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

May 6,1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cancer Assessment Review Committee Meeting on Thiabendazole

FROM: Sanjivani Diwan Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addressees

Attached for your review is a package on Thiabendazole prepared by Pat Gaunt.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday May 26,1999 at 1:00 pm in Room 813, CM2.

Addressees

K. Baetcke
L. Brennecke
L. Brunsman
W. Burnam
M. Copley
K. Dearfield
V. Dellarco
V. Dobozy
R. Hill
M. Ioannou
N. McCarroll
E. Rinde
J. Rowland
J. Stewart
C. Swentzel
L. Taylor
Y. Woo

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TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Thiabendazole: Evaluation of Carcinogenic Potential

TO: Sanjivani Diwan, PhD
Executive Secretary, Cancer Assessment Review Committee
Health Effects Division (7509C)

FROM: Patricia S. Gaunt, DVM, PhD Patricia S. Gaunt 5.4.99
Toxicologist, RRB4
Health Effects Division (7509C)

THROUGH: Susan Hummel, PhD Susan Hummel 5/4/99
Senior Scientist, RRB4
Health Effects Division (7509C)

Attached is the Cancer Assessment Document for thiabendazole.

The issue of concern is the occurrence of thyroid follicular cell adenomas in male and female rats. The Registrant has conducted a mechanistic study to elucidate the mechanism involved in the induction of thyroid tumors in rats.

The discussion of the use of the threshold model for evaluation of thyroid tumors in the weight of Evidence section of this document has been revised from the previous format to reflect wording contained in the current Agency Science Policy for assessment of thyroid tumors.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

THIABENDAZOLE

DRAFT REPORT

26-MAY-1999

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: _____
Patricia S. Gaunt, Toxicologist

DOCUMENT PREPARATION: _____
Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke _____

William Burnam _____

Marion Copley _____

Vicki Dellarco _____

Virginia Dobozy _____

Linda Taylor _____

Esther Rinde _____

Joycelyn Stewart _____

Clark Swentzel _____

Yin Tak Woo _____

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

Luke Brennecke, Pathology Consultant _____

Lori Brunsman, Statistical Analysis _____

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CONTENTS

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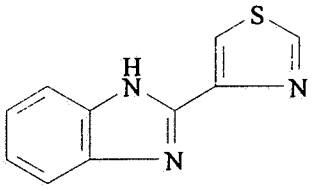
EXECUTIVE SUMMARY

I. Introduction

II. Background Information:

Chemical Name: 2-Thiazol-4-yl)benzimidazole
Synonym: 2-(4-thiazolyl)benzimidazole; TBZ
2-(4-thiazolyl)benzimidazole; TBZ
CAS #: 148-79-8
The chemical structure is shown below:



Thiabendazole is a systemic fungicide used to control fruit and vegetable diseases such as mold, rot, blight, and stain. It is also active against storage diseases and Dutch Elm disease. In livestock, thiabendazole is also applied to treat roundworms.

Thiabendazole binding to soil increases with increasing soil acidity. It is quite persistent and is not expected to leach readily from soil.

III. Evaluation of Carcinogenicity Evidence:

1. Rat 104-Week Dietary Chronic/Carcinogenicity Study

Reference: Squibb R.E. (1993). Thiabendazole: 106-Week Dietary Toxicity/Carcinogenicity Study in rats. Study conducted by Hazleton Washington, Inc. Study Identification Number 618-67/TT #90-9009. **MRID** # 43593201. Unpublished study.

a. Experimental Design

Groups of 50 Sprague-Dawley Crl:CD BR rats/sex/dose received thiabendazole (>98.9%) at dosages of 0,10, 30, or 90 mg//kg/day (achieved average doses of 0, 10.1, 30.2, or 91.8 mg/kg/day) for 104 weeks [See attachment 1 for DER]. All sacrifices were made at 104 weeks.

b. Discussion of tumor Data and comparison with historical control data

A treatment-related increased incidence of thyroid follicular cell adenoma was detected in the mid- (5/50, 10%) and high-dose (6/50; 12%) males (vs 0/50 in controls) and the high-dose (5/50; 10%) females (vs 1/50 and 2/50 in controls). The increase in the incidence was statistically significant ($p \leq 0.05$) in the high-dose males compared to concurrent controls (Table 1). The incidences for the mid- and high-dose males and high-dose females exceeded the range for the historical controls (Table 2). Also, in the male rats, there was a statistically significant trend ($p \leq 0.05$) in the incidence of thyroid follicular cell adenomas with increasing dose. No statistically significant trend in the incidence of any other neoplasm in either sex was observed.

Table 1: Incidence of selected neoplastic lesions[a]

Observation	Dose Group (mg/kg/day)				
	Control 1	Control 2	10	30	90
Males					
Thyroid, Follicular Cell					
Adenoma	0/50[¥](0)[b]	0/50 (0)	1/50 (2)	5/50 (10)	6/50* (12)
Carcinoma	0/50 (0)	1/50 (2)	0/50 (0)	0/50 (0)	1/50 (2)
Females					
Thyroid, Follicular Cell					
Adenoma	1/50 (0)	2/50 (4)	0/50 (0)	1/50 (2)	5/50 (10)
Carcinoma	1/50 (0)	1/50 (2)	0/50 (0)	0/50 (0)	0/50 (0)

a Data were extracted from study report, page 2127

b () = %

* Statistically significant from controls at $p \leq 0.05$. Also, there was a statistically significant trend ($p \leq 0.05$) in the incidence of thyroid follicular cell adenomas with increasing dose in the male rats.

For HED statistical analysis of tumor data refer to attachment 2

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Table 2 Historical control data from Sprague-Dawley rats from MRID No. 43904806 conducted by Life Science Research Limited, Eye, Suffolk, England in 1990-1991.

Males *Historical controls*[a]		
	Mean (%)	Range (%)
Thyroid		
Follicular cell adenoma	3.04	0-6.4
Follicular cell carcinoma	–	--
Females *Historical controls*[a]		
Thyroid		
Follicular cell adenoma	0.72	0-2.0
Follicular cell carcinoma	–	--

[a] Historical control incidence data from 8 studies, 440 males and 420 females

c. Non-neoplastic lesions and other findings:

Thiabendazole had no significant effect on the survival , clinical signs, food consumption, ophthalmoscopic findings, urinalysis, or gross pathology. Systemic signs of toxicity in the treated groups were as follows: 1) decreased body weight gain were generally lower (↓7%-30%) throughout the study for the mid- and high-dose males and high-dose females. Reduced body weight gains (↓15%, 28%, and 19%, $p \leq 0.05$) for the mid- and high-dose males and high-dose females, respectively, compared to the controls were observed at week 103. A reduced body weight gain (↓10%, not statistically significant) was also noted at this time for the mid-dose females.

Significant increases (↑36%-79%, $p \leq 0.05$) in total serum cholesterol observed in the high-dose group were judged to be treatment-related. The incidence of selected non-neoplastic lesions is shown in Table 3. In the high-dose males, increased (↑29%, $p \leq 0.05$) relative (to body) liver weights and an increased incidence of centrilobular hepatocellular hypertrophy (28/50 treated vs 0/50 controls) were also detected. Centrilobular hepatocellular hypertrophy was also observed in 7/50 mid-dose males. In the high-dose females, an increased (↑45%, $p \leq 0.05$) relative thyroid weights and increased incidences of thyroid focal cystic follicular cell hyperplasia (6/50 treated vs 2/50 controls) and diffuse follicular cell hypertrophy (2/50 treated vs 0/50 controls) were

observed. Thyroid diffuse follicular cell hypertrophy was also observed (4/50 treated vs 0/50 controls) in the high-dose males. Based on these findings, the (LOAEL) was 30 mg/kg/day based on reduced body weights and body weight gains and liver hypertrophy (males); the (NOAEL) was 10 mg/kg/day.

Table 3: Incidence of selected non-neoplastic lesions in rats dosed with thiabendazole for 104 weeks[a]

Observation	Dose Group (mg/kg/day)				
	Control 1	Control 2	10	30	90
Males					
Thyroid					
Diffuse follicular cell hypertrophy	0/50 (0)	0/50 (0)	0/50 (0)	1/50 (2)	4/50 (8)
Liver					
Centrilobular hypertrophy	0/50 (0)	0/50 (0)	0/50 (0)	7/50 (14)	28/50 (56)
Females					
Thyroid					
Diffuse follicular cell hypertrophy	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	2/50 (4)
Focal cystic follicular cell hyperplasia	2/50 (0)	1/50 (2)	0/50 (0)	3/50 (6)	6/50 (12)

a Data were extracted from study report, page 2127

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

In light of the systemic effects observed, dosing was considered to be adequate for assessing the carcinogenic potential of thiabendazole in the rat. The body weight gain was decreased throughout the study for the mid- and high-dose males and high-dose females. The following evidence of liver pathology was observed: 1) increased incidence of relative liver weights in high-dose males (↑29%), 2) increased incidence of centrilobular hepatocellular hypertrophy in mid- and high-dose males (7/50 and 28/50, respectively vs. 0/50, controls) . The following evidence of thyroid pathology was observed: 1)increased relative thyroid weights in high-dose females (↑45%); 2) thyroid diffuse follicular cell hypertrophy was also observed in the high-dose males (4/50 treated vs. 0/50 controls) and high dose females (2/50 treated vs. 0/50 controls); 3) increased incidences of thyroid focal cystic follicular cell hyperplasia in high-dose females (6/50 treated vs. 2/50 controls).

2. Mouse Life-time Carcinogenicity Study

Reference: Bagdon W, Bokeman B , and Zwickey R (1980) : Thiabendazole: Lifetime Carcinogenic Study in Mice. Study conducted by Merck Institute for therapeutic Research (West Point, Pennsylvania), Laboratory report number TT #77-014-0, Accession # 242116. Unpublished study.

a. Experimental Design

In a carcinogenicity study, Thiabendazole (98.9%) was administered to 50 mice (Charles River CD-1)/sex /dose in diet at dose levels of 0, 31-42, 63-121, 184-372 mg/kg/day for males and 0, 94-131, 209-368, and 534-1005 mg/kg/day for females for 105 weeks. [See Attachment 2 for DER] All sacrifices were made at 105 weeks.

b. Discussion of Tumor Data

There were no treatment-related neoplastic lesions detected in the animals when compared to controls. However, this study was considered unacceptable due to several deficiencies.

c. Non-neoplastic lesions and other findings:

There was an increase in mortality in all dose groups. Body weight gains were significantly lower in high dose females (28%) and males (18%). There was an increase in the absolute liver weight of high-dose females, and an increase in the relative liver weight in mid-dose females and high-dose males and females. There was an increase in the relative liver: brain weight ratio in high-dose males and females.

d. Adequacy of dosing for Assessment of Carcinogenic Potential

Adequacy of dosing could not be determined due to increased mortality at all dose levels (18-36%).

IV. Toxicology Data

1. Metabolism

In a rat metabolism study (MRID 42114701), [phenyl-U-^{14}C] thiabendazole (99.1% a.i.) was administered to five Crl:CD BR strain rats/sex/dose by gavage as a single dose at 25 or 400 mg/kg or as a single dose at 25 mg/kg following a 14-day pretreatment with unlabeled

thiabendazole at 25 mg/kg.

[^{14}C]Thiabendazole was readily absorbed by male and female rats following oral dosing. The rate of urinary excretion for both sexes in the high dose groups was lower during the initial 24 hours compared to the low dose groups. For all test groups, ~51-73% of the dose was excreted in the urine during the first 48 hours. Dose rate and pretreatment with thiabendazole had no apparent effect on absorption. Within 168 hours of dosing at 25 mg/kg (with or without pretreatment) or 400 mg/kg, 94.3-98.9% of the administered dose was recovered from both sexes, of which 67.3-74.6% was in the urine, 21.3-26.7% was in the feces, and 0.3-2.5% was in the cage washes. Based upon data from a preliminary study, significant levels of radioactivity were not expected in organic volatiles. For all dose groups, concentrations of radioactivity were highest in the cellular fraction of the blood. Residue levels in tissues/organs were generally highest in heart, lungs, spleen, kidneys, and liver, and lowest in fat (soluble and insoluble).

The metabolic profile in urine was similar between the test groups; no unchanged thiabendazole was detected in urine. The majority of the administered dose was recovered in the urine and was isolated by HPLC and identified by enzymatic reactions as the glucuronide conjugate of 5-hydroxythiabendazole (7.3-21.3% of dose) and the sulfate conjugate of 5-hydroxy thiabendazole (23.7-44.9% of dose). Males produced lower amounts of the glucuronide conjugate and higher amounts of the sulfate conjugate than females. Minor amounts ($\leq$1% of dose) of free 5-hydroxythiabendazole (HTBZ) were present in urine from rats from all dose groups. HPLC analyses fecal extracts isolated minor amounts of thiabendazole from males in the preconditioned low dose group and from both sexes treated in the high dose groups, and minor amounts of free HTBZ in all dose groups.

The data indicate that renal excretion is the primary pathway for the elimination of thiabendazole from rats. At the low dose level, it was shown that thiabendazole oxidizes to form 5-hydroxythiabendazole, followed by conjugation to form glucuronide and sulfate conjugates of 5-hydroxythiabendazole.

2. Mutagenicity

The acceptable genetic toxicology studies on thiabendazole indicate that it is non-genotoxic/mutagenic *in vivo* and *in vitro* assays. The available studies although acceptable are inadequate to fulfill the guideline requirements. A data gap exists for two *in vitro* studies namely, *in vitro* mammalian gene mutation and *in vitro* chromosome aberration assay. In contrast to the negative findings from the acceptable studies, data from the open literature show that thiabendazole-induced micronuclei in somatic cells (mouse bone marrow) but not chromosomal aberrations. As seen in germ cells, it can induce aneuploidy at toxic doses ($\geq$150 mg/kg). When taken together, thiabendazole can be considered at most a weak aneugen *in vivo* in both somatic and germ cells. (The published studies are currently being reviewed by Irv Mauer and

his evaluation will be presented at the CARC meeting) . Benomyl, also a benzimidazole compound, induced micronuclei *in vivo* but is not clastogenic (CARC, 1999).

The studies submitted to the Agency are summarized below.

a) In an *in vivo* bone marrow chromosome aberration assay (MRID 43328304), male CRL:CD-1 mice were given a single oral dose of Thiabendazole (99.8% purity) in methylcellulose at levels of 200, 667, and 2,000 mg/kg bodyweight. Bone marrow was sampled 6, 24, and 48 hours after treatment. An acute toxicity test indicated that 2000 mg/kg (approximately one half the LD_{50} for male mice) was a suitable top dose.

All mice survived to their scheduled termination and clinical signs of toxicity were noted at 667 and 2,000 mg/kg. **There were no significant increases in the incidence of chromosome aberrations at any sampling time.** The positive control induced significant increases in cells with chromosome aberrations.

b) **In a plate incorporation assay (MRID No. 42361801), five doses of thiabendazole, ranging from 100 ug/plate to 6000 ug/plate +/- S9, did not induce mutations 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 ug/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 ug/plate +/- S9). Based on these findings, it was concluded that thiabendazole was tested over an appropriate range of concentrations and was not genotoxic in this bacterial test system.

c) In a DNA damage/repair assay, the test material was first assayed in a cytotoxicity test (MRID No. 41170103) employing trypan blue exclusion as a measure of cell viability in cultures exposed for 3 hours at concentrations up to precipitating levels (ca. 1.3 mM) in culture medium (Leibowitz, L-15). Concentrations selected for testing in the main assay were 0.3, 0.7, 1.0, and 1.3 mM, applied for 3 hours to duplicate monolayer cultures of hepatocytes, following which cells were gently scraped from culture dishes and suspended in fresh medium. Cell viability was determined from a small aliquot, and the remainder lysed and fractionated under tetrapropyl ammonium hydroxide, then eluted for fluorometric determinations of DNA according to conventional (published) procedures. Aflatoxin B1 (AFL, 1 uM) in DMSO served as the positive control.

Data from these fractions were transformed into elution slopes, which were then compared to known standards, according to the following criteria for defining positive results.

At none of the concentrations tested (0.3 to 1.3 mM) did the test material produce a significant

(at least threefold) increase in elution slope relative to concurrent negative control. By contrast, the positive control, AFL produced a twentyfold increase, indicating that the cells were responding to a known strand-breaking mutagen.

Based on these results the author concluded that thiabendazole did not induce DNA strand breakage in primary rat hepatocytes exposed to concentrations up to the level of its insolubility in culture medium. The study was acceptable/guideline

3. Structure-Activity Correlations

Benomyl and thiabendazole are both benzimidazoles. Benomyl causes hepatocellular adenomas and carcinomas in two genetically related strains of mice (CD-1 and Swiss SPF). Benomyl has been classified as a group C chemical EPA (1998). Thiophanate-methyl is not a benzimidazole but is converted into benzimidazole group structure when metabolized. It causes hepatocellular adenomas in male and female mice and thyroid adenomas in male and female rats (CARC 1999).

4. Subchronic/Chronic Toxicity Studies

i. Fourteen Week Oral Toxicity (Gavage) Study in Rats

In an oral toxicity (gavage) study in rats, thiabendazole (98.9%) was administered to Crl:CD (SD) albino rats (20/sex/dose) by gavage at dosages of 0, 25, 100, or 400 mg/kg/day for 14 weeks. Very slight to slight centrilobular hypertrophy was observed in the livers of 13/20 males and 11/20 females, and absolute and relative liver weights were increased 42-77%. Slight to moderate follicular cell hyperplasia was observed in the thyroids of 188-19/20 rats per sex; absolute and relative thyroid weights were increased 56-64% and 104-136%, respectively. Centrilobular hypertrophy was observed in the livers of 2/20 males and 5/20 females; follicular cell hyperplasia was observed in the thyroids of 8/20 males and 8/20 females. Absolute liver weights females only) and relative liver, kidney, and thyroid weights were increased 10-29%.

The LOAEL for this study is 100 mg/kg/day, based on histopathological changes of the liver and thyroid. The NOAEL is 25 mg/kg/day. The study was acceptable/guideline.

ii. Fourteen Week Oral Toxicity (Feeding) Study in Rats

In a 14 week oral toxicity (feeding) study in rats (MRID 42942802), thiabendazole (99.4% a.i.) was administered to Crl:CD(SD) albino rats (10/sex/dose) in the diet at nominal dose

levels of 10, 40, 160, or 320 mg/kg/day (achieved doses: 1, 9.4, 37, 149, and 302 mg/kg/day for males; 0, 9.4, 38, 152, and 302 mg/kg/day for females) for 13 weeks.

There were statistically significant increases in liver and thyroid weights with increasing dosages. Statistically significant increases in absolute liver weights were observed at 160 mg/kg/day and higher in females and relative liver weights at 40 mg/kg/day in females and 160 mg/kg/day and higher in males and females. Statistically significant increases in absolute thyroid weights were observed in females dosed at 160 mg/kg/day and higher and relative thyroid weights in males and female rats dosed at 160 mg/kg/day and higher. Histopathological examination revealed treatment-related changes in the liver and thyroid. At 40, 160, and 320 mg/kg/day, centrilobular hypertrophy was observed in the livers of fmales(7/10, 9/10, and 9/10, respectively) and females (1/10, 9/10, and 9/10, respectively). At these same doses, follicular cell hypertrophy was observed in the thyroids of males (1/10, 2/10, and 6/10, respectively) and females (3/10, 10/10, and 10/10, respectively).

The LOAEL for this study is 40 mg/kg/day (37 mg/kg/day), based on reduced body eight gains and histopathological changes in the bone marrow, liver, and thyroid. The NOAEL is 10 mg/kg/day (9.4 mg/kg/day). The study was acceptable/guideline.

iii. Chronic toxicity Study in dogs

In a chronic toxicity study (MRID 42809701), thiabendazole (99% a.i.) was administered orally in capsules to four beagle dogs/sex/dose at dose levels of 0, 10, 40 or 160 mg/kg/day for 52 weeks [See Attachment # 4 for DER].

Dogs lost weight during the first half of the study primarily due to emesis. One mid-dose male dog died of acute hepatitis after two weeks of treatment.

Clinical pathology revealed treatment-related changes in some of the hematology parameters; clinical chemistry and urinalysis parameters were unaffected by treatment. Both sexes were mildly anemic, with decreased red blood cell counts, hematocrit, and hemoglobin values, and had increased activated partial thromboplastin time (10-14%) and platelet counts (51-65%). However, none of the values were outside of the historical control range. There was also a higher incidence of polychromasia and hypochromia compared to the controls during weeks 4, 12, and 26.

At terminal sacrifice, treatment-related changes in organ weights and incidence of histopathological findings were observed. The absolute and relative (% of body weight) liver weights were statistically significantly ($p \leq 0.05$) higher in mid- (14 and 20%, respectively, combined sexes) and high- (37 and 41%, respectively, combined sexes) dose animals. In high-

dose animals, absolute thyroid weights were increased by 22% (not significant), while relative thyroid weights were increased by 33% ($p \leq 0.05$).

