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

005601

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Thiabendazole - EPA Registration No. 618-75

Tox. Chemical No. 849A

FROM: Carlos A. Rodriguez
Review Section VI
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Lois Rossi, PM 21
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THRU: Judith Hauswirth, Ph.D., Head, Section VI
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Judith W. Hauswirth 11/26/86

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Requested Action:

Review miscellaneous toxicity data (teratology, subchronic, subacute and mutagenicity) and made part of the files for EPA Registration No. 618-75.

Conclusions:

The submitted miscellaneous toxicity studies (teratology, subchronic feeding, and mutagenicity) have been reviewed and made part of the files for EPA Registration No. 618-75.

1. The teratology study (Study Report No. 292,112-115, 1978) of Thiabendazole (TBZ) suspended in olive oil and administered orally to (JCL-TCR) mice from days 7 to 15 of gestation is

classified "Supplementary Study." A systemic or developmental toxicity NOEL was not demonstrated from the dose levels selected.

Levels tested: 700, 1300, and 2400 mg/kg/day.

2. The teratology study of TBZ suspended in gum arabic solution and administered orally to JCL-JCR strain of mice once on day 9 of gestation is considered "Supplementary Study." The test material should be administered orally covering the period of organogenesis.

Levels tested: 1157, 1389, 1667, 2000, and 2400 mg/kg/day.

3. The comparative teratology study (Study No. R123-12) of TBZ suspended in olive oil and administered orally once on day 9 of gestation to ICR strain of mice from three different commercial breeders in classified "Supplementary Study." The test material should be administered orally covering the period of organogenesis.

Levels tested: 1157 or 1389 mg/kg/day.

4. The subchronic (13 weeks) feeding study is classified "Supplementary Study." Legible tables of laboratory results must be submitted to support the findings reported.

NOEL = 500 ppm
LEL = 1000 ppm (dec. weight gain, anemia)

Levels tested: 500, 1000, 2000, 4000, and 8000 ppm.

5. The pathological study on rats fed TBZ for 13 weeks is classified Core-Supplementary study.

Tables in Study No. 4 (F344/DuCrJ rats) subchronic feeding study are illegible for clinical chemistries and hematology.

Levels tested: 0.05% (500 ppm), 0.1% (1000 ppm), 0.2% (2000 ppm), 0.4% (4000 ppm), and 0.8% (8000 ppm).

6. The subacute feeding study (1 or 6 weeks) (Study No. 80-84, 1980) of TBZ is classified Supplementary Study. No gross pathological or histopathological examinations of tissues were made and only two doses with only five animals per dose level were on test.

7. The mutagenicity evaluations of TBZ in Salmonella/Microsome Assays is considered unacceptable. The materials and methods used do not comply with the EPA Health Effects Test Guidelines No. 560/6-83-001 for conducting the Ames Mutagenicity Assay.

REVIEW OF TOXICITY DATA SUBMITTED

I. Study Type: Teratology in Mice (JCL-JCR)

Study Report No.: 292, 112-115, 1978

Accession No.: 260307

MRID No.: N/A

Laboratory: Tokyo Metropolitan Research Laboratory of Public Health

Date: 1978

Test Material: Thiabendazole (TBZ), 98.5% Purity

Method:

Sexually mature (JCL-JCR) virgin female mice weighing between 24.0 and 31.0 grams and reaching 8 to 13 weeks old were used in this study. One female was mated with one male from the same strain (1:1 ratio), and those showing the vaginal plug on the next morning were regarded as pregnant and considered to be at Day 1 of gestation. The test material was orally administered once a day for 9 days from Days 7 to 15 of gestation to four groups (A through D) each composed of 16 pregnant mice. Group A received TBZ 24% oil solution (2400 mg/kg/day), Group B received TBZ 13% oil solution (1300 mg/kg/day), Group C received TBZ 7% oil solution (700 mg/kg/day) and the control group received the vehicle, olive oil, in the same dosing volume (10 mL/kg/day) as the test material treated groups. Body weights were recorded daily. On day 18 of pregnancy all mice were sacrificed and the uterine contents examined.

The following data were recorded: number of fetuses *in utero*, number of live and dead fetuses, sex ratio, implantations, number of corpora lutea, external alterations. All the fetuses were fixed in 95% ethanol, stained with alizarin red and examined for skeletal alterations.

Laboratory Investigations:

Mortality (Maternal) - There were ten treatment related deaths at the highest dose group tested (2400 mg/kg/day).

Body Weight (Maternal) - A dose-related decrease in body weight gain was observed in all TBZ-treated groups who were administered TBZ during the period of organogenesis.

Gross Necropsy - Necrosis was observed in the kidneys of pregnant and nonpregnant mice in all treated groups. No histology data are provided in the report.

Organ Weights - Significant increase ($p < 0.01$) in liver weight to body weight ratios for all dose groups tested was observed when compared to the control group. Significantly greater increase ($p < 0.001$) in lung weight to body weight ratio for Group B (1300 mg/kg/day) was observed when compared to the control group. Significantly greater increases ($p < 0.001$) in heart, spleen, and kidney weights to body weight ratios for Group B (1300 mg/kg/day) and Group C (700 mg/kg/day) were noted when compared to control group.

