CASWELL FILE

010290

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

MAY 27 1993

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Thiabendazole:** Review of a Mutagenicity Study.

EPA ID# 060102-000618
Case No. 807285

DP Barcode D171805
Chem. ID No. 060101

FROM: John E. Whalan, D.A.B.T., Toxicologist
Section 1, Toxicology Branch I
Health Effects Division (H7509C)

John E Whalan
4-6-93

TO: Barbara Briscoe (PM Team # 51)
Special Review and Reregistration Division (H7508W)

THRU: Roger L. Gardner, Section Head
Section 1, Toxicology Branch I
Health Effects Division (H7509C)

Roger Gardner 5-19-93
KB 5/24/93

Merck & Co., Inc. submitted the following study for review:

> Phase 4 Response: Reformat of MRID No. 98002. Subacute Dominant Study of Thiabendazole in the Mouse; Study No. TT#76-703-0; August 6, 1991; MRID No. 420853-01.

The study was reviewed by Clement Associates and Irving Mauer and classified **Unacceptable**. Thus, this study does not satisfy Guideline requirement 84-2b for structural chromosome aberrations. There were no clear signs of overt toxicity in the treated males, so it is doubtful that an adequate high-dose was used.

FINAL

DATA EVALUATION REPORT

THIABENDAZOLE

Study Type: Mutagenicity: Dominant Lethal Assay in Mice

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S. Date 3/18/93

Independent Reviewer Kristin Jacobson, MSPH Date 3/18/93

QA/QC Manager Sharon Segal, Ph.D. Date 3/18/93

Contract Number: 68D10075
Work Assignment Number: 2-50
Clement Number: 148
Project Officer: Caroline Gordon

GUIDELINE § 84: MUTAGENICITY
DOMINANT LETHAL

EPA Reviewer: Irving Mauer, Ph.D.
Immediate Office HED (H-7509C)

Signature: [signature] 03, 23, 93
Date:

EPA Section Head: Marion Copley, DVM, DABT
EPA Review Section IV, Toxicology Branch
I/HED (H-7509C)

Signature: [signature]
Date: 3/23/93

RAS. 5-19-93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Dominant lethal assay in mice

EPA IDENTIFICATION Numbers:

PC Code: 060101

Tox Chem. Number: 849A

MRID Number: 420853-01

TEST MATERIAL: Thiabendazole

SYNONYM(S): None listed

SPONSOR: Merck & Co., Inc., Agricultural Research & Development, Three Bridges, NJ

STUDY NUMBER: TT #76-703-0

TESTING FACILITY: Merck Sharp & Dohme Research Laboratories, West Point, PA

TITLE OF REPORT: Subacute Dominant Study of Thiabendazole in the Mouse

AUTHORS: Zwickey, R. and Lankas, G.R.

REPORT ISSUED: Original study: February 12, 1976; Reformatted study: August 7, 1991

CONCLUSIONS--EXECUTIVE SUMMARY: Ten male mice/group received daily oral gavage administrations of 125, 250, or 500 mg/kg/day thiabendazole for 5 days and were sequentially mated with untreated females (1:1) for 8 consecutive weeks. The findings of the assay provided no convincing evidence that thiabendazole adversely affected the reproductive performance or induced a dominant lethal effect in the germinal cells of the treated males. However, the study was compromised for the following reasons:

1. There were no clear signs of overt toxicity in the treated males; it is doubtful, therefore, whether an adequate high dose was selected for investigation.

2. The number of mated females (10) and the number of successful pregnancies were below the number generally recommended (30 females/group/mating) to ensure an adequate sample size.[1]

3. Analytical determinations were not performed to determine actual concentrations.

4. Data from a concurrent or recently tested positive control were not included with the study report.

5. A quality assurance statement was not provided.

Based on the above considerations, we conclude that the study is unacceptable and should be repeated in accordance with the Health Effects Testing Guidelines found in 40 CFR Part 798 and the U.S. EPA Gene-Tox Program's published procedures for the dominant lethal assay.[2]

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosome Aberrations.