Histopathological evaluations identified lesions in the liver, thyroid, gallbladder, kidney, urinary bladder and spleen. Livers exhibited slight to moderate bile duct vacuolation in mid- (4/4 males; 2/4 females) and high- (3/4 males; 3/4 females) dose animals. Thyroids had very slight follicular enlargement in high-dose females (1/4), while very slight to slight follicular cell hypertrophy was observed in high-dose males (1/4) and females (2/4). Dogs in the 10, 40 and 160 mg/kg/day treatment groups had gallbladders which exhibited cytoplasmic lipid vacuolation and discolored foci of the mucosa; dose-related increases in severity (very slight to marked) were observed. However, kidneys of mid- (3/4) and high- (4/4) dose females showed very slight to slight distal tubule vacuolation, compared to 1/4 females each in the control and low-dose groups. Urinary bladders of all high-dose dogs had very slight to slight epithelial cytoplasmic inclusions; this finding was also observed in 3/4 males and 2/4 females in the mid-dose group. However, the toxicological significance of the above findings could not be determined. Spleens exhibited very slight to slight increases in erythropoiesis in mid- (1/4 males; 1/4 females) and high- (2/4 males; 3/4 females) dose animals; hemosiderin deposits were observed in mid- (2/4 males; 2/4 females) and high- (1/4 males; 4/4 females) dose animals.

The LOAEL is 40 mg/kg/day, based on increased liver weight, as well as splenic erythropoiesis and hemosiderosis in both sexes. The NOAEL is 10 mg/kg/day. The study was acceptable/guideline.

5. Mechanistic Study

In a thyroxine clearance study (MRID 43593202), thiabendazole (99.8% a.i.) was administered to 35 Crl:CD(SD) BR male rats/dose in the diet at nominal dose levels of 0, 10, 90, or 270 mg/kg/day (actual 0, 10.15, 91.14, and 235.23 mg/kg/day). After 13 weeks of treatment, 15 rats/dose were sacrificed for pathological evaluation (liver and thyroid only). During week 14 of the study, blood samples from 5 rats/dose were used for evaluation homeostasis of thyroid hormones and thyroid stimulating hormone; animals were discarded after the final blood sampling. All remaining rats (14 to 15/dose) were sacrificed after 13 weeks of the treatment-free recovery phase and evaluated pathologically (liver and thyroid only).

Mortality and clinical signs of treated animals were unaffected by treatment.

After 13 weeks of dosing, mean body weights of the mid- and high- dose animals were 12% and 32% lower than controls, respectively. At the end of the recovery phase, their mean body weights for each of these dose groups were 13% lower than the controls.

Pathological evaluations of the thyroid and liver showed increased absolute and relative organ weights and increased incidence of microscopic lesions. In the mid- and high-dose animals, absolute thyroid weights were increased by 20 and 40%, respectively, and relative (to body) thyroid weights, by 26% and 103%, respectively. Relative liver weights were also increased by 7% and 25%, in mid- and high-dose animals, respectively (Table 4). Histopathological examination revealed dark foci in the thyroids of the 6/15 high-dose animals and very slight to slight diffuse follicular cell hyperplasia in the thyroid of 10/15 mid- and 12/16 high-dose animals. Very slight to slight hepatocellular centrilobular hypertrophy was detected in all (15/15) mid-dose animals and 15/16 high-dose animals. The thyroid and liver lesions were not observed in any of the low-dose and control main study animals or in any of the recovery phase animals.

Table 4: Absolute and relative (to body) organ weights (g) in 15 male rats/dose treated with thiabendazole for 13 weeks. [a]

Organ Weight [b]		Dose (mg/kg/day)			
		0	10	90	270
Thyroid	Absolute	0.02	0.02	0.024	0.028
	Relative	0.0038	0.0040	0.0048	0.0077
Liver	Absolute	15.36	14.78	15.42	13.15
	Relative	2.88	2.89	3.09	3.59

[a] These data were extracted from study report Table B-2, page 97.
[b] Relative organ weights listed parenthetically.

Thyroid homeostasis (serum T_3, T_4 and TSH) was evaluated during treatment weeks 2, 4, 8, and 13 and weeks 6 and 13 of the recovery phase. T_3 levels decreased by 5-10% in mid-dose and 11-19% in high-dose animals compared to controls; TSH levels were increased by 66-160% in mid-dose and 65-189% in high-dose animals (Table 5). Evaluations carried out during week 14 of the study, showed effects in mid- and high-dose animals. For high-dose animals, statistically significant increases in thyroxine clearance (44%, $p=0.001$), volume of distribution (V_d, 64%,$p<0.001$), rate of elimination (k_{el}, 13%, $p<0.001$), and half-life ($T_{1/2}$, 16%, $p<0.001$). At the mid-dose significant increases in k_{el} (18%, $p<0.001$), $T_{1/2}$ (23%, $p<0.001$) and in V_d (22%, $p=0.004$) were observed, while no differences in thyroxine clearance was observed (Table 6). At the end of the recovery period, TSH levels in the mid- and high-dose animals were comparable to control values while T_3 serum levels were higher (9%-19%). T_4 were comparable to controls throughout both the treatment and recovery periods.

Table 5. Mean T_3 and TSH [a]

Study Group	Week	Nominal Dose (mg/kg/day)			
		0	10	90	270
T_3 (ng/dL)					
Main Study	Pretest	101	106	108	102
	2	97	97	87	79
	4	109	111	102	93
	8	81	85	77	66
	13	104	106	97	92
Recovery Phase	Pretest	122	120	122	122
	19	90	103	100	106
	26	92	96	100	109
TSH (µU/mL)					
Main Study	Pretest	50	64	77	71
	2	46	56	88	75
	4	28	37	72	80
	8	96	91	159	190
	13	73	65	128	144
Recovery Phase	Pretest	50	59	63	62
	19	75	72	61	59
	26	88	98	86	86

[a] These data were extracted from study report Tables A-9 and A-11, pages 78 through 81 and 86 through 89, respectively.

Table 6. Thyroxine elimination rate constant (k_{el}), half-life ($T_{1/2}$), volume of distribution (V_d), and systemic clearance (Cl_s) determined in 5 male rats/dose treated with thiabendazole for 13 weeks.[a]

Variable	Nominal Dose (mg/kg/day)			
	0	10	90	270
K_{el}	-0.0374 ± 0.0008 [b]	-0.0393 ± 0.0025	-0.0306 ± 0.0008 ‡‡	-0.0325 ± 0.0012 ‡‡
$T_{1/2}$	18.50 ± 0.39	17.66 ± 1.12	22.67 ± 0.61 ‡‡	21.36 ± 0.78 ‡‡
V_d [c]	15.65 ± 0.45	16.63 ± 0.33	19.07 ± 0.57 ‡	25.52 ± 1.72 ‡‡
Cl_s [c]	0.59 ± 0.01	0.65 ± 0.04	0.58 ± 0.02	0.83 ± 0.08 ‡‡

[a] These data were extracted from study report Table 2, page 175.
[b] Values are geometric mean ± S.E. (n = 5)
[c] Normalized for 100 g body weight.
‡, ‡‡ Statistically significant increasing trend at p=0.004 and p≤0.001, respectively.

These data support the hypothesis that thiabendazole alters thyroid hormone homeostasis in male rats resulting in hypothyroidism. The study authors contend that the primary effect of thiabendazole is on the liver, resulting in hepatocellular hypertrophy [microsomal enzyme induction presumed, but not measured in the study]. Enhanced hepatic metabolism of the thyroid hormones leads to decreased serum levels. The decreased serum levels of the hormones causes an increased release of TSH. The higher serum TSH levels ,in tern, causes thyroid hypertrophy and hyperplasia. The study was classified as Acceptable/nonguideline [See Attachment # 5 for DER].

V. Weight-of-Evidence Considerations

The Health Effects Division Carcinogenicity Peer Review Committee is asked to consider the following toxicology data in determining the carcinogenic potential of thiabendazole:

1) In the rat carcinogenicity study, administration of thiabendazole in the diet at 0, 10, 30, or 90 mg/kg/day for 104 weeks was associated with a treatment-related increased incidence of thyroid follicular cell adenoma in the mid- and high-dose males. The increase incidence was statistically significant in the high-dose males. Also, in the male rats, there was a statistically significant trend in the incidence of thyroid follicular cell adenomas with increasing dose.

Centrilobular hepatocellular hypertrophy was observed in mid-dose males. In the high-

dose females, an increased relative thyroid weights and increased incidences of thyroid focal cystic follicular cell hyperplasia and diffuse follicular cell hypertrophy were seen. Thyroid diffuse follicular cell hypertrophy was also observed in the high-dose males.

In light of the systemic effects observed, dosing was considered to be adequate for assessing the carcingenic potential of thiabendazole in the rat. The body weight gain was decreased throughout the study for the mid- and high-dose males and high-dose females. The following evidence of liver pathology was observed: a) increased incidence of relative liver weights in high-dose males, b) increased incidence of centrilobular hepatocellular hypertrophy in mid- and high-dose males . The following evidence of thyroid pathology was observed: a) increased relative thyroid weights in high-dose females ; b) thyroid diffuse follicular cell hypertrophy was also observed in the high-dose males and high dose females ; c) increased incidences of thyroid focal cystic follicular cell hyperplasia in high-dose females .

2) In the mouse carcinogenicity study, administration of thiabendazole in the diet was not associated with a significant increase in thyroid tumors. There was an increase in the absolute liver weight of high-dose females, and an increase in the relative liver weight in mid-dose females and high-dose males and females. There was an increase in the relative liver: brain weight ratio in high-dose males and females. However, this study was inadequate to assess the carcinogenic potential of thiabendazole.

3) In the chronic dog study, the absolute and relative liver weights were statistically significantly ($p \leq 0.05$) higher in mid- (14 and 20%, respectively, combined sexes) and high- (37 and 41%, respectively, combined sexes) dose animals. In high-dose animals, absolute thyroid weights were increased by 22% (not significant), while relative thyroid weights were increased by 33% ($p \leq 0.05$). On histopathological evaluation the thyroid had very slight follicular enlargement in high-dose females (1/4), while very slight to slight follicular cell hypertrophy was observed in high-dose males (1/4) and females (2/4).

Consideration of the Use of the Threshold Model for Thiabendazole

When evaluating thiabendazole, the Committee is asked to consider whether use of the threshold model for thyroid neoplasms is appropriate.

The quotations which follow are taken from the Agency's Policy Document entitled "Assessment of Thyroid Follicular Cell Tumors", March 1998 (EPA/630/R-97/002):

"Tumors of the thyroid gland follicular cells are fairly common in chronic studies of chemicals in rodents. Experimental evidence indicates that the *mode of action* for these rodent

thyroid tumors involves (a) changes in the DNA of thyroid cells with the generation of mutations, (b) disruption of thyroid-pituitary functioning, or (c) a combination of the two. The only verified cause of human thyroid cancer is ionizing radiation, a *mutagenic* insult to which children are more sensitive than adults.

...Treatments of rodents that cause *thyroid-pituitary disruption* result in chronic reduction in circulating thyroid hormone levels, increase in TSH levels and the development of increased cell division, increased size and numbers of thyroid cells, increased thyroid gland weight and, finally, tumors of the thyroid. In some cases, there is also an increase in tumors of the pituitary cells that produce TSH. Cessation of treatment early in the process before tumor development results in reversal of processes back towards normal."

When assessing tumors of the thyroid, "For those cases where thyroid tumors arise from chemically induced disturbances in thyroid-pituitary functioning, tumors are considered to be secondary to the adverse effects on the thyroid gland function that precede them. As exposures to such agents decrease, the likelihood of cancer decreases; risks may be seen as minimal at doses where there is no effect on thyroid-pituitary homeostasis. Generally, homeostasis is considered to apply when serum T4, T3 and TSH levels and thyroid and pituitary morphology and growth are within their normal limits."

In the Science Policy Guidance section of this document, factors that should be considered in making this determination are discussed.

"Most of the focus in implementing this policy is devoted to answering the following questions: (1) Does an agent that shows thyroid carcinogenic effects have antithyroid activity? (2) Can modes of action other than thyroid-pituitary carcinogenic effects have antithyroid activity? (3) How can one express thyroid dose-response relationships?" The occurrence of tumors in tissues other than the thyroid is also considered in determining mechanism of carcinogenesis.

Determination of whether neoplasms are due to thyroid-pituitary imbalance

The Science Policy Guidance discusses the types of information necessary to characterize the mechanism of thyroid carcinogenesis. These are addressed as they apply to thiabendazole, as follows:

Consideration of whether the thyroid tumors associated with administration of thiabendazole can be attributed to disruption of the thyroid-pituitary hormonal balance (antithyroid activity). In addressing this point, the Policy lists eight areas of inquiry for evidence demonstrating antithyroid activity (for additional details on the results described below, see individual study

summaries presented earlier in this document or attached DERs for carcinogenicity and mechanistic studies):

a. Increases in cellular growth *in vivo* (evidence required):

In the chronic toxicity/carcinogenicity study in Sprague-Dawley rats, treatment with thiabendazole resulted in increased relative thyroid weights at dietary concentrations of 90 mg/kg/day in females. This increase was due to both follicular cell hypertrophy and hyperplasia, based on microscopic evaluation of the thyroid. Thyroid follicular cell hypertrophy was also seen in males treated at 90 mg/kg/day. Increased thyroid weight and follicular cell hypertrophy were also observed in both sexes in the rat subchronic (feeding) toxicity study and increased thyroid weight and hyperplasia were seen in both sexes in the 90 day (gavage) rat study. In the special mechanistic study, there was an increase in the absolute and relative thyroid weights and hyperplasia. Increases in thyroid weight and thyroid follicular cell hypertrophy were reported in both sexes in the dog chronic toxicity study at high-dosage treatment.

b. Hormone changes (e.g., reduced thyroid hormones T3, T4 and increased TSH; evidence required):

In the special mechanistic study, rats treated with 90 and 270 mg/kg/day thiabendazole had increased thyroid and liver weights, decreased circulating T3 levels and increased TSH levels.

c. Site of action (intra thyroidal, peripheral tissues, liver or other sites; evidence required):

The available toxicology data suggest that the primary site of action may be the liver, and to a lesser extent, the thyroid. The mechanistic study concludes that the primary effect of thiabendazole is on the liver, resulting in hepatocellular hypertrophy [microsomal enzyme induction presumed, but not measured in the study]. Enhanced hepatic metabolism of the thyroid hormones leads to decreased serum levels. The decreased serum levels of the hormones causes an increased release of TSH. The higher serum TSH levels ,in tern, causes thyroid hypertrophy and hyperplasia.

d. Dose correlations (evidence required):

The available data (2-year rat chronic toxicity, subchronic feeding rat, subchronic [gavage] rat, chronic dog and special mechanistic study) indicate that the increase in thyroid follicular cell tumors is correlated with perturbance of thyroid hormone levels, hypertrophy and hyperplasia in both sexes. Increases in thyroid tumors were only observed at dose levels causing these effects.

e. Reversibility (evidence required):

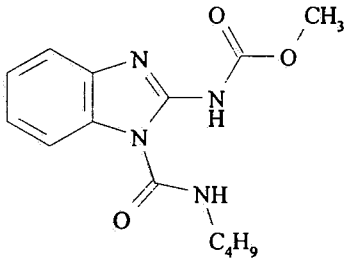
The special mechanistic study demonstrated that after the 13th week in the recovery phase, there was no treatment-related differences in organ weights relative to controls. Also, at the end of the recovery period, TSH levels in the mid- and high-dose animals were comparable to control values while T_3 serum levels were higher (9%-19%). T_4 were comparable to controls throughout both the treatment and recovery periods. After the 13th week recovery phase, there were no treatment-related morphological findings in the liver or thyroid.

f. Lesion progression (evidence desirable):

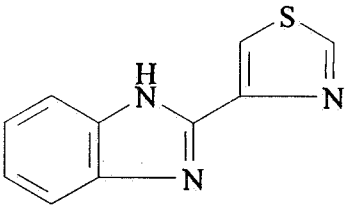
Some evidence exists for lesion progression. In the rat subchronic feeding study, follicular cell hypertrophy was observed and increased thyroid weights in males and females at ≥ 160 mg/kg/day. In the rat subchronic gavage study, thyroid follicular cell hyperplasia along with increased thyroid weights was seen at >100 mg/kg/day. In the chronic dog study there was follicular cell hypertrophy and increased thyroid weight at ≥ 160 mg/kg/day.
In the rat 2 year chronic toxicity/carcinogenicity study adenomas were seen in males at ≥ 30 mg/kg/day and in females at 90 mg/kg/day. Also in male rats, there was a statistically significant trend in the incidence of thyroid follicular cell adenomas with increasing dosages. At ≥ 90 mg/kg/day in females there was an increase in relative thyroid weights and increased incidences of thyroid follicular cell hyperplasia and follicular cell hypertrophy. In males at ≥ 90 mg/kg/day there was thyroid follicular cell hypertrophy. In the special Mechanistic Study, there were increased thyroid weights and follicular cell hyperplasia at ≥ 90 mg/kg/day.

g. Structure-activity analysis (evidence desirable):

Both thiabendazole and benomyl are benzimidazole compounds.



BENOMYL



THIABENDAZOLE

Benomyl causes hepatocellular adenomas and carcinomas in two genetically related strains of mice (CD-1 and Swiss SPF). Benomyl has been classified as a group C chemical (EPA, 1999). Thiophanate-methyl is not a benzimidazole but is converted into benzimidazole group structure when metabolized. It causes hepatocellular adenomas in male and female mice and thyroid adenomas in male and female rats (CARC, 1999).

h. Other studies (evidence desirable):

Based on the overall judgement of the findings listed above, it may be concluded that there are sufficient data to determine whether or not there is suggestive evidence that the thyroid tumors in the rat associated with administration of thiabendazole may be due to disruption in the thyroid-pituitary status.

Consideration of the extent to which genotoxicity may account for the observed tumor effects.

The studies in the published literature indicate that Thiabendazole may have a mutagenic potential.

Consideration of the dose-response.

In the chronic toxicity/carcinogenicity study in rats, thyroid effects were observed at the same dosages at which increases in thyroid tumors were observed (follicular cell hypertrophy ♂ ≥ 30 mg/kg/day and follicular cell hyperplasia ♀ ≥ 30 mg/kg/day). However, the only statistically significant increase (pair-wise comparison to controls) was observed in males at high-dose treatment (90 mg/kg/day). Liver toxicity (hypertrophy) was also observed at these dose levels in males. The special mechanistic study provided evidence for perturbation of thyroid-pituitary homeostasis secondary to the effects on the liver resulting in hepatocellular hypertrophy (microsomal enzyme induction presumed, but not measured). Enhanced hepatic metabolism of thyroid hormones leads to decreased serum levels. The decreased serum levels of hormones causes an increased release of TSH. The higher serum TSH levels in turn cause thyroid hypertrophy and hyperplasia.

Conclusions: As indicated above, based on the overall judgement of the 8 types of data evaluating evidence for thyroid activity, it may be concluded that there are sufficient data to determine whether or not there is suggestive evidence that the thyroid tumors in the rat associated with administration of thiabendazole may be due to a disruption in the thyroid-pituitary homeostasis. In addition to evidence supporting disruption of the thyroid-pituitary homeostasis, the following factors should therefore be considered in evaluating the carcinogenic potential of thiabendazole: (1) the incidence of thyroid tumors in rats was statistically significantly increased above controls and above the range observed in available historical

control range only in males at 90 mg/kg/day; at 30 mg/kg/day, although the incidence was not statistically significant compared to controls it was biologically significant ; (2) thiabendazole did not demonstrate mutagenic potential in a gene mutation assay, chromosomal aberration, or DNA damage/repair assay but it may be a weak aneugen *in vivo* in both somatic and germ cells; (3) Liver tumors were observed in mice treated with the pesticide benomyl, which along with thiabendazole belongs to the benzimidazole compounds. Benomyl is mutagenic. However, benomyl did not affect the thyroid or induce thyroid tumors in rats.

When considered together, the available information suggests that although thiabendazole appears to disrupt thyroid-pituitary homeostasis, the carcinogenic effect to the thyroid is relatively weak. If genotoxicity plays a role in initiation of thyroid carcinogenesis by thiabendazole, it would appear to be limited given the low rate of tumor formation. The Committee is asked to determine whether (1) despite evidence supporting perturbance of the thyroid-pituitary homeostasis, the low incidence of thyroid tumors at non-excessive doses indicates lack of significant carcinogenic potential.