Data extracted from page 13 (Table 1) of the report.

Pregnancy - The number of pregnant female mice have been proportionately decreased with increased dose levels tested.

Embryotoxicity - A dose response was noted as evidenced by increase incidence of dead and resorbed fetuses and decreased mean fetal body weight with increased dose levels tested.

Data extracted from page 14 (Table 2) of the report.

Fetal Sex Ratio - Was similar in all groups tested.

Fetal Malformation - A dose related effect in cleft palate from TBZ-treated groups was observed as follows: Group A (2400 mg/kg/day) three fetuses in one dam (100%); Group B (1300 mg/kg/day) 23%; Group C (700 mg/kg/day) 11%, and Group D (oil solvent control) 1.34%. Open eyelids were seen in two fetuses (two dams) in Group (1300 mg/kg/day), in four fetuses in Group C (700 mg/kg/day) and in one fetus in Group D (oil solvent control). No data of number of fetuses examined in dose groups B, C, and D were recorded.

Legible tables must be submitted to support the findings reported.

Skeletal Malformation - No significant dose-related effect in the number of fetuses with thoracic, vertebral and rib malformations was seen. The incidence as reported was seen in two fetuses in Group B (1300 mg/kg/day) and in two fetuses in Group C (700 mg/kg/day). A significant delay in fetal ossification was seen in Group B (1300 mg/kg/day) and Group C (700 mg/kg/day). An increased incidence of other minor skeletal variations was seen in Group B (1300 mg/kg/day) when compared to the control group value.

Conclusions:

1. There were treatment related toxic effects to the dams (mice) as evidenced by increase maternal mortality in the high dose (2400 mg/kg/day) and maternal body weight decreases observed in all treated groups during gestation. Necrosis in the kidneys of pregnant and nonpregnant mice in all treated groups was observed.

2. There were treatment related maternal effects in all treated groups as evidenced by significant increases in liver, lung, heart, spleen, and kidney weights to body weight ratio when compared to the control group value.

3. Significant dose-related effects in dead and resorbed fetuses and fetal body weight gain decreases were observed in fetuses of all treated groups when compared to the control group value. A delay in fetal ossification in TBZ-dosed groups may be related to the lower fetal weight observed.

4. A fetal malformation was noted as evidenced by a significant increase in cleft palate from all treated groups when compared to the control group. The relationship for this effect appears to be dose-related.

5. No statistically significant or dose-related effects on the total number of fetuses showing skeletal malformations among groups were observed.

Maternal NOEL < 700 mg/kg/day (LDT).

Maternal LEL = 700 mg/kg/day (body weight gain decreased, kidney necrosis, increase in heart, spleen, and kidney weight to body weight ratio).

Developmental Toxicity NOEL < 700 mg/kg/day (LDT).

Developmental Toxicity LEL = 700 mg/kg/day (decreased body weight gain, mortality, cleft palate, open eyelids, delayed ossification).

Core-Classification:

Supplementary. The teratology study in mouse is classified as Supplementary Data. A systemic or developmental NOEL cannot be demonstrated from the dose levels selected and no visceral examinations were performed.

II. Study Type: Teratology in Mice (JCL-JCR)

Accession No.: 260307

MRID No.: N/A

Laboratory: Tokyo Metropolitan Research Laboratory of Public Health

Date: 1982

Test Material: Thiabendazole (TBZ), 98.5% Purity

Method:

Sexually mature (JCL-JCR) virgin female mice weighing between 24.0 and 31.0 grams and reaching 8 to 13 weeks of age were used in this study. One female was mated with one male of the same strain (1:1 ratio), and those confirmed showing the vaginal plug on the next morning were regarded as pregnant and considered to be at Day 1 of gestation. The test material was suspended in 0.5% gum arabic and administered orally once on Day 9 of gestation to groups of pregnant females as follows:

Group	No. of Mice	Dose Level
1	9	2400 mg/kg
2	10	2000 mg/kg
3	9	1667 mg/kg
4	10	1389 mg/kg
5	10	1157 mg/kg
6 (Control)	10	10 mg/kg (0.5% gum arabic)

Observations of body weights and general appearance were made daily.

On the 18th day of pregnancy all the animals were sacrificed, autopsy was performed on each animal and the following observations made: number of implantations, dead and viable fetuses, sex ratio, resorptions, number of corpora lutea, and gross abnormalities. All surviving fetuses were fixed in 95% ethanol, stained with alizarin red and examined for skeletal alterations.

Laboratory Investigations (Maternal):

1. Two mortalities occurred, 1/10 animals in the control group and 1/9 animals in the 1667 mg/kg dosed group. Mortality was comparable between the treated animals and control group.

Mortality data extracted from p. 11 of 12 (Appendix 3a, Table 2 of Report).

