Data Evaluation Report
Ecological Effects Branch

1. **Chemical:** Thiabendazole

2. **Test Material:** Technical grade Thiabendazole of 99.6% purity.
   Lot no. PRM-029 received 8/25/88.

3. **Study Type:** Acute Toxicity - Flow Through
   Shell Deposition with *Crassostrea virginica*.

4. **Study Identification:**
   - **Study Author:** Surprenant, Donald
   - **Laboratory:** Springborn Life Sciences, Inc.
   - **Study Dates:** March 2-6 and 16-20, 1989
   - **Lab Identification:** SLS Study No. 359.0888.6107.504
   - **Sponsor:** Merck Sharp and Dohme Research Labs.
     Rahway, N.J.
   - **EPA Identification:** MRID 411920-04

5. **Reviewed by:** Brian Montague, Fisheries Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division

6. **Approved by:** Ray Matheny, Supervisory Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division (H7505C)

7. **Conclusions:** The study has not fulfilled guideline
   requirements as the laboratory has not achieved a true EC₅₀
   or NOEL point. The study does indicate that thiabendazole
   may reduce oyster shell deposition at levels below 21 ppb.

8. **Recommendations:** The registrant must submit a study which
   establishes the necessary data needed in risk assessment
   (EC₅₀ and NOEL), or demonstrate the EC₅₀ to be greater than
   100 ppm. The testing laboratory for any additional studies
   may wish to test alternative solvents and their effects on
   solubility or to test levels of triethylene glycol which
   will cause no effect on oyster shell growth yet allow for
   solution of higher concentrations of thiabendazole.
9. Submission Purpose: To support registration data requirements.


Test Organisms: Eastern oysters, Crassostrea virginica were obtained form Aquacultural Research Corporation in Dennis, Mass. The oysters were reared on seawater obtained from the same general area as the dilution seawater used by Springborn Laboratories. No mortality was reported during the 14 days prior to study initiation. Temperature ranged from 15-19°C, pH from 7.3-8.0, salinity from 27-30 ppt, and dissolved O₂ from 89-97% of saturation during the acclimation period. Marine algae at 37 x 10⁶ cells/ml density was fed to the oysters at this time. Due to problems in obtaining sufficient dose response a second study was conducted with oysters from the same source. Acclimation conditions were very similar for these oysters as for the first group tested. Transport time to the laboratory was 1.5 hours and the oysters were delivered 48 hours prior to test initiation where they were held in a wooden epoxy-painted tray. Mean valve height was 36±3 mm in group I and 37±4 mm in group II. No mortality occurred during the 48 hour observation period prior to initiation.

Dilution Water and Test Solution: Natural unfiltered seawater was used as dilution and control water. The seawater was obtained from the Cape Cod Canal near Bourne, Massachusetts and transported to the laboratory's epoxy-coated concrete reservoir. Salinity of the dilution water was 32-33 ppt with salinity of 7.7-7.9. No pesticide or PCB contamination was reported, however no summary of the water analysis has been provided in the report to allow review of other water quality characteristics.

Test solutions for the first test were prepared with triethylene glycol as the solvent. Stock solutions of 2.01 mg ai/L were prepared by addition of 0.6030 gms active ingredient to 300 ml of the solvent. A stock solution at 0.0283 ml/minute rate was introduced to 375 ml/minute of dilution water to produce the highest concentration of 150 μg ai/L. Solvent control solutions contained 75 microliters/L of triethylene glycol which was equivalent to the amount contained in the highest concentration.

Stock solutions for the second test were prepared to a concentration of 6.58 mg ai/L by addition of 2.3027g/ml ai of Thiabendazole to 350 mg of triethylene glycol. The syringe
pump delivered 0.0285 ml/minute of the stock solution to 375 ml/minute of dilution water to produce the highest nominal concentration of 500 ppb. The solvent control contained 76 ppb of solvent which was equivalent to the solvent concentration contained in the highest test level.

**Test Material and Methods:** A continuous flow serial diluter as described by Benoit (1982) delivered the test solutions to 14 glass test aquaria partially immersed in a thermoregulated water control bath. Aquaria were equipped with a 10 cm standpipe to allow maintenance of a constant 18 liter volume of test solution in each test vessel. The flow was 75 ml/minute which provided 6 volume additions per 24 hour period. An external plastic lined impeller pump on each aquarium provided additional recirculation of the test solution within the test vessel, thus aiding distribution of the algal food cells.

The selected test concentrations for first definitive test were 150, 90, 54, 32, and 19 µg/L with two replicates of each concentration, the solvent control and the dilution control. Selected concentrations for the second test were 500, 300, 180, 110, and 65 µg/L and were based on the results obtained in the first test.