Factors to be Considered in Determining Method to be Used in Estimating the Risks of thiabendazole

Guidance given in the EPA policy for proceeding with the quantitation of risk when evaluating thyroid tumors is as follows:

"Some chemicals that have produced thyroid follicular cell tumors in laboratory rodents appear to work by producing a derangement in thyroid-pituitary homeostasis; others appear to act primarily through a mutagenic mode of action; and still others seem to show a combination of both modes of action. The question then becomes how to evaluate the risks of thyroid tumors for humans given exposure to any of these chemicals. If the animal tumors are due to chemical doses that produce imbalances in thyroid-pituitary functioning, it is anticipated that the chance of cancer is minimal under conditions of hormonal homeostasis. Tumors seeming to arise from relevant mutagenic influences...without perturbation in thyroid-pituitary status may pose some chance of cancer across a broader range of doses. Consequently, until such time that biologically based models and data become available, EPA adopts the following science policy for conducting dose-response assessments of chemical substances that have produced thyroid follicular cell (and related pituitary) tumors in experimental animals:

1. A linear dose-response procedure should be assumed when needed experimental data to understand the cause of thyroid tumors are absent and the mode of action is unknown...
2. A linear dose-response procedure should be assumed when the mode of action underlying thyroid tumors is judged to involve mutagenicity alone.
3. A margin of exposure dose-response procedure based on nonlinearity of effects

should be used when thyroid-pituitary disruption is judged to be the sole mode of action of the observed thyroid and related pituitary tumors...Thyroid-pituitary perturbation is not likely to have carcinogenic potential in short-term or highly infrequent exposure conditions. The margin of exposure procedure generally should be based on thyroid-pituitary disruptive effects themselves, in *lieu* of tumor effects, when data permit...

4. Consistent with EPA risk characterization principles, both linear and margin of exposure considerations should be assumed when both mutagenic and thyroid-pituitary disruption modes of action are judged to be potentially at work...The weight of evidence for emphasizing one over the other should also be presented...
5. Dose-response relationships for neoplasms other than the thyroid (or pituitary) should be evaluated using mode of action information bearing on their induction and principles laid out in current EPA cancer risk assessment guidelines. There is an association between thyroid and liver tumors in rodent cancer studies (McConnell, 1992; Haseman and Lockhart, 1993). The reason(s) for this relationship has not been generically established but should be carefully assessed for chemicals on a case-by-case basis. Some may be due to induction of hepatic microsomal enzymes."

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ATTACHMENTS

ATTACHMENT 1

(27)

DATA EVALUATION RECORD

THIABENDAZOLE

Study Type: 83-5a; Combined Chronic/Oncogenicity Study
with Thiabendazole in the Rat

Work Assignment No. 3-04H (MRID 43592301)

Prepared for

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

Prepared by

Pesticides Health Effects Group
Sciences Division
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Date: 11/21/97

Quality Assurance:
William Spangler, Ph.D.
Signature: William J. Spangler
Date: 11/20/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Robert F. Fricke, Ph.D.
Reregistration Branch 2 (7509C)
Robert F. Fricke, Date 12/16/98

EPA Work Assignment Manager: Sanjivani Diwan, Ph.D.
Toxicology Branch 2 (7509C)
Sanjivani Diwan, Date 2/27/99

DATA EVALUATION RECORD

STUDY TYPE: Combined Chronic/Oncogenicity Study in Rats
OPPTS Number: 870.4300 OPP Guideline Number: §83-5a

DP BARCODE: D214192 SUBMISSION CODE: S485189
P.C. CODE: 060101 TOX. CHEM. NO.: 849A

TEST MATERIAL (PURITY): Thiabendazole Technical (>98.9% a.i.)

SYNONYMS:2-(4'-Thiazolyl)benzimidazole, Arbotect, Bioguard

CITATION: Robert E. Squibb (1993). Thiabendazole: 106-Week Dietary Toxicity/Carcinogenicity Study in Rats. Hazleton Washington, Inc. Study Identification Number 618-67/TT #90-9009, MRID 43593201. Unpublished

SPONSOR: Merck Research Laboratories, West Point, PA

EXECUTIVE SUMMARY: In a combined chronic/oncogenicity study (MRID 43593201), thiabendazole (>98.9% a.i.) was administered to 50 Sprague-Dawley Crl:CD BR rats/sex/dose in the diet at dose levels of 0, 10, 30, or 90 mg/kg/day (achieved average doses of 0, 10.1, 30.2, or 91.8 mg/kg/day) for 104 weeks.

There were no treatment-related effects on survival, clinical signs, food consumption, ophthalmoscopic findings, urinalysis, or gross pathology. Body weights and body weight gains were generally lower (↓7-30%) throughout the study for the mid- and high-dose males and high-dose females. Reduced body weight gains (↓15, 28, and 19%, $p \leq 0.05$) for the mid- and high-dose males and high-dose females, respectively, compared to the controls were observed at week 103. A reduced body weight gain (↓10%, not statistically significant) was also noted at this time for the mid-dose females.

Significant increases (↑36-79%, $p \leq 0.05$) in total serum cholesterol observed in the high-dose group were judged to be treatment-related. In the high-dose males, increased (↑29%, $p \leq 0.05$)

relative (to body) liver weights and an increased incidence of centrilobular hepatocellular hypertrophy (28/50 treated vs 0/50 controls) were also detected. Centrilobular hepatocellular hypertrophy was also observed in 7/50 mid-dose males. In the high-dose females, a increased (↑45%, $p \leq 0.05$) relative thyroid weights and increased incidences of thyroid focal cystic follicular cell hyperplasia (6/50 treated vs 2/50 controls) and diffuse follicular cell hypertrophy (2/50 treated vs 0/50 controls) were observed. Thyroid diffuse follicular cell hypertrophy was also observed (4/50 treated vs 0/50 controls) in the high-dose males.

The systemic LOAEL is 30 mg/kg/day based on reduced body weights and body weight gains and liver hypertrophy (males). The systemic NOAEL is 10 mg/kg/day.

An increase in benign thyroid follicular cell adenoma was observed in the mid-dose (5/50) and high-dose (6/50) males (vs 0/50 controls) and the high-dose females (5/50 treated vs 2/50 controls). The increase was statistically significant ($p \leq 0.05$) in the high-dose males. Also, in the male rats, there was a statistically significant trend ($p \leq 0.05$) in the incidence of thyroid follicular cell adenomas with increasing dose. No statistically significant trend in the incidence of any other neoplasm in either sex was observed. Thiabendazole may affect the rat thyroid indirectly by altering thyroxine clearance via increased hepatic metabolism. This mechanism is specific to the rat. The Sponsor has submitted for Agency review a 14 week thyroxin clearance study (MRID 43592302) in support of this hypothesis.

The study demonstrated that thiabendazole induces thyroid adenomas at dosages of ≥30 mg/kg/day.

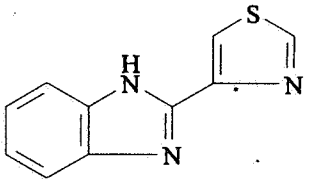
This study is classified as **acceptable (§83-5a)** and satisfies the guideline requirements for a chronic/oncogenicity study in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Thiabendazole technical
   Description: White powder
   Lot/Batch #: L-585, 216-000S159
   Purity: >98.9% a.i.
   Stability of compound: Reported to be stable for the study period
   CAS Reg No: 148-79-8
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Rat
   Strain: Sprague-Dawley, Crl:CD BR
   Age at study initiation: Approximately 6 weeks
   Weight at study Initiation: 216-280g (males) and 130-194g (females)
   Source: Charles River Laboratories, Inc., Raleigh, NC
   Housing: Individually in suspended stainless-steel, wire mesh cages
   Diet: Purina Certified Rodent Chow #5002, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 65 to 78.1 °F
   Humidity: 22-78%
   Air changes: ≥10/hr
   Photoperiod: 12 hr dark/12 hr light
   Acclimation period: approximately 2 weeks

B. STUDY DESIGN:

1. In life dates - Start: 8/23/90 End: 8/28/92

2. Animal assignment: Animals were assigned to treatment groups as indicated in Table 1 using a computerized weight randomization program.

Table 1. Study design

Test Group	Target Dosage Level (mg/kg/day)	Number of Animals	
		Male	Female
Control 1	0	50	50
Control 2	0	50	50
Low	10	50	50
Mid	30	50	50
High	90	50	50

3. Dose Selection: The rationale for dose selection was based on a 14-week dietary toxicity study (Lab. ID TT No. 90-9002; not included with the current submission). Sprague-Dawley Crl: BR rats (10/sex/dose) were fed thiabendazole (purity not reported) at 0, 10, 40, 160, or 320 mg/kg/day.

At ≥40 mg/kg/day in males and ≥160 mg/kg/day in females, there were dose-related decreases (17-70%, $p \leq 0.05$) in body weight gain and decreased food consumption. Centrilobular hepatic hypertrophy and thyroid follicular cell hypertrophy were present in both sexes at ≥40 mg/kg/day. Statistically significant increases ($p \leq 0.05$) in relative liver and thyroid weights were also observed in both sexes at ≥160 mg/kg/day. Thymic atrophy was noted in males at ≥160 mg/kg/day and in females at 320 mg/kg/day. Slight renal pelvic mineralization in females occurred at ≥160 mg/kg/day. In males at 320 mg/kg/day, skeletal muscle atrophy secondary to decreased body weight gain was noted.

Based upon the results of the 14-week study, the doses summarized in Table 1 were selected for the submitted rat chronic/oncogenicity study.

C. METHODS:

1. Preparation of Test Diets: Diets were prepared and adjusted weekly, based on the most recent body weight and food consumption data. A premix of basal feed and the appropriate amounts of test substance was added to the required amounts of additional feed. The diets were stored at room temperature. Homogeneity (top, middle, and bottom) from the control, low-, and high-dose formulations was determined prior to initiation of dosing. Concentration analyses were performed on all diets prepared for study weeks 1, 2, 3, 4, 10, 16, 28, 40, 52, 65, 78, 91, and 104. Stability information was obtained from prior studies. All analyses were performed by the sponsor and the data were not included with the present study.

2. Observations: Animals were inspected twice daily for mortality and once daily for signs of toxicity. Complete physical examinations were performed at each weighing.

3. Body weight: Animals were weighed prior to treatment and weekly thereafter. Body weight data were evaluated statistically at 4-week intervals.

4. Food consumption and compound intake: Food consumption for each animal was recorded weekly and analyzed statistically at 4-week intervals. Compound intake values were calculated from the mean food consumption and the targeted concentration in the diet and expressed as mg/kg body weight/day.

5. Ophthalmoscopic examination: Ophthalmoscopic examinations were performed on each animal prior to treatment and during weeks 52 and 104.

6. Clinical Pathology

a. Blood was collected at weeks 14, 27, 53, 79, and 105 from 15 animals/sex/group, and (when possible) from animals sacrificed *in extremis*. The animals were fasted overnight prior to scheduled blood sampling.

b. Hematology

X	Hematocrit	X	Leukocyte differential count
X	Hemoglobin	X	Mean corpuscular hemoglobin
X	Corrected leukocyte count	X	Mean corpuscular hemoglobin concentration
X	Erythrocyte count	X	Mean corpuscular volume
X	Platelet count	X	Red cell morphology
	Blood clotting measurements		
X	Activated partial thromboplastin time		
X	Prothrombin time		

c. Clinical Chemistry:

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
X	Phosphorus	X	Blood urea nitrogen
X	Potassium	X	Total Cholesterol
X	Sodium	X	Globulins
		X	Glucose
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase	X	Total serum protein
X	Creatine phosphokinase	X	Albumin/globulin ratio
X	Alanine aminotransferase		
X	Aspartate aminotransferase		

d. Urinalysis: Urine was collected at weeks 14, 27, 53, 79, and 105 from 15 animals/sex/group. Sampling occurred during an overnight fast. The following CHECKED (X) parameters were examined.

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	Sediment (microscopic)*	X	Blood
X	Protein		

7. Sacrifice and Pathology: All animals that died or were killed *in extremis* and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. Additionally, the (XX) organs were weighed from 10 animals/sex/group. A full complement of tissues from all rats was examined microscopically.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Oral cavity	X	Aorta	XX	Brain
X	Tongue	XX	Heart	X	Peripheral nerve
X	Salivary glands	X	Bone marrow	X	Spinal cord (3 levels)
X	Esophagus	X	Lymph nodes	XX	Pituitary
X	Stomach	X	Spleen	X	Eyes (with optic nerve)
X	Duodenum	X	Thymus		_____
X	Jejunum		_____		GLANDULAR
X	Ileum		UROGENITAL	XX	Adrenal gland
X	Cecum	XX	Kidneys	X	Harderian gland
X	Colon	X	Urinary bladder		Lacrimal gland
X	Rectum	XX	Testes	X	Mammary gland
X	Liver	XX	Epididymides	XX	Parathyroids
X	Gall bladder	XX	Prostate	XX	Thyroids
	Pancreas	X	Seminal vesicles		_____
X	_____	XX	Ovaries		OTHER
	RESPIRATORY	XX	Uterus	X	Bone
	Trachea	X	Vagina	X	Skeletal muscle
X	Lung	X	Preclitoral	X	Skin
X	Nose	X	Preputial	X	All gross lesions and masses
	Pharynx			X	Head
	Larynx and nasopharynx				

8. Statistics - Cumulative survival data were analyzed using the National Cancer Institute Statistical Package. Body weight, food consumption, clinical pathology, and organ weight data were analyzed using analysis of variance, Levene's test for homogeneity, and Dunnett's test. The data for treated groups were compared to the combined data for the 2 control groups. However, when statistical differences between data from the control groups were found, the data for the treated groups were compared to each control group separately. Histopathology data were analyzed for trend using the extended Mantel-Haenszel procedure, and an exact test was used when the number of tumor bearing animals was ≤10.

II. RESULTS

A. Analytical Chemistry:

Homogeneity Analysis: This was determined from control, low-, and high-dose formulations prior to initiation of dosing. The dose formulations were reported to be homogeneous.

Stability Analysis: Thiabendazole was reported to be stable in rodent feed for at least 3 weeks at the concentration range used in this study.

Concentration Analysis: All concentrations were reported to be within ±20% of nominal concentrations.

The reported results (data not presented) indicated that the mixing procedure was adequate for preparing test diets and that the variance between nominal and actual dosage to the animals was acceptable.

B. Clinical Observations

1. Toxicity - The general condition, behavior, and appearance of treated animals was unaffected by treatment.

2. Mortality - No statistically significant differences were observed in survival rates in either sex of the treated groups throughout the study when compared to the respective control groups. There were 7 accidental deaths during the study, none of which occurred in the controls. At the end of the study, survival rates for controls 1 and 2, 10, 30, and 90 mg/kg/day groups (adjusted for accidental deaths) were respectively, 62%, 70%, 64%, 49%, and 74% for males and 36%, 46%, 33%, 45%, and 51% for females. The low survival in the control females, particularly the first control group, is not addressed in the study report.

C. Body weight and body weight gain - Mean body weights and body weight gains were often lower in the mid-dose males (↓7-17%) and high-dose animals (↓13-30%) compared to controls (Table 2). Statistically significant differences were, however, sporadic. At week 103, just prior to termination of treatment (week 104), body weight gains were decreased (↓15, 28, and 19%, $p \leq 0.05$) for the mid- and high-dose males and high-dose females, respectively, compared to the controls. A decrease of 10% (not statistically significant) was also observed in the body weight gain for the mid-dose females. Body weight gains at the low dose in both sexes was comparable to the controls.

Table 2. Selected mean body weight and body weight gains[a]

Observations	Study Week	Dose Group (mg/kg/day)				
		Control 1	Control 2	10	30	90
Males						
Body Weight (g)	1	251	251	254	248	251
	52	682	686	687	635*(7,7)[b]	588*(14,14)
	96	737	705	702	625*(15,11)	594*(19,16)
	104	680	659	686	607*(11,8)	548*(19,17)
Body Weight Gain (g)	1 - 51	431	435	433	388*(10,11)	337*(22,23)
	1- 103	429	409	435	355*(17,13)	300*(30,27)
Females						
Body Weight (g)	1	165	161	161	161	159
	52	403	413	414	400	345*(14,16)
	96	473	479	490	468	409*(14,15)
	104	474	472	478	443	411*(13,13)
Body Weight Gain (g)	1 - 51	238	252	253	239	186*(22,26)
	1- 103	312	313	314	280	254*(19,19)

a Data extracted from the study report Tables 3 and 4B, pages 91, 96, 102, 103, 126, and 132
b Values in parentheses are the % decreases from control values (Control 1, Control 2)
* Statistically different from controls, $p \leq 0.05$

D. Food consumption and compound intake

1. Food consumption - Mean food consumption was generally lower for the high-dose males and females (↓3-6%) compared to the controls. The differences were statistically significant ($p \leq 0.05$) for the high-dose animals and the mid-dose males at various intervals during the study, however, because the differences were small and sporadic, and because there were no differences between treatment groups and controls in food efficiency, the decreases in food consumption were judged to be not treatment-related.

2. Food efficiency - Relative food consumption (g/kg body weight/day) was not affected by treatment.

3. Compound intake - Time-weighted average compound intake values (mg/kg/day) are summarized in Table 3.

Table 3: Achieved Compound Intake (Time Weighted Average for the Entire Study)[a]

Test Group	Target Dose (mg/kg/day)	Achieved Dose (mg/kg/day)[a]	
		Male	Female
Low	10	10.1	10.1
Mid	30	30.1	30.3
High	90	91.4	92.2

a Data from study report Table 6, page 161

D. Ophthalmoscopic examination: Ocular changes noted at 104 weeks were typical of older rats and were unrelated to treatment.

E. Clinical Pathology:

1. Hematology - Changes observed in hematology parameters were small and occurred inconsistently throughout the study. Significant decreases ($p \leq 0.05$) were noted in erythrocyte counts (↓4-7%), hemoglobin (↓4-7%) and hematocrit (↓5-8%) for high-dose males at weeks 14 and 53; hematocrit (↓12%) for mid-dose males at week 105; hemoglobin (↓4-7%) for high-dose females at weeks 14 and 53; and hematocrit (↓5%) for mid- and high-dose females at week 53. All other hematology values were comparable for treated and control groups.

2. Clinical Chemistry - Significant increases ($p \leq 0.05$) in total cholesterol were noted for high-dose males at weeks 14 (↑53%), 53 (↑79%), and 105 (↑36%); high-dose females at weeks 14 (↑77%), 27 (↑53%), 53 (↑54%), 79 (↑62%), and 105 (↑54%); and mid-dose females at week 14 (↑26%). At week 79, the values for the low- and mid-dose females were higher (↑36-37%) than one of the two control groups (Table 4).

3. Urinalysis - No treatment related effects were observed after 14, 27, 53, 79, or 105 weeks.

Table 4: Total cholesterol (mg/dL)[a]

Week of study	Dose Group (mg/kg/day)			
	0[b]	10	30	90
Males				
14	62	73	77	95*
53	80	98	101	143*
105	121	129	120	165*
Females				
14	74	79	93*	131*
27	91	105	110	139*
53	105	117	127	161*
79	102	121[c]	122[c]	165*
105	109	145	126	168*

a Data extracted from the study report Table 9, pages 193-194
b Mean of the two control groups
c Significantly different from one of two control groups
* Significantly different from controls, $p \leq 0.05$

G. Sacrifice and Pathology:

1. Organ weights - At terminal sacrifice, absolute organ weights were comparable for treated and control groups. Relative (to body) liver weights were increased (↑29%, $p \leq 0.05$) in the high-dose males and relative (to body) thyroid/parathyroid weights were increased (↑45%, $p \leq 0.05$) in the high-dose females (Table 5).

Table 5: Absolute and Relative (to body weight) Liver and Thyroid/Parathyroid Weights [a].

Observation		Dose Group (mg/kg/day)				
		Control 1	Control 2	10	30	90
Males						
Liver Weight	Absolute (g)	15.17	16.34	17.19	16.04	16.93
	Relative (%)	2.396	2.622	2.593	2.869	3.237*
Females						
Thyroid/Parathyroid	Absolute (g)	0.038	0.037	0.038	0.043	0.044
	Relative (%)	0.0091	0.0078	0.0083	0.0100	0.0123*

a Data extracted from the study report Table 11, pages 243, 244, 257, and 258
* Statistically different from controls, $p \leq 0.05$

2. Gross pathology: No treatment-related gross findings were detected. The observed necropsy findings occurred with comparable frequency in the control and treated groups.

3.Microscopic pathology

a) Non-neoplastic - An increased incidence of centrilobular hepatocellular hypertrophy was detected in the mid-dose (7/50) and high-dose (28/50) males (vs 0/50 in the controls) (Table 6). Increased incidences of thyroid diffuse follicular cell hypertrophy in the high-dose males (4/50 treated vs 0/50 controls) and the high-dose females (2/50 treated vs 0/50 controls) and of focal cystic follicular cell hyperplasia in the high-dose females (6/50 treated vs 2/50 controls) were also observed. Historical control data were not included with the current submission.

An increased incidence of renal pelvic epithelial hyperplasia was detected in the low-dose (22/50), mid-dose (27/50), and high-dose (32/50) males (vs 19/50 controls) and the high-dose females (44/50 treated vs 35/50 controls). The Sponsor indicated that this type of lesion is a common age-related change in this strain of rat. In addition, there were no corroborative data indicating a toxicological effect on the kidneys.

The incidence of all histopathological findings is attached to this review (Appendix 1).

