UNDATED

DATA EVALUATION RECORD

1. **CHEMICAL:** Molinate.
Shaughnessey No. 041402.

2. **TEST MATERIAL:** Arrosolo 3-3E; 33.5% molinate; Lot No. WRC 11877-1-1; a dark-brow formulated product.

3. **STUDY TYPE:** Mollusc 96-Hour Flow-Through Shell Deposition Study. Species Tested oyster (*Crassostrea virginica*).

4. **CITATION:** Dionne, E. 1990. Molinate: Acute Toxicity of Arrosolo 3-3EE to Easte *Crassostrea virginica*). Performed by Springborn Laboratories, Inc., Wareham, M by ICI Americas Inc. EPA MRID No. 417053-02.

5. **REVIEWED BY:**
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7. **CONCLUSIONS:** This study is scientifically sound but does not meet the requiremen deposition study. ICI did not submit the raw datd for growth, therefore, an AN correct statistical test) could not be done.

Unser the conditions of the test, the 96-hour EC_{50} of Arrosolo 3-3E for eas oyster would have been 4.5 mg/l (mean measured) with a 95% confidence interva 4.2-4.8 mg/l. Therefore, Arrosolo 3-3E would be classified as moderately toxic oyster. The NOEC given by the author of Arrosolo 3-3E for eastern oyster was 2.2

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

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5. **REVIEWED BY:**

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

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a deposition study. The 96-hour EC_{50} of Arrosolo 3-3E for eastern oyster was 4. measured) with a 95% confidence interval of 4.2-4.8 mg/l. Therefore, Arrosolo classified as moderately toxic to eastern oyster. The NOEC given by the author for eastern oyster was 2.2 mg/l.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

    A. **Test Animals:** Eastern oysters (Crassostrea virginica) were obtained from a supplier in Dennis, MA. The supplier held the oysters in flowin (Massachusetts Bay) with a temperature of 19°-22.5°C, a salinity of 30-thousand (ppt), and a dissolved oxygen (D.O.) concentration 82-90% of sat oysters were fed marine algae which had an average density of 29 x 10^3 cell Forty-eight hours before test initiation, the oysters were transported styrofoam containers to the laboratory. The oysters were inspected for

maturity, were similar in age, and had a mean valve height of 43 ±4 mm.

During the last 48 hours of acclimation to the laboratory, the oysters w diet of Isochrysis galbana and Tetraselmis maculata. The temperature w salinity was 31 ppt, the pH was 7.9, and the D.O. concentration was saturation. No mortality occurred during holding.

Twenty-four hours prior to testing, 3-5 mm of new peripheral shell removed by grinding the shell with a grinding wheel. The oysters overnight and examined for signs of stress. Any oysters which app than optimal were discarded. Immediately prior to test initiation, of the shell was buffed with an emery board to remove any new shell g

B. **Test System:** A continuous flow serial diluter with a dilution factor used to deliver 5 Arrosolo 3-3E concentrations and a dilution wa Twelve aquaria, two replicate aquaria per concentra-tion, were positioned in a temperature controlled water bath set to maintain 20 glass aquarium measured 60 x 30 x 30 cm, was equipped with a standpipe, and had a total test volume of approximately 18 L. The aquarium (75 mL/minute) provided six volume replacements every 24 Recirculation of the test solution was provided in each individual a a flow rate of about 5 L/oyster/hour. During the exposure, the oys supplemental feedings of 180 mL of algal suspension (I. galba maculata, 10^5 cells/mL) per aquarium three times daily. Overhead flu lighting was maintained on a 16-hour light photoperiod.

Natural, unfiltered seawater was used as dilution water. The se pumped from Cape Cod Canal, Bourne, MA, into a large fiberglass hol before distribution to the diluter. The salinity and pH of the seaw and 7.8-8.1, respectively.

