

US EPA ARCHIVE DOCUMENT

(5-12-92)

S398327
SUBMISSION #

060101
SHAUGHNESSY NO.

REVIEW NO.

EEB REVIEW

DATE: IN 6-27-91

DATE: OUT 5/12/92

FILE OR ID NO. 060101-000618

PETITION OR EXP. NO. _____

DATE OF SUBMISSION 12-03-90

DATE RECEIVED BY EFED 6-27-91

RD REQUESTED COMPLETION DATE 10-25-91

EEB ESTIMATED COMPLETION DATE 04-27-92

RD ACTION CODE/TYPE OF REVIEW Data Evaluation Record
48-hour Acute Flow-Through Study
for Daphnids

TYPE OF PRODUCT(S) : I,D,H,F,N,R,S Fungicide

DATA ACCESSION NO(S). _____

PRODUCT MANAGER (NO.) Barbara Briscoe

PRODUCT NAME(S) Thiabendazole

COMPANY NAME Merck Sharp and Dohme Research Laboratories

SUBMISSION PURPOSE Meet EEB Study requirements

SHAUGHNESSY NO.	CHEMICAL & FORMULATION(S)	% A.I.
-----------------	---------------------------	--------

<u>060101</u>	_____	_____
---------------	-------	-------

_____	_____	_____
-------	-------	-------



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: Thiabendazole -- 48-hour Flow Through Study for Daphnids

From: Doug Urban, Acting Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C) *Doug Urban* 5/12/02

To: Barbara Briscoe, Product Manager
Reregistration Branch
Special Review and Reregistration Division (H7508 W)

The 48-hour flow through study for Daphnids was reviewed by EEB. This study reflects laboratory derived toxicity values (EC_{50}) which provides support for the reregistration of Thiabendazole. The EC_{50} value for daphnids is 0.85 mg a.i./L. Therefore, Thiabendazole is considered very highly toxic to freshwater invertebrates.

If you have any questions concerning the above, please feel free to contact Regina Hirsch (305-5366).

DATA EVALUATION RECORD

1. **CHEMICAL:** Thiabendazole
2. **TEST MATERIAL:** Thiabendazole (Lot No. MRM 526; 90RTS051), 99.8% active ingredient, white powder.
3. **TEST TYPE:** Acute Toxicity Test for Freshwater Invertebrates using Daphnia magna.
4. **Citation:** Holmes, C.M., D.C. Bellantoni, and G.T. Peters, Ph.D. Thiabendazole: a 48-hour flow-through toxicity with the Cladoceran (Daphnia magna). Study performed by: Wildlife International Ltd., 305 Commerce Drive, Easton, Maryland 21601. Submitted to: Merck Sharp and Dohme Research Laboratories Agricultural Research, Hillsborough Road, Three Bridges, New Jersey 08887. Wildlife International Ltd. project number: 105A-101. Accession number: 417094-01.
5. **REVIEWED BY:**
Regina M. Hirsch, Wildlife Biologist *R. Hirsch* 5/7/92
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C)
6. **APPROVED BY:**
Les Touart, Section Head *L. Touart* 5/7/92
Ecological Effects Branch
Environmental Fate and Effects Division (H7507 C)
7. **CONCLUSIONS:** This study fulfills the guideline requirements (72-2) for an Acute flow through test with daphnia. The estimated $EC_{50} = 0.85$ mg a.i./L and the $NOEC \geq 0.75$ mg a.i./L. Thiabendazole is considered ~~very~~ highly toxic to Daphnia.

submitted
in 1990
==

Janet

M

8. MATERIALS AND METHODS:

A. Test Organisms:

Species -- daphnid (Daphnia magna).

Supplier -- Wildlife International Ltd. Cultures, 322 Brooks Drive, Easton, Maryland 21601.

Acclimation Period -- Neonates were obtained for testing by transferring individual adult daphnids to dilution water 24 hours prior to test initiation. The progeny produced by three or more adults during this 24-hour period were used as the test organisms.

B. Test System:

Invertebrates -- 140 juvenile daphnids, less than 24 hours old at test initiation, were used in this study.

Test vessels -- ten glass cylinder compartments (5 cm diameter & 10 cm length) were suspended in Teflon-lined, 8-L polyethylene test chambers filled with 6.5-L of test solution. The depth of the test solution in each test compartment was approximately 6.5 cm.

Photoperiod -- 16 hours of light and 8 hours of darkness.

Source of dilution water -- freshwater was obtained from a well 45 meters deep located on Wildlife International property.

Hardness -- $\text{CaCO}_3 = 148 \text{ mg/L}$

Alkalinity -- 186 mg/L as CaCO_3

Conductivity -- 350 umhos/cm

Temperature -- $20 \pm 1^\circ\text{C}$

pH -- 7.8

Loading -- not available

Dissolved oxygen -- not available

Feeding -- Daphnids were fed a yeast, Cerophyll, and trout chow, as well as a suspension of the freshwater green alga, Selenastrum capricornutum. Isolated adults were fed during the 24-hour period to test initiation, but neonates were not fed during the test.