2. There was a decreased body weight gain in the 2400 and 2000 mg/kg dose groups on Days 12 and 13 of pregnancy. The decrease continued throughout the study.

Body weight data extracted from p. 10 of 12 (Appendix 3a, Figure 1 of Report).

3. An increased heart weight ($p < 0.05$) and adrenal (right) weight ($p < 0.001$) was observed in the groups receiving the highest dose level (2400 mg/ kg/day).

Organ weight data extracted from p. 10 of 12 (Appendix 3a, Table 1 of Report).

Laboratory Investigations (Fetal):

1. The conception rate was comparable between the control and the various dosage groups.

2. No significant differences were observed between the control group and the treated groups in the distribution of viable and dead fetuses, resorbed fetuses, sex ratio, litter size, and implantation sites.

3. The body weight gain decreased in male fetuses in the 2400 mg/kg group and in fetuses of both sexes in the 1667 mg/kg group which were statistically significant ($p < 0.005$) when compared to the control group value.

Data extracted from p. 11 of 12 (Appendix 32, Table 2 of the Report).

4. External malformations observed:

Group 2400 mg/kg - 1/100 fetuses examined showed cleft palate.

- 2/100 fetuses examined showed open eyelids.

- 1/100 fetuses examined showed brachyury.*

Group 2000 mg/kg - 1/81 fetuses examined showed cleft palate.

- 1/81 fetuses examined showed open eyelids.

Group 1667 mg/kg - 2/106 fetuses examined showed open eyelids.

Group 1389 mg/kg - 2/109 fetuses examined showed open eyelids.

Group 1157 mg/kg - 3/115 fetuses examined showed open eyelids.

Control group (gum arabic) - 2/96 fetuses examined showed open eyelids.

Data extracted from p. 11 of 12, Table 3, Appendix 3a of Report.

5. Skeletal Malformations:

Group 2400 mg/kg - 11/100 fetuses examined showed fusion of vertebral arches.

- 5/100 fetuses examined showed fusion of vertebral body.

Group 2000 mg/kg - 3/81 fetuses examined showed fusion of vertebral arches.

- 1/81 fetuses examined showed fusion of vertebral body.

- 1/81 fetuses examined showed fusion of ribs.

Group 1667 mg/kg - 1/106 fetuses examined showed fusion of vertebral body.

* We are not familiar with the term "brachyury" used under external malformations outlined on page 11 of 12, Table 2, Appendix 3a of Report. Please define.

Group 1389 mg/kg - 1/109 fetuses examined showed fusion of vertebral arches.

- 7/109 fetuses examined showed fusion of ribs.

Group 1157 mg/kg - 2/115 fetuses examined showed fusion of vertebrae arches.

- 1/115 fetuses examined showed fusion of vertebral body.

- 1/115 fetuses examined showed fusion of ribs.

The incidence of skeletal malformations was 16% (Group 2400 mg/kg); 6.0% (Group 2000 mg/kg); 0.9% (Group 1667 mg/kg); 7.4% (Group 1389 mg/kg) and 0% for control group.

Data extracted from page 12 of 12. Table 4, Appendix 3a.

Skeletal Variations:

There was no significant difference in skeletal variations in all treated groups when compared to the control groups. However, a dose dependent progression of ossification in TBZ-treated groups was observed.

Data extracted from page 12 of 12, Table 4, Appendix 3a.

Conclusions:

1. There were treatment related toxic effects to the dams (mice) as evidenced by maternal body weight decreases in the 2400 and 2000 mg/kg/day dose groups on days 12 and 13 of pregnancy. This body weight decrease continued throughout the study.

2. There were treatment related maternal effects as evidenced by an increased heart weight and right adrenal weight observed in the 2400 mg/kg/day dose group.

3. A significant body weight gain decrease was observed in male fetuses in group 2400 mg/kg/day, and in fetuses of both sexes in Group 1667 mg/ kg/day when compared to the control group.

4. Fetal external malformations consisted of 1/100 fetuses examined showing cleft palate at 2000 mg/kg/day and 1/81 fetuses examined at 2400 mg/kg/ day. The incidence of open eyelids appears in all test groups and was comparable to the control group. These external malformations appears to be of low incidence and according to the testing laboratory, TMRL, no statistically significant differences were noted between the treated groups and the control group.

5. The skeletal malformations showed an increase in the number of fetuses with fusion of vertebral arches in the Group 2400 mg/kg/day when compared to the control value.

6. Skeletal variations showed no significant differences in the treated groups when compared to the control group. A dose dependent progression of ossification in treated groups was observed.

Maternal NOEL = 1667 mg/kg/day.

Maternal LEL = 2000 mg/kg/day (decreased body weight gain).

Developmental Toxicity NOEL = 1389 mg/kg/day.

Developmental Toxicity LEL = 1667 mg/kg/day (body weight decreased in both sexes).

$$\text{A/D ratio} = \frac{\text{Maternal NOEL}}{\text{Developmental NOEL}} = \frac{1667}{1389} = 1.2$$

Study Classification:

Supplementary. The dosed period did not cover the period of major organogenesis. The test material was given orally once only on Day 9 of gestation.

