4-15-86

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

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005038

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

To: Michael McDavit, PM #61
Special Review Branch
Registration Division (TS-767)

From: Judith W. Hauswirth, Ph.D.
Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769)

Thru: Reto Engler, Ph.D., Chief
Mission Support Staff
Toxicology Division
Hazard Evaluation Division (TS-769)

and

Theodore M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Subject: Mancozeb; Data Call-In Notice of April 30, 1985. Submission of Subchronic Feeding Studies of ETU in Rats and Mice. Rohm and Haas Company. Accession No: 259838.

Caswell #'s 913A, 443AA, 539

Action Requested: Review of study entitled "Dithane M-45 and Ethylene-thiourea (ETU) 3 Month Dietary Study in Mice". Report No. 80R-124. by G. P. O'Hara and L. J. Di Donato.

Background: In the Data Call-In Notice of April 30, 1985 the Agency requested a subchronic feeding study of ETU in mice under several of the EBDC's for NRDC reassessment. Rohm and Haas has submitted this study to fulfill this requirement to support their registered products containing mancozeb and maneb.

Conclusions:

1. Administration of Dithane M-45 (mancozeb) to Charles River CD-1 mice for 90 days resulted in an increased incidence of thyroid follicular cell hyperplasia and hypertrophy in male and female mice and a decrease in

1 of 13

hepatic aminopyrine N-demethylase in male mice at levels of 1000 ppm and higher. The NOEL for these effects was 100 ppm. This level was equivalent to 18.13 mg/kg/day in male mice and 21.68 mg/kg/day in female mice.

2. Administration of ethylenethiourea (ETU) to Charles River CD-1 mice for 90 days resulted in increased relative thyroid weights in female mice and an increased incidence of follicular cell hyperplasia in male and female mice at levels of 100 ppm and higher in the diet. The NOEL for these effects was 10 ppm. The level was equivalent to 1.72 mg/kg/day in male mice and 2.38 mg/kg/day in female mice.

Classification:

Acceptable - This study is acceptable for the purposes of the DCI on ETU. ETU has been shown to cause liver tumors in mice. This study was designed to determine a NOEL for the effects of ETU on mouse liver. A NOEL of 100 ppm for liver toxicity was established based upon centrilobular hepatocytic hypertrophy seen at 1000 ppm ETU. However, the NOEL for the entire study was 10 ppm based upon increased relative thyroid weights in female mice and an increased incidence of thyroid follicular cell hyperplasia in male and female mice at higher dosages of ETU. Considering the sensitivity of mouse thyroid to ETU and using hindsight, some determination of thyroid function in these animals would have been advantageous. This study was not part of the DCI on Dithane M-45 (mancozeb).

Review

Study Title: Dithane M-45 and Ethylenethioura (ETU) 3 Month Dietary Study in Mice.

Protocol No.: 80P-15

Report No.: 80R-124

Authors: G. P. O'Hara and L. J. DiDonato

Conducted By: Toxicology Department
Rohm and Haas Company

Test Substance:
1. Dithane M-45 (zinc coordination compound of manganese ethylenebisdithiocarbamate). Lot No. 2-8767, 83.1% A.I. Sample No. 80-10.
2. Purified ethylenethiourea (ETU) Matheson, Coleman and Bell. Recrystallized. Sample No. 80-11.

Dosages: Dithane 0, 10, 100, 1000 and 10,000 ppm
ETU 0, 1, 10, 100 and 1000 ppm

Route of Administration : Dietary

Animals: Charles River CD-1 mice (approx. 27 days old).

Acclimation : Two weeks

005038

No. of Animals: 15 mice/sex/group (two control groups were run).

Diet Preparation: Analyzed weekly and at the time of preparation for Dithane M-45 and sTU.

Observations: Mice were observed twice daily for clinical signs.

Body Weights: Individually recorded one week prior to the initiation of dosing and weekly to termination.