C. **Definitive Test:**

Groups -- 5 test groups and 2 control groups (one solvent control) were used in this study.

Number of test organisms -- 20 (10 per replicate) daphnids per treatment group and control group were used.

Dosage form -- test substance was supplied to the test vessels by a continuous flow, proportional diluter. Two syringe pumps were used to deliver the test substance to mixing chambers where the test substance was mixed with dilution water, the flow was controlled by rotameters. After mixing, test solutions were split to each replicate chamber.

The diluter was adjusted so that each test chamber received at least 5 volume additions of test solution every 24 hours.

Test concentration -- test substance was dissolved in dimethylformamide to form a stock solution that was subsequently mixed in dilution water. The test substance stock concentration was adjusted to correct target test concentrations to 100% active ingredient. The solvent, dimethylformamide, was reagent grade and its concentration in the test solutions was ≤ 0.12 ml/L.

Water samples (1 L) were collected from each test chamber at 0, 24, and 48 hours for concentration analyses of the test substances. Nominal concentrations of test substance were 0.00 (control), 0.22, 0.37, 0.61, 1.02, and 1.70 mg a.i./L Thiabendazole.

Study duration -- 48 hours of exposure to Thiabendazole.

Organism observations -- all aquaria were examined at 24 hour intervals for: number of survivors; sublethal effects (loss of equilibrium, erratic behavior, loss of reflex, excitability, discoloration, or change in behavior); and dead organisms.

Physical observations -- dissolved oxygen, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live daphnids. The temperature in one negative control vessel was recorded continuously throughout the test.

9. REPORTED RESULTS:

Statistics: EC₅₀ values were interpreted by standard statistical techniques. The probit analysis, moving average angle method, or binomial method was used to calculate the LC₅₀ and EC₅₀. In this study, the binomial method with non linear interpolation was used to evaluate mortality and immobilization at 24 hours and the probit method was used to evaluate mortality and immobilization at 48 hours. The NOEC was determined by visual observation of the mortality and toxicity data.

48-hour EC₅₀: 0.81 mg/L with confidence interval of 0.72-0.91 mg/L.

NOEC: ≥ 0.75 mg a.i./L

Test conditions: Nominal concentrations and mean measured concentrations were quite different:

<u>Nominal</u>	<u>Mean measured</u>
(mg a.i./L)	
0.22	0.48
0.37	0.75
0.61	1.19
1.02	2.36
1.70	3.72

Observations: Daphnids were observed to be floating on the surface of the water at 24 hours in the solvent control and in the 1.19 and 2.36 mg a.i./L concentrations. However, this was not considered an adverse effect. In addition, the low levels of mortality (≤ 5%) observed in the solvent control and 0.48 mg a.i./L concentrations were not considered significant.

Loading rate: can not be calculated.

pH: 7.95 (7.9-8.0)

Conductivity: 348 umhos/cm (320-380 umhos/cm)

Dissolved oxygen: 8.3-8.7 mg/L

Temperature: 19.7-19.9°C

10. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

Quality Assurance and Good Laboratory Practice regulation Statements were included in the report.

11. REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY:

A. **Test Procedures:** Test procedures deviated from the protocols recommended by the guidelines by having the mean measured concentrations at least 2 times greater than the intended nominal concentrations.

B. **Statistical Analysis:** A probit analysis test was used to determine the EC_{50} of Thiabendazole on Daphnia magna. Using the conservative 95% confidence intervals of 0.76 and 0.96 the EC_{50} was calculated to be 0.85. This number is comparable with the EC_{50} determined by the study authors.

C. **Discussion/Results:** This study fulfills the guideline requirements (72-2) for an Acute flow through test with daphnia. The $EC_{50} = 0.85$ mg a.i./L (0.76-0.96) and the NOEC ≥ 0.75 mg a.i./L. Therefore, Thiabendazole is considered very highly toxic to Daphnia.

D. **Adequacy of Test:**

1. **Validation Category:** Core
2. **Rationale:** N/A
3. **Repairability:** N/A

12. COMPLETION OF ONE-LINER FOR TEST: Yes

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

rhirsch thiabendazole Acute 48-hour flow-through daphnid study

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3.72	19.5	19.5	100	1.348699E-04
2.36	19.5	19.5	100	1.348699E-04
1.19	19.5	18.5	94.87181	2.697398E-03
.75	19.5	4.5	23.0769	.6792048
.48	19.5	.5	2.5641	1.348699E-04

THE BINOMIAL TEST SHOWS THAT .75 AND 1.19 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .8788007

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4			.921322	.763512
1.078785		4.317336E-02		

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
7	.1792912	1
.680548		

SLOPE = $\frac{9.864252}{5.687453}$ AND 14.04105

LC50 = .8518741
 95 PERCENT CONFIDENCE LIMITS = .7571036 AND .9614869

LC10 = .6333265
 95 PERCENT CONFIDENCE LIMITS = .4910738 AND .7197876
