

UNDATED

DATA EVALUATION RECORD

1. **CHEMICAL:** Molinate.
Shaughnessey No. 041402.
2. **TEST MATERIAL:** Ordram 8E; 90.3% active ingredient; Lot No. WRC 11877-6-1; an a liquid 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 Ordram-8E to Eastern O (*Crassostrea virginica*). SLI Report No. 90-10-3513; Performed by Springborn Lab Wareham, MA. Submitted by ICI Agrochemicals, Haslemere, Surrey, UK. EPA M 417053-01.
5. **REVIEWED BY:**
James J. Goodyear, Ph.D. **Signature:**
Biologist, Section 1
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Environmental Fate and Effects Division (H7507C)
6. **APPROVED BY:**

Leslie W. Touart, Ph.D. **Signature:**
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Henry T. Craven, M.S. **Signature:**
Supervisor, EEB/HED
USEPA **Date:**
7. **CONCLUSIONS:** This study is scientifically sound but does not meet the requiremen deposition study. ICI did not submit the raw data for growth, therefore, an AN correct statistical test) could not be done.
Under the conditions of the test, the 96-h ~~Ordram 8E~~ ^{Ordram 8E} for eastern oysters would have been 5.3 mg/l (mean measured) with a 95% confidence interval of 4.7 mg/l. Therefore, Ordram 8E would be classified as moderately toxic to eastern The NOEC given by the author for Ordram 8E was 2.1 mg/l.
8. **RECOMMENDATIONS:** Submit the growth data. Although it is not required, the review speeded if the data were submitted on a DOS or DOS diskette in ASCII format a paper.
9. **BACKGROUND:** Phase V submission.
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

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5. **REVIEWED BY:**

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KBN Engineering and **Date:**
Applied Sciences, Inc.
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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. Under the conditions of the test, the 96-hour EC₅₀ of Ordram oyster was 5.3 mg/l (mean measured) with a 95% confidence interval of 4.7-6.2 mg Ordram 8E would be classified as moderately toxic to eastern oyster. The NOEC author for Ordram 8E was 2.1 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 15°-22°C, a salinity of 30-3 thousand (ppt), and a dissolved oxygen (DO) concentration of 86-97% of sat oysters were fed marine algae which had an average density of 48 x 10³ cell Forty-eight hours before test initiation, the oysters were transported ou styrofoam containers to the laboratory. The oysters were inspected for maturity, were similar in age, and had a mean valve height of 41 ±5 mm.

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During the 48 hours of acclimation to the laboratory, the oysters were fed Isochrysis galbana. The temperature was 18°C, the salinity was 31 ppt,

7.9-8.0, and the DO concentration was 87-97% of saturation.

Twenty-four hours prior to testing, 3-5 mm of new peripheral shell growth by grinding the shell with a grinding wheel. The oysters were held examined for signs of stress. Any oysters which appeared less than o discarded. Immediately prior to test initiation, the outer edge of the sh an emery board to remove any new shell growth.

- B. **Test System:** A continuous flow serial diluter with a dilution factor of 60 deliver 5 Ordram 8E concentrations, a solvent (acetone) control and a d control. Fourteen aquaria, two replicate aquaria per concentration, w positioned in a temperature controlled water bath set to maintain $20 \pm 2^\circ\text{C}$ aquarium measured 60 x 30 x 30 cm, was equipped with a 10 cm standpipe, a total test volume of approximately 18 L. The flow to each aquarium (7 provided six volume replacements every 24 hours. Recirculation of the tes provided in each individual aquarium to give a flow rate of about 5 L/oyste During the exposure, the oysters received supplemental feedings of 180 suspension (*I. galbana*, 10^5 cells/mL) per aquarium three times daily. fluorescent lighting was maintained on a 16-hour light photoperiod.

Natural, unfiltered seawater was used as dilution water. The seawater was Cape Cod Canal, Bourne, MA, and held in an epoxy-coated concrete holding before distribution to the diluter. The salinity and pH of the seawater w 7.8-8.1, respectively.

