UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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

FROM William Dykstra
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TO Henry Jacoby
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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#77-014-0, 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 5 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 27.3 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 fifty-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 II: 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 mice or both given the middle and high concentrations.

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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 concentration female mouse. Although it is an occasionally encounted incidental lesion, because of the greater incidence in the above groups, it was considered due to 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 control groups, although the incidence of Type "B" hepatocellular neoplasms was slightly greater in females given the highest concentration. The latter difference was 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 were combined, as shown in Table I.

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	13	6	3	3	6	4
WITH BENIGN NEOPLASMS	9	12	18	13	11	11	13	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	11	12	16	13	6	11
OF NEOPLASMS	18	16	25	19	21	21	21	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	-	-	-	-	-

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%

CONTINUED
TABLE

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)	-	-	-	-	-	-	[illegible]	-	-	-	-	-
ADENOMA (LACRIMAL GLAND)	-	1	1	1	-	1	-	1	-	3	-	2
FIBROSARCOMA (TAIL)	-	-	1	-	-	-	-	-	-	-	-	-
LEUKEMIA (PRIMARY SITE UNDETERMINED)	5	2	3	-	3	3	[illegible]	2	-	1	1	2
RETICULUM CELL SARCOMA (PRIMARY SITE UNDETERMINED)	-	-	1	-	1	-	[illegible]	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%