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WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

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

EPA ID# 060101-000618
Case No. 807285

DP Barcode D180333
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 4-22-93

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 5/3/93

KB 5/4/93

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

Thiabendazole Microbial Mutagenesis Assay; Study Nos TT# 91-8039 and TT# 91-8042; MRID No. 423618-01.

The study, which was a *Salmonella typhimurium/Escherichia coli*/mammalian microsome mutagenicity assay, was reviewed by Clement Associates and Irving Mauer and classified **Acceptable.** Thus, this study satisfies Guideline requirement 84-2a for gene mutation. There was a negative response for induction of gene mutation in two bacterial species (*Salmonella* TA strains and *E. coli* WP2) exposed up to cytotoxic precipitating levels (300-1000 μg/plate) with and without activation.

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DATA EVALUATION REPORT

THIABENDAZOLE

Study Type: Mutagenicity: Salmonella typhimurium/Escherichia coli/
Mammalian Microsome Mutagenicity Assay

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	Kristin Jacobson Kristin Jacobson, MSPH	Date 3/25/93
Independent Reviewer	Nancy E. McCarroll Nancy E. McCarroll, B.S.	Date 3/25/93
QA/QC Manager	Sharon Q. Segal Sharon Segal, Ph.D.	Date 3/25/93

Contract Number: 68D10075
Work Assignment Number: 2-50
Clement Number: 149
Project Officer: Caroline Gordon

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GUIDELINE §84: MUTAGENICITY
SALMONELLA/E. COLI

MUTAGENICITY STUDIES

EPA Reviewer: Irving Mauer, Ph.D. Signature: 4-02-93
Immediate Office, TS, Date:
Health Effects Division (H7509C)
EPA Section Head: Marion Copley, DVM, DABT Signature: Marion Copley
Review Section IV, Toxicology Branch I Date: 4/7/93
Health Effects Division (H7509C)

R.A. 5/3/93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/Escherichia coli/mammalian microsome mutagenicity assay

EPA IDENTIFICATION NUMBERS:

PC Code: 060101

Tox Chem. Number: 849A

MRID Number: 423618-01

TEST MATERIAL: Thiabendazole

SYNONYM(S): None indicated

SPONSOR: Merck and Co., Inc., Three Bridges, NJ

STUDY NUMBERS: TT# 91-8039; TT# 91-8042

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

TITLE OF REPORT: Thiabendazole Microbial Mutagenesis Assay

AUTHORS: G.R. Lankas and J.F. Sina

REPORT ISSUED: March 4, 1992

CONCLUSIONS--EXECUTIVE SUMMARY: Under the conditions of a microbial/mammalian microsome plate incorporation assay, five doses of thiabendazole, ranging from 100 µg/plate to 6000 µg/plate +/- S9, were not mutagenic in Salmonella typhimurium strains TA1535, TA97A, TA98, or TA100 and Escherichia coli strains WP2, WP2 uvrA, or WP2 uvrA pKM101. Compound precipitation and cytotoxicity for the majority of strains was observed at levels ≥1000 µg/plate +/- S9. Similar results were obtained in a repeat assay conducted in three strains (S. typhimurium TA97A and E. coli WP2 uvrA and WP2 uvrA pKM101) with a lower dose range (3-300 µg/plate +/- S9). Based on these findings, we conclude that thiabendazole was tested over an appropriate range of concentrations and was not genotoxic in this bacterial test system.

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STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2a) for genetic effects Category I, Gene Mutations.

A. MATERIALS:

1. Test Material: Thiabendazole

Description: Not provided
Identification number: L-585,216-000S159
Purity: >99.5%
Receipt date: Not reported
Stability: Stable for the duration of the study
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: Neither storage conditions for the test material nor frequency of dosing solution preparation were reported. Dosing solutions and test material stability were verified analytically.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/0.1 mL/plate

Positive:

Nonactivation:

The four Salmonella typhimurium tester strains (TA1535, TA100, TA97A, and TA98) were exposed to the following positive control compounds:

