DATA EVALUATION RECORD

1. **CHEMICAL:** Benefin (or Benfluralin). Shaughnessey No. 084301.

2. **TEST MATERIAL:** Benefin; N-Butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-ρ-toluidine; Lot No. 231 EF4; CAS No. 1861-40-1; 96.6% active ingredient; a yellow powder.

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

4. **CITATION:** Dionne, E. 1990. (Benefin) - Acute Toxicity to Eastern Oysters (*Crassostrea virginica*) Under Flow-Through Conditions. SLI Report No. 90-07-3355. Performed by Springborn Laboratories, Inc., Wareham, MA. Submitted by DowElanco Products Company. EPA MRID No. 416138-03.

5. **REVIEWED BY:**

Alvaro A. Yamhure
Aquatic Biologist, EEB/EFED
USEPA

Signature: 2/2/94
Date:

6. **APPROVED BY:**

Daniel Rieder,
Head Section 3
EEB/EFED

Signature:
Date: 2-2-94

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a 96-hour flow-through mollusc shell deposition study. Control and solvent control oysters demonstrated lower shell growth - under 2.0 mm - than should be expected (i.e., 1.7 and 1.4 mm, respectively) by test termination. In addition, the measured concentrations increased considerably during the 96-hour exposure. Under these test conditions the actual concentrations to which the test organisms were exposed could not be properly determined. Under the conditions of the test, the reported 96-hour EC_{50} was 103 µg a.i./l and the NOEC 44 µg a.i./l (mean measured concentrations). These toxicity levels classify benefin as highly toxic to *Crassostrea virginica*. This test is rated as **supplemental** and the data obtained can be used for risk assessment purposes.

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 Massachusetts. The supplier held the oysters in flowing seawater (Massachusetts Bay) at a temperature of 17-20°C, a salinity of 31-32 parts per thousand (ppt), and a dissolved oxygen concentration of 80-88% of saturation. The oysters were continuously fed a combination of marine algae (average density of 24 x 10^3 cells/ml). Forty-eight hours prior to test initiation, oysters were transported to the testing laboratory out of water (1.5 h). Upon arrival at the facility, the oysters were inspected for parasites and maturity. The oysters were held in water at a temperature of 18-19°C, a salinity of 30-31 ppt, a pH of 7.5-8.0, and a dissolved oxygen concentration of 65-96% of saturation. The oysters were fed a supplementary algal diet of *Isochrysis galbana* and *Tetraselmis maculata*. No mortality occurred during holding. The oysters were of similar age and size and had a mean valve height of 37 ±5 mm.

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

B. **Test System:** A continuous flow serial diluter with a dilution factor of 50% was used to deliver 5 benefin concentrations, a solvent control, and a dilution water control. Fourteen aquaria, two replicate aquaria per concentration, were randomly positioned in a temperature-controlled water bath maintained at 20 ±2°C. Each glass aquarium (60 x 30 x 30 cm) was equipped with a 10-cm high standpipe which maintained a test solution volume of approximately 18 l. The flow to each aquarium (75 ml/minute) provided six volume replacements every 24 hours. Recirculation of the test solution was provided in each aquarium to give a flow rate of about 5 l/oyster/hour. During the exposure, the oysters received supplemental feedings of 180 ml of

algal suspension (*I. galbana* and *T. maculata*, 10^5 cells/ml) per aquarium three times daily. Overhead fluorescent lighting was maintained on a 16-hour light photoperiod.

Natural, unfiltered seawater was used as dilution water. The seawater was pumped from Cape Cod Canal, Bourne, MA, into a large fiberglass holding tank before distribution to the diluter. The salinity and pH of the seawater were 30-31 ppt and 7.9-8.0, respectively.

A diluter stock solution (2.43 mg a.i./l) was prepared by dissolving 2.52 g of test material in acetone to a total volume of 1000 ml. A syringe pump delivered 0.0986 ml/minute of the stock solution into the chemical mixing chamber which also received 300 ml/minute of seawater. The resulting solution was equivalent to the highest nominal test concentration (800 µg/l). A portion of this solution was diluted to produce the next four lower concentrations. The pump also delivered 0.0986 ml/minute of an acetone stock solution (500 µl/ml) to a diluter mixing cell where it was mixed with 150 ml/minute seawater. The calibration of the diluter system was confirmed prior to test initiation and at termination.

C. **Dosage:** Ninety-six-hour flow-through acute test. Based on preliminary testing, five nominal concentrations were tested (50, 100, 200, 400, and 800 µg a.i./l) were tested. A dilution water control and a solvent control (330 µl acetone/l) were also included.

D. **Design:** Twenty oysters were impartially selected and distributed to each aquarium for a total of 40 oysters per concentration. Oysters were placed equidistant from each other with their valves facing towards the flow of water from the recirculator.

The pH, temperature, salinity and dissolved oxygen concentration were measured daily in each replicate aquarium. Temperature was also monitored continuously in one replicate. The diluter function was checked twice daily during the test.

Every 24 hours, the oysters and test solutions were observed for visible abnormalities and solution characteristics. After 96 hours, new shell growth was measured microscopically to the nearest 0.1 mm using a calibrated micrometer.

Water samples were removed from each replicate of the exposure solutions and the controls on days 0 and 4 for analysis of benefin by gas chromatography.

E. **Statistics:** The 96-hour EC_{50} value and its 95% confidence limits were determined by fitting untransformed and transformed data to a best fit linear regression curve based on least squares. The mean shell growth measurement of 40 individual oysters for each of the five exposure concentrations were expressed as percentage of the control oyster growth.

The growth data were analyzed for homogeneity of variance and the no-observed-effect concentration (NOEC) determined using Williams' test. There was no significant difference between the control and solvent control, therefore the control data were pooled for analysis.