Table 6: Incidence of selected non-neoplastic lesions in rats dosed with thiabendazole for 104 weeks[a]

Observation	Dose Group (mg/kg/day)				
	Control 1	Control 2	10	30	90
Males					
Thyroid					
Diffuse follicular cell hypertrophy	0/50 (0)	0/50 (0)	0/50 (0)	1/50 (2)	4/50 (8)
Liver					
Centrilobular hypertrophy	0/50 (0)	0/50 (0)	0/50 (0)	7/50 (14)	28/50 (56)
Females					
Thyroid					
Diffuse follicular cell hypertrophy	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	2/50 (4)
Focal cystic follicular cell hyperplasia	2/50 (0)	1/50 (2)	0/50 (0)	3/50 (6)	6/50 (12)

a Data were extracted from study report, page 2127

b) Neoplastic - A treatment-related increased incidence of thyroid follicular cell adenoma

(39)

was detected in the mid- (5/50) and high-dose (6/50) males (vs 0/50 in controls) and the high-dose (5/50) females (vs 1/50 and 2/50 in controls). The increase incidence was statistically significant ($p \leq 0.05$) in the high-dose males compared to controls (Table 7). Also, in the male rats, there was a statistically significant trend ($p \leq 0.05$) in the incidence of thyroid follicular cell adenomas with increasing dose. No statistically significant trend in the incidence of any other neoplasm in either sex was observed.

The incidences of all neoplastic findings are attached (Appendix 2).

Table 7: Incidence of selected neoplastic lesions[a]

Observation	Dose Group (mg/kg/day)				
	Control 1	Control 2	10	30	90
Males					
Thyroid, Follicular Cell					
Adenoma	0/50 (0)	0/50 (0)	1/50 (2)	5/50 (10)	6/50* (12)
Carcinoma	0/50 (0)	1/50 (2)	0/50 (0)	0/50 (0)	1/50 (2)
Females					
Thyroid, Follicular Cell					
Adenoma	1/50 (0)	2/50 (4)	0/50 (0)	1/50 (2)	5/50 (10)
Carcinoma	1/50 (0)	1/50 (2)	0/50 (0)	0/50 (0)	0/50 (0)

a Data were extracted from study report, page 2127

* Statistically significant from controls at $p \leq 0.05$. Also, there was a statistically significant trend ($p \leq 0.05$) in the incidence of thyroid follicular cell adenomas with increasing dose in the male rats.

III. DISCUSSION

A. Investigators Conclusions - Thiabendazole administered to rats for 2 years at a dose of 90 mg/kg/day resulted in increased liver and thyroid weights, and increased incidences of thyroid follicular cell hypertrophy, hyperplasia, and benign thyroid adenomas. A non statistically significant increase in benign thyroid adenomas was also noted in males at 30 mg/kg. The results are consistent with other studies in the rat, which show that many compounds that increase liver weight also increase thyroxine clearance and thus, increase the incidence of thyroid hyperplasia and adenoma by inducing increased TSH levels. The systemic LOAEL is 30 mg/kg/day. The systemic NOAEL is 10 mg/kg/day.

The oncogenic LOAEL is 30 mg/kg/day based on the increased incidence of thyroid adenomas. The oncogenic NOAEL is 10 mg/kg/day.

B. Reviewer's Discussion/Conclusions - Male and female mice were fed diets containing thiabendazole at 0, 10, 30, or 90 mg/kg/day for 104 weeks.

There were no treatment-related effects on survival, clinical signs, food consumption, ophthalmoscopic findings, urinalysis, or gross pathology. For both sexes, survival in the high-dose groups exceeded that of the controls (70% vs 63% in males and 48% vs 38% in females). Since laboratory historical control data were not included with the submission, it could not be determined whether control group survival in the current study was typical for this strain of rat.

Body weights and body weight gains were generally lower (↓7-30%) throughout the study for the mid- and high-dose males and high-dose females. Reduced body weight gains (↓15, 28, and 19%, $p \leq 0.05$) for the mid- and high-dose males and high-dose females, respectively, compared to the controls were observed just prior to termination of treatment. A reduced body weight gain (↓10%, not statistically significant) was also noted at this time for the mid-dose females. Food consumption was generally lower for the high-dose animals. The differences, however, were small (3-6%) and occurred sporadically. In addition, food efficiency was comparable for treated and control groups. Therefore, the slight decreases in food consumption were judged to be not treatment-related.

The changes in hematological parameters in the mid- and high-dose animals (↓4-12%, $p \leq 0.05$), while statistically significant, were minimal and occurred inconsistently throughout the study. They were judged to be not of toxicological concern.

Significant increases (↑36-79%, $p \leq 0.05$) in total serum cholesterol observed in the high-dose group were judged to be treatment-related. In the high-dose males, increased (↑29%, $p \leq 0.05$) relative liver weights and an increased incidence of centrilobular hepatocellular hypertrophy (28/50 treated vs 0/50 controls) were also detected. Centrilobular hepatocellular hypertrophy was also observed in 7/50 mid-dose males. In the high-dose females, an increased (↑45%, $p \leq 0.05$) relative thyroid weights and increased incidences of thyroid focal cystic follicular cell hyperplasia (6/50 treated vs 2/50 controls) and diffuse follicular cell hypertrophy (2/50 treated vs 0/50 controls) were observed. Thyroid diffuse follicular cell hypertrophy was also observed (4/50 treated vs 0/50 controls) in the high-dose males.

The systemic LOAEL is 30 mg/kg/day based on reduced body weights and body weight gains and liver hypertrophy. The systemic NOAEL is 10 mg/kg/day.

A treatment-related increased incidence of thyroid follicular cell adenoma was detected in the mid-dose (5/50) and high-dose (6/50) males (vs 0/50 in the controls) and the high-dose (5/50) females (vs 2/50 in the controls). The increase incidence was statistically significant ($p \leq 0.05$) only in the high-dose males compared to controls. Also, in the male rats, there was a statistically significant trend ($p \leq 0.05$) in the incidence of thyroid follicular cell adenomas with increasing dose. No statistically significant trend in the incidence of any other neoplasm in either sex was observed. Thiabendazole may affect the rat thyroid indirectly by altering thyroxine clearance via increased hepatic metabolism. This mechanism is specific to the rat. The Sponsor has submitted a 14 week thyroxin clearance study (MRID 43592302) in support of this hypothesis.

The dose levels employed in this study were adequate to characterize the carcinogenic potential of thiabendazole in both sexes of the rat.

The study demonstrated that thiabendazole induces thyroid adenomas at dosages of ≥30 mg/kg/day.

C. Study deficiencies - Analysis of the test compound for stability and the dose preparations for uniformity, stability, and actual concentrations were reported to be the sponsor's responsibility, and analysis data were not included with the current submission. However, the test compound was tested at doses that resulted in toxicity and carcinogenicity, and the LOEL and NOEL were established; therefore, these deficiencies would not be expected to alter the interpretation of the study.

The submitted study is classified as **acceptable (§83-5a)** and satisfies the guideline requirements for a combined chronic/oncogenicity study in rats.

ATTACHMENT NO. 2: Thiabendazole Qualitative Risk Assessment Based on Sprague-Dawley Crl:Cd BR Rat Dietary Study.

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

MEMORANDUM

May 07,1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Thiabendazole Risk Assessment Based On Sprague-Dawley Rat Dietary Study

MRID # 43593201, P.C. Code 060101

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Background

A carcinogenicity study in Sprague-Dawley rats was conducted by Hazleton Laboratories, Vienna, Virginia, for Merck Research Laboratories, West Point, Pennsylvania dated 29 September, 1993 (HWA 284-172; MRID No. 43593201).

The study design allocated groups of 50 rats/sex/dose group. Dose levels of 0, 10, 30, or 90 mg/kg/day of Thiabendazole were administered via the diet for 106 weeks.

The methodology for the analysis was an extension of the usual HED procedure including additional statistical tests and the presentation of graphical results.

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Preliminary Analyses

The two control groups were reported to be not significantly different by the registrant. They were thus combined for use in the subsequent analyses leaving 100 rats in each of the female and male control groups.

Survival Analyses

The Cochran-Mantel-Haenzel Test was run to test the existence of survival disparity among the dose groups. A test of linear trend on mean scores across the levels of dose (nonzero correlation) was found non significant for both the male and female rats with two-tailed p-values of 0.625 and 0.626 respectively.

The statistical evaluation of mortality was also tested using statistics calculated in the computer program of Thomas, Breslow and Gart. An unadjusted survival analysis trend test of mortality (Cochran-Armitage) indicated a non-significant decreasing trend with increasing doses of Thiabendazole in both male and female rats. See Tables 1 and 3 for mortality test results for male and female rats.

Tumor Analyses

All tumors are considered to be mortality independent in this analysis.

Trend tests of tumor rates in male rats were conducted for adenomas, carcinomas and a combination of both. The results are summarized in table 2. The exact randomization trend test had p-values of 0.0006, 0.3289 and 0.0004. There were significant differences in the pair-wise comparisons of the 30 and 90 mg/kg/day dose groups with the controls, for thyroid adenomas both at $p < .01$ and for the combined adenomas/carcinomas at $p < 0.05$ for 30 mg/kg/day and at $p < 0.01$ for 90 mg/kg/day.

The statistical analyses of the male rats were based upon the Exact Randomization Trend Test and Fisher's Exact Test for Pairwise Comparisons since there was not a statistically significant positive trend for mortality with increasing doses of Thiabendazole in male rats. See Table 2 for tumor analysis results.

Trend tests of tumor rates in female rats were conducted for adenomas, carcinomas and a combination of both. The results are summarized in table 4. The exact randomization trend test had p-

values of 0.0233, 0.3210 and 0.0433. There were non significant differences in the pair-wise comparisons of the 10, 30 and 90 mg/kg/day dose groups with the controls, for thyroid adenomas and for the combined adenomas/carcinomas.

The statistical analyses of the female rats were based upon the Exact Randomization Trend Test and Fisher's Exact Test for Pairwise Comparisons since there was not a statistically significant positive trend for mortality with increasing doses of Thiabendazole in female rats. See Table 4 for tumor analysis results.

Graphical Analyses

Mortality and tumor rate analyses are summarized in figures 1 and 2 for the males and in figures 3 and 4 for the female rats.

Table 1. Thiabendazole Sprague-Dawley Male Rat Study
Mortality Rate Analysis
Approximate Test for Unadjusted Negative Trend
Exact Test of Pairwise Comparisons

	WEEK					
DOSE(Mg/Kg/Day)	1-26	27-52	53-78	79-106[f]	TOTAL	1-tail p-Values
0.000	0/100 (0)	3/100 (3)	6/97 (6)	28/91 (31)	37/100 (37)	0.1843
10.000	2/50 (4)	2/48 (4)	3/46 (7)	13/43 (30)	20/50 (40)	0.4274
30.000	1/50 (2)	1/48[a] (2)	7/47 (15)	17/40 (42)	26/49 (53)	0.0574
90.000	1/50 (2)	1/48[b] (2)	2/47 (4)	9/44[c] (20)	13/48 (27)	0.1217

*Number of animals that died during interval/Number of animals alive at the beginning of the interval.

There were no interim sacrifices

[f]Final sacrifice at week 106.

[a]1 accidental death at week 43, dose 30 mg/kg/day
[b]1 accidental death at week 37, dose 90 mg/kg/day
[c]1 accidental death at week 79, dose 90 mg/kg/day

( )Percent.

Note:

Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 2. Thiabendazole Sprague-Dawley Rat Study
Male Thyroid Follicular Cell Tumor Rates+

Exact Randomization Trend Test
Fisher's Exact Test for Pairwise Comparisons

DOSE (Mg/Kg/Day)	0.0000	10.0000	30.0000	90.0000
Adenomas	0/76	1/37	5/44	6[a]/34
(%)	(0)	(3)	(11)	(18)
	p= 0.0006**	p= 0.3274	p= 0.0057**	p= 0.0006**
Carcinomas	1/74	0/34	0/42	1[b]/33
(%)	(1)	(0)	(0)	(3)
	p= 0.3289	p= 0.6852[N]	p= 0.6379[N]	p= 0.5237
Combined	1/76	1/37	5/44	7/34
(%)	(1)	(3)	(11)	(21)
	p= 0.0004**	p= 0.5496	p= 0.0245*	p= 0.0010**

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 14 for adenomas and combined, and before week 53 for carcinomas.

[a]First adenoma observed at week 14, hi-dose group - 90 mg/kg/day.

[b]First carcinoma observed at week 105, hi-dose group -90 mg/kg/day.
[N]Negative change from control.

The number of animals with multiple tumors are 0 for the control, 10, 30, and 90 mg/kg/day dose groups.

The number of male rats not examined for thyroid follicular cell tumor was 24, 12, 6 and 16 for the control, 10,30, and 90 mg/kg/day dose groups respectively.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Figure 1

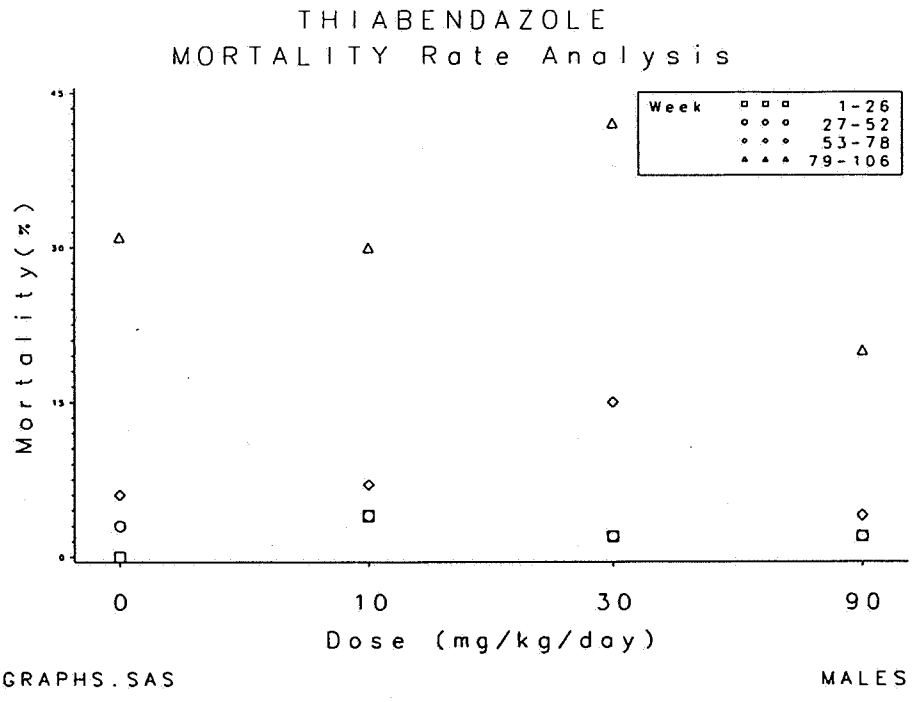

Figure 2

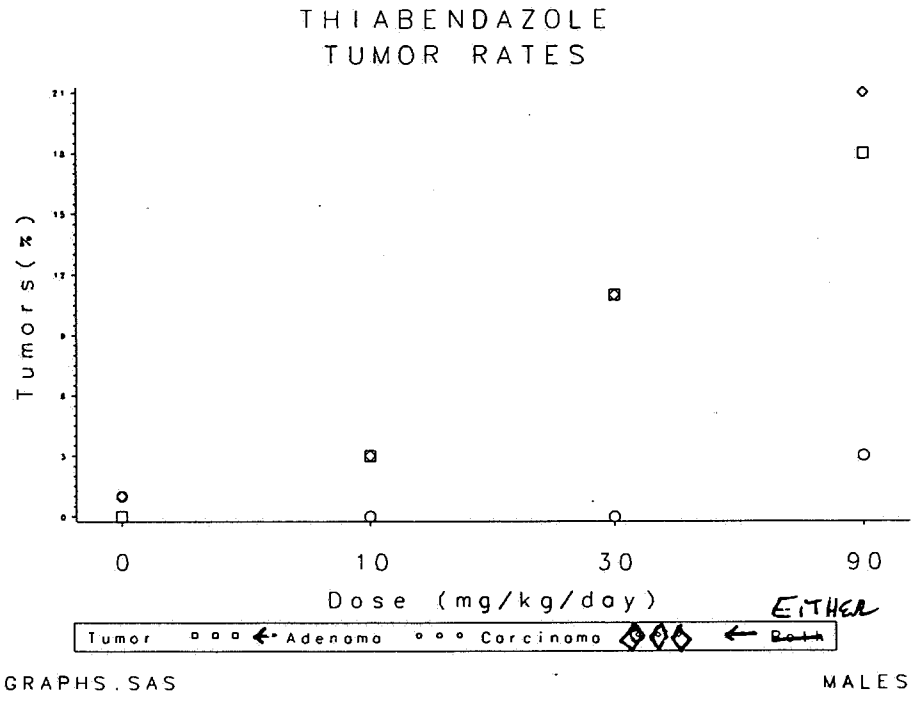

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Table 3. Thiabendazole Sprague-Dawley Female Rat Study
Mortality Rate Analysis
Approximate Test for Unadjusted Negative Trend
Exact Test of Pairwise Comparisons

DOSE(Mg/Kg/Day)	WEEK 1-26	27-52	53-78	79-106[f]	TOTAL	1-tail P-value
0.000	0/100 (0)	3/100 (3)	15/97 (15)	44/82 (54)	62/100 (62)	0.1306
10.000	0/50 (0)	2/50 (4)	9/48 (20)	21/37[a] (57)	32/48 (67)	0.3578
30.000	0/50 (0)	0/50 (0)	6/50 (12)	21/43[b] (49)	27/49 (55)	0.2641
90.000	1/50 (2)	1/48[c] (2)	3/47 (6)	20/44 (45)	25/49 (51)	0.1357

[+]Number of animals that died during interval/
Number of animals alive at the beginning of the interval.

[i]No interim sacrifices

[I]Final sacrifice at week 106.

[a] 2 accidental deaths at week 79, dose 10 mg/kg/day
[b] 1 accidental death at week 79, dose 30 mg/kg/day
[c] 1 accidental death at week 52, dose 90 mg/kg/day

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Figure 3

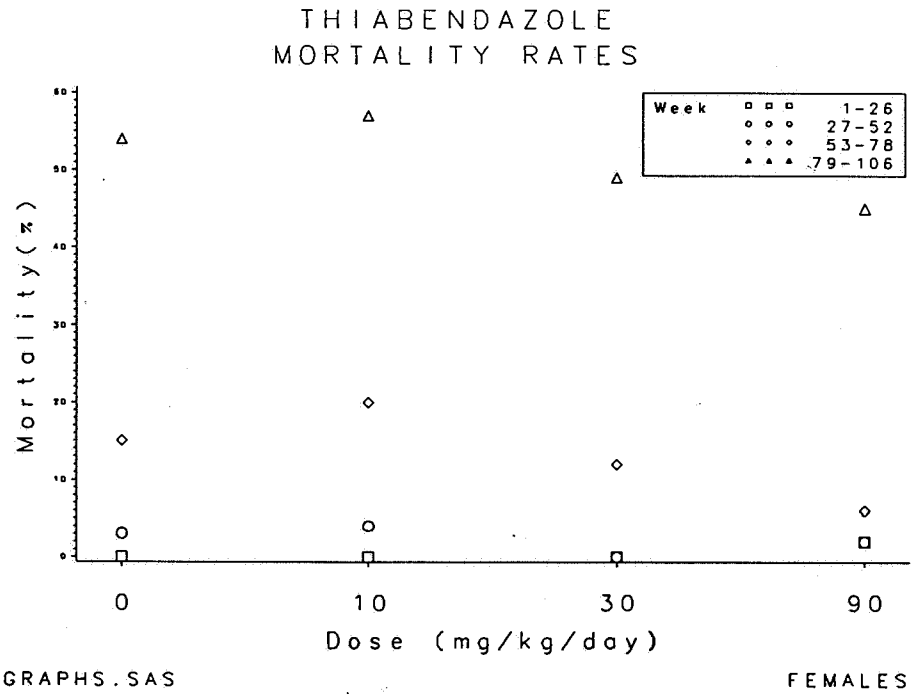

Figure 4

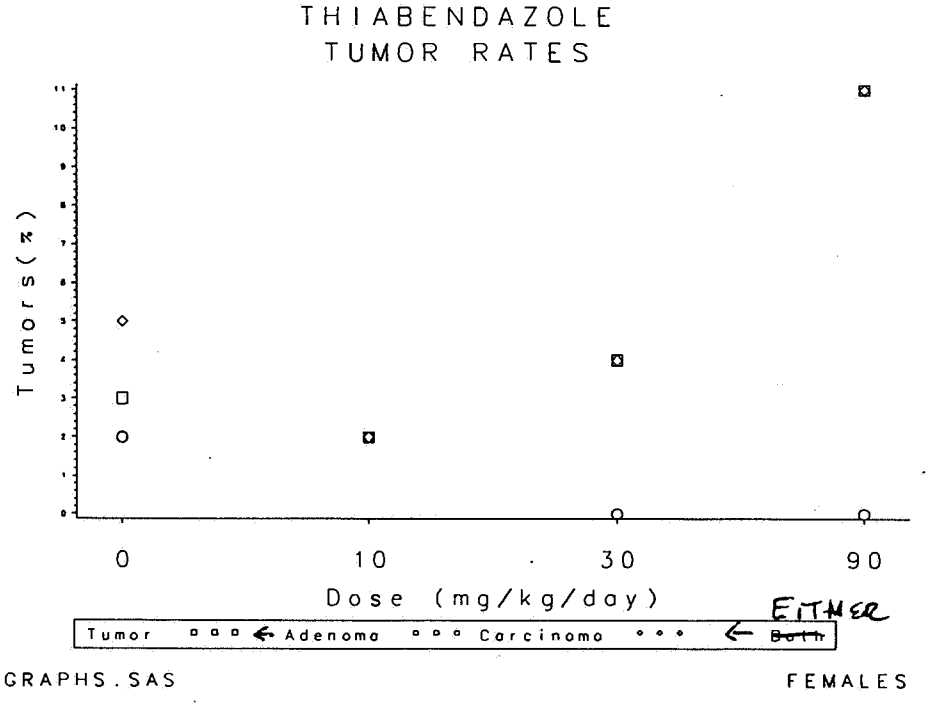

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Table 4. Thiabendazole Sprague-Dawley Rat Study
Female Thyroid Follicular Cell Tumor Rates

Exact Randomization Trend Test
Fisher's Exact Test for Pairwise Comparisons

DOSE (Mg/Kg/Day)	0.0000	10.0000	30.0000	90.0000
Adenomas (%)	3[a]/96 (3)	1/46 (2)	1/48 (4)	5/47 (11)
	p= 0.0234*	p= 0.6095[N]	p= 0.5926	p= 0.0773
Carcinomas (%)	1[b]/96 (2)	1/46 (2)	0/48 (0)	0/47 (0)
	p= 0.3210	p= 0.5445	p= 0.6667[N]	p= 0.6713
Combined (%)	4/96 (5)	1[c]/46 (2)	1/48 (4)	5/47 (11)
	p= 0.0433*	p= 0.4775[N]	p= 0.4586[N]	p= 0.1304

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a]First adenoma observed at week 81, control group - 0 mg/kg/day.

