

3-17-90

Accession No. 408851-01

72-3

DATA EVALUATION RECORD

1. CHEMICAL: Dithane F-45.
Shaughnessey No. 014504.
2. TEST MATERIAL: Dithane F-45, TD #87-138, Lot #2-4262, a yellow viscous liquid, 37 percent active ingredient.
3. STUDY TYPE: Mollusc Flow-Through Shell Deposition Study.
Species Tested: Eastern Oyster
(Crassostrea virginica).
4. CITATION: Manning, C.S. 1988. Dithane F-45: Acute Toxicity on Shell Growth of the Eastern Oyster (Crassostrea virginica). Conducted by Environmental Science and Engineering, Inc., Gainesville, FL. ESE No. 89328-0300-2130. Submitted by Rohm and Haas Company, Spring House, PA. EPA Accession No. 408851-01.

TEP
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5. REVIEWED BY:

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Staff Toxicologist
KBN Engineering and
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Signature: P. Kosalwat

Date: January 20, 1989

6. APPROVED BY:

Isabel C. Johnson, M.S.
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Signature: Isabel C. Johnson

Date: January 30, 1989

Henry T. Craven, M.S.
Supervisor, EEB/HED
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Signature:

Date:

C.E.T. 3-7-90

Henry T Craven
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 1.53 mg a.i./L mean measured concentration, Dithane F-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.

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 20 days in a cage submerged in water at Marineland, FL. They were then transferred to Environmental Science and Engineering, Inc. (ESE) and maintained at $26 \pm 1^{\circ}\text{C}$ for an additional 3 days in unfiltered seawater. Their length was approximately 26 to 51 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.

- S 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 $26 \pm 1^{\circ}\text{C}$ 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 5.04 g of Dithane F-45 into 315 L of unfiltered seawater contained within a 400 L fiberglass reservoir. 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., 2.1, 3.5, 5.8, 9.6, and 16.0 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 F-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 F-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 F-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 with a single malfunction on Day 1 when the flow to the chemical cell became obstructed resulting in all treatments (other than the 16.0-mg/L level which received no dilution water) receiving only dilution water for approximately four hours. The measured concentrations of Dithane F-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 all test concentrations. The 96-hour EC50 value was 5.88 mg/L whole product (1.89 mg a.i./L measured concentration) with 95% confidence limits of 0.92 to 37.6 mg/L whole product (0.16 to 21.6 mg a.i./L measured concentration).

At test termination, control oysters ranged from 26 to 51 mm in total valve height (excluding the new shell growth) and averaged 1.18 g/oyster on a shucked wet weight basis. Loading was calculated to be 0.047 g/L of test solution or dilution water passing through the test aquaria in 24 hours.

During the test, temperature was maintained at $26 \pm 1^{\circ}\text{C}$ with an occasional reading of 28°C (less than 5% of the total test duration) and the salinity of the seawater ranged from 30 to 35 parts per thousand. Dissolved oxygen concentrations remained ≥ 6.0 mg/L ($\geq 90\%$ of saturation) and pH ranged from 7.9 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. Also, the flow to the chemical cell was obstructed for approximately 4 hours on Day 1, reducing

the exposure concentrations in all aquaria (except the highest level).

- 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 similar results to those performed by the author. The 96-hour EC50 values for both whole material and mean measured concentration (active ingredient) were calculated using EPA's Toxanal computer program. The results were also similar to those calculated by the author. All printouts are attached.
- C. **Discussion/Results:** With a 96-hour EC50 value of 1.53 mg a.i./L mean measured concentration (95% C.L. = 0.40-3.43 mg/L), Dithane F-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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Page _____ is not included in this copy.

Pages 6 through 7 are not included.

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.
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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	S.D.
*			119	0.7218	0.5918
1		Control	20	1.2900	0.4400
2		0.53	20	1.0300	0.3854
3		1.20	20	0.8200	0.7537
4		1.80	19	0.4789	0.4379
5		3.10	20	0.3200	0.3443
6		5.10	20	0.3800	0.4034

Fmax for testing homogeneity of between subjects variances: 4.79
 Number of variances= 6 df per variance= 19.

Analysis of Variance Dependent variable: GROWTH

Source	df	SS (H)	MSS	F	P
Between Subjects	118	41.3232			
C (CONC)	5	15.2356	3.0471	13.199	0.0000
Subj w Groups	113	26.0876	0.2309		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.290	6	0.380
2	1.030		
3	0.820		
4	0.479		
5	0.320		

Comparison	Tukey-A*	Bon-ferroni	Dunnnett
1 > 2			
1 > 3 ✓	0.0500	0.0376	0.0500
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			N.A.
2 > 4	0.0100	0.0079	N.A.
2 > 5	0.0100	0.0000	N.A.
2 > 6	0.0100	0.0007	N.A.
3 > 4			N.A.
3 > 5	0.0500	0.0202	N.A.
3 > 6	0.1000	0.0682	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 Dunnnett's test only the P-values .05 and .01 are possible
 and only for comparisons with the control mean (level 1).

mean measured concentration

KOSALWAT DITHANE F-45 CRASSOSTREA VIRGINICA 01-19-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
5.1	100	69	69	0
3.1	100	77	77	0
1.8	100	62	62	0
1.2	100	38	38	0
.53	100	23	23	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 1.469694

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	8.472743E-02	1.372533	1.046554	1.708159

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2	.649783	4.297104	4.877329E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.475263
95 PERCENT CONFIDENCE LIMITS = .2860667 AND 2.66446

LC50 = 1.527696
95 PERCENT CONFIDENCE LIMITS = .4000804 AND 3.426989

LC10 = .2104619
95 PERCENT CONFIDENCE LIMITS = 2.753297E-05 AND .6040005

Whole material

KOSALWAT DITHANE F-45 CRASSOSTREA VIRGINICA 01-19-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
16	100	69	69	0
9.600001		100	77	77
5.8	100	62	62	0
3.5	100	38	38	0
2.1	100	23	23	0

THE BINOMIAL TEST SHOWS THAT 3.5 AND 5.8 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 4.505553

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	8.472743E-02	4.497763	3.52678	5.472747

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.7024679	4.614057	3.127813E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.58879
95 PERCENT CONFIDENCE LIMITS = .2571718 AND 2.920407

LC50 = 4.996682
95 PERCENT CONFIDENCE LIMITS = 1.21688 AND 11.45962

LC10 = .7931057
95 PERCENT CONFIDENCE LIMITS = 2.74646E-05 AND 2.150004