Sodium azide	1.5 µg/plate
ICR-191	1.0 µg/plate
Daunomycin	5.0 µg/plate
Methyl methanesulfonate	2.0 µL/plate
2-Aminoanthracene	2.0 and 5.0 µg/plate

The three E. coli tester strains (WP2, WP2 uvrA, and WP2 uvrA pKM101) were exposed to the following positive control compounds:

Methyl methanesulfonate	2.0 µL/plate
2-Aminoanthracene	5.0 and 10 µg/plate

Activation:

All Salmonella typhimurium tester strains:

2-Aminoanthracene	2.0 and 5.0 µg/plate

E. coli tester strains WP2 uvrA and WP2 uvrA pKM101:

2-Aminoanthracene	5.0 and 10 µg/plate

E. coli tester strain WP2:

Hydrazine sulfate	500 and 1000 µg/plate WP2

3. Activation: S9 derived from 200-325-g male CRCD Crl:CD®(SD) BR Sprague-Dawley

___ Aroclor 1254 _x_ induced _x_ rat _x_ liver
___ phenobarbital ___ noninduced ___ mouse ___ lung
___ none ___ hamster ___ other
_x_ other: phenobarbital and β-naphthoflavone

For induction, rats received four daily intraperitoneal (i.p.) injections of sodium phenobarbital (75 mg/kg/day) and one i.p. injection of β-naphthoflavone (80 mg/mL) on day 3; on day 5, animals were sacrificed, livers were excised, and homogenates were prepared.

Note: The combined injection of phenobarbital and β-naphthoflavone is considered a safe and effective alternative to Aroclor 1254 induction[1].

The rat liver S9 homogenate was prepared by the testing laboratory and assigned lot number PN91-2. The S9 mix was prepared as follows:

Component:	Amount/mL
Dulbecco's PBS with 25 mM sucrose	0.2 mL
Sodium phosphate buffer (pH 7.4)	0.5 mL
Glucose-6-phosphate (50 mM)	0.1 mL
NADP (40 mM)	0.1 mL
Stock salts (0.4 M $MgCl_2$, 1.65 M KCl)	0.02 mL
S9	0.1 mL

Note: The reported final concentration of S9 was 50 µL/plate.

4. Test Organism Used: S. typhimurium strains

_x_ TA97A _x_ TA98 _x_ TA100 _____ TA102 _____ TA104
_x_ TA1535 _____ TA1537 _____ TA1538

Others: E. coli strains WP2, WP2 uvrA, and WP2 uvrA pKM101

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

[1]Maron, D.M. and Ames, B.N. (1983). Revised methods for the Salmonella mutagenicity test. Mutat Res 113:173-215.

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5. Test Compound Concentrations Used:

Initial mutation assay: Five doses (100, 300, 1000, 3000, and 6000 µg/plate +/- S9) were evaluated in all tester strains; triplicate plates were prepared per dose, per strain, per condition. In addition, a single supplemental plate was prepared per strain, per dose, per condition, as a growth control.

Repeat mutation assay: Because of the high cytotoxicity observed in the initial mutation assay in S. typhimurium strain 97A and E. coli strains WP2 uvrA and WP2 uvrA pKM101, the assay was repeated in these three tester strains, using a lower dose range (3, 10, 30, 100, and 300 µg/plate +/- S9).

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
- x Standard plate test
- ___ Pre-incubation (_____) minutes
- ___ "Prival" modification
- ___ Spot test
- ___ Other (describe)

2. Mutation assays: The selected concentrations of the test material, solvent or positive control compounds in 0.1-mL volumes and 0.5 mL buffer (nonactivated conditions) or S9 mix (activated conditions) were added to 2 mL of top agar containing 0.1 mL of 12-hour broth cultures of the appropriate tester strain. The tubes were mixed, and poured onto minimal Vogel-Bonner medium E containing 1.67 µg/mL biotin and either 1.39 µg/mL L-histidine (S. typhimurium cultures) or 10 µg/plate L-tryptophan (E. coli cultures). Triplicate plates were prepared per dose, per strain, per condition; in addition, one supplemental plate containing excess L-histidine (0.1 mg/mL) or L-tryptophan (48 µg/mL) was prepared as a growth control per strain, per dose, per condition. Plates were incubated at 37°C for 48 hours and scored immediately or held at 4°C until they were evaluated. Revertant colonies were counted, and means and standard deviations were determined. Supplemental plates were evaluated for inhibition of the background lawn of growth or for contamination.