[b]First carcinoma observed at week 70, control group -0 mg/kg/day.

[c] One animal in the 10 mg/kg/day dose group had both an adenoma and a carcinoma.

[N]negative change from control.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Supplement to Document No. 001980 - DER for Accession No.242116 (MRID 00031447): Thiabendazole: Lifetime Carcinogenicity Study in Mice. This supplement provides an Executive Summary to upgrade the original DER.

EPA Reviewer: Patricia S. Gaunt, DVM, PhD David Kryon for Date 10/12/99
Reregistration Branch 4, HED (7509C)
EPA Secondary Reviewer: Sanjivani Diwan, PhD S Diwan, Date 10/12/99
Reregistration Branch 4, HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity feeding in mice; OPPTS 870.3200 [§83-2b]

DP BARCODE: D214192 SUBMISSION CODE: S485189
P.C. CODE: 060101 TOX. CHEM. NO.: 849A

TEST MATERIAL (PURITY): Thiabendazole Technical (>98.9% a.i.)

SYNONYMS: 2-(4'-Thiazolyl)benzimidazole, Arbotect, Bioguard

CITATION: Bagdon W, Bokeman D, and Zwickey R. (1980) Thiabendazole: Lifetime Carcinogenic Study in Mice. Merck Institute for Therapeutic Research (West Point, Pennsylvania). Laboratory report number TT #77-014-0, January 2, 1980. MRID 00031447. Unpublished

SPONSOR: Merck Sharpe & Dohme, West Point, Pennsylvania

EXECUTIVE SUMMARY:

In a 105-week carcinogenicity toxicity study (Accession No. 242116), Thiabendazole, 98.5% a.i. was administered to 50 mice (Charles River CD-1)/sex /dose in diet at dose levels of 0, 5.6-8.3, 31-42, 63-121, 184-372 mg/kg/day for males and 0, 5.7-9.9, 94-131, 209-368, and 534-1005 mg/kg/day for females.

There was an increase in mortality in all dose groups. Body weight gains were significantly lower in high dose females (28%) and males (18%). There was an increase in the absolute liver weight of high-dose females, and an increase in the relative liver weight in mid-dose females and high-dose males and females. There was an increase in the relative liver: brain weight ratio in high-dose males and females.

The LOAEL for systemic toxicity is 209-368 mg/kg/day for females and 63-121 mg/kg/day for males, based on decreased body weight gains and increased liver weights. The NOAEL is 5.7-9.9 mg/kg/day for females and 5.6-8.3 mg/kg/day for males.

It was noted that there were sufficient number of animals alive at 15 and 18 month intervals to assess the carcinogenic response. However, no treatment-related increase in tumor incidence above background level was observed. Although the dosing was variable, and assuming that the animals received the lowest dose of the range for each dose group, a compound-related effect (i.e. increased mortality) was noted in both sexes at the mid- and high dose at 18 month and at the high dose at 15 month. Therefore, the dosing was considered to be adequate and the study was acceptable and satisfies the guideline requirement for a carcinogenicity study (83-5) in mice.

At the doses tested, there was no treatment related increase in tumor incidence when compared to controls in this strain of mice.

COMPLIANCE: Signed and dated GLP and Quality Assurance. Data Confidentiality and Flagging statements were not provided because the study was conducted prior to the establishment of FIFRA guidelines.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

APR 29 1980

(45)

EPA Reg. ~~#618-75~~; Thiabendazole, ~~Lifetime Carcinogenic~~ Study in Mice
CASWELL~~#849A~~ Accession#242116

William Dykstra
Toxicology Branch, HED (TS-769)

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WRD 4/15/80

Recommendations:

1) Thiabendazole was not oncogenic in this mouse feeding study. The study is acceptable as core-minimum data.

Review

Thiabendazole: Lifetime Carcinogenic Study in Mice (Merck Sharp & Dohme Research Laboratories, TT#77-014-C, January 2, 1980)

Test Material: Technical Thiabendazole, Lot. No. E94851

Six hundred albino mice of the Charles River CD-1 (HaM/ICR) strain were selected for a study of the carcinogenic potential of thiabendazole. The study was performed at the Merck Sharp & Dohme Research Laboratories, West Point, Pa., with the exception that microscopic examinations of the tissues after January 31, 1979, were examined at the facilities of Dr. Richard Jensen, a consultant pathologist, 62 West State Street, Doylestown, Pa. This study started March 29, 1977, and was terminated March 28, 1979. Prior to this time, Dr. Jensen was an employee of Merck Sharp & Dohme Research Laboratories and the pathologist for the study.

Litters containing 6 males or 6 females were used with one animal from each litter placed in each of three control and three drug-treated groups. The animals were approximately four weeks old when the study started; the males weighed 14.0 to 27.8 gm. and the females weighed 12.0 to 25.0 gm.

Each animal was randomly assigned to one of six groups. The mice were ear-notched and had digits removed for identification. They were housed 2 or 3 per wire-covered plastic box and box was placed in a random pattern on laminar air-flow racks (Carworth) in a climate-controlled room.

The animals had free access to powdered Purina Lab Chow admixed with 1 percent by weight of edible vegetable oil (Wesson Oil, Hunt-Wesson Foods, Inc.). Prior to necropsy the mice were fasted a minimum of 16 hours. Water was available at all times.

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Thiabendazole was mixed in the diet each week. This compound was assayed prior to the start of the study, periodically during the study, and at termination. The material was chemically acceptable prior to the start of the study and remained chemically unchanged during the study.

Initially, the males were given concentrations of 0.022 percent, 0.066 percent, or 0.2 percent, and the females were given concentrations of 0.066 percent, 0.2 percent, or 0.533 percent.

Different concentrations were used for the male and female mice based on results of the range-finding study (TT#77-004-0) in an effort to have a concentration which would be the maximally tolerated for each sex. Starting in the seventh week, the lowest concentrations for the male and female drug-treated mice were both reduced to 0.006 percent.

Food consumption was determined for a six-day period on 30 male and 30 female mice group per group (3 animals/food dish) weekly.

Fifty males and 50 females were used in each dosage group and in three control groups. The animals in the control groups received the diet without drug and were identical with respect to treatment.

Females given the highest concentration (0.533 percent) were killed in the eighty-first week when the number of survivors was 10. The remaining groups were also killed and necropsied when the number of survivors approached this number. The males given the highest concentration (0.2 percent) were killed in eighty-fifth week, males and females given the middle concentrations (0.2 percent for females and 0.066 percent for males) were killed in the ninety-third week, and males given the lowest concentration (0.022 percent reduced to 0.006 percent in week 7) were killed the 101st week.

At these times, selected control animals from each of the three control groups were also killed; the number killed and their selection was determined by the Biometrics Department. The 17 surviving females given the lowest concentration (0.066 percent reduced to 0.006 percent in week 7) and the remaining 31 female controls were killed in the 105th week.

Observations for physical signs of drug effect were made daily, although less detailed examinations were made on weekends and holidays. All animals were palpated for massess generally once a week during the study. The mice were weighed pretest and once a week during the study.

Ophthalmologic examination with an indirect ophthalmoscope were performed on all surviving mice in the eighty-second or eighty-third and 101st weeks. Individual animals with overt lesions were examined in the thirteenth, twenty-eight, and firty-eighth weeks. One or two drops of 1% tropicamide ("Mydriacyl", Alcon) were placed in each eye to dilate the pupils.

Animals sacrificed because of morbidity or a scheduled necropsy were anesthetized by the administration of ether vapor and killed by exsanguination.

Terminal body weights and weights of liver, spleen, heart, kidney, brain and testes were recorded for mice killed at scheduled necropsies. Statistical analyses were performed on terminal body weights and on organ weights.

The carcasses (after necropsy) of 5 males and 5 females from each of the drug-treated groups killed at scheduled group necropsies were frozen in toto and saved for possible future analysis. Five females from each control group and 3 males from control group I, 2 males from control group II, and 4 males from control group III were also frozen and retained.

Hematoxylin - and eosin-stained sections of paraffin - embedded tissue samples were routinely used for microscopic examinations. Special staining procedures were used as required. The following tissues and organs were routinely examined from each mouse, lung (with main stem bronchi), heart, liver (three different lobes), kidney, urinary bladder, gallbladder, spleen, thymus, lymph nodes (mesenteric, mediastinal, and submandibular), adrenal, thyroid, parathyroid (if present in the thyroid section), pituitary, pancreas, bone, joint, bone marrow, reproductive organs (testes, epididymes, and prostate or ovary and uterus corpus and cervis), salivary gland, stomach, small intestine, large intestine, brain (three levels), spinal cord (three levels), peripheral nerve, skeletal muscle, eye (with intraorbital glands), skin (from the region of the mammary gland), mammary gland (if present in the skin section), blood smear (only from mice killed at scheduled necropsies), trachea, and esophagus.

In addition, paranasal sinues and middle ear were examined from 5 males and 5 females from each group killed at scheduled necropsies and microscopic examination was also performed upon all findings of uncertain gross character at necropsy.

Dr. Henry Pitot of the McArdle Institute in Madison, Wisconsin, was consulted about the morphologic criteria used in the classification of neoplastic and hyperplastic hepatocellular changes.

Dr. Pitot stated that he used the terminology of Walker et al (Walker, A.I.T., Thorpe, E. and Stevenson, D.E. (1972); The Toxicology of Dieldrin (HEOD). 1. Long-term toxicity studies in mice, FD Cosmet toxicol 11: 415-432) and that he placed special importance on the loss of the usual orderly pattern of hepatocytic architecture, i.e., foci in which a relative lack of normal architecture was evident were considered to be Type B nodules regardless of the prominence of other characteristics. His recommendations were followed in the evaluation of liver sections.

Results:

The only physical sign related to treatment was a higher mortality in male and female mice given the middle and highest concentrations of thiabendazole compared to controls. Myocardial thrombosis affecting the atrium (determined at necropsy) was the primary reason for the decreased survival rate of the males (0.2 percent) and the females (0.533 percent) given the highest concentrations and the females given the middle concentration (0.2 percent).

The greater mortality in males given the middle concentration (0.066 percent) cannot be explained.

Prior to being found dead or killed, these animals were generally less active than normal. The female mice given the highest concentration (0.533 percent) had a greater mortality than the controls beginning near the end of the first year, which continued until this group was killed in the eighty-first week when the number of survivors was 10. Similarly, shortly after the first year of treatment, males given the highest concentration (0.2 percent) had a greater mortality than controls and this group was terminated in the eighty-fifth week. Male and female mice given the middle concentrations (0.066 percent males and 0.2 percent females) also had greater mortality than controls after the sixtieth week, and these groups were killed in the ninety-third week. In the 101st week, males given the lowest concentration of thiabendazole (0.022 percent reduced to 0.006 percent in Week 7) were also terminated because of only 12 survivors, but the mortality in this group was similar to controls.

The number of masses palpated each week was similar in drug-treated and control animals. These masses were in similar locations and, if present at the time of necropsy, were examined.

No drug-induced ocular changes were seen during the study. Retinal degeneration, an inherited condition, was seen similarly in both drug-treated and control animals. Other changes seen similarly in both drug-treated and control animals included cataracts, corneal calcification, and synechia.

Male and female mice given the highest concentrations of thiabendazole in the diet (females 0.533 percent and males 0.2 percent), and female mice given the middle concentration (0.2 percent) had lower average weight gains compared to controls during the study. Male mice given the middle concentration (0.066 percent) had lower average weight gains compared to the controls for the first three months of the study; thereafter, the average weight gain was similar to controls until the eighty-first week, when again the average weight gain was less. This remained so until the group was killed in the ninety-third week. Female mice given the lowest concentration (0.066 percent reduced to 0.006 percent in Week 7) and males given the lowest concentration (0.022 percent reduced to 0.006 percent in Week 7) had lower average weight gains compared to controls during the first three months of the study, but thereafter their average weight gains were similar to controls.

The average food consumption values of male and female mice generally correlated with body weight changes. Although male mice given the highest concentration of thiabendazole (0.2 percent) initially had decreased average food consumption compared to the controls, after the sixtieth week of the study their average food consumption was greater than the controls; however, this was insufficient to raise the average body weights to be similar to the controls.

No changes considered due to treatment were seen in male and female mice given the lowest concentration of thiabendazole in the diet. Treatment-related changes were seen in male or female

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These changes consist of an increased mortality, atrial thrombosis, lower terminal body weight, and organ weight differences of the liver and kidneys.

The incidence of atrial thrombosis affecting the left atrium is shown below:

Group	Males	Females
I (control)	3	0
II (control)	3	1
III (control)	3	0
IV (Low-dose)	0	1
V (Mid-dose)	3	19
VI (High-dose)	24	33

Atrial thrombosis was first seen in week 31 and occurred in a high concentrat female mouse. Although it is an occasionally encounted incidental lesion, because of the greater incidence in the above groups, it was considered due t treatment in group V and VI.

The incidence of treatment-related statistically significant ($P \leq .050$) terminal body weight and organ weight difference as compared to controls is shown below:

	Groups					
	IV		V		VI	
	F	M	F	M	F	M
Terminal Body wt.	a	a	<	a	a	<
Liver - grams	a	a	a	a	>	a
Liver - % Body wt.	a	a	>	a	>	>
Liver - % Brain wt.	a	a	a	a	>	>
Kidney - grams	a	a	<	a	<	a
Kidney - % Body wt.	a	a	a	<	<	a
Kidney - % Brain wt.	a	a	a	<	<	a

a = not statistically significant from controls
> = statistically significantly increased
< = statistically significantly decreased

There were no gross or microscopic changes associated with these organ weight changes. All other changes seen were considered incidental and were not attributed to the administration of thiabendazole. The incidence and time of onset of neoplasms in the drug-treated animals were similar to the controls. The number of hepatocellular neoplasms was similar in drug-treated and contro groups, although the incidence of Type "B" hepatocellular neoplasms was sligh greater in females given the highest concentration. The latter difference wa not considered biologically significant, however, since Type "A" and Type "B" lesions are considered not qualitatively different and the incidence of hepatocellular neoplasms was similar in each group when males and females wer combined; as shown in Table I.

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Conclusion: Thiabendazole was not oncogenic in this mouse feeding study.

Classification: Core-Minimum Data

	GROUP 1 F	GROUP 1 M	GROUP 2 F	GROUP 2 M	GROUP 3 F	GROUP 3 M	GROUP 4 F	GROUP 4 M	GROUP 5 F	GROUP 5 M	GROUP 6 F	GROUP 6 M
NUMBER NECROPSIED	50	50	50	50	50	50	50	50	50	50	50	50
WITH MALIGNANT NEOPLASMS	9	4	5	5	9	8	10	6	3	3	6	4
WITH BENIGN NEOPLASMS	9	12	18	13	11	11	11	10	15	11	6	10
WITH NEOPLASMS	17	14	23	17	18	18	19	14	17	14	11	14
OF MALIGNANT NEOPLASMS	9	5	6	5	9	9	10	6	3	3	6	4
OF BENIGN NEOPLASMS	9	13	19	14	12	12	13	12	16	13	6	11
OF NEOPLASMS	18	18	25	19	21	21	23	18	19	16	12	15
ADENOMA (SALIVARY GLAND)	-	-	-	-	-	1	-	-	-	-	-	-
PAPILLOMA (STOMACH)	-	-	-	-	-	-	1	-	1	-	-	-
SQUAMOUS CELL CARCINOMA (STOMACH)	-	-	-	2	-	-	-	-	-	-	-	-
ADENOCARCINOMA (SMALL INTESTINE)	-	-	1	-	-	-	-	-	-	-	-	-
FIBROSARCOMA (SMALL INTESTINE)	-	-	-	-	1	-	-	-	-	-	-	-
HEMANGIOMA (LIVER)	-	-	-	-	-	-	-	-	-	-	-	1
HEMANGIOSARCOMA (LIVER)	1	-	-	1	1	1	-	-	-	-	-	-
NEOPLASM TYPE A (LIVER)	1	-	1	1	1	1	3	-	-	-	1	1
NEOPLASM TYPE B (LIVER)	-	2	-	1	-	3	-	2	1	-	4	1
ADENOMA (ADRENAL)	-	-	-	1	-	-	-	1	-	-	-	-
BENIGN PHEOCHROMOCYTOMA (ADRENAL)	-	1	-	-	-	1	1	2	-	2	-	-
ADENOMA (PITUITARY)	-	-	-	-	1	-	-	-	-	-	-	-
ADENOCARCINOMA (THYROID)	-	-	-	-	-	-	-	-	-	-	-	1
CARCINOMA (THYROID)	-	1	-	-	-	-	-	-	-	-	-	-
LEIOMYOMA (UTERUS)	-	-	3	-	1	-	3	-	1	-	-	-
LEIOMYOSARCOMA (UTERUS)	1	-	-	-	1	-	-	-	-	-	-	-
POLYP (UTERUS)	-	-	-	-	1	-	-	-	-	-	-	-
BENIGN INTERSTITIAL CELL TUMOR (TESTIS)	-	1	-	1	-	-	-	1	-	-	-	1
FIBROMA (SKIN)	1	-	-	-	-	-	-	-	-	-	-	-
FIBROSARCOMA (SKIN)	1	-	-	-	-	1	-	-	-	-	-	-
PAPILLOMA (SKIN)	-	-	-	-	-	-	-	1	-	-	-	-
ADENOCARCINOMA (MAMMARY GLAND)	-	-	-	-	1	-	-	-	-	-	-	-
ADENOMA (MAMMARY GLAND)	1	-	1	-	2	-	-	-	1	-	-	-
ADENOCARCINOMA (LUNG)	-	-	-	-	1	-	1	1	1	1	1	-
ADENOMA (LUNG)	6	9	13	10	6	7	7	6	13	8	5	6
FIBROSARCOMA (PLEURA)	-	-	-	-	-	1	-	-	-	-	-	-
HEMANGIOMA (SPLEEN)	-	-	-	-	-	1	-	-	-	-	-	-
HEMANGIOSARCOMA (SPLEEN)	-	-	-	1	-	-	2	-	-	-	-	-
HEMANGIOMA (LYMPH NODE)	-	-	-	-	-	-	2	-	-	-	-	-

NOTE: GROUP 1 = CONTROL I
GROUP 2 = CONTROL II
GROUP 3 = CONTROL III
GROUP 5 = MALES 0.066% FEMALES 0.200%
GROUP 6 = MALES 0.200% FEMALES 0.533%

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CONTINUED
TABLE 1.