III. Study Type: Teratology in Mice

Accession No.: 260307

Test Material: Thiabendazole (TBZ)

Synonyms: N/A

Study No.: R123-12

005601

Sponsor: Merck Sharpe & Dohme

Testing Facility: Tokyo Metropolitan Research Laboratory of Public Health

Title of Report: Comparative Teratogenicity Using the ICR Strain Mice From Three Different Breeders

Authors: Kubo, Ando, Ogata, and Hiraga

Report Issued: 1982

A. Materials:

1. Test compound: Thiabendazole (TBZ), Batch No. BZA-539, Purity 98.5%

2. Test animals: Species: Mice, strain ICR

Age: 8 to 13 weeks

Weight: 25.0 to 33.0 g

Source: Japan Clea, Japan Charles River, Shizuoka Agricultural, and Cooperative Union

B. Study Design:

Sexually mature JCL:ICR, CRJ:CD-1 (ICR-derived strain), and Slc:ICR female mice weighing between 25.0 and 33.0 g and reaching 8 to 13 weeks of age were selected in this study. One female was mated with one male of the same strain (1:1 ratio), and the next morning those confirmed for the vaginal plug were regarded pregnant and considered to be at Day 1 of gestation. TBZ was administered orally as a suspension in olive oil to each strain group on Day 9 of gestation. A concurrent control group was used for each strain group as follows:

rain (Mice):	JCL:ICR			CRJ:CD-1			Slc-ICR		
ses (mg/kg)	1389	1157	Control (10 mL/kg olive oil)	1389	1157	Control (10 mL/kg olive oil)	1389	1157	Control (10 mL/kg olive oil)
. of pregnant females group	13	15	14	18	18	17	18	13	18

Observations of body weight and general appearance were made daily.

On the 18th day of pregnancy all the animals were sacrificed, autopsy was performed on each animal and the following observations made: number of implantations, dead and viable fetuses, sex ratio, resorptions, number of corpora lutea, and gross abnormalities. Living fetuses were fixed in 95% ethanol, stained with alizarin red and examined for skeletal abnormalities.

Laboratory Investigations (Maternal):

Mortality

Six mortalities occurred as follows:

Group 1389 mg/kg (JCL:ICR) - 1/15 animals on test died.

Group 1389 mg/kg (CRJ:CD-1) - 2/18 animals on test died.

Group 1157 mg/kg (CRJ:CD-1) - 1/18 animals on test died.

Group 1389 mg/kg (Slc:ICR) - 2/18 animals on test died.

Mortality data obtained from p. 16 of 18, Table 2 of Report.

Body Weight

Immediately following treatment, all the treated groups showed significant body weight decrease when compared to the control group; however, there was no dose response, since higher weight inhibition was seen in lower dosed groups. These changes in body weight gain decreases observed in all dosed groups were statistically significantly different from the control groups with the exception of Group 1389 mg/kg (Slc:IRC) which was not considered significantly different from the control group value.

Data on body weight obtained from p. 14 of 18, Figure 1, 2, and 3 of the Report.

Organ Weight

Group 1389 mg/kg (JCL:IRC) - Statistically significant decreases are seen in absolute liver weight ($p < 0.01$), relative liver weight ($p < 0.001$) to body weight ratio and relative kidney weight ($p < 0.05$) to body weight ratio.

Group 1389 mg/kg (CRJ:CD-1) - Statistically significant increases are seen in absolute and relative liver weight ($p < 0.05$), relative spleen weight ($p < 0.05$) and in absolute and relative right kidney weight ($p < 0.01$) to body weight ratio.

Group 1157 mg/kg (CRJ:CD-1) - Statistically significant decreases are seen in relative liver weight ($p < 0.05$), absolute and relative right ovary weight ($p < 0.05$) to body weight ratio and a statistically significant increase in absolute and relative spleen weight ($p < 0.05$) to body weight ratio.

Group 1389 mg/kg (JCL:IRC) - A statistically significant decrease is seen in absolute liver weight ($p < 0.05$) to body weight ratio and statistically significant increase in absolute heart and right kidney weights ($p < 0.01$) to body weight ratio.

Organ weight data obtained from p. 15 of 18, Table No. 1, Appendix 4a of the Report.

Laboratory Investigations (Fetal):

1. The conception rate and sex ratio were comparable between the corresponding control group and the various dosages groups.

2. Embryotoxicity and fetotoxicity are evidenced by statistically significant increases in the number of resorptions and dead fetuses ($p < 0.001$) in Groups JCL:ICR and CRJ:CD-1. Fetotoxicity in Slc:ICR mice was not significantly different from corresponding control group. The number of implantation sites was decreased in Group CRJ:CD-1 group ($p < 0.05$) and statistically significantly increased ($p < 0.001$) in Group Slc:ICR.

Data obtained from page 16 of 18, Appendix 4a, Table 2 of the Report.