Food Consumption: Weekly

Laboratory Studies: Blood samples were taken from 10 mice/sex/group at week thirteen for analysis. Mice were fasted overnight and anesthetized with ether.

o Hematology

packed cell volume	red cell count
hemoglobin	white cell count
red cell morphology	
differential white cell count	

o Clinical Chemistry

serum urea nitrogen
serum glutamic pyruvic transaminase

Liver samples were taken at thirteen weeks for determination of hepatic mixed function oxidase (MFO) activity on the postmitochondrial fraction.

o MFO Activities

p-nitroanisole O-demethylation
aminopyrine N-demethylation
aniline hydroxylation

Necropsy and Organ Weights:

The following organ weights were recorded for each mouse:

adrenals*	muscle, skeletal
bone marrow and smear*	muscle, peripheral
brain (3 sections)*	nerve, peripheral
esophagus	ovaries
eyes (2)	pancreas*
gall bladder	pituitary*
gross lesions*	prostate
heart*	salivary gland
intestine	seminal vesicles
colon, jejunum	skin
duodenum, rectum	spinal cord (2)
ileum, cecum	spleen*

kidneys (2)*	stomach*
larynx	testes*
liver (2 sections)*	trachea
lung (2)*	thymus
lymph gland (mesenteric)*	thyroid/parathyroid*
mammary gland	urinary bladder
masses*	uterus

*These tissues were examined for all mice in the study.

Statistical Methodology:

According to the report the following statistical analyses were done:

"Weekly body weight and food consumption data were analyzed by analysis of covariance. The covariate utilized was the week 0 value for body weight and food consumption respectively. A two-way analysis (group X sex) and separate analyses for each sex were performed. The two control groups were found not to be significantly different and were combined. Treated groups were compared to the combined control using linear contrasts.

Organ weights, clinical chemistry, and hematology with the exception of WBC differential were analyzed utilizing two-way (group by sex) analysis of variance and separate one-way analyses of variance for each sex. The two control groups were found not be significantly different and were combined. Treated groups were compared to the combined control groups utilizing Duncans multiple range test.

WBC differential data were analyzed using the modified Jonckheere trend test.

A probability level of less than 0.05 was considered significant throughout".

Results:

Clinical Observations: None due to treatment.

Mortality: None due to treatment.

Body Weights:

Terminal Body Weights(g)

Group(ppm)	Male	Female	Treatment
0	38.9	29.8	none
0	40.3	29.8	none
10	39.5	29.1	Dithane M-45
100	39.2	29.9	Dithane M-45
1000	38.5	29.4	Dithane M-45
10000	36.5**	28.1*	Dithane M-45
1	37.7*	28.8	ETU
10	38.3*	27.8	ETU
100	37.7	29.1	ETU
1000	37.9*	28.7*	ETU

* p<0.05 when compared to combined control group
** p<0.01 when compared to combined control group

The decrease in body weight in the 10,000 ppm Dithane M-45 group is considered to be treatment related in both male and female mice and was seen consistently throughout the treatment period.

In the ETU treated groups a slight effect on body weight was seen sporadically throughout the treatment period. This reviewer does not consider it to be related to ETU treatment since no dose-response was seen.

Food Consumption:

Group	Terminal Food Consumption Values (g) Males	Females	Treatment
0	6.11	6.28	none
0	6.01	6.51	none
10	5.75	6.09	Dithane M-45
100	5.85	5.35*	Dithane M-45
1000	5.33	6.21	Dithane M-45
10000	5.19*	4.85*	Dithane M-45
1	5.00**	5.69	ETU
10	5.40	6.05	ETU
100	6.09	6.19	ETU
1000	5.45	5.80	ETU

* p<0.05 when compared to combined controls
** p<0.01 when compared to combined controls

The decrease in food consumption seen in the 10,000 ppm Dithane M-45 group is considered to be treatment related in both males and females. This decrease was also accompanied by a statistically significant decrease in body weight.

The sporadic effects on food consumption seen in the ETU groups are not considered, by this reviewer, to be related to treatment.