THIABENDAZOLE: LIFETIME CARCINOGENIC STUDY IN MICE. TT#77-01--0
SUMMARY OF PRIMARY NEOPLASMS

	GROUP 1 F	GROUP 1 M	GROUP 2 F	GROUP 2 M	GROUP 3 F	GROUP 3 M	GROUP 4 F	GROUP 4 M	GROUP 5 F	GROUP 5 M	GROUP 6 F	GROUP 6 M
FIBROMA (BONE)	-	1	-	-	-	-	-	-	-	-	-	-
OSTEOSARCOMA (BONE)	-	-	-	-	-	-	1	-	-	-	-	-
ADENOMA (LACRIMAL GLAND)	-	1	1	1	-	1	-	1	-	3	-	2
FIBROSARCOMA (TAIL)	-	-	1	-	-	-	-	-	-	-	-	-
LEUKEMIA (PRIMARY SITE UNDETERMINED)	5	2	3	-	3	3	4	2	-	1	1	2
RETICULUM CELL SARCOMA (PRIMARY SITE UNDETERMINED)	-	-	1	-	1	-	2	1	1	1	-	-
UNDIFFERENTIATED SARCOMA (PRIMARY SITE UNDETERMINED)	1	-	-	-	-	-	-	-	-	-	-	-

KEY: GROUP 1 = CONTROL I

GROUP 2 = CONTROL II

GROUP 3 = CONTROL III

GROUP 4 = 0.006% (FEMALES - CONCENTRATION REDUCED FROM 0.06% TO 0.006% DRUG WEEK 7;
MALES - CONCENTRATION REDUCED FROM 0.02% TO 0.006% DRUG WEEK 7)

GROUP 5 = MALES 0.06% FEMALES 0.20%

GROUP 6 = MALES 0.20% FEMALES 0.53%

TOXICOLOGY BRANCH REVIEWER'S COMMENTS: Thiabendazole was administered to male and female mice at dietary levels of 0 ,31-42, 63-121, 184-372 mg/kg/day for males and 0, 94-131, 209-368, and 534-1005 mg/kg/day for females. The doses were selected based on results of the range-finding study (TT#77-004-0). Starting in the seventh week, the lowest concentrations for the male and female treated mice were both reduced to 5.6-8.3 mg/kg/day for males and 5.7-9.9 mg/kg/day for females (Table 1).

Table 1. Concentration of Thiabendazole in the diet[a]

Male		Female	
Dose Group	mg/kg/day[d]	Dose Group	mg/kg/day[d]
Low	5.6 - 8.3[b]	Low	5.7 - 9.9[b]
Low	31 - 42[c8]	Low	94 - 131[c]
Mid	63 - 121	Mid	209 - 368
High	184 - 372	High	534 - 1005

[a] = The doses expressed in mg/kg/day using conversion factor of 7 and actual test consumption data from the study report page 28.
[b] = Started week 7
[c] = First six weeks of study
[d] = Information obtained from chemical analysis chart in study page 28.

Mortality was high at all dose levels (Table 2a and b). However, contrary to the reported mortality in the DER, mortality was noted to be higher only in low and high dose males.

Table 2a. Mortality of female mice during lifetime oncogenicity study with Thiabendazole

FEMALES	Controls			Diet Concentrations		
	I	II	III	Low	Middle	High
% found dead during study	30	24	20	28	26	18
% Sacrificed during study	24	32	28	38	50	62
% Sacrificed at interim autopsies	22	24	34	-	24	20
% Sacrificed at termination	24	20	18	34	-[a]	-[a]

[a] = None survived to study termination

Table 2b. Mortality of male mice during lifetime oncogenicity study with Thiabendazole

MALES	Controls			Diet Concentrations		
	I	II	III	Low	Middle	High
% found dead during study	32	30	26	36	32	36
% Sacrificed during study	32	36	32	40	46	40
% Sacrificed at interim autopsies	36	34	42	24	22	24
% Sacrificed at termination	-[a]	-[a]	-[a]	-[a]	-[a]	-[a]

[a] = None survived to study termination

Body weight gain was decreased in the high-dose group females (-28%) and males (-18%) (Tables 3a and b). Body weight gain in females was decreased (-28%). The mid-dose males had decreased body weight gain only through week 84 (-7%).

Table 3a. Average body weight changes (grams) for Female Mice during lifetime oncogenicity study with Thiabendazole

Treatment Group	Week 80	Week 92	Week 104
Control I	14.4	14.8	14.7
Control II	15.3	14.8	14.1
Control III	15.6	13.8	12.4
Low[a,b]	15.5 (2.6%)[c]	14.4 (-1%)	14.6 (6.6%)
Mid[a]	13.0 (-14%)	10.5 (-28%)	-
High[a]	10.9 (-28%)	-	-

[a] = Concentration of thiabendazole in the diet
[b] = Concentration of thiabendazole reduced from 94-131 mg/kg/day to 5.7-9.9 mg/kg/day at week 7
[c] = % decrease or increase

Table 3b. Average body weight changes (grams) for male Mice during lifetime oncogenicity study with Thiabendazole

Treatment Group	Week 84	Week 92	Week 101
Control I	16.7	17.1	15.9
Control II	16.7	6.3	13.1
Control III	16.1	15.0	14.4
Low[a,b]	15.9 (-4%)[c]	16.2 (26%)	15.5 (6.5%)
Mid[a]	15.4 (-7%)	13.6 (6.2%)	-
High[a]	13.6 (-18%)	-	-

[a] = Concentration of thiabendazole in the diet
[b] = Concentration of thiabendazole reduced from 31-42 mg/kg/day to 5.6-8.3 mg/kg/day at week 7
[c] = % decrease or increase

Animals were sacrificed when the number of survivors approached ten. High-dose females were sacrificed in the eighty-first week. High-dose males were sacrificed in the eighty-fifth week; mid-dose males and females in the ninety-third week, low-dose males were killed in the 101st week, and low-dose females were sacrificed in the 105th week. **There were three sets of control groups for each of the treatment groups.** On post mortem

examination, there was a significant but slight decrease in terminal body weight for mid- and high-dose females (3.5% and 7.8%, respectively) and high-dose males (7.5%). Absolute liver weight was significantly increased in high-dose females (18%) compared to controls. Relative liver weight was significantly increased in high-dose males (18.5%) and females (24.4%) and in mid-dose females (14%) (Refer to Tables 4a, 4b, 4c). The absolute kidney weights were significantly decreased in mid-dose (-12%) and high-dose females (-17%). Relative kidney weights were significantly decreased in mid-dose males (-15.9%) and high-dose females (13.7%).

Table 4a. Terminal body weight and organ weight (absolute and relative) changes for Low-dose Treated Mice during lifetime oncogenicity study with Thiabendazole

.	Control I		Control II		Control III		Low-dose	
	F	M	F	M	F	M	F	M
Terminal body weight (g)	29.5	34	30.5	32	28.9	33	30.7 (-3.5%)[b]	34 (3%)[b]
Absolute Liver weight--grams	1.44	1.59	1.45	1.62	1.48	1.69	1.65 (12%)[b]	1.61 (0.6%)[b]
Relative Liver weight (%)	4.88	4.64	4.25	6.02	5.11	5.13	5.38 (9.1%)[b]	4.72 (-11.5%)[b]
Liver weight relative to brain weight (%)	302	325	299	335	322	347	339 (8.8%)[b]	342 (2%)[b]

[a] = statistically significant p = ≤ 0.05
[b] = % increase or decrease; calculated by reviewer
Table extracted from page 434 of MRID 242116

Table 4b. Terminal body weight and organ weight (absolute and relative) changes for Mid-dose Treated Mice during lifetime oncogenicity study with Thiabendazole

	Control I		Control II		Control III		Mid-dose	
	F	M	F	M	F	M	F	M
Terminal body weight (g)	28.0	34	28.9	32	29.0	33	26.7 [a] (-3.5%)[b]	34.2 (3.3%)[b]
Absolute Liver weight-grams	1.33	1.65	1.52	1.65	1.37	1.77	1.52 (7.5%)[b]	1.64 (-3%)[b]
Relative Liver weight (%)	4.75	4.95	5.26	4.92	4.71	5.47	5.68[a] (14%)[b]	4.80 (-6.8%)[b]
Liver weight relative to brain weight (% brain weight)	265	334	315	348	272	377	310 (8.4%)[b]	338 (-4.4%)[b]

[a] = statistically significant p = ≤ 0.05
[b] = % increase or decrease; calculated by reviewer
Table extracted from page 436 of MRID 242116

Table 4c. Terminal body weight and organ weight (absolute and relative) changes for for High-dose Treated Mice during lifetime oncogenicity study with Thiabendazole

	Control I		Control II		Control III		High-dose	
	F	M	F	M	F	M	F	M
Terminal body weight (g)	29.1	35.5	29.5	34.7	30.7	34.0	27.5 (-7.8%)[b]	32.1[a] (-7.5%)[b]
Absolute Liver weight--grams	1.32	1.66	1.22	1.56	1.45	1.55	1.62[a] (18%)[b]	1.79 (12%)[b]
Relative Liver weight (%)	4.52	4.65	4.14	4.50	4.73	4.57	5.90[a] (24.4%)[b]	5.60[a] (18.5%)[b]
Liver weight relative to brain weight (% brain weight)	253	320	247	315	295	308	325[a] (23.7%)[b]	376 (9.8%)[b]

[a] = statistically significant p = ≤ 0.05
[b] = % increase or decrease; calculated by reviewer
Table extracted from page 438 of MRID 242116

There were no gross or microscopic changes associated with these organ weight changes.

Dietary administration of Thiabendazole did not increase the incidence of spontaneous tumors in this strain of mice. Although the dosing was variable, and assuming that the animals received the lowest dose of the range for each dose group, a compound-related effect (i.e. increased mortality) was noted in both sexes at the mid- and high dose at 18 months and at the high dose at 15 months. Therefore, the dosing was considered to be adequate.

The LOAEL for systemic toxicity is 209-368 mg/kg/day for females and 63-121 mg/kg/day for males, based on decreased body weight gain and increase in liver weight. The NOAEL is 5.7-9.9 mg/kg/day for females and 5.6-8.3 mg/kg/day for males.

19

Attachment 4

(58)

DATA EVALUATION RECORD

THIABENDAZOLE

Study Type: Special Mechanistic; Fourteen-Week Thyroxine Clearance Study in Rats

Work Assignment No. 3-04B (MRID 43593202)

Prepared for

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

Prepared by

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Primary Reviewer:
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Signature: Mary L Menetrez
Date: 12/9/97

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Date: 12/12/97

Quality Assurance:
Steve Brecher, Ph.D.

Signature: Steven Brecher
Date: 12/12/97

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

THIABENDAZOLE

Special Mechanistic: Thyroxine Clearance in Rats

EPA Reviewer: Robert F. Fricke, Ph.D.
Reregistration Branch 2 (7509C)

Robert Fricke, Date 12/17/98

EPA Work Assignment Manager: Sanjivani Diwan, Ph.D.
Toxicology Branch 2 (7509C)

Sanjivani Diwa Date 3/1/99

DATA EVALUATION RECORD

STUDY TYPE: Thyroxine Clearance Study in the Rat

OPPTS Number: None, Special Study

OPP Guideline Number: None, Special Study

DP BARCODE: D214192
P.C. CODE: 060101

SUBMISSION CODE: S485189
TOX. CHEM. NO.: 849A

TEST MATERIAL (PURITY): Thiabendazole, Technical (99.8% a.i.)

SYNONYMS: 2-(4'-Thiazolyl)benzimidazole

CITATION: Lankas, George R., (1995) Fourteen-Week Dietary Thyroxine Clearance Study in Rats with a 14-Week Recovery Period. Merck Research Laboratories, West Point, Pennsylvania. Study Identification No. TT No. 94-024-0, February 16, 1995. MRID 43593202. Unpublished.

SPONSOR: Merck Research Laboratories, Merck & Co., Inc. PO Box 450, Hillsborough Road, Three Bridges, NJ

EXECUTIVE SUMMARY:

In a special study (MRID 43593202), thiabendazole (99.8% a.i.) was administered to 35 Crl:CD(SD)BR male rats/dose in the diet at nominal dose levels of 0, 10, 90, or 270 mg/kg/day (actual 0, 10.15, 91.14, and 235.23 mg/kg/day). After 13 weeks of treatment, 15 rats/dose were sacrificed for pathological evaluation (liver and thyroid only). During week 14 of the study, blood samples from 5 rats/dose were used for evaluation homeostasis of thyroid hormones and thyroid stimulating hormone; animals were discarded after the final blood sampling. All remaining rats (14 to 15/dose) were sacrificed after 13 weeks of the treatment-free recovery phase and evaluated pathologically (liver and thyroid only).

Mortality and clinical signs of treated animals were unaffected by treatment.

After 13 weeks of dosing, mean body weights of the mid- and high- dose animals were 12% and 32% lower than controls, respectively. At the end of the recovery phase, their mean body weights for each of these dose groups were 13% lower than the controls.

Pathological evaluations of the thyroid and liver showed increased absolute and relative organ weights and increased incidence of microscopic lesions. In the mid- and high-dose animals, absolute thyroid weights were increased by 20 and 40%, respectively, and relative (to body) thyroid weights, by 26% and 103%, respectively. Relative liver weights were also increased by 7% and 25%, in mid- and high-dose animals, respectively. Histopathological examination revealed dark foci in the thyroids of the 6/15 high-dose animals and very slight to slight diffuse follicular cell hyperplasia in the thyroid of 10/15 mid- and 12/16 high-dose animals. Very slight to slight hepatocellular centrilobular hypertrophy was detected in all (15/15) mid-dose animals and 15/16 high-dose animals. The thyroid and liver lesions were not observed in any of the low-dose and control main study animals or in any of the recovery phase animals.

Thyroid homeostasis (serum T_3, T_4 and TSH) was evaluated during treatment weeks 2, 4, 8, and 13 and weeks 6 and 13 of the recovery phase. T_3 levels decreased by 5-10% in mid-dose and 11-19% in high-dose animals compared to controls; TSH levels were increased by 66-160% in mid-dose and 65-189% in high-dose animals. Evaluations carried out during week 14 of the study, showed effects in mid- and high-dose animals. For high-dose animals, statistically significant increases in thyroxine clearance (44%, $p=0.001$), volume of distribution (V_d, 64%,$p<0.001$), rate of elimination (k_{el}, 13%, $p<0.001$), and half-life ($T_{1/2}$, 16%, $p<0.001$). At the mid-dose significant increases in k_{el} (18%, $p<0.001$), $T_{1/2}$ (23%, $p<0.001$) and in V_d (22%, $p=0.004$) were observed, while no differences in thyroxine clearance was observed. At the end of the recovery period, TSH levels in the mid- and high-dose animals were comparable to control values while T_3 serum levels were higher (9%-19%). T_4 were comparable to controls throughout both the treatment and recovery periods.

These data support the hypothesis that thiabendazole alters thyroid hormone homeostasis in male rats resulting in hypothyroidism. The study authors contend that the primary effect of thiabendazole is on the liver, resulting in hepatocellular hypertrophy [microsomal enzyme induction presumed, but not measured in the study]. Enhanced hepatic metabolism of the thyroid hormones leads to decreased serum levels. The decreased serum levels of the hormones causes an increased release of TSH. The higher serum TSH levels ,in tern, causes thyroid hypertrophy and hyperplasia.

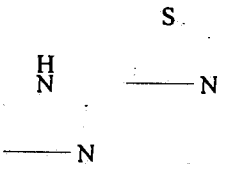
The submitted special study is classified as **acceptable/nonguideline**

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Thiabendazole, technical
   Description: White powder (obtained from MRID 43592301)
   Batch No.: L-585,216-000S159
   Purity: 99.8% a.i.
   Stability of compound: Compound is stable in the feed for up to 21 days at room temperature.
   CAS Reg No.: 148-79-8
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Male Rat
   Strain: Crl:CD(SD)BR
   Age and weight at study initiation: 59 days; 249-366 g
   Source: Charles River Laboratories, Raleigh, NC
   Housing: Individually in stainless steel wire cages
   Diet: Powdered Purina Certified Rodent Diet *ad libitum*
   Water: Drinking water (source not specified), *ad libitum*
   Environmental conditions:
   Temperature: Not specified
   Humidity: Not specified
   Air changes: Not specified
   Photoperiod: 12 hr dark/12 hr light
   Acclimation period: Not reported

B. STUDY DESIGN:

1. The study was designed to determine if dosing with thiabendazole affects thyroxine clearance and thyroid stimulating and thyroid hormone levels.

2. In life dates - Start: 3/14/94 End: 1/17/95

3. Animal assignment: Male rats were randomly assigned to treatment groups as indicated in Table 1. Main study consisted of 20 animals/dose; 15 animals/dose were necropsied after 13-weeks of treatment and the remaining 5 animals/dose used for determination of thyroxine clearance assay in week 13/14. The 15 animals/dose in the recovery phase of the study were

treated for 13 weeks and necropsied after a 13-week treatment-free period.

Table 1. Study Design

Test Group	Nominal Dose (mg/kg/day)	Number of Male Animals[a]	
		Main Study	Recovery Phase
Control	0	20	15
Low	10	20	15
Mid	90	20	15
High	270	20	15

[a] Main study animals were dosed for 13 weeks then sacrificed. Recovery phase animals were dosed for 13 weeks and then carried over into a 13 week treatment-free period.

4. Dose Selection: The rational for dose selection was based on a previously conducted chronic/oncogenicity study (MRID 43592301) in which thiabendazole (>98.9% a.i.) was administered to 50 Sprague-Dawley Crl:CD BR rats/sex/dose in the diet at dose levels of 0, 10, 30, or 90 mg/kg/day (achieved average doses of 0, 10.1, 30.2, or 91.8 mg/kg/day) for 104 weeks.

There were no treatment-related clinical signs, mortality, or ophthalmoscopic findings. , , urinalysis, or gross pathology.

Body weights and body weight gains were lower (7-30%) compared to the controls throughout the study for the mid- and high-dose males and high-dose females. At week 103. Reduced body weight gains of 15% were observed in mid-dose males and 28 and 19% ($p \leq 0.05$) in high-dose males and high-dose females, respectively. Food consumption was unaffected by treatment.

In the high-dose males, increased (29%, $p \leq 0.05$) relative to body liver weights and in high-dose females, an increased (45%, $p \leq 0.05$) relative thyroid weights.

Increased incidence of centrilobular hepatocellular hypertrophy was noted in 7/50 mid- and 28/50 high-dose males, compared to 0/50 controls. In high-dose females there was increased incidences of thyroid focal cystic follicular cell hyperplasia (6/50 treated vs 2/50 controls) and diffuse follicular cell hypertrophy (2/50 treated vs 0/50 controls). Diffuse follicular cell hypertrophy in the thyroids was observed in 4/50 high-dose males, compared to 0/50 for controls.

Benign thyroid follicular cell adenoma was observed in 5/50 mid- and 6/50 ($p \leq 0.05$) high-dose males and 5/50 high-dose females, compared to 0/50 control males and 2/50 control females. In addition, a statistically significant trend ($p \leq 0.05$) in the incidence of thyroid

follicular cell adenomas was observed in males. No statistically significant trend in the incidence of any other neoplasm in either sex was observed. The systemic and oncogenic LOAELs and NOAELs for rats were determined to be 30 mg/kg/day and 10 mg/kg/day, respectively.

It was postulated by the Sponsor that thiabendazole may affect the rat thyroid indirectly by altering thyroxine clearance via increased hepatic metabolism.

Based upon the results of this study, the doses summarized in Table 1 above were selected for the submitted special mechanistic study.

C. METHODS

1. Diet Preparation and Analysis: Diets were prepared once a week based on the previous week's mean food consumption, body weight, and the desired nominal dose for each treated group. Homogeneity was tested in samples taken from the top, middle, and bottom of test formulations prepared at concentrations of 112, 1006, and 3038 ppm. Homogeneity was also tested in test formulations prepared at concentrations of 87.3 and 6834 ppm. Stability of the three diet formulations (112, 1006, and 3038 ppm) was determined after 7 days of storage at room temperature; 87.3 and 6834 ppm diet formulations were tested for stability after 7, 14, and 21 days of storage at room temperature. Concentration analyses were performed on diet formulations prepared at 155.4, 1455, and 4300 ppm for study week 7 and on diet formulations prepared at 193.3, 1798.2, and 4500 ppm for study week 13.

2. Observations: Animals were inspected daily for viability and signs of toxicity.

3. Body weight: All the animals were weighed prior to dosing and at weekly intervals thereafter.

4. Food consumption and compound intake: Daily food consumption for 15 rats/dose was determined weekly based on a 6-day consumption period. The concentration of the test compound was adjusted weekly to achieve the target doses on a mg/kg/day basis.

5. Serum Biochemical Determinations: Blood from non-fasted animals was collected pretest (30 rats/dose), in weeks 2, 4, 8, and 13 (15 rats/dose), and during weeks 6 and 13 of the recovery phase (14-15 rats/dose). Serum levels of thyroid stimulating hormone (TSH), thyroxine (T_4), and tri-iodothyronine (T_3) were determined.

6. Thyroxine Clearance Determinations: Thyroxine clearance was determined in week 14 of the study. Five non-fasted rats/dose were intravenously injected with approximately 160 µCi/kg of ^{125}I-thyroxine (specific activity, approximately 4400 Ci/mmol; 10 ml/minute); blood samples were taken at approximately 8, 22, 34, 48, and 72 hours after injection. The animals were discarded without necropsy.