3. A statistically significant fetal body weight decrease ($p < 0.001$) was noted in all treatment groups as compared with the corresponding control group.

Data obtained from p. 16 of 18, Appendix 4a, Table No. 2 of the Report.

4. All treatment groups showed evidence of external malformations. Treatment related malformations seen were reduction deformity of the forelimbs, and atresia, tail malformations, exencephalia, and club foot. Cleft palate and open eyelids were also observed; these two malformations were comparable with the

corresponding control group. The incidence of malformations was lower in Group SLC:IRC in comparison with the other treated groups.

Data obtained from p. 18 of 18, Appendix 4a, Table 4 of the Report.

5. Skeletal examination showed statistically significant increases ($p < 0.001$) in the incidence of fusion of vertebral arches, body and ribs. The incidence ranged from 23.5 percent to 45.5 percent in the treated groups and 8.9 percent in the corresponding control groups.

Statistically significant increases in retarded ossification were noted in all treatment groups as compared to the corresponding control group.

The incidence of skeletal variations was lower in group Slc:ICR, when compared to the other treated groups, but the difference was not statistically significant.

Data obtained from page 18 of 18, Appendix 4a, Table 4 of the Report.

Conclusions:

The results of this study using ICR-derived strain of mice from three different breeders showed similar variations relative to fetal appearance, external malformations and skeletal deformities to high dosages of administered TBZ. The incidences were decreased in Slc:ICR mice.

Study Classification:

Supplementary. The dosed period did not cover the period of major organogenesis. That test material was given orally once only on Day 9 of gestation.

IV. Study Type: Subchronic Feeding

Accession No.: 260377

Test Material: Thiabendazole

Synonyms: N/A

Tox. Chem. No.: 849A

MRID No.: N/A

005601

Study No.: Not available

Sponsor: Merck Sharpe & Dohme

Testing Facility: Tokyo Metropolitan Research Laboratory of Public Health

Title of Report: Subacute Toxicity of Thiabendazole by Food Administration to F344/ DuCrj Rats

Authors: Mikuriya, Hayashida, Takahashi, Iochi, and Hiraga

Report issued: May 15, 1981

A. Materials:

- Test compound: Thiabendazole (TBZ), Batch No. BZA-539, Purity 98.5%

B. Study Design:

1. Animals assignment - Animals were assigned at random to the following test groups:

Test Group	Dose in Diet (ppm)	Study (13 Weeks) Male	Female
Control	0 (diet only)	10	10
1	500 (0.05%)	9	10
2	1000 (0.1%)	10	10
3	2000 (0.2%)	10	10
4	4000 (0.4%)	10	10
5	8000 (0.8%)	10	10

2. Diet preparation - Diet was prepared by adding Japan Clea's powder food CE-2 to the test material and solidified. This solid food was given freely and water ad libitum to the rats for 13 weeks.

C. Methods and Results:

Animals were observed for signs of toxicity and general appearance daily. Body weight was obtained twice a week. Food and water intake was measured weekly for the first 4 weeks, followed by every other week thereafter. At the end of the feeding period, the following laboratory investigations were made at termination of study.

Urinalysis: Fresh urine samples were collected and estimation of the following parameters was performed: pH, glucose, ketones, bilirubin, urobilinogen, and occult blood pigments.

Hematology - Samples of blood were withdrawn and the following parameters examined: WBC, RBC, hemoglobin (Hgb), hematocrit, MCV (mean RBC volume), MCH (mean RBC hemoglobin), and MCHC (mean RCB hemoglobin concentration).

Blood Chemistry - The following parameters were examined: SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvic transaminase), TB (total protein), SAP (serum alkaline phosphatase), and UN (urea nitrogen).

Results:

Mortality - One death occurred in one male rat at 12 weeks of treatment in the 500 ppm (0.05%) dose group. No other deaths were seen in any other group.

Clinical Signs - Emaciation was seen in all males and females in treatment groups 4000 (0.4%) and 8000 ppm (0.8%). No other changes were reported.

Body Weight - Statistically significant decreases ($p < 0.050$ in body weight gain were observed in groups 4000 (0.4%) and 8000 ppm (0.8%) during the initiation of the study. Male rats recovered after 4 weeks to the level of pretreatment weight, but the females in this dosage group remained below the pretreatment weight throughout the study. A decrease in body weight gain (10%) was observed in the 1000 ppm (0.1%) and 2000 ppm (0.2%) groups when compared with the control group.

Food Consumption - Males and females receiving 8000 ppm (0.8%) and males receiving 4000 ppm (0.4%) of the test material had lower food intake than controls ($p < 0.05$ when compared with control values) during the first 3 to 4 weeks of treatment. The tendency of lower food intake was increased in the group 8000 ppm (0.8%) thereafter.

Food intake was comparable to controls in the other treatment groups at termination of the study.

Water Consumption - No marked differences between individual groups.

Urinalysis - No significant difference between individual groups in any of the parameters examined.

