(9-15-92)

MRID No. 418695-09

DATA EVALUATION RECORD

1. **CHEMICAL:** Cimectacarb.
Shaughnessey No. 112602.

2. **TEST MATERIAL:** CGA-163935 Technical; Batch No. FL-891393; 92.2% active ingredient; a dark amber liquid.

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

4. **CITATION:** Dionne, E. 1991. CGA-163935 Technical - Acute Toxicity to Eastern Oysters (*Crassostrea virginica*). SLI Report No. 91-03-3701. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA MRID No. 418695-09.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
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Signature: Rosemary Graham Mora
Date: 7/24/92

Brian Montague
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6. **APPROVED BY:**

Louis M. Rifici, M.S.
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Signature: P. Kosalwat for LMR
Date: 7/24/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
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7. **CONCLUSIONS:** This study is scientifically sound and does meet the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test. Concentrations tested were less than 100 ppm but not high enough to produce a precise EC_{50}. The NOEC could not be determined.

8. **RECOMMENDATIONS:** ~~N/A.~~ Study should be repeated at dosages which produces 50% shell reduction and reliable Confidence Limits. Bpm

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Eastern oysters (*Crassostrea virginica*) were obtained from Aquacultural Research Corporation, Dennis, MA. Once received in the laboratory, the animals were held in wooden epoxy-painted trays in flowing seawater and examined for suitability in the test. The oysters were held for 2 days prior to test initiation and acclimated to a salinity of 31 parts per thousand (ppt) and a temperature of 18-20°C. The oysters were fed a supplementary algal diet of *Isochrysis galbana* and *Tetraselmis maculata*.

The oysters were of similar age and size and had a mean valve height of 36 ±3 mm. No mortality was noted during the holding period at the testing laboratory.

B. **Test System:** The test was conducted in a continuous flow serial diluter system (60% dilution factor). This system provided a flow rate of 75 ml/minute to each aquarium, providing six volume replacements/day. The glass test aquaria (60 x 30 x 30 cm) were each equipped with a 10-cm standpipe to regulate solution volume at 18 l. Circulation (flow rate of 1.75 l/minute) within each aquarium provided an even distribution of algae and test solution. The flow rate of the recirculated volume of test solution was 5 l/oyster/hour.

A temperature-controlled water bath was used to maintain test temperature (20 ±2°C). The test was conducted under fluorescent lighting on a 16-hour light and 8-hour dark photoperiod.

The dilution water was natural unfiltered seawater collected from Cape Cod Canal, Bourne, MA. The seawater had a pH range of 7.8-7.9 and a salinity range of 31-32 ppt.

A diluter stock solution (181 mg a.i./ml) was prepared by dissolving 196 g (181 g as a.i.) of the test material in dimethylformamide (DMF) to a final volume of 1000 ml. This diluter stock solution was then mixed with dilution water in the chemical mixing chamber to provide the highest test concentration (85 mg a.i./l). Serial dilutions of this solution provided the remaining test concentrations.

C. **Dosage:** Ninety-six-hour flow-through acute test. Based on preliminary testing, five nominal test concentrations (11, 18, 31, 51, and 85 mg a.i./l), a

dilution water control, and a solvent control (0.47 ml DMF/l) were used.

D. **Design:** One day prior to test initiation, 3-5 mm of the new peripheral shell growth of each oyster were removed by grinding the shell to a blunt edge. Immediately prior to test initiation, the outer shell edge was buffed to remove any new shell deposition.

Fourteen aquaria (two aquaria/treatment) were positioned in the water bath using random selection. The test was initiated by impartially selecting and positioning 20 oysters in each test aquarium (40 oysters/treatment). During the exposure period, the oysters were fed a supplementary algal diet of *Isochrysis galbana* and *Tetraselmis maculata* three times daily.

At test initiation and every 24 hours thereafter, the oysters were observed for visible abnormalities and the physical characteristics of the test solutions were observed. 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 using a calibrated micrometer.

The temperature, salinity, pH, and dissolved oxygen concentration were measured daily in each aquarium. The temperature was also continuously monitored in one replicate of the control. Analytical determination (high performance liquid chromatography) of CGA-163935 Technical was performed on samples collected on days 0 and 4.

E. **Statistics:** The EC_{50} values (with 95% confidence limits) were determined by fitting untransformed and transformed data to a best fit linear regression curve based on least squares. Four linear regression curves were computed and the best fit of the untransformed or transformed data was selected based on the highest associated coefficient of determination (i.e., r^2). This regression equation was then applied to calculate the EC_{50} and 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1969). An SLI computer program was used to assist in the calculation.

Williams' (1971, 1972) test method was used to determine the NOEC. There was a significant difference between the control and solvent control, therefore, the solvent control data were used for analysis.

12. **REPORTED RESULTS:** Mean measured concentrations were 8.4, 16, 34, 52, and 85 mg a.i./l (Table 2, attached). The mean measured concentrations averaged 95% of nominal concentrations. No undissolved test material was observed in any test chamber.

No mortality was observed in any treatment group or the controls at test termination. Based on new shell growth reduction of treatment oysters compared to the new shell growth of the solvent control oysters, the EC_{50} (95% confidence interval) was 89 (50-180) mg a.i./l mean measured concentration (Table 3, attached). Compared to the solvent control, shell growth in all exposures were significantly reduced, therefore, the NOEC was <8.4 mg a.i./l.

During the test period, the pH was 7.5-7.9, the dissolved oxygen concentration was 6.0-7.8 mg/l, the temperature was 19-21°C, and the salinity was 31-32 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based on criteria established by US EPA (1985) CGA-163935 would be classified as slightly toxic to Eastern oysters (*Crassostrea virginica*)."

Good Laboratory Practice Compliance and quality assurance statements were included in the report, indicating that the study was conducted in accordance with the EPA Good Laboratory Practice regulations (40 CFR Part 160) except for the stability, characterization, and verification of the test substance identity.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, but deviated as follows:

The report does not indicate whether 15- to 30- minute dawn/dusk simulation periods were used during the study as recommended.

In this study, the flow rate of the "recirculating" test solution was about 5 l/oyster/hour. According to protocols recommended by the SEP (APHA, 1981 and Anonymous, 1976), each oyster should receive a minimum of 5 l of "once-through" flow through test solution per hour. However, for this study, it is probably acceptable since a supplemental algal diet was added and control oysters met the minimum new shell growth requirement (2 mm).

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 96-hour EC_{50} value (printouts, attached). Since no concentration produced a 50% reduction in new shell growth, no reliable EC_{50} could be determined.

The author's analysis of an NOEC for new shell growth could not be verified since individual new shell growth data were not presented in the report.

C. **Discussion/Results:** This study is scientifically sound and ~~but~~ does ~~not~~ meet the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test. Concentrations tested were less than 100 ppm but not high enough to produce a precise EC_{50}. The NOEC could not be determined. However the highest concentration 89 ppm produced 48% mortality level.

D. **Adequacy of the Study:**

(1) **Classification:** ~~Supplemental.~~ Core

(2) **Rationale:** No precise EC_{50} was determined by this study.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, July 21, 1992.

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