To determine thyroxine clearance, internal standards (thyroxine/triiodothyronine) were mixed

with blood plasma and the mixture was passed through a solid C_{18} extraction bed. The sorbed radiolabelled thyroxine and standards were eluted with methanol, reduced to dryness by nitrogen convection, and the residue reconstituted in methanol and water prior to HPLC analyses. The extracts were analyzed for the presence of ^{125}I-thyroxine metabolites, triiodothyronine (T3), and thyroxine. Sample analyses were accomplished with an HPLC system using an isocratic mixture of methanol (57%) and phosphate/HxSA buffer (pH 2.5) gradient and equipped with UV (254 nm) and radioactivity detectors. Sample chromatograms were not submitted. The clearance of ^{125}I-thyroxine was determined by regression analysis of ^{125}I-thyroxine remaining in the plasma over time; the elimination rate constant (k_{el}) was determined from the slope of the regression line. Thyroxine half-life ($t_{1/2}$), volume of distribution (V_d), and systemic clearance (Cl_s) were also determined. Sample calculations were submitted.

7. Sacrifice and Pathology: Gross pathological and microscopic examinations of the thyroid and liver were performed on 15 rats/dose after 13 weeks of dosing and on all the surviving rats (14-15/dose) after an additional 13 weeks of recovery. Additionally, the brain, thyroid and liver from all necropsied animals were weighed.

8. Statistics: Statistical analyses of the thyroxine elimination rate constant (k_{el}), half-life ($t_{1/2}$), volume of distribution (V_d), and systemic clearance (Cl_s) were performed. An analysis of variance was performed on the log transformed data and the No Statistical Significance of Trend method (NOSTASOT) was applied.

II. RESULTS

A. Analytical Chemistry

1. Concentration analysis: The average concentrations of the analyzed diets during weeks 7 and 13 were 98.8-104% of the intended concentrations (cv, 1.34-3.53%).

2. Homogeneity analysis: The mean concentrations of 6 diet samples from various positions in the mixer were 102% of nominal (cv, 1.19%) for the 112 ppm diet, 106% (cv, 1.83%) for the 1006 ppm diet, and 114% (cv, 2.87%) for the 3038 ppm diet. Homogeneity of the 87.3 and 6834 ppm diet samples were 90 to 98.3% of nominal.

3. Stability analysis: After 7 days of storage at room temperature, 98-112% of nominal (mean cv, 5.84%) were recovered from the three diet formulations. After 21 days of storage at room temperature, 94.8 and 98.4% of nominal were recovered from the 87.3 and 6834 ppm diet formulations, respectively.

The analytical data indicated that the mixing procedure was adequate for preparing trial diets and that the variance between nominal and actual dosage to the animals was acceptable.

B. Clinical signs and mortality: The general condition and appearance of treated animals was

unaffected by treatment. Except for one accidental death of a high-dose rat, no deaths occurred during the study.

C. Body weight- Mean body weights of the mid- and high- dose animals were lower than concurrent controls throughout the study (Table 2). After 8 weeks of dosing, mean body weights of the mid-and high-dose animals were 10 and 30% lower than controls, respectively; after 13 weeks of dosing, their respective weights were 12 and 32% lower than controls. At the end of the recovery phase, mean body weights of the mid- and high-dose animals were each 13% lower than the controls. The data were not analyzed for statistical significance.

Table 2. Mean body weights (g) at selected intervals in male rats dosed with thiabendazole for 13 weeks (study weeks 1 through 13) followed by a 13-week recovery phase (study weeks 14 through 26).

Study Group	Study Week	Dose (mg/kg/day)			
		0	10	90	270
Main Study	1	339	338	327	300
	8	517	513	466	363
	13	592	580	520	404
Recovery Phase	21	675	672	588	573
	26	707	704	616	614

[a] Data extracted from study report, Table A-2, pages 34-36. 35 rats/dose were weighed during weeks 1, 8, and 13; 15 rats/dose were weighed during weeks 21 and 26.

D. Food consumption and compound intake

1. Food consumption - Food consumption in the high-dose animals was decreased (25-48%) compared to controls throughout the 13 week dosing period. After an initial increase of 11% in food consumption during the first week of recovery, food consumption by the high-dose animals remained 2-8% less than the controls through to the end of recovery phase (week 26). The sponsor stated that the decreased food consumption was related to the poor palatability of the dosed diets. Food consumption in the mid- and low-dose groups was comparable to the controls.

2. Compound consumption - Average compound consumption values (mg/kg/day) are summarized in Table 3.

Table 3: Achieved Doses

Test Group	Dose (mg/kg/day)	
	Nominal	Achieved
Low	10	10.2
Mid	90	91.1
High	270	235

E. Serum Biochemical Determinations: Throughout the treatment period (weeks 1 through 13), compared to control values mean T_3 were decreased in the 5 to 10% in mid-dose animals and 11 to 19% in high-dose animals (Table 4). During the same period, TSH levels were increased (mid-dose, 66-160%; high-dose, 65-189%); the pretest values in the mid- and high-dose animals were also increased over the controls (55 and 41%, respectively). During the recovery phase (weeks 19 and 26 of the study), T_3 serum levels were increased compared to controls, (mid-dose, 9-11% and high-dose, 18-19%). TSH levels in the mid- and high-dose animals were comparable to control values at the end of the recovery phase (week 26). Serum levels of T_4 were comparable to controls throughout the treatment and recovery phases.

Table 4. Mean T_3 and TSH [a]

Study Group	Week	Nominal Dose (mg/kg/day)			
		0	10	90	270
T_3 (ng/dL)					
Main Study	Pretest	101	106	108	102
	2	97	97	87	79
	4	109	111	102	93
	8	81	85	77	66
	13	104	106	97	92
Recovery Phase	Pretest	122	120	122	122
	19	90	103	100	106
	26	92	96	100	109
TSH (µU/mL)					
Main Study	Pretest	50	64	77	71
	2	46	56	88	75
	4	28	37	72	80
	8	96	91	159	190
	13	73	65	128	144
Recovery Phase	Pretest	50	59	63	62
	19	75	72	61	59
	26	88	98	86	86

[a] These data were extracted from study report Tables A-9 and A-11, pages 78 through 81 and 86 through 89, respectively.

E. Thyroxine Clearance: High-dose animals had statistically significant in trend for thyroxine clearance (Cl_s; 44%, $p \leq 0.001$), elimination rate constant (k_{el}; 13%, $p \leq 0.001$), half-life ($T_{1/2}$; 16%, $p \leq 0.001$) and in the thyroxine volume of distribution (V_d; 64%,$p \leq .001$). In the mid-dose group, significant differences from concurrent controls were observed in k_{el} (18%, $p \leq 0.001$), half-life ($T_{1/2}$; 23%, $p \leq 0.001$) and in V_d (22%, $p \leq 0.004$). There was no increase in clearance in the mid-dose group (Table 5).

Table 5. Thyroxine elimination rate constant (k_{el}), half-life ($T_{1/2}$), volume of distribution (V_d), and systemic clearance (Cl_s) determined in 5 male rats/dose treated with thiabendazole for 13 weeks.[a]

Variable	Nominal Dose (mg/kg/day)			
	0	10	90	270
K_{el}	-0.0374 ± 0.0008 [b]	-0.0393 ± 0.0025	-0.0306 ± 0.0008 ‡‡	-0.0325 ± 0.0012 ‡‡
$T_{1/2}$	18.50 ± 0.39	17.66 ± 1.12	22.67 ± 0.61 ‡‡	21.36 ± 0.78 ‡‡
V_d [c]	15.65 ± 0.45	16.63 ± 0.33	19.07 ± 0.57 ‡	25.52 ± 1.72 ‡‡
Cl_s [c]	0.59 ± 0.01	0.65 ± 0.04	0.58 ± 0.02	0.83 ± 0.08 ‡‡

[a] These data were extracted from study report Table 2, page 175.
[b] Values are geometric mean ± S.E. (n = 5)
[c] Normalized for 100 g body weight.
‡, ‡‡ Statistically significant increasing trend at p=0.004 and p≤0.001, respectively.

F. Sacrifice and Pathology:

1. Organ weights - Absolute and relative (to body) organ weights following 13 weeks of dosing are presented in Table 6. Absolute thyroid weights were increased in mid- and high-dose animals by 20 and 40% , respectively, and relative (to body weight) thyroid weights, by 26 and 103%, respectively. Relative liver weights were increased in the mid- and high-dose animals by 7 and 25%, respectively. In mid-dose animals, absolute liver weights were comparable to the concurrent controls while in the high-dose animals a decrease of 14% was observed.

After the 13 week recovery phase, there were no treatment-related differences in organ weights relative to controls.

Table 6: Absolute and relative (to body) organ weights (g) in 15 male rats/dose treated with thiabendazole for 13 weeks. [a]

Organ Weight [b]		Dose (mg/kg/day)			
		0	10	90	270
Thyroid	Absolute	0.02	0.02	0.024	0.028
	Relative	0.0038	0.0040	0.0048	0.0077
Liver	Absolute	15.36	14.78	15.42	13.15
	Relative	2.88	2.89	3.09	3.59

[a] These data were extracted from study report Table B-2, page 97.
[b] Relative organ weights listed parenthetically.

2. Gross pathology - Dark foci were observed in the thyroids of 6/15 high-dose animals, compared to 0/15 control animals. No other treatment related gross finding was detected during the course of the study.

3. Microscopic pathology: Following 13 weeks of dosing, an increased incidence of very slight to slight hepatocellular centrilobular hypertrophy was detected in the all (15/15) mid- and 15/16 high-dose animals; this lesion was not observed in control animals. Very slight to slight diffuse follicular cell hyperplasia of the thyroid was observed in the 10/15 mid- and 12/16 high-dose animals compared to 0/15 control animals.

Other lesions observed following dosing, such as focal cellular infiltration or necrosis of the hepatocytes, were not considered to be treatment related due to a lack of dose-response trend and/or because they were considered to be similar to those commonly seen in aging/aged rats.

After the 13 week recovery phase, there were no treatment related findings in the liver or thyroid were observed.

III. DISCUSSION

A. Investigators Conclusions - Male rats were treated for 14 weeks with thiabendazole at dose levels of 10, 90, or 270 mg/kg/day in the diet. Compared to concurrent controls, the 90 and 270 mg/kg/day males had moderate to marked decreases in body weight gain; a slight to moderate decrease still remained after the recovery phase. The difference in body weight gain in the high-dose group was associated with a decrease in food consumption throughout the dosing period. Centrilobular hepatocellular hypertrophy, increased relative (to body) liver weights, increases in thyroid weight and diffuse thyroid follicular cell hyperplasia were observed in the mid- and high-dose groups. These organ changes were not observed in any treated group after the recovery phase.

Dosing with thiabendazole induced very slight to slight decreases in serum T_3 levels, which were associated with moderate to marked increases in TSH levels in the mid- and high-dose groups. These differences in hormone levels were associated with changes in thyroxine clearance and/or the thyroxine volume of distribution in the mid- and high-dose groups. At the end of the recovery phase, TSH serum levels were similar to control values in all treated animals while T_3 levels were slightly increased in the high-dose group.

These data support the hypothesis that the thiabendazole-induced changes observed in the thyroid gland in the carcinogenicity study (MRID 43592301) are due to indirect effects of dosing or are mediated by changes in thyroid hormone homeostasis. TSH has been shown to act as a rat thyroid growth stimulator and tumor promoter. This mechanism is specific to rats; alterations in

thyroid homeostasis do not produce increases in thyroid tumors in humans [Hill et al. Fund. and Appl. Toxicol., 12 (1989), 629-697]. These changes are therefore not relevant for risk assessment for human exposure.

B. Reviewer's Discussion/Conclusions - Male rats were treated with thiabendazole at dose levels of 10, 90, or 270 mg/kg/day in the diet for 13 weeks.

Mortality and clinical signs of dosed animals were unaffected by treatment.

Mean body weights of the mid- and high- dose animals were lower than concurrent controls throughout the study; after 13 weeks of dosing, their respective weights were 12 and 32% lower than controls. At the end of the recovery period, mean body weights of the mid- and high-dose animals were each 13% lower than the controls. Food consumption in the high-dose animals was decreased (↓25-48%) compared to controls throughout the 13 week dosing period and remained 2-8% less than the controls through to the end of recovery period (week 26). The decreased food consumption observed in the high-dose may have been related to the poor palatability of the dosed diets. Following 13 weeks of dosing, absolute thyroid weights in the mid- and high-dose animals were increased (20 and 40%, respectively) and relative (to body) thyroid weights were increased by 26% and 103%, respectively. In addition, dark foci were observed in the thyroid of the high-dose group (6/15 treated vs 0/15 controls). Relative liver weights were increased in the mid- and high-dose animals by 7 and 25%, respectively; these differences could have been related to their decreased body weights. However, following 13 weeks of dosing, an increased incidence compared to concurrent controls of very slight to slight hepatocellular centrilobular hypertrophy was detected in the mid-dose animals (15/15 treated vs 0/15 controls) and the high-dose animals (15/16). In addition, very slight to slight diffuse follicular cell hyperplasia of the thyroid was observed in the mid-dose group (10/15 treated vs 0/15 controls) and in the high-dose group (12/16).

Throughout the treatment period (weeks 1 through 13), mean T_3 blood serum levels were decreased compared to controls in the mid- and high-dose animals (↓5-10% mid-dose; ↓11-19% high-dose). During the same period, TSH levels were increased (mid-dose, ↑66-160%; high-dose, ↑65-189%); the pretest values in the mid- and high-dose animals were also increased over the controls (↑55 and 41%, respectively). In week 14 of the study, the high-dose group showed statistically significant differences from concurrent controls in Cl_s (↑44%, $p=0.001$), k_{el} (↑13%, $p<0.001$), $T_{1/2}$ (↑16%, $p<0.001$) and in V_d (↑64%,$p<0.001$). The mid-dose group displayed significant differences from concurrent controls in k_{el} (↑18%, $p<0.001$), $T_{1/2}$ (↑23%, $p<0.001$) and in V_d (↑22%, $p=0.004$). However, there was no increase in thyroxine clearance in the mid-dose group.

At the end of the recovery period, TSH levels in the mid- and high-dose animals were comparable to control values while T_3 serum levels were higher (↑9%-19%). Serum levels of T_4 were comparable to controls throughout both the treatment and recovery periods.

Dosing for 13 weeks resulted in minimal signs of toxicity in the mid- and high-dose groups, however, the changes were consistent with those observed in the chronic/onco study (MRID 43592301). Dosing also resulted in increased TSH levels along with decreased mean T_3 blood serum levels and in statistically significant increases in thyroxine volume of distribution in the two highest dose groups and in thyroxine clearance in the high-dose group.

The submitted study is classified as acceptable/nonguideline as it is not a required guideline study. It is acceptable for the purposes for which it was intended.

C. Study deficiencies - None observed.

DATA EVALUATION RECORD

THIABENDAZOLE

Study Type: 83-1b; 53-Week Oral (Capsule) Toxicity Study in Dogs

Work Assignment No. 3-04G (MRID 42809701)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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DATA EVALUATION RECORD

STUDY TYPE: 53-Week chronic toxicity [capsule] - dog
OPPTS Number: 870.4100

OPP Guideline Number: §83-1b

DP BARCODE: D192557
P.C. CODE: 060101

SUBMISSION CODE: S443207
TOX. CHEM. NO.: 849A

TEST MATERIAL (PURITY): Thiabendazole (99% a.i.)

SYNONYMS: 2-(4′-thiazolyl)-benzimidazole; TBZ; L-585,216-000S

CITATION: Lankas, G.R. (1993) Thiabendazole fifty-three-week oral toxicity study in dogs. Merck Institute of Therapeutic Research. Merck Research Laboratories, Merck & Co., Inc., West Point, PA. Laboratory Project ID: TT #91-068-0. January 20, 1993. MRID 42809701. Unpublished.

SPONSOR: Merck Research Laboratories, Merck & Co., Inc., West Point, PA 19486

EXECUTIVE SUMMARY:

In a chronic toxicity study (MRID 42809701), thiabendazole (99% a.i.) was administered orally in capsules to four beagle dogs/sex/dose at dose levels of 0, 10, 40 or 160 mg/kg/day for 52 weeks.

Dogs lost weight during the first half of the study primarily due to emesis. One mid-dose male dog died of acute hepatitis after two weeks of treatment

Clinical pathology revealed treatment-related changes in some of the hematology parameters; clinical chemistry and urinalysis parameters were unaffected by treatment. Both sexes were mildly anemic, with decreased red blood cell counts, hematocrits, and hemoglobin values, and had increased activated partial thromboplastin time (10-14%) and platelet counts (51-65%). However, none of the values were outside of the historical control range. There was also a higher incidence of polychromasia and hypochromia compared to the controls during weeks 4, 12, and 26.

At terminal sacrifice, treatment-related changes in organ weights and incidence of histopathological findings were observed. The absolute and relative (% of body weight) liver weights were statistically significantly ($p \leq 0.05$) higher in mid- (14 and 20%, respectively,

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combined sexes) and high- (37 and 41%, respectively, combined sexes) dose animals. In high-dose animals, absolute thyroid weights were increased by 22% (not significant), while relative thyroid weights were increased by 33% ($p \leq 0.05$).

Histopathological evaluations identified lesions in the liver, thyroid, gallbladder, kidney, urinary bladder and spleen. Livers exhibited slight to moderate bile duct vacuolation in mid- (4/4 males; 2/4 females) and high- (3/4 males; 3/4 females) dose animals. Thyroids had very slight follicular enlargement high-dose females (1/4), while very slight to slight follicular cell hypertrophy was observed in high-dose males (1/4) and females (2/4). Dogs in the 10, 40 and 160 mg/kg/day treatment groups had gallbladders which exhibited cytoplasmic lipid vacuolation and discolored foci of the mucosa; dose-related increases in severity (very slight to marked) were observed. The kidneys of mid- (3/4) and high- (4/4) dose females showed very slight to slight distal tubule vacuolation, compared to 1/4 females each in the control and low-dose groups. Urinary bladders of all high-dose dogs had very slight to slight epithelial cytoplasmic inclusions; this finding was also observed in 3/4 males and 2/4 females in the mid-dose group. The toxicological significance of the above findings could not be determined. Spleens exhibited very slight to slight increases in erythropoiesis in mid- (1/4 males; 1/4 females) and high- (2/4 males; 3/4 females) dose animals; hemosiderin deposits were observed in mid- (2/4 males; 2/4 females) and high- (1/4 males; 4/4 females) dose animals.

No neoplastic changes were observed at any dose level.

The LOAEL for this study is 40 mg/kg/day, based on increased liver weight, splenic erythropoiesis and hemosiderosis in both sexes. The NOAEL is 10 mg/kg/day.

This 53-week chronic toxicity study is classified **acceptable** and satisfies the Subdivision F guideline requirement for a chronic toxicity study (83-1b) in non-rodents.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

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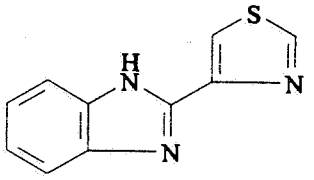
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Thiabendazole
   Description: Not provided
   Lot/Batch No: L-585, 216-000S159
   Purity: 99% a.i. [determined by TLC]
   Stability of compound: It was stated that "adequate stability of thiabendazole under the conditions employed within this study has been demonstrated" [page 24]. Data were not provided.
   CAS No.: 148-79-8

   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Dog
   Strain: Purebred beagle
   Age at study initiation: 34-37 Weeks of Age
   Weight at study initiation: Males, 8.9-12.3 kg; Females, 7.8-10.7 kg
   Source: Marshall Farms, North Rose, NY
   Housing: Individually housed in stainless steel cages
   Diet: Pelleted Purina Certified Canine Chow, approximately 350 g, was provided once daily. Food was withdrawn overnight prior to scheduled venipunctures and necropsy
   Water: *ad libitum* (not further described)
   Environmental conditions:
      Temperature: Not reported
      Humidity: Not reported
      Air Changes: Not reported
      Photoperiod: 12-Hour light/dark cycle
   Acclimation period: Not reported

B. STUDY DESIGN:

1. In life dates - Start: 6/13/91 End: 6/11-12/92

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2. Animal assignment

Four dogs of each sex were assigned to the test groups in Table 1 using a random allocation scheme. Group assignments were based on pretest ophthalmic, hematologic, serum biochemical and urinalysis values, electrocardiographic parameters, and general health. Littermates were not assigned to the same dosage group.

TABLE 1. STUDY DESIGN[a]

Test Group	Dose to Animal (mg/kg/day)	Animals Assigned	
		Male	Female
1	0	4	4
2	10	4	4
3	40	4	4
4	160	4	4

[a] The rationale for dose selection was not provided.