A. MATERIALS:

1. Test Material: Thiabendazole

Description: Not provided
Identification number: Lot number: F291764, SP1492
Purity: 99.86%
Receipt date: Not reported
Stability: Reported to be stable in the vehicle for >24 hours at room temperature
Contaminants: None listed
Vehicle used: 0.5% Aqueous methylcellulose (MC)
Other provided information: The storage conditions of the test material were not reported. Dosing solutions were prepared daily but were not analyzed for actual test material concentrations.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: 0.5% MC was administered once daily for 5 days by oral gavage; the dosing volume was 10 mL/kg.

Positive/final concentration/route of administration: None

---

[1]Green, S., Auletta, A., Fabricant, J., Kapp, R., Manemdhar, M., Shea, C., Springer, J., and Whitfield, B. (1985). Current status of bioassays in genetic toxicology--The dominant lethal assay. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 154:49-67.

[2]Ibid.

3. Test Compound:

Route of administration: Oral gavage; once daily for 5 days

Dose levels used: 125, 250, and 500 mg/kg/day

Note: The rationale for dose selection was not provided.

4. Test Animals:

(a) Species: Mouse Strain: CF_1S Age (at initiation): ≈10 weeks
Weight range (at initiation): 27.7-38.1 g (males)
Source: Carworth Farms, New York, NY

(b) Number of animals used per dose: Males: 10/treatment group
10/two vehicle control groups
Females: 10/group/mating interval

(c) Animals properly maintained? Yes.

B. TEST PERFORMANCE:

1. Dominant Lethal Assay:

(a) Compound administration/animal observations: Groups of 10 male mice received single oral gavage administrations of the vehicle (0.5% MC) or the selected test material doses (125, 250, or 500 mg/kg/day) for 5 consecutive days. Animals were observed daily for mortality and other signs of compound toxicity; body weights were determined on days 1, 3, and 5 of dosing.

(b) Mating: Immediately following administration of the final dose, individual males in the treatment and vehicle control groups were mated with single untreated females for 1 week. At the end of the mating period, females were replaced with untreated females and the 1:1 mating sequence was continued over a total of 8 consecutive weeks. Females were examined at unspecified intervals for the presence of a vaginal plug; the day on which the plug was found was designated gestation day (GD) 1.

(c) Examination of uterine contents: On GD 14, females were sacrificed and the uteri were examined for total implants, live and dead implants, and early and late resorptions.

2. Statistical analysis: Male body weight data were analyzed using analysis of variance and Dunnett's t-test at $p<0.05$. Resorptions and live and dead implants were analyzed using nonparametric analysis at $p<0.05$.

3. Evaluation criteria: No criteria were provided to evaluate assay validity, a positive response, or the biological significance of the findings.

4. Protocol: None provided.

C. REPORTED RESULTS

1. Animal Observations: No deaths or clinical signs of compound toxicity were seen in males receiving 5 daily administrations of 125, 250, or 500 mg/kg thiabendazole. Slight decreases in male body weight were reported for the three treatment groups and the body weight change for high-dose males (days 1-3 but not days 1-5) was significantly ($p<0.05$) lower than the pooled vehicle control. Although the study authors concluded that body weight differences between vehicle and exposure groups were compound related, our reviewers disagree. The differences in body weight were very slight (i.e., ≤3%) and did not increase with time; the data are, therefore, insufficient to conclude that thiabendazole adversely affected male body weight.

2. Dominant Lethal Assay: Representative findings from the dominant lethal assay are presented in Table 1. As shown, exposure to the three selected doses of thiabendazole did not appear to have an effect on the reproductive performance of the treated males. Similarly, there was no indication that the test material induced a dominant lethal effect. However, the number of mated females (10/group/mating interval) and the number of successful pregnancies, which ranged from a low of 6 (week 3, 125 mg/kg/group) to a high of 10 provided no confidence in the results or the outcome of the study. The study authors, nevertheless, concluded that "thiabendazole is not mutagenic based on the lack of dominant lethal mutations in the germ line of male mice."