A syringe pump delivered 0.0040 mL/minute of the test material (377 directly into the chemical mixing chamber which also received 375 m seawater. This resulted in a solution which was equivalent to the hi test concentration of 4.0 mg a.i./L. A portion of this solution produce the lower concentrations.

C. **Dosage:** Ninety-six-hour acute flow-through toxicity test. Based on preliminary test, five nominal concentrations (0.52, 0.86, 1.4, 2. a.i./L), and a dilution water control were chosen for the defini concentrations of Arrosolo 3-3E were based on the percent active present in the test material.

D. **Design:** Twenty oysters were impartially distributed to each aquarium of 40 oysters per concentra-tion. Oysters were placed equidistant other with their valves facing towards the flow of water from the rec

The calibration of the diluter system was confirmed prior to test i termination. The diluter function was completely checked twice dai test. The pH, temperature, salinity, and D.O. of the test solutions in each replicate aquarium every 24 hours. Temperature was also continuously in one replicate of the dilution water control.

Every 24 hours, the oysters were observed for visible abnormalitie hours, new shell growth was measured microscopically to the neares

using a calibrated micrometer.

Water samples were removed from each replicate exposure solution controls on days 0 and 4 for analysis of molinate by gas chromatograp

E. **Statistics:** For each observation period, the EC_{50} value and its 95% limits were determined by linear regression of response (percent shell growth as compared with controls) vs. mean measured ex concentration over the range of test concentrations. Various m manipulations (logarithm and probit transformations) were used concentration and response data to get the linear regression with coefficient of determination (r^2). The regression equation was used the EC_{50} and confidence interval (C.I.) using the method of inverse p The growth data were analyzed for homogeneity of variance an no-observed-effects concentration (NOEC) determined using William's t

12. **REPORTED RESULTS:** The mean measured concentrations were 0.50, 0.75, 1.2, 2 and 3.8 mg a.i./L (Table 2, attached). These values were fairly consi sampling days and averaged 90% of nominal. The diluter system was operati throughout the test.

No mortality occurred during the test. Oyster shell deposition decreased Arrosolo 3-3E concentration (Table 3, attached). The 96-hour EC_{50} of Arro eastern oysters was calculated as 1.5 mg a.i./L (95% C.I. = 0.91-2.50 mg a exposed to the three highest concentrations exhibited reduced feeding an shell growth reductions in comparison to the controls. The NOEC was 0.75 the percent active ingredient is applied to the final results (EC_{50} and NOE would be 4.5 mg of Arrosolo 3-3E/L and the NOEC would be 2.2 mg of Arrosolo

The temperature during the test ranged from 19° to 22°C. The pH was 7.8 t D.O. ranged from 6.0 to 7.1 mg/L or 60 to 71% of saturation at 20°C and a ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the author.

Good laboratory practice and Quality Assurance Unit statements were incl report indicating compliance to EPA Good Laboratory Practice Standards Federal Insecticide, Fungicide, and Rodenticide Act.

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

A. **Test Procedure:** The test procedures were generally in accordance wit protocols recommended by the guidelines with the following deviations

In this study, the flow rate of the "recirculating" test soluti L/oyster/hour. According to the protocols recommended by the SEP, e should receive a minimum of 5 L of "once-through," flow-through test hour. However, the above method is considered acceptable beca supplemental diet was added.

A 30-minute dawn and dusk simulation is recommended. The report d state whether a dawn and dusk simulation was used.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to det the 96-hour EC_{50} as 1.5 mg a.i./L (95% C.I. = 1.4-1.6 mg a.i./L) b method (see attached printout). Raw shell deposition data were not therefore, no NOEC could be recalculated.

C. **Discussion/Results:** The 96-hour EC_{50} of Arrosolo 3-3E for eastern oy 4.5 mg/L with a 95% confidence interval of 4.2-4.8 mg/L. Therefor 3-3E would be classified as moderately toxic to eastern oyster. T Arrosolo 3-3E for eastern oyster was determined to be 2.2 mg/L.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 6-17-91.

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