Twenty four hours prior to the definitive test 3-5 mm of peripheral shell growth were removed from each test oyster. They were held overnight for observation and fed for a new growth the next day. Twenty oysters were placed in each test aquarium thus providing 40 oysters per test level. The oysters received 180 ml of concentrated (10^7 cells/ml) *Isochrysis galbana* and *Tetraselmis maculata* algae 3 times per day. Algal density within the aquaria was estimated to be 10^5 cells/ml.

Observation of the specimens and their feeding response as well as measurement of water quality parameters was made every 24 hours.

Samples were removed from mid-water level in each replicate aquarium on day 0 and day 4 for analysis of actual toxicant concentration by HPLC analysis methods. Average recovery was 88±11% of the nominal concentration levels.

**Statistical Analysis:** Four linear regression curves were selected based on the highest coefficient of determination. Using Sokal and Rohlf's method of inverse prediction an EC_{50} was estimated to be greater than the highest concentration tested.

11. **Reported Test Results:** In preliminary testing a 91% and 61%
reduction in shell growth was observed at 100 ppm and 0.16 ppm treatment levels, respectively. Undissolved test material was, however, observed at the 3 highest test levels.

The analysis of actual test concentrations in the first definitive test yielded 98 ppb for the 150 ppb level, 63 ppb for the 90 ppb level, 45 ppb for the 54 ppb level, 35 ppb for the 32 ppb level and 21 ppb for the 19 ppb nominal concentration. No malfunction of the diluter system was observed during the 96 hour test period. Shell growth was reduced 25%, 14% and 11% at the 3 highest treatment levels and 7% at the 2 lower concentrations.

Shell growth in the controls during this first study averaged 2.7 mm for the controls and 2.9 mm for the solvent controls. No mortality or abnormal reaction was noted in this first definitive study.

The second definitive study was conducted March 16-20. Mean measured concentrations were 260, 180, 130, 98 and 64 μg/L of active ingredient. A surface film of thiabendazole was observed in the diluter box (but not in the test aquaria) indicating the highest test concentration may have reached the limit of solubility. Again no mortality or abnormal behavior was displayed by the test animals. Average Shell growth for the controls in the second test was 3.0 mm and 2.7 mm of shell growth was recorded for the solvent controls oysters. Reduction in shell growth was 22% and 19% for the 260 ppb and 180 ppb concentrations and 15% for the 3 remaining concentrations.

12. Study Authors Conclusion: "Based on the results of the two definitive exposures it was established that Thiabendazole, at concentrations of >260 μg AI/L, reduced the shell deposition of Eastern oysters by ≤ 25%. Due to the material's behavior in seawater and effect on the exposed organisms, a well-defined concentration effect relationship could not be established at the treatment levels tested (260-21 mg AI/L thiabendazole) .... Based on these data, it was determined that additional testing (ie >500 ug ai/L thiabendazole, nominal) would not accurately define the toxicity of the test material to Eastern oysters. Therefore, the EC50 for thiabendazole and Eastern oysters was empirically estimated as being greater than the highest mean measured test concentration (ie, >260 μg ai/L). Statistical analysis (William's Test) of the effects (reduction in shell growth) established the NOEC for this study as 65 μg ai/L."
13. Reviewers Discussion: The study has followed guideline requirements for methodology in most areas. Some areas of deviation which were noted are as follows:

1. Oysters were not actually acclimated for 10 days at the laboratory itself. Instead they were held at the commercial supplier and then transported 1.5 hours out of water to reach Springborn labs where they were acclimated for 48 hours at Springborn. This is not considered a 10 day acclimation to the laboratory dilution water.

2. Growth for controls was less than the optimum which might be expected for 96 hours.

3. An unexplained drop in dissolved oxygen occurred at the 24 hour point in the first & second tests and subsequently went back to prior levels at 48 and 72 hours. This may have been an effect from the chemical or due to a possible reduction in the volume of dilution water being introduced during that 24 hour period.

4. An EC₅₀ has not been established by this study. Though it was stated that the solubility was the factor which prevented this it has been noted that in prior studies using the same solvent higher concentrations were kept in solution. A higher level of solvent might have allowed the concentration level needed to obtain a 50% effect. Also no mention is made in this study of alternative solvent testing.

5. Raw data concerning individual shell growth has not been included for review, only mean shell growth summarizations.

6. Reduction of shell deposition is also seen at lower concentrations in the first definitive test, thus compromising the use of the NOEC observed in the second test. Reduction in shell growth was 11% at 45 μg/L and 7% at 35μg/L and 21μg/L in the first test thus indicating that shell growth may be inhibited at below 21 ppb.
Adequacy of Study:

**Classification**: Supplemental

**Rationale**: Though the study has generally followed EPA testing guidelines a satisfactory EC_{50} or NOEL has not been established. The data does, however, indicate that the NOEL is below 21\mu g/L as shown by the 7% reduction in shell deposition shown in the first definitive test.

**Repairability**: Not repairable

14. **One liner**: N/A