Dogs in the treatment groups received an oral dose of thiabendazole via gelatin capsules. The appropriate dose was administered based on the most recent body weight. Control dogs were orally administered an empty gelatin capsule.

3. Statistics

Group means were calculated for all data. Absolute and relative weights of liver, kidney, adrenal and thyroid glands were analyzed using trend (dose-response) analysis. The absolute and relative organ weights of the combined sexes at each dose level were compared to the corresponding control weights. Significance was determined at the 5% confidence level.

C. METHODS:

1. Observations

All animals were observed for clinical signs and mortality once daily, with less detailed examinations conducted on weekends and holidays.

2. Body weight

Animals were weighed pretest and once weekly throughout the study.

3. Food consumption

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Food consumption for each animal was measured based on an approximate 4-day intake once weekly during weeks 1-13 and every 4 weeks for the remainder of the study. Animals were presented with approximately 350 g of food.

4. Ophthalmoscopic examination

Ophthalmological examinations were performed on all animals prior to study initiation and on all surviving animals during study weeks 27 and 50. Indirect ophthalmoscopy and a slit-lamp examination were performed on each animal.

5. Clinical Pathology

a) Blood collection: Blood was collected from the jugular vein of each test animal prior to treatment initiation, and during weeks 4, 12, 26, and 52 of dosing for hematology and clinical analyses. Blood was also collected during week 7 from one male and one female in the high dose groups due to the appearance of physical signs (lethargy and pole mucous membranes). The dogs were fasted prior to blood collection. The CHECKED (X) parameters were examined.

b) Hematology: The following parameters were evaluated:

Hematocrit (HCT)*	Leukocyte differential count*
Hemoglobin (HGB)*	Platelet count*
Leukocyte count (WBC)*	Mean corpuscular HGB (MCH)
Erythrocyte count (RBC)*	Mean corpusc. HGB conc.(MCHC)
Prothrombin time	Mean corpusc. volume (MCV)
Activated partial thromboplastin time	

c) Clinical Chemistry: The following parameters were evaluated:

ELECTROLYTES	OTHER
Calcium*	Albumin*
Chloride*	Blood creatinine*
Phosphorus*	Blood urea nitrogen*
Potassium*	Total Cholesterol
Sodium*	Glucose*
ENZYMES	Direct bilirubin
Alkaline phosphatase (ALP)	Total bilirubin
Serum alanine aminotransferase*	Total serum protein (TP)*
Serum aspartate aminotransferase*	Triglycerides
	A/G Ratio

d) Urinalysis

Urine was collected overnight from the test animals prior to treatment initiation and during study weeks 4, 12, 26, and 52 of dosing. The CHECKED (X) parameters were examined.

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Appearance	Glucose
Volume	Ketones
Specific Gravity	Bilirubin
pH	Blood
Sediment (microscopic)	Urobilinogen
Protein	

6. Electrocardiograms

Electrocardiograms were recorded from all dogs, pretest and during weeks 14, 25, and 50, in lateral recumbency approximately 3-6 hours after dosing. Recordings were made from leads I, II, III, AVR, AVL, AVF, CV_5RL, and V10.

7. Sacrifice and Pathology

All test animals that were sacrificed on schedule at study termination were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL		
X	Colon*			XX	Adrenal gland*
X	Rectum*	XX	Kidneys*		Lacrimal gland
XX	Liver*	X	Urinary bladder*	X	Mammary gland
X	Gall bladder*	XX	Testes*	XX	Thyroids with parathyroids*
X	Pancreas*	X	Epididymides		
		X	Prostate		OTHER
	RESPIRATORY	X	Ovaries*		
		X	Uterus*	X	Bone*
X	Trachea*	X	Cervix	X	Skeletal muscle*
X	Lung*	X	Vagina	X	Skin*
	Nose			X	All gross lesions and masses*
	Pharynx				
	Larynx				

* Required for chronic toxicity studies.

II. RESULTS

A. Observations

1. Mortality - One male in the 40 mg/kg/day treatment group died during study week 3.

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The cause of death was not determined; there was no clear relationship between this death and treatment.

2. Clinical Signs - Both sexes in the 160 mg/kg/day treatment group exhibited an increased incidence of emesis during the first half of the study. Emesis was most pronounced in the 160 mg/kg/day group during weeks 1-3 when 7 or 8/8 dogs were emetic for 1-5 days per week compared to 1-3 dogs in each of the other test groups that were emetic for 1 or 2 days per week. The incidence of emesis in the 160 mg/kg/day group dogs declined somewhat during weeks 4-26 when feeding procedures were altered, so that food was withheld 1-5 hours prior to and 5-6 hours following dosing. No other changes in appearance or behavior in any of the treatment groups were considered to be treatment-related. No differences between males and females within the same test group were observed. Excess salivation observed primarily in the 160 mg/kg/day group dogs throughout the study was considered a reflex response to anticipation of dosing, rather than a treatment-related effect. The 40 mg/kg/day male that died prematurely exhibited lateral recumbency and slowness of breath, and was inactive, unresponsive, and cold to the touch prior to death. No other clinical signs observed in the treatment groups were considered to be treatment-related.

B. Body weight and weight gain

No treatment-related differences in body weights were observed between males and females within the same test group. Mean body weight gains of dogs in the 160 mg/kg/day treatment groups (combined sexes) were 0.3-0.7 kg lower than the control weight gains through week 26, then were similar to the controls through the end of the study. Decreased body weights of dogs in the 160 mg/kg/day treatment groups during the initial 26 study weeks were attributed to emesis in both sexes and weight loss in two females. Mean body weights of dogs in the 10 or 40 mg/kg/day treatment groups (combined sexes) were similar to the control weights throughout the study. After 52 weeks of treatment, mean body weight gains of the combined sexes were 2.2 kg for the control group, and 2.8 kg for the 10 mg/kg/day, 1.8 kg for the 40 mg/kg/day, and 1.9 kg for the 160 mg/kg/day treatment groups.

C. Food consumption

Food consumption (g/animal/day) was not affected by treatment.

D. Ophthalmoscopic examination

No treatment-related ophthalmoscopic abnormalities were observed in any of the treatment groups.

E. Clinical Pathology

1. Hematology - Dogs in the 160 mg/kg/day treatment group (combined sexes) exhibited

possible treatment-related differences in some of the hematology parameters throughout the study; no differences were observed between the sexes. Red blood cell counts, hematocrits, and hemoglobin were 14-17% lower at week 52 compared to the controls (Table 2). Activated partial thromboplastin time (APTT) was 14% higher and platelet counts were 61% higher at week 52 compared to the controls. An increased incidence of polychromasia was observed in the 160 mg/kg/day dogs; 1-3 dogs were affected during weeks 4, 12, 26, and 52 compared to only 1-2 dogs in each of the other test groups only during week 52. Hypochromia was observed in the 160 mg/kg/day treatment group only, affecting 1-2 dogs during weeks 12 and 26. No other differences in hematology parameters between the treatment and control groups appeared to be treatment-related. None of the values, however, were outside of the historical control range.

Table 2. Selected hematology parameters combined for male and female dogs following 52 weeks of treatment with thiabendazole.[a,b]

Dose (mg/kg/day)	RBC count (10^6/mm^3)	Hemoglobin (g/100 mL)	Hematocrit (%)	APTT[c] (sec)	Platelet count (1,000/mm^3)
0	7.61	18.1	50	10.6	274
10	7.11	16.8	46	11.2	360
40	7.77	18.1	51	11.6	334
160	6.48	15.1	43	12.1	441
Historical Control Median (range)	7.10 (5.40 - 9.00)	16.7 (12.7 - 21.2)	46 (35 - 61)	10.9 (9.4 - 13.6)	300 (0 - 600)

[a] Data obtained from Tables A-6 through A-32, pages 100-151, in the study report.
[b] Mean for each parameter was reported for the combined sexes in each test group.
[c] APTT - Activated partial thromboplastin time.

2. Clinical Chemistry - No differences in clinical blood chemistry in dogs from any of the treatment groups were considered to be treatment-related.

3. Urinalysis: No treatment-related differences in urinalysis parameters were observed in any of the treatment groups.

F. Electrocardiograms

No treatment-related differences in electrocardiograms of dogs in any of the treatment groups were observed.

G. Sacrifice and Pathology

1. Organ weight - Dogs in the 160 mg/kg/day treatment group (combined sexes) had absolute and relative liver weights that were 37 and 41% higher ($p \leq 0.05$), respectively, and a relative thyroid weight that was 33% higher than the corresponding control weights (Table 3). The absolute thyroid weight of the 160 mg/kg/day treatment group was 22% higher, but not statistically different, compared to the controls. Dogs in the 40 mg/kg/day treatment group (combined sexes) had absolute and relative liver weights that were 14 and 20% higher ($p \leq 0.05$), respectively, than the control weights.

Table 3. Absolute (g) and relative (% of body wt) liver and thyroid weights of dogs (combined sexes; 4 dogs/sex/dose) following 52 weeks of treatment with thiabendazole.[a]

Dose (mg/kg/day)	Liver weight		Thyroid weight	
	Absolute	Relative	Absolute	Relative
0	286	2.49	0.74	0.006
10	345	2.68	0.81	0.006
40	327*	2.98*	0.73	0.007
160	392*	3.52*	0.90	0.008*

[a] Data obtained from Tables B-3 and B-4, pages 318 - 321, in the study report.
* Significantly different from the control, $p \leq 0.05$.

No other differences in absolute or relative organ weights were considered to be treatment-related in any of the treatment groups. An increased relative kidney weight for the 160 mg/kg/day treatment groups (combined sexes; 11% higher) compared to the controls was due to a high absolute kidney weight in one dog that was outside the historical control range (also noted for one control dog). The single high value and lack of associated histological kidney alterations indicate that the increased relative kidney weight was not treatment-related.

2. Gross pathology: An increased incidence of discolored foci of the gallbladder mucosa was observed in all dogs in the 160 and 40 mg/kg/day treatment groups (4/sex/group), compared to the 10 mg/kg/day treatment (1/4 males; 2/4 females) and control (1/4 males; 1/4 females) groups. All other findings occurred randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic: Dogs in the 40 and 160 mg/kg/day treatment groups exhibited treatment-related abnormalities of the gallbladder, liver, kidney, urinary bladder, and spleen (Tables 4 and 5). The incidence and severity of the abnormalities were, in general, concentration-dependent.

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All treated dogs, except for one 40 mg/kg/day group female, exhibited epithelial cytoplasmic vacuolation of the gallbladder. The severity of vacuolation increased from very slight to slight in the 10 mg/kg/day treatment group, from to very slight to moderate in the 40 mg/kg/day treatment group, and from very slight to marked in the 160 mg/kg/day treatment group. The effect in the low-dose animals was not considered a treatment-related effect since this lesion was also observed in 1/4 control males.

In the 160 mg/kg/day treatment group, slight to moderate bile duct vacuolation was observed in the livers of 3/4 males and 3/4 females. In the 40 mg/kg/day treatment group, the livers of 4/4 males and 2/4 females had very slight to moderate bile duct vacuolation.

In the 160 mg/kg/day treatment group, urinary bladders had very slight to slight epithelial cytoplasmic inclusions in all males (4/4) and females (4/4). In the 40 mg/kg/day treatment group, urinary bladders had epithelial cytoplasmic inclusions in 3/4 males (very slight) and 2/4 females (very slight to slight).

The kidneys of females, but not males, had increased incidences distal tubule vacuolation. This lesion was observed in 4/4 (very slight to slight) high-dose females and 3/4 (very slight) mid-dose females.

Thyroid follicles were very slightly enlarged in 1/4 females and exhibited cellular hypertrophy in 1/4 males (slight) and 2/4 females (very slight to slight).

The spleens of mid- and high-dose animals had increased incidence of hemosiderosis and/or erythropoiesis. Erythropoiesis was observed in, 2/4 (very slight) males and 3/4 (very slight to slight) females dosed at 160 mg/kg/day, and 1/4 (very slight) males and 1/4 (very slight) females dosed at 40 mg/kg/day. Hemosiderin deposits were very slightly increased in the spleens of 2/4 males and 2/4 females treated at 40 mg/kg/day and 1/4 males (very slight) and 4/4 (very slight to slight) females treated at 160 mg/kg/day. Additionally, the bone marrow of 3/4 females in the 160 mg/kg/day treatment group was very slightly erythropoietic.

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Table 4. Microscopic findings in male dogs (affected/total) fed thiabendazole in capsules at 10, 40 or 160 mg/kg/day for 52 weeks.[a]

Observation	Dose (mg/kg/day)			
	0	10	40	160
Gallbladder				
Epithelial cytoplasmic vacuolation				
Very slight	1/4	2/4	0/4	0/4
Slight	0/4	2/4	3/4	2/4
Moderate	0/4	0/4	1/4	1/4
Marked	0/4	0/4	0/4	1/4
Liver				
Bile duct vacuolation				
Very slight	0/4	0/4	2/4	0/4
Slight	0/4	0/4	0/4	2/4
Moderate	0/4	0/4	2/4	1/4
Thyroid				
Follicle enlargement				
Very slight	0/4	0/4	0/4	0/4
Slight	0/4	0/4	0/4	0/4
Follicular cell hypertrophy				
Very slight	0/4	0/4	0/4	0/4
Slight	0/4	0/4	0/4	1/4
Urinary bladder				
Epithelial cytoplasmic inclusions				
Very slight	0/4	0/4	3/4	2/4
Slight	0/4	0/4	0/4	2/4
Spleen				
Erythropoiesis				
Very slight	0/4	0/4	1/4	2/4
Slight	0/4	0/4	0/4	0/4
Hemosiderosis				
Very slight	0/4	2/4	2/4	0/4
Slight	0/4	0/4	0/4	1/4

[a] Data obtained from Tables B-7 and B-8, pages 327-337, in the study report.

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Table 5. Microscopic findings in female dogs (affected/total) fed thiabendazole in capsules at 10, 40 or 160 mg/kg/day for 52 weeks.[a]

Observation		Doses (mg/kg/day)			
		0	10	40	160
Gallbladder,					
Epithelial cytoplasmic vacuolation	Very slight	0/4	3/4	1/4	2/4
	Slight	0/4	1/4	1/4	0/4
	Moderate	0/4	0/4	1/4	1/4
	Marked	0/4	0/4	0/4	1/4
Liver					
Bile duct vacuolation	Very slight	0/4	0/4	1/4	1/4
	Slight	0/4	0/4	0/4	1/4
	Moderate	0/4	0/4	1/4	1/4
Thyroid					
Follicle enlargement					
	Very slight	0/4	0/4	0/4	1/4
	Slight	0/4	0/4	0/4	0/4
Follicular cell hypertrophy					
	Very slight	0/4	0/4	0/4	1/4
	Slight	0/4	0/4	0/4	1/4
Kidney,					
Distal tubular vacuolation		1/4	1/4	3/4	3/4
	Very slight	0/4	0/4	0/4	1/4
	Slight				
Urinary bladder					
Epithelial cytoplasmic inclusions	Very slight	0/4	0/4	1/4	3/4
	Slight	0/4	0/4	1/4	1/4
Spleen					
Erythropoiesis	Very slight	0/4	0/4	1/4	2/4
	Slight	0/4	0/4	0/4	1/4
Hemosiderosis	Very slight	0/4	0/4	2/4	3/4
	Slight	0/4	0/4	0/4	1/4
Bone Marrow, Erythropoiesis, Very slight		0/4	0/4	0/4	3/4

[a] Data obtained from Tables B-7 and B-8, pages 327-337 , in the study report.

No other microscopic changes observed in any of the treatment groups were considered to be treatment-related. Histological changes in the testes and pancreas of the 40 mg/kg/day male that died prematurely were attributed to the dog's poor condition and not to treatment. All other tissue abnormalities appeared to occur randomly and sporadically in all study groups.

b) Neoplastic - No neoplastic tissue was observed in dogs from any test group.

III. DISCUSSION

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A. Investigator's Conclusions

The study author concluded that the toxicological NOAEL for this study is 10 mg/kg/day. The author did not consider epithelial cytoplasmic vacuolation of the gallbladder in the 10 mg/kg/day group dogs to be toxicologically significant, since the condition was not considered progressive, has no apparent functional significance, and was also observed in several control dogs. The study author stated that the observed lipid vacuolation of the gallbladder did not progress during the study, citing a previous 3-month subchronic dog study in which identical findings were observed. The study author also cited a 2-year chronic study in which gallbladder lipid vacuolation was observed in the control as well as treated dogs. In the 40 and 160 mg/kg/day treatment groups, increased liver weight, splenic erythropoiesis and hemosiderosis, and lipid vacuolation of the urinary bladder, kidney, and hepatic bile ducts were considered to be treatment-related.

B. Reviewer's Discussion

We agree with the study author that the NOAEL for this study is 10 mg/kg/day. Gallbladders of dogs in all treatment groups appeared to be affected by treatment; however, the toxicological significance of the alterations cannot be determined from the data provided in this study.

Gallbladders of all treated dogs (4/sex/group) exhibited epithelial cytoplasmic vacuolation, except for one 40 mg/kg/day group female; the severity increased from very slight to slight in the 10 mg/kg/day group dogs to very slight to moderate in the 40 mg/kg/day group dogs, and very slight to marked in the 160 mg/kg/day group dogs. In the controls, one male exhibited gallbladder epithelial cytoplasmic vacuolation. An increased incidence of inspissated bile was observed in dogs from all treatment groups (3-4/sex) compared to the controls (2 females only). Macroscopically, discolored foci were observed in the gallbladder mucosa of all dogs treated at 40 and 160 mg/kg/day (4/sex/group), compared to the 10 mg/kg/day (1/4 males; 2/4 females) and control (1/4 males) groups.

Thiabendazole affected the livers of dogs in the 40 and 160 mg/kg/day treatment groups. Slight to moderate bile duct vacuolation in the 40 mg/kg/day (4/4 males; 2/4 females) and 160 mg/kg/day (3/4 males; 3/4 females) treatment groups. Absolute and relative liver weights (combined sexes) were 14-20% and 37-41% higher in the 40 and 160 mg/kg/day treatment groups, respectively.

Urinary bladders were affected by treatment at the 40 and 160 mg/kg/day treatment levels. Very slight to slight epithelial cytoplasmic inclusions were observed in the 40 mg/kg/day (3/4 males; 2/4 females), and 160 mg/kg/day (4/4 dogs/sex) treatment groups.

Spleens of dogs treated at 40 and 160 mg/kg/day also exhibited treatment-related effects, as evidenced by increased erythropoiesis and hemosiderosis. Erythropoiesis (very slight or slight) was observed at the 40 mg/kg/day (1/4 males; 1/4 females) and 160 mg/kg/day (2/4 males; 3/4 females) treatment levels. Increased hemosiderin deposits (very slight or slight)

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were also observed in the 40 mg/kg/day (2/4 males; 2/4 females) and 160 mg/kg/day (1/4 males; 4/4 females) treatment groups.

Thiabendazole affected body weights, hematology, and thyroids of dogs in the 160 mg/kg/day treatment. Decreased body weights during the initial 26 study weeks were attributed to emesis in both sexes and weight loss in two females. Both sexes exhibited mild anemia, based on decreased red blood cell counts, hematocrits, and hemoglobin compared to the controls throughout the study. Increased activated partial thromboplastin time and platelet counts were observed throughout the study. Polychromasia and hypochromia were observed at an increased incidence compared to the control dogs during the study. Thyroid changes were very slight follicular enlargement (1/4 females), very slight to slight cell hypertrophy (1/4 males; 2/4 females), and increased absolute and relative weights (22-33%) compared to the controls. Bone marrow in 3/4 females exhibited very slight erythropoiesis.

The toxicological significance of the clinical signs observed in the 40 mg/kg/day male that died prematurely is uncertain since they were not observed in other test dogs. No other dogs died during the study. No treatment-related differences in food consumption, ophthalmoscopic, urinalysis or electrocardiographic parameters were observed. No neoplastic tissue was observed. The LOAEL for this study is 40 mg/kg/day, based on increased liver weight, splenic erythropoiesis and hemosiderosis, and lipid vacuolation of the urinary bladder, kidney, and hepatic bile ducts in both sexes. The NOAEL is 10 mg/kg/day.

IV. Study Deficiencies

No significant deficiencies or deviations from Subdivision F were noted in this study.

All animals were provided 350 g pelleted food throughout the treatment period; the quantity of feed was not increased as the animals grew. Several dogs in each test group consumed 100% of the offered food during various weeks throughout the study period. The limited food availability during those weeks could have depressed body weight gains and may have masked additional treatment-related effects on body weight. However, this would not be expected to significantly impact the study results or their interpretation.

In addition, the data were reported in terms of the combined sexes within each test group, rather than in terms of the individual sexes. Statistical analyses were performed on absolute and relative organ weights for the combined sexes at each dose level and not for each sex. Presentation of the data for each sex allows for comparisons to be made between the sexes and to determine if effects are more pronounced in one sex within a test group. However, since no treatment-related differences between males and females within a test group were observed, presentation of the data in terms of the combined sexes within a test group does not impact interpretation of the study results.

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