8-16-91

ECOLOGICAL EFFECTS BRANCH
DATA EVALUATION REPORT

1. **Chemical:** Bromacil

2. **Test Material:** Technical grade, 95.1%

3. **Study Type:** 48 Hour Embryo-Larvae Acute Toxicity Test with Crassostrea virginica under static conditions

4. **Study Identification:**

Study Director: Boeri, Robert L.
Study Laboratory: Enseco, Inc., Marblehead, Ma.
Study Dates: March 14-16, 1989
Study Identification: Project DP 2988
Sponsor: E.I. duPont de Nemous & Co.
EPA Identification: MRID 415887-03

5. **Reviewed by:** Brian Montague, Fisheries Biologist
Ecological Effects Branch
Environmental Fate & Effects Division
Brian Montague 8/16/91

6. **Approved by:** Les Touart, Supervisory Biologist
Ecological Effects Branch
Environmental Fate & Effects Division
LT 9/16/91

7. **Conclusions:** The report has estimated the EC_{50} of Bromacil to oyster larvae be 130 mg/L. Based on the reductions in numbers of normally developed shells in resulting larvae the LOEL is felt to be $\leq$ 100 mg/L. Due to the fact that 23% of the control larvae also failed to produce normal shells the study does not fully fulfill guideline requirements. The EC_{50} would not have been affected by this factor and may be used in risk assessment.

8. **Recommendation:** Repeat study with new broodstock and achieve better control performance in order to establish an NOEL.

9. **Submission Purpose:** Submitted to satisfy reregistration guideline requirements.

10. **Protocol and Methodology:** Protocol followed Enseco Marblehead Study Protocol DP 2988 Aquatic Toxicological Laboratory SEP manual which is based on ASTM (1980) standard procedures.

Test Organisms: Eastern oyster embryos were obtained from National Marine Fisheries Milford Laboratory on March 12, 1989. Spawning was achieved by removing oysters for 2 hours, placement in culture dishes and raising the dilution water from 20 to 28°C over a one hour period. Sperm was added at this time and, after successful spawn, sperm and eggs were combined for 15 minutes. The resulting embryos were utilized within 1 hour of spawning.

Dilution Water and Test Solutions: Dilution water utilized in the test was obtained from the Atlantic Coast near Marblehead. Water was tested for organic and inorganic contaminants. Measured pH was 8.0, TOC was 0.6 mg/L, and NH_3 was 0.2 mg/L.

Test solutions were derived by first preparing a stock solution of 300 mg/L of Bromacil (1.26 gms ai added to 4000 ml dilution water). From this stock solution 150,100,60,40, and 25 mg/L nominal test concentrations were prepared. Nine hundred ml aliquots of test solution were added to 1 liter glass beakers.

Test Methods and Materials: Three replicates were used at each test concentration and 4 were used for controls. Within one hour of fertilization and addition of test solutions to the test vessels approximately 30,000 embryos/L were randomly distributed to the test beakers. Developing larvae were maintained under a 16D/8N photoperiod at 400 fc light intensity. Water temperature was maintained at 20 ± 1°C in an environmental chamber. Five ml subsamples of each replicate were removed, preserved in 5% buffered formalin solution, and counted by microscopic examination. Larvae were considered normal if a complete shell was formed - regardless of size and shape.

Water quality parameters were measured from 0, 24, and 48 hour samples from each replicate.

11. **Reported Test Results:** Water quality parameters remained consistent throughout the study with pH 7.7 - 7.9, temperature at 19°C, D.O. of 8.3 - 7.3 mg/L, and salinity at 30 ppt. Measured concentrations analysis corresponded closely to estimated nominals with 26, 41, 62, 100, and 150 mg/L concentrations being reported.

Survival was over 98% in control populations. A very slight, almost insignificant reduction in number of larvae per ml is seen at the 100 ppm concentration with a much more significant reduction at 150 mg/L. Mean average populations were 23.4, 23.7, 23.4, 23.2, 21.6, and 7.8 larvae per ml for respective control, 26, 41, 62, 100 and 150 mg/L concentration groups.

Production of abnormal larvae (larvae without shells complete) was 6.95, 6.46, 6.86, 7.3, 8.6, and 22.06 larvae/ml for controls, 26, 41, 62, 100, and 150 mg/L concentrations, respectively. Very little difference was seen in total number of larvae produced.

12. **Study Author's Conclusions:** "The EC_{50} based on percentage reduction of normal oyster larvae after 548 hours of exposure to Haskell Sample Number 16,473 is 130 mg/L, active ingredient."

13. **Reviewer's Discussion:** Control organisms in this study showed over 23% abnormality in shell development. This exceeds Agency criteria of 10% abnormal development in controls. Concentration division among test groups was less than the recommended 60% dilution factor in most cases. The laboratory has failed to report any incidence of misshapen or malformed shell development.

Water quality parameters and test procedures were generally acceptable. Analysis of Bromacil in controls was limited to a detection limit of 12.5 mg/L. A lower level of certainty would have been prefered. The dose response curve is somewhat apparent, though the % of abnormal development of shells within the controls compromises the results and is not explained by the laboratory.

Adequacy of Study

1. **Classification:** Supplemental

2. **Rationale:** Poor performance of control larvae compromises the results.

3. **Repairability:** Not Repairable.