

**Data Evaluation Report  
Ecological Effects Branch**

1. **Chemical:** Thiabendazole
2. **Test Material:** Technical grade of 99.6% purity Received  
8/25/88 and 4/6/89 - Lot No PRM-029
3. **Study Type:** 96 Hour Acute Toxicity under Flowthrough  
conditions with Cyprinodon variegatus

4. **Study Identification:**

**Study Author:** Surprenant, Donald  
**Laboratory:** Springborn Life Sciences, Wareham, Mass  
**Study Dates:** March 9-13, 1989  
**Lab Identification:** Proj. no.359.0888.6106.505  
**Sponsor:** Merck Sharp and Dohme Research, Rahway, N.J.  
**EPA Identification:** MRID 262949

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

*Brian Montague*

6. **Approved by:** Ray Matheny, Supervisory Biologist  
Ecological Effects Branch  
Environmental Fate and Effects Division (H7507C)

*Ray Matheny 3-9-90*

7. **Conclusions:** The study has failed to establish an LC<sub>50</sub> value due to the inability of the laboratory to achieve solubility of the test material in the test solution. The NOEL value was estimated to be 3.6 mg/L.
8. **Recommendations:** Registrant must submit a new study to fulfill this guideline requirement.

9. **Submission Purpose:** Submitted to support registration guideline requirements.
10. **Study Design and Protocol:** The protocol used was Springborn's Protocol for Conducting Flow-through Acute Toxicity Test with Sheepshead Minnow (Cyprinodon variegatus) which is closely based on ASTM 1980 standard testing procedures.

**Test Organisms:** Sheepshead minnows Cyprinodon variegatus were obtained from a commercial supplier in Louisiana and assigned Lot no. 89A10 by Springborn. The fish were held in 22-23°C natural seawater 14 days prior to testing. Salinity was 32-35 ppt, pH 7.5-7.8 and dissolved oxygen within 83-88% of saturation. No mortality occurred during acclimation. Sample wet body weights averaged 0.10 (0.02-0.24) grams and body length averaged 18(12-22) mm. Fish were fed commercial dry pellet food up to 48 hours prior to test initiation.

**Dilution Water and Test Solution:** The dilution water was prepared from natural seawater collected at the Cape Cod Canal near Bourne, Mass. Before use the seawater was circulated through 20 and 5 micron polypropylene filters and activated carbon filtration.

Test solutions were prepared with triethylene glycol as the solvent. Maximum solvent concentration was 0.35 ml/L. Stock solutions of 40.4 mg ai/ml were prepared by diluting 40.4479 gms of active ingredient to 1000 ml of triethylene glycol.

The stock solution was analyzed and contained 50% of the nominal or 20 mg/ml. This stock solution was introduced into a mixing chamber located inside a sonicating water bath located over a magnetic stirrer.

**Test Material and Methods:** The diluter delivered 500 ml of solution per cycle to each of the 14 replicate aquariums. The test aquaria were housed in a constant temperature water bath and maintained at a constant volume of 11 L. This provided approximately 6.6 volume additions per 24 hour period.

Test vessels and organisms were observed every 24 hours and at test initiation. Dissolved oxygen, pH, temperature, and salinity were monitored daily. Temperature was constantly monitored in one replicate control vessel throughout the period. Samples were removed from mid-level in each aquarium

on day 0 and 4 for analysis of actual toxicant concentration. Samples were analyzed using HPLC methods.

11. **Reported Test Results:** The water quality parameters remained consistent with temperature ranging from 21-22°C, a salinity of 31 ppt, D.O. from 7.1-8.2 mg/L, and pH from 7.7-7.9.

The mortality in the test concentrations was inconsistent with concentration levels.

In no test concentration was 50% mortality obtained for both replicate chambers. Behavioral observations included lethargy, extended abdomens, surface breathing, and loss of equilibrium. There was no mortality in any of the control vessels. The following table is a summarization of mortalities.

<u>Measured Concentration</u>	<u>48 Hour % Mortality</u>		<u>96 Hour</u>	
10 mg ai/L	A-20	B-0	A-50	B-10
6.0 mg ai/L	A-0	B-10	A-10	B-10
3.6 mg ai/L	A-0	B-0	A-0	B-0
2.2 mg ai/L	A-0	B-20	A-0	B-40
1.5 mg ai/L	A-0	B-10	A-0	B-10
Solvent Control	A-0	B-0	A-0	B-0
Control	A-0	B-0	A-0	B-0

12. **Study Authors Conclusion:** "Toxicant-related sublethal effects (e.g. lethargy, loss of equilibrium) were observed among surviving fish in the two highest concentrations tested (10 and 6.0 mg A.I./L). Based on the results of the data, the 96-hour LC<sub>50</sub> for sheepshead minnow exposed to thiabendazole was empirically estimated to be greater than 10 mg AI/L, the highest mean measured concentration tested. The limited solubility of thiabendazole in organic solvents and seawater prevented testing at higher treatment levels. The No Observed Effect Concentration (NOEC) for sheepshead minnow exposed to Thiabendazole was determined to be 3.6 mg AI/L."

13. **Reviewers Discussion:** The study report has noted difficulties in obtaining complete solvency of the test material in triethylene glycol despite solvent levels above EPA accepted guidelines. According to the report the maximum nominal stock concentration of 20 mg/L yielded a measured concentration of only 10 mg/L in the highest

concentration tested.

The study has followed procedural guidelines in most other areas, though one deviation noted by the author may have caused mortality and behavioral abnormalities unrelated to the toxicant. This was the failure to remove mortalities at 48 and 72 hour periods. This might lead to high  $\text{NH}_3$  or  $\text{NO}_2$  levels over the 48+ hours the mortalities were allowed to decompose in the test vessels. No deviation was noted in  $\text{O}_2$  or pH levels, as a result of the oversight. It should be noted, however, that 10 and 40% mortality were seen in two of the lowest concentration test vessels.

The test has failed obtain mortality above 50% in any of the test levels therefore making it impossible to obtain an accurate  $\text{LC}_{50}$  value. The NOEC level of 3.6 mg/L may be of use in risk assessment procedures.

Other deviations from accepted ASTM and EPA guidelines include:

- 1) Use of test fish under .50 grams wet weight. No age data or source (wild caught or commercial raised?) information was included.
- 2) Though no precipitate was noted some loss in concentration was seen in the 2 highest concentrations. Also measured concentration was over 30% higher than estimated nominal concentration at these test levels.

**Adequacy of Study:**

**Classification:** Supplemental

**Rationale:** The NOEL maybe of some use in risk determination however an  $\text{LC}_{50}$  value has not been established.

**Repairability:** Not repairable