A diluter stock containing 86.4 mg a.i./mL was prepared by diluting 28.7 Ordram 8E to 300 mL in acetone. A syringe pump delivered the stock to th second syringe pump delivered an acetone solution to the solvent control which resulted in a solvent concentration equal to the concentration of s the highest test solution (0.08 mL acetone/L).

- C. **Dosage:** Ninety-six-hour acute flow-through toxicity test. Based on a prel five nominal concentrations (0.93, 1.6, 2.6, 4.3, and 7.2 mg a.i./L), a so a dilution water control were chosen for the definitive test. The concent 8E were based on the percent active ingredient present in the test material
- D. **Design:** Twenty oysters were impartially distributed to each aquarium for a oysters per concentration. Oysters were placed equidistant from each ot valves facing towards the flow of water from the recirculator.

The calibration of the diluter system was confirmed prior to test in termination. The diluter function was completely checked twice daily duri pH, temperature, salinity, and D.O. of the test solutions were measured in aquarium every 24 hours. Temperature was also monitored continuously in o of the dilution water control.

Every 24 hours, the oysters were observed for visible abnormalities. Afte shell growth was measured microscopically to the nearest 0.1 mm using a micrometer.

Water samples were removed from each replicate exposure solution and the c days 0 and 4 for analysis of molinate by gas chromatography (GC).

- E. **Statistics:** For each observation period, the EC_{50} value and its 95% confid were determined by linear regression of response (percent reduction of sh compared with controls) vs. mean measured exposure concentration over the test concentrations. Various mathematical manipulations (logarithm transformations) were used on the concentration and response data to ge

regression with the highest coefficient of determination (r^2). The regress was used to calculate the EC_{50} and confidence interval (C.I.) using the met inverse prediction. The growth data were analyzed for homogeneity of var no-observed-effects concentration (NOEC) determined using William's test.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.84, 1.2, 1.9, 3.2, and mg a.i./L (Table 2, attached). Measured concentrations were fairly consistent days and averaged 76% of nominal. The diluter system was operating properly t test.

No mortality or sublethal effects were observed among the oysters. Oyster s decreased with increasing Ordram 8E concentration (Table 3, attached). The 96-Ordram 8E for eastern oysters was calculated as 4.9 mg a.i./L (95% C.I. = 4.0 Oysters in the two highest exposure concentrations exhibited significant shell in comparison to the controls. Growth in the dilution water control and solve significantly different, so the results were pooled prior to NOEC determination 1.9 mg a.i./L. Based on the total product, the EC_{50} was 5.4 mg of Ordram 8E/L a was 2.1 mg of Ordram 8E/L.

The temperature during the test ranged from 19° to 21°C. The pH was 7.8 to 8. ranged from 6.2 to 7.5 mg/L or 62.0 to 75.0% of saturation at 20°C and a salinity

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

Good laboratory practice and Quality Assurance Unit statements were included indicating compliance to with EPA Good Laboratory Practice Standards under Insecticide, Fungicide, and Rodenticide Act.

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

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

In this study, the flow rate of the "recirculating" test solution was ab According to the protocols recommended by the SEP, each oyster should minimum of 5 L of "once-through," flow-through test solution per hour. above method is considered acceptable because a supplemental diet was added

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

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine 96-hour EC_{50} as 4.8 mg a.i./L (95% C.I. = 4.2-5.6 mg a.i./L) by the probi attached printout). Based on total product, the EC_{50} of Ordram 8E would Since this number is more conservative than the author's, it will be taken EC_{50} value. Raw shell deposition data were not submitted, therefore, no NO recalculated.

- C. **Discussion/Results:** The greatest percentage reduction in shell deposition 4.8 mg a.i./L (mean measured). The EC_{50} generated is therefore an extrapol on the regression equation from the probit analysis. To be considered vali should be bracketed by two test concentrations. However, in this test, the generated is probably a good approximation of the actual EC_{50} .

Under the conditions of the test, the 96-hour EC_{50} of Ordram 8E for eastern 5.3 mg/L with a 95% confidence interval of 4.7-6.2 mg/L. Therefore, Ordram

classified as moderately toxic to eastern oyster. The NOEC of Ordram 8E oyster was determined to be 2.1 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.