

at all test concentrations, including 0.71 mg/L, when compared to the control ($p \leq 0.05$).

The 96-hour EC50 value was calculated using EPA's Toxanal computer program. The result was similar to that calculated by the author. All printouts are attached.

C. **Discussion/Results:** With a 96-hour EC50 value of 1.60 mg a.i/L mean measured concentration (95% C.L. = 1.40-1.80 mg/L), Dithane M-45 is considered moderately toxic to eastern oyster (Crassostrea virginica). Since shell growth at all test concentrations was significantly reduced when compared to the control ($p \leq 0.05$), the no-observed-effect concentration (NOEC) could not be determined.

D. **Adequacy of the Study:**

(1) Classification: Core

(2) Rationale: N/A

(3) Repairability: N/A

15. **COMPLETION OF ONE-LINER:** Yes, January 20, 1989.

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Analysis of Variance File: dithoys2 Date: 01-20-1988

FILTER: None

N's, means and standard deviations based on dependent variable: GROWTH

* Indicates statistics are collapsed over this factor

Factors: C	Measured Conc. (mg a.i./L)	N	Mean (mm)	S.D.
*		119	0.6815	0.6803
1	Control	20	1.3250	0.7559
2	0.71	20	1.1200	0.6288
3	1.40	20	0.6050	0.5286
4	2.10	20	0.4550	0.6100
5	3.20	20	0.2850	0.3167
6	4.00	19	0.2789	0.3706

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Fmax for testing homogeneity of between subjects variances: 5.70

Number of variances= 6 df per variance= 19.

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Analysis of Variance Dependent variable: GROWTH

Source	df	SS (H)	MSS	F	P
Between Subjects	118	54.6193			
C (CONC)	5	19.4937	3.8987	12.542	0.0000
Subj w Groups	113	35.1256	0.3108		

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Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.325	6	0.279
2	1.120		
3	0.605		
4	0.455		
5	0.285		

Comparison	Tukey-A*	Bon-ferroni	Dunnett
1 > 2			
1 > 3	0.0100	0.0014	0.0100
1 > 4	0.0100	0.0000	0.0100
1 > 5	0.0100	0.0000	0.0100
1 > 6	0.0100	0.0000	0.0100
2 > 3	0.1000	0.0633	N.A.
2 > 4	0.0100	0.0041	N.A.
2 > 5	0.0100	0.0000	N.A.
2 > 6	0.0100	0.0000	N.A.
3 > 4			N.A.
3 > 5			N.A.
3 > 6			N.A.
4 > 5			N.A.
4 > 6			N.A.
5 > 6			N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000).
A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

KOSALWAT DITHANE M-45 CRASSOSTREA VIRGINICA 01-20-89

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
4	100	77	77	0
3.2	100	77	77	0
2.1	100	62	62	0
1.4	100	54	54	0
.71	100	15	15	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.313595

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.942913E-02	1.496679	1.28939	1.698213

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	4.021041E-02	1	5.852825E-02

SLOPE = 2.308158

95 PERCENT CONFIDENCE LIMITS = 1.845313 AND 2.771001

LC50 = 1.598989

95 PERCENT CONFIDENCE LIMITS = 1.399895 AND 1.800062

LC10 = .4504256

95 PERCENT CONFIDENCE LIMITS = .3066643 AND .5863703

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