Hematology - At termination of study a significant decrease ($p < 0.05$) in WBC was observed in rats in group receiving 8000 ppm (0.8%). RBC, Hgb, and Hct were decreased in groups 8000 ppm (0.8%) and 4000 ppm (0.4%). A tendency of a slight dose-related anemia was observed in groups 1000 ppm (0.1%) and higher.

Blood Chemistry - A statistically significant dose-related decrease ($p < 0.05$) in SGOT and SGPT was observed in male rats at doses of 1000 ppm (0.1%) and above. The decrease was more markedly observed in the 8000 ppm (0.8%) and 4000 ppm (0.4%) groups. Significant increases ($p < 0.05$) in SGOT and SGPT in females at 8000 ppm (0.8%) group were observed. Also, alkaline phosphatase was elevated and total protein (TP) decrease in females in this group was seen.

According to the investigator, these changes seem to correspond to the degeneration or necrosis observed in hepatic cells of all male and female rats treated with TBZ at dose levels of 2000 ppm (0.2%) and above.

Conclusions:

1. There were treatment toxic effects by the signs of emaciation observed in the treatment groups 4000 ppm (0.4%) and 8000 ppm (0.8%).

2. A treatment toxic related effect was observed by the significant body weight gain decrease in both sexes at dose levels of 1000 ppm (0.1%) and above.

3. Mild anemia at doses of 1000 ppm (0.1%) and above was observed due to the significant decrease in RBC (red blood cells), Hgb (hemoglobin), HcT (hematocrit) and WBC (white blood cells).

4. An increase in SGPT and SGOT was observed in female rats at dose levels of 8000 ppm (0.8%) (HDT). Alkaline phosphatase increase and total protein decrease was observed in female rats at 8000 ppm (0.8%) (HDT).

Systemic NOEL = 500 ppm (0.05%) (LDT).

Systemic LEL = 1000 ppm (0.1%) (body weight gain decreased, anemia).

Study Classification:

Supplementary. Legible tables of laboratory results must be submitted to support the findings reported.

005601

V. Study Type: Pathological Study

Accession No.: 26037

Test Material: Thiabendazole

Synonyms: N/A

Tox. Chemical No.: 849A

MRID No.: N/A

Study No.: Not available

Sponsor: Merck Sharpe & Dohme

Testing Facility: Tokyo Metropolitan Research Laboratory of Public Health

Title of Report: Pathological Study on Rats Fed Thiabendazole for 13 Weeks

Authors: Fujii, Fukumori, Nagasawa, Mikuriya, Hayashida, Takahashi, Yano, Yuzawa, and Hirage

Report Issued: Date not available

A. Materials:

1. Test Compound: Thiabendazole (TBZ), microfine MSD (Lot No. BZA-539)

2. Test Animals: Species: Rats

Strain: F344, DuCrj

Age: Five weeks

Source: Japan Charles River

B. Study Design:

1. Animal Assignments - Rats were assigned randomly to the following test groups.

Test Group	Dose in Diet (ppm)	Study (13 Weeks) Male	Female
Control	0 (diet only)	10	10
Group 1	500 (0.05%)	9	10
Group 2	1000 (0.1%)	10	10
Group 3	2000 (0.2%)	10	10
Group 4	4000 (0.4%)	10	10
Group 5	8000 (0.8%)	10	10

2. Diet preparation - Diet was prepared by adding the test material to Japan Clea's powder food CE-2 and solidified. This solid food was given freely with water ad libitum to the rats for 13 weeks.

At the end of the treatment animals were sacrificed and subjected to gross pathological examinations.

Organ Weight - The following organs were weighed: brain, pituitary, thymus, heart, lung, liver, spleen, adrenal, testes, prostate, ovary, uterus, and urinary bladder.

The following tissues were collected for histological examination:

Eyeball
Hardenian gland
Parathyroids gland
Submaxillary gland
Thyroids (including epithelocorpuscle)
Trachea
Escphagus
Stomach
Large intestine
Small intestine
Pancreas
Spermatocyst
Testes
Lymph node
Femur

The tissues were preserved in 10% buffered formalin, sectioned and stained with hematoxylin-eosin. The liver, kidney, and spleen were stained with Berlin blue and ribofustin. Microscopic examinations were made.

Results: (Appendix 5b, Tables 1 and 2)

Body Weight - Marked body weight gain decrease was observed in all treated groups except at 500 ppm (0.05%) group. A statistically significant difference in body weight decrease

($p < 0.001$) was observed at dose levels of 2000 ppm (0.2%), 4000 ppm (0.4%) and 8000 ppm (0.8%) when compared to the control group value. The present weight loss is considered to be due to the increase of dosage among the treated groups and the result of taste dislike to the test material.

Organ Weight - Marked organ weight decreases and organ weight changes relative to body weight were observed in the heart, kidneys, lungs, spleen, and adrenal in both sexes at dose levels of 1000 ppm (0.1%) and above. The observed changes in organ weights are apparently due to biochemical (SGPT, SGOT, AP, and TP) changes reported in the subacute feeding to F344, DuCrj related to treatment with TBZ.