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: We assess that, while the findings of this dominant lethal assay provided no evidence of dominant lethal activity or adverse effects on reproductive performance, the study was compromised for the following reasons:

1. No deaths or convincing signs of compound toxicity were observed in the treated males. The differences in body weight of treated versus control males were too slight (i.e., ≤3%) to conclude that thiabendazole had an adverse effect on body weight. We assess, therefore, that the results did not establish that an appropriate high dose was administered.

2. The low number of mated females (10/group/mating) and the number of successful pregnancies were below the number generally recommended (30 females/group/mating) to provide an adequate sample size.[3]

[3]Green, S., et al. (1985). Mutat. Res. 154:49-67.

TABLE 1. Representative Results from Selected Mating Weeks of the Dominant Lethal Assay in Male Mice Orally Exposed to Thiabendazole

Mating Interval	Substance	Dose/kg/day	No. of Pregnant Females/ No. mated	Percent Pregnancies[a]	Total Implants per female	Live Implants per female[a]	Total Dead Implants per female[a]	Early Fetal Deaths per female[a]	Late Fetal Deaths per female[a]	Dominant Lethal Index[a,b]
1	0.5% Methyl-cellulose	10 mL[c]	18/20	90	13.4	12.2	1.2	1.1	0.06	0.09
	Thiabendezole	125 mg	9/9	100	13.0	11.8	1.2	1.1	0.1	0.09
		250 mg	8/9	89	13.9	12.8	1.1	1.1	0.0	0.08
		500 mg	9/10	90	14.7	12.8	1.9	1.7	0.2	0.13
2	0.5% Methyl-cellulose	10 mL[c]	14/19	74	13.6	12.6	1.0	1.0	0.0	0.07
	Thiabendazole	125 mg	8/9	89	12.9	11.6	1.3	1.3	0.0	0.10
		250 mg	10/10	100	14.4	13.1	1.3	1.2	0.1	0.09
		500 mg	9/10	90	13.1	12.3	0.8	0.8	0.0	0.06
3	0.5% Methyl-cellulose	10 mL[c]	19/20	95	14.2	12.9	1.2	1.2	0.1	0.09
	Thiabendazole	125 mg	6/7	86	14.3	14.2	0.2	0.2	0.0	0.01
		250 mg	8/9	89	14.7	14.0	0.8	0.8	0.0	0.05
		500 mg	8/9	89	15.5	14.0	1.5	1.4	0.1	0.10
4	0.5% Methyl-cellulose	10 mL[c]	17/19	89	14.3	12.8	1.6	1.4	0.2	0.11
	Thiabendezole	125 mg	8/9	89	14.6	13.9	0.8	0.6	0.1	0.05
		250 mg	9/10	90	13.6	12.8	0.8	0.8	0.0	0.06
		500 mg	7/8	88	11.0	10.1	0.9	0.9	0.0	0.08

[a]Calculated by our reviewers

[b]Dominant Lethal Index = $\frac{\text{Dead Implants per Female}}{\text{Total Implants per Female}}$; calculated by our reviewers.

[c]Data from the two vehicle control groups were combined by our reviewers.

NOTE: Values for the test material groups during mating intervals 5 through 8 did not suggest a dominant lethal effect.

Data were extracted from the study report pp. 13-14 (summary tables) and pp. 15-54 (primary data).

3. Data from a concurrent or recently tested positive control were not presented as recommended by Guidelines.

4. Dosing solutions were not analyzed for actual concentrations.

Based on the above considerations, we conclude that the study is unacceptable and should be repeated in accordance with the Health Effects Testing Guidelines found in 40 CFR Part 798 and the U.S. EPA Gene-Tox Program's published procedures for the dominant lethal assay.[4]

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? No. The report included an undated statement indicating that the study was conducted prior to implementation of FIFRA GLP guidelines and therefore "does not fall under its requirements." A subsequent statement, signed and dated August 19, 1991 claimed that the study reformat was in compliance with FIFRA GLPs. A quality assurance statement was, however, not provided.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84-2) for genetic effects, Category II, Structural Chromosome Aberrations.

[4]Green, S., et al. Mutat. Res. 154:49-67.