3. Evaluation Criteria: The test material was considered positive in the assay if it induced a ≥2-fold reproducible, dose-related increase in revertant colonies, relative to solvent controls.

C. REPORTED RESULTS: Compound precipitation occurred at nonactivated and S9-activated levels ≥1000 µg/plate; the study authors stated that the precipitation did not interfere with the scoring of revertant colonies. Representative results from the initial mutation assay are presented in Table 1. Marked cytotoxicity was observed in several strains (S. typhimurium strain TA97A and all three E. coli strains) at levels ≥1000 µg/plate, with or without S9 activation. However, there were no

appreciable dose-related increases in revertant colonies of any tester strain at any noncytotoxic dose either with or without S9 activation. Owing to cytotoxicity, a repeat assay was conducted with S. typhimurium strain 97A and E. coli strains WP2 uvrA and WP2 uvrA pKM101, using a lower range of doses (3-300 µg/plate +/- S9). The results of the repeat assay indicated that the high dose (300 µg/plate +/- S9) was slightly cytotoxic in all strains. In agreement with the earlier findings, there were no appreciable increases in mutant colonies of any strain. By contrast, the diagnostic positive control compounds induced the expected response in each bacterial strain.

Based on these findings, the study authors concluded that thiabendazole was not mutagenic in this bacterial mutation assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study authors' interpretation of the data was correct. Thiabendazole was tested over a range of concentrations that included levels that were insoluble and cytotoxic in the majority of the tester strains (≥1000 µg/plate +/- S9), but did not increase the frequency of revertant colonies. Although the reporting of fold-increases rather than actual colony counts is not encouraged, the strain specific responses induced by the nonactivated and S9-activated positive controls clearly demonstrated the sensitivity of the test system to detect mutagenesis. It was concluded, therefore, that thiabendazole was tested over an adequate range of concentrations and was negative in this microbial mutation assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A signed quality assurance statement was included with the report.)

CORE CLASSIFICATION: Acceptable; the study satisfies the data Guideline requirements (§84.2a) for genetic effects Category I, Gene Mutations.

Table 1: Representative Results of the Initial Microbial/Mammalian Microsome Mutation Assay with Thiabendazole

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]						
			S. typhimurium				E. coli		
			TA1535	TA97A	TA98	TA100	WP2	WP2 uvrA	WP2 uvrA pKM101
Solvent Control									
Dimethyl sulfoxide	0.1 mL	-	10.8±3.7	99.3 ±10.7	18.8±5.5	62.1±9.3	29.6±5.1	28.8±4.2	85.1±11.3
	0.1 mL	+	12.6±4.7	133.7±15.3	26.4±3.7	70.2±11.9	26.6±3.2	30.6±3.8	75.3±3.3
Positive Controls									
Methyl methanesulfonate[b]	2.0 µL	-	8.4[c]	3.8[c]	2.4[c]	23.7[c]	11.2[c]	30.1[c]	24.7[c]
2-Aminoanthracene[d]	5.0 µg	+	202.3±45.0	1398.7±108.2	1901.7±73.2	2091.0±339.4	--	91.0±14.4	651.0±72.2
Hydrazine sulfate[e]	500.0 µg	+	--	--	--	--	204.7±17.6	--	--
Test Material									
Thiabendazole	100 µg	-	11.3±5.5	101.7±4.9	20.7±6.4	57.3±13.7	25.0±1.0	22.7±3.1	71.3±3.5
	300 µg	-	9.3±2.3	99.0±10.8	15.7±0.6	49.7±2.1	21.7±7.5	20.0±0.0	39.7±10.0
	1000 µg[f]	-	7.7±2.3	4.3±2.3	17.0±3.6	53.0±7.8	12.7±5.8	12.3±4.5	19.0±4.0
	100 µg	+	9.0±3.6	114.7±6.8	27.3±0.6	65.7±3.8	28.3±3.5	30.7±6.7	56.3±8.1
	300 µg	+	8.3±5.1	108.0±13.5	27.0±4.0	63.0±7.2	25.7±7.2	19.0±4.4	36.7±5.9
	1000 µg[f]	+	10.0±1.7	11.0±2.6	21.0±6.2	61.3±2.3	14.3±2.9	6.3±2.1	10.0±2.0