Mortality - One death occurred in group 500 ppm (0.05%) at 12 weeks after treatment. No histological or gross observations were observed in this animal.

Histology - The microscopic examination of tissues revealed variations described as atrophy of thymus, thyroid, and bone marrow tissue. Lobular centralized hepatocellular degeneration, necrosis, and phagocytes were observed. These changes were classified very slight-to-slight and more commonly seen in the dietary dose levels of 0.2 percent (2000 ppm) and above.

Hemosiderin deposits were seen in the spleen at dose levels of 0.2 percent (2000 ppm) and above. This correlates with the mild anemia observed at dose levels of 0.1 percent (1000 ppm) and above in the subchronic rat feeding Study No. 4.

Conclusions:

Dietary levels of 0.2 percent (2000 ppm) and above resulted in microscopic tissue variations described as atrophy of thymus, thyroid, and bone marrow, degeneration of hepatic cells, necrosis and hemosiderin deposits in the spleen.

NOEL = 500 ppm
LEL = 1000 ppm (decreased body weight gain, anemia)

Core-Classification:

Supplementary because tables of clinical chemistries and hematology are illegible.

VI. Study Type: Subacute Feeding

Accession No.: 260307

Test Material: Thiabendazole [2-(4-thiazolyl) benzimidazole]

Synonyms: N/A

Tox. Chemical No.: 849A

MRID No.: N/A

Study No.: 80-84, 1980

Sponsor: Merck Sharpe & Dohme

Testing Facility: Tokyo Metropolitan Research Laboratory of Public Health

Title of Report: Subacute Toxicity Study of TBZ in Rats

Authors: Yoneyama, Ikawa, and Hiraga

Report Issued: Published in 1970

A. Materials: Thiabendazole (TBZ), Tokyo Kasei's Lot No. AN03

B. Test Animals: Species - Rats
Strain - Wistar JCL

Age - 4 weeks

Source - Shizuoka Experimental Animal Cooperative Society

C. Study Design:

- Animal assignment: Rats were assigned to the following test groups.

Test Group	Dose in Diet (ppm)	Number of Males	Number of Females
1 Control	0 (diet only)	5	5
2 Low	2500 (0.25%)	5	5
3 High	5000 (0.50%)	5	5

TBZ was mixed with Japan Clea's food, CE-Z, to make the concentrations of 0.25 percent (2500 ppm) and 0.5 percent (5000 ppm) which were given to the rats for either 1 or 6 weeks. Body weight, food, sample, and water intake were recorded.

At termination of the feeding period, the animals were sacrificed and hematological and biochemical tests were performed. Liver and kidney weights were also recorded.

The hematology parameters examined were: WBC, RBC, Hgb, HcT, MCV, and MCHC.

The biochemical parameters examined were: SGOT, SGPT, glucose, UN, total cholesterol, alkaline phosphatase (AP) and choline esterase (CHE).

The following liver enzymes were determined: glucose 6-phophatase, LDH, SGPT, SGOT, total acid phosphatase (TACP).

Kidney enzymes determined were: glucose 6-phosphatase, Na, K-AT phosphatase, total acid phosphatase, alkaline phosphatase, ICDH, MDH, glucose dehydrogenase (GIDH).

Results:

Body Weight: Body weight was significantly reduced in both male and female rats at the highest dose level 5000 (0.5%) ppm (Table No. 1 of Report).

Food Consumption: Food intake decreased in both male and female rats at the highest dose level 5000 ppm (0.5%) when compared with the control value (Table No. 1 of Report).

Hematology: Decreased values for RBC were observed at week 6 for male and female rats receiving the highest dose 5000 ppm (0.5%) TBZ ($p < 0.001$ when compared with control value). MCV and MCH decreased significantly in male rats receiving the highest dose 5000 ppm (0.5%) TBZ ($p < 0.001$ when compared with control value) (Table No. 2 of Report).

Serum Biochemical Measurements:

Cholesterol (CHO) and total protein (TP) were significantly increased at 6 weeks after treatment for both male and female rats receiving 2500 ppm (0.25%) or 5000 ppm (0.5%).

Serum glucose concentration decreased and an increased in SGPT activity was seen at 1 week after treatment at the highest dose level 5000 ppm (0.5%) group. The reduction in glucose concentration is likely the result of the decreased food consumption reported.

Organ Weight: Significant increases in the relative liver and kidney weights to body weight ratio were observed in male and female rats at 6 weeks after treatment at the highest dose level 5000 ppm (0.5%) when compared with the control value. The increase in liver weights appears to be of some toxicological significance as an increase in SGPT discussed above appears to be treatment related.

Liver and Kidney Enzymes: Glucose 6-phosphatase activity in both liver and kidney was reduced significantly in male rats at 5000 ppm (0.5%) group. This change may be associated with the lower food consumption reported 1 week after treatment.

Other changes seen in liver and kidney enzymes between treated and control groups appear to be slight and not biologically significant.

Classification: Supplementary Study. No gross pathological or histopathological examination of tissues was made and only two doses were studied with only five animals per dose level.