12. **REPORTED RESULTS:** Mean measured concentrations were 44, 59, 100, 120, and 260 µg a.i./l (Table 2, attached). The measured concentrations averaged 30-88% of nominal concentrations. "Undissolved material was observed in the diluter cells and mixing chambers. The thickness of the film increased with increasing test material concentration. No undissolved material was observed in the test aquaria during the exposure."

At test termination, one mortality was observed at the 120 µg a.i./l mean measured test concentration and in the solvent control and two mortalities were observed at the 100 µg a.i./l test concentration. No mortalities were observed at test concentrations ≤59 µg a.i./l and the dilution water control. Sublethal effects (reduced feeding and fecal production) were observed at test concentrations ≥100 µg a.i./l.

At test termination, shell growth was reduced by 75, 69, 56, and 25% at the four highest measured concentrations compared to the pooled control group (Table 3, attached). Shell deposition was not significantly reduced at the lowest treatment level (44 µg a.i./l). The control and solvent control oysters had a mean new shell growth of 1.7 and 1.4 mm, respectively. Based on mean measured concentrations, the 96-hour EC_{50} value was 100 (95% C.I. = 27-400) µg a.i./l. The NOEC was 44 µg a.i./l.

During the test period, the pH was 7.8-8.0, the dissolved oxygen concentration was 5.8-7.5 (59-76% of saturation at

20°C and 30 ppt), the temperature was 19-20°C, and the salinity was 30-32 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions, other than those presented above, were made by the author.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Regulations (40 CFR Part 160). Maintenance of records on the test substance, including stability, characterization and verification of the test substance identity is the responsibility of the test sponsor.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following:

Control and solvent control oysters demonstrated insufficient growth (i.e., 1.7 and 1.4 mm new shell deposition, respectively) by test termination. As stated in an amendment to the SEP (dated 9/90) a minimum of 2 mm of shell deposition is required. The SEP states that with acceptable water conditions, 25 mm and larger oysters deposit as much as 1.0 mm of peripheral new shell per day. Therefore, the quality and\or quantity of food used in the test may not have been sufficient to sustain the oysters.

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 EPA, 1976) each oyster should receive a minimum of 5 l of "once-through" flow through test solution per hour.

The report did not indicate whether 15- to 30-minute dawn and dusk simulation periods were provided during the study as recommended.

Each test concentration was 50% of the next highest concentration. The SEP recommends that the test concentrations be at least 60% of the next highest concentration.

The amount of solvent used in the solvent control and the highest treatment level (0.330 ml/l) exceeded the amount recommended by the SEP (0.1 ml/l).

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 96-hour EC_{50} value (95% confidence interval) based on percentage reduction relative to the solvent control. Based on mean measured concentrations, the 96-hour EC_{50} was 103 (95-115) µg a.i./l (printout, attached). This value is similar to the author's.

Homogeneity and normality of the data were tested using Hartley test and chi-square test, respectively. The reviewer determined the NOEC using analysis of variance with Bonferroni's test. The reviewer's analysis demonstrated a significant difference from the solvent control at the three highest mean measured concentrations (100, 120, and 260 µg a.i./l). Therefore, the NOEC was 59 µg a.i./l. The author's analysis compared treatment levels to a pooled control which may explain the difference in NOEC results.

C. **Discussion/Results:** This study is scientifically sound but because of the noted deficiencies does not meet the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test. Control and solvent control oysters demonstrated insufficient shell growth (i.e., 1.7 and 1.4 mm, respectively) by test termination. A minimum of 2 mm of shell deposition should have occurred. In addition, the measured concentrations increased considerably during the 96-hour exposure. Therefore, the actual concentrations to which the test organisms were exposed are unknown. Under the conditions of the test, the 96-hour EC_{50} was 103 µg a.i./l and the NOEC 59 µg a.i./l (mean measured concentrations). These results classify benefin as being highly toxic to *Crassostrea virginica*.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Control and solvent control oysters demonstrated low shell growth (i.e., 1.7 and 1.4 mm, respectively) by test termination. The measured concentrations increased considerably during the 96-hour exposure making it difficult to determine the actual exposure concentrations.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, June 1, 1992.

DATA EVALUATION RECORD

1. **CHEMICAL:** Benefin (or Benfluralin). Shaughnessey No. 084301.

2. **TEST MATERIAL:** Benefin; N-Butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-ρ-toluidine; Lot No. 231 EF4; CAS No. 1861-40-1; 96.6% active ingredient; a yellow powder.

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

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11. **MATERIALS AND METHODS:**

A. **Test Animals:** Eastern oysters (*Crassostrea virginica*) were obtained from a supplier in Massachusetts. The supplier held the oysters in flowing seawater (Massachusetts Bay) at a temperature of 17-20°C, a salinity of 31-32 parts per thousand (ppt), and a dissolved oxygen concentration of 80-88% of saturation. The oysters were continuously fed a combination of marine algae (average density of 24 x 10^3 cells/ml). Forty-eight hours prior to test initiation, oysters were transported to the testing laboratory out of water (1.5 h). Upon arrival at the facility, the oysters were inspected for parasites and maturity. The oysters were held in water at a temperature of 18-19°C, a salinity of 30-31 ppt, a pH of 7.5-8.0, and a dissolved oxygen concentration of 65-96% of saturation. The oysters were fed a supplementary algal diet of *Isochrysis galbana* and *Tetraselmis maculata*. No mortality occurred during holding. The oysters were of similar age and size and had a mean valve height of 37 ±5 mm.

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D. **Adequacy of the Study:**

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(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, June 1, 1992.