[a]Means and standard deviations of the counts from three plates

[b]S. typhimurium tester strains were exposed to 1.5 µg/plate sodium azide, 1.0 µg/plate ICR-191, 5.0 µg/plate daunomycin, 2.0 µL/plate methyl methanesulfonate, and 2.0 and 5.0 µg/plate 2-aminoanthracene -S9; E. coli strains were exposed to 2.0 µL/plate methyl methanesulfonate and 5.0 and 10.0 µg/plate 2-aminoanthracene -S9; results for exposure of each strain to 2.0 µL/plate methyl methanesulfonate were selected as representative.

[c]Value represents fold-increase in revertant colonies in positive control cultures relative to solvent controls; primary data were not provided in the study report.

[d]S. typhimurium strains were exposed to 2.0 and 5.0 µg/plate 2-aminoanthracene +S9, and E. coli strains were exposed to 5.0 or 10.0 µg/plate +S9; findings from the 5.0-µg/plate cultures for all strains were selected as representative.

[e]E. coli strain WP2 was exposed to 500.0 and 1000.0 µg/plate +S9 hydrazine sulfate; data from the 500.0-µg/plate cultures were selected as representative.

[f]Compound precipitation and cytotoxicity for all strains were observed at this dose and higher levels (3000 and 6000 µg/plate +/-S9).

Abbreviations used: S. typhimurium = Salmonella typhimurium; E. coli = Escherichia coli

Note: Data were extracted from the study report, pp. 21-23.

TABLE 1

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Table 2: Representative Results of the Repeat Microbial/Mammalian Microsome Mutation Assay in Three Bacterial Tester Strains with Thiabendazole

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] S. typhimurium TA97A	E. coli WP2 uvrA	E. coli WP2 uvr pKM101
Solvent Control					
Dimethyl sulfoxide	0.1 mL	-	86.5±9.0	25.3±5.0	62.6±15.5
	0.1 mL	+	116.2±17.3	26.3±2.3	75.3±6.5
Positive Controls					
Methyl methanesulfonate[b]	2.0 µL	-	3.3[c]	24.9[c]	25.5[c]
2-Aminoanthracene[d]	5.0 µg	+	1479.7±90.4	62.7±6.8	447.7±25.1
Test Material					
Thiaberdazole	300 µg[e]	-	65.0±7.8	18.3±1.5	27.7±6.8
	300 µg[e]	+	80.7±13.6	15.0±3.5	44.7±3.1

[a]Means and standard deviations of the counts from three plates

[b]S. typhimurium tester strains were exposed to 1.5 µg/plate sodium azide, 1.0 µg/plate ICR-191, 5.0 µg/plate daunomycin, 2.0 µL/plate methyl methanesulfonate, and 2.0 and 5.0 µg/plate 2-aminoanthracene -S9; E. coli strains were exposed to 2.0 µL/plate methyl methanesulfonate, and 5.0 and 10.0 µg/plate 2-aminoanthracene -S9; results for exposure of each strain to 2.0 µL/plate methyl methanesulfonate were selected as representative.

[c]Value represents fold-increase in revertant colonies in positive control cultures relative to solvent controls; primary data were not provided in the study report.

[d]S. typhimurium strain TA97A was exposed to 2.0 and 5.0 µg/plate 2-aminoanthracene +S9, and E. coli strains were exposed to 5.0 and 10.0 µg/plate +S9; findings from the 5.0-µg/plate cultures were selected as representative.

[e]Highest level assayed; results for lower doses (3, 10, 30, and 100 µg/plate +/- S9) did not suggest a mutagenic effect.

Abbreviations used: S. typhimurium = Salmonella typhimurium; E. coli = Escherichia coli

Note: Data were extracted from the study report, pp. 24-26.