VII. Study Type: Mutagenicity Evaluation in Salmonella/Microsome Test

Accession No.: 260307

Test Material: Thiabendazole (TBZ)

Synonyms: N/A

Study Nos.: 32-2, 25-27, 1981

Sponsor: Merck Sharpe & Dohme

Testing Facility: Tokyo Metropolitan Research Laboratory of Public Health

Title of Report: Cumutagenic Effect of Antioxidants on the Mutagenicity of Thiabendazole in the Salmonella/Microsome Test

Authors: Hiroshi Fugita, Kogo Hiraga

Report Issued: 1981

A. Materials: Thiabendazole (TBZ) [2-(4-thiazolyl)-benzimidazole]/antidoxidants (butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), Ascorbic acid and alpha-tocopherol.

B. Study Design: The materials and methods used in the assay do not comply with the EPA Health Effects Test Guidelines No. 560/6-83-001 for conducting the Ames Mutagenicity Assay.

The following inconsistencies were noted:

1. Only Salmonella typhimurium strain TA98 was used. At least four strains should be tested.

2. The technical material should be tested alone.

3. Strain specific positive controls were not used.

4. The test should be conducted with and without metabolic activation.

5. The dose levels used and the rationale for selecting them was not stated.

6. Individual plate counts, mean number and revertant colonies per plate should be provided.

7. Statistical evaluation.

8. Detailed study results in tabulated form.

Core-Classification: Unacceptable, because of the reasons discussed in the study design.

VIII. Study Type: Mutagenicity Evaluation in Salmonella/ Microsome Test

Accession No.: \260307

Test Material: Thiabendazole (TBZ)

Synonyms: N/A

Study No.: Not available

Sponsor: Merck Sharpe & Dohme

Testing Facility: Tokyo Metropolitan Research Laboratory of Public Health

Title of Report: Effect of Butylated Hydroxytoluene (BHT) on Mutagenicit, of Thiabendazole in Salmonella/Microsome Test

Authors: Hiroshi Fujita and Kogo Hiraga

Report Issued: Date not available

A. Materials: Thiabendazole [2-(4-thiazolyl)-benzimidazole] and butylated hydroxytoluene (BHT) combinations in the Ames Salmonella assay.

B. Protocol: Varying dose levels of TBZ (1000, 500, 100 ug/0.05 mL) and BHT (100, 50 0, 5, and 1 ug/0.05 mL) were dissolved in DMSO and each dilut poured in test tubes. S-9 (50 uL) alachlor 1254 metabolic activation was added to each tube in the presence of Salmonella strain TA98 precultured at 37 °C for 20 minutes. Warmed soft agar (45 °C) was added and mixed before pouring into plates. After cultured for 48 hours at 37 °C the colonies were counted.

Neither TBZ nor BHT were found to be mutagenic.

BHT 10 ug/plate in combination with 500 or 1000 ug/plate TBZ in the presence of S-9 phenobarbital-induced metabolic activation caused an increase in reversions of approximately twofold over controls.

In view of this, increased combinations of 30 ug BHT/plate with both 500 and 1000 ug/plate TBZ in the presence of increasing concentrations of S-9 were investigated. A threefold increase in revertants over controls was obtained and the increase seemed a dose-response with increasing S-9 concentrations.

The authors (TMRL) concluded that the BHT-induced mutagenesis of TBZ is related to metabolic activation and the possibility that BHT will affect the formation of this substance.

Conclusions:

TBZ, or BHT by itself, was found to be nonmutagenic in this assay.

The weak positive results obtained with TBZ and BHT occurs only in the presence of phenobarbital-induced and not alachlor-induced S-9.

The weak positive results with TBZ/BHT combination is directly related to the level of metabolic enzyme used.

Study Classification: Unacceptable.

1. Only Salmonella typhimurium strain TA98 was used. At least four strains should be tested.

2. The dose levels used and the rationale for selecting them was not stated.

3. Detailed study results in tabulated form was not performed.

IX. Study Type: Mutagenicity Evaluation in Salmonella/ Microsome Test

Accession No.: 260307

Test Material: Thiabendazole (TBZ), O-phenylphenol (OPP), diphenyl (DP) and parahydroxy-butylbenzoate (PHBB).

Study No.: Not available

Sponsor: Merck Sharpe & Dohme

Testing Facility: Tokyo Metropolitan Research Laboratory of Public Health

Study Design:

The material and methods used in the assay do not comply with the EPA Health Effects Test Guidelines, No. 560/6-83-001 for conducting the Ames Mutagenicity Assay.

Combinations of TBZ and the above fungicides OPP, DP, or PHBB were tested for revertant induction; however, considering the assay conditions and the high concentrations of TBZ necessary to demonstrate a very weak positive result, the results are not relevant to assess the mutagenicity of TBZ.

Title of Report: Mutagenicity of Paired Fungicide Mixtures in the Salmonella/Microsome Test

Classification: Unacceptable, because of the reasons discussed in the study design.