Compound Intake:

The following table was taken directly from the report.

Group No.	Test Substance	Dietary Conc. (ppm A.I.)	Thirteen Wk. Mean Compd. Intake (mg/kg/day) Males	Females
3	Dithane M-45	10	1.78	2.34
4	Dithane M-45	100	18.13	21.68
5	Dithane M-45	1000	166.9	233.8
6	Dithane M-45	10000	1662.5	2160.2
7	ETU	1	0.16	0.22
8	ETU	10	1.72	2.38
9	ETU	100	18.18	24.09
10	ETU	1000	168.2	231.1

005038
005039

Laboratory Studies:

Hematology: No treatment related effects.

Clinical chemistry: No treatment related effects.

Hepatic Mixed Function Oxidase Activity (MFO Activity):

Test Substance	Group	N	MFO Activity AH[1]	AP[2]	PNA[3]
			Males		
none	0	12	221±40	1295±243	439±139
Dithane M-45	10	6	215±56	1248±371	358±103
Dithane M-45	100	6	193±32	1307±98	532±254
Dithane M-45	1000	6	164±33	803±209*	288±118
Dithane M-45	10000	6	98±26*	854±395*	289±169
ETU	1	6	180±35	982±298	379±205
ETU	10	6	184±35	1102±327	399±271
ETU	100	6	227±36	1099±335	483±137
ETU	1000	6	355±82*	1105±318	810±284*
			Females		
none	0	12	216±42	1610±561	454±193
Dithane M-45	10	6	226±59	1576±527	333±169
Dithane M-45	100	6	200±37	1149±256	342±155
Dithane M-45	1000	6	206±10	1141±333	456±296
Dithane M-45	10000	6	112±5*	981±544	322±51
ETU	1	6	221±31	1209±523	289±109
ETU	10	6	193±36	1396±557	457±129
ETU	100	6	214±58	1089±226	311±80
ETU	1000	6	270±57	1048±308	512±322

* $p<0.05$ when compared to control group

1 aniline hydroxylase: nmoles p-aminophenol produced per gram liver per 4.8 min. at 37°C.

2 aminopyrine N-demethylase: nmoles formaldehyde formed per gram liver per 4.8 min. at 37°C.

3 p-nitroanisole O-demethylase: nmoles p-nitrophenol formed per gram liver per 4.8 min. at 37°C.

Dithane M-45 treatment resulted in a statistically significant increase in aniline hydroxylase activity in male and female mice at 10,000 ppm in the diet and in aminopyrine N-demethylase activity in male mice at 1000 ppm and 10000 ppm in the diet. ETU administration resulted in a statistically significant decrease in aniline hydroxylase activity and p-nitroanisole O-demethylase activity in male mice at 1000 ppm in the diet.

005038

Organ Weights:

Treatment related effects on organ weights are summarized in the table below.

Group	Treatment	Mean Organ Weights Thyroid absolute(g)	Thyroid relative	Liver absolute(g)	Liver relative
		Males			
0	none	0.0044	1.28	1.50	439
0	none	0.0049	1.39	1.54	439
10	Dithane M-45	0.0042	1.25	1.50	440
100	Dithane M-45	0.0053	1.59	1.50	448
1000	Dithane M-45	0.0044	1.39	1.47	457
10000	Dithane M-45	0.0082*	2.79**	1.45	493**
1	ETU	0.0048	1.45	1.43	436
10	ETU	0.0045	1.34	1.46	441
100	ETU	0.0045	1.42	1.47	457
1000	ETU	0.0124**	3.81**	1.74**	531**
		Females			
0	none	0.0043	1.68	1.19	463
0	none	0.0047	1.83	1.20	471
10	Dithane M-45	0.0042	1.63	1.16	457
100	Dithane M-45	0.0049	1.92	1.15	448
1000	Dithane M-45	0.0054	2.18	1.15	456
10000	Dithane M-45	0.0073**	3.09**	1.22	516**
1	ETU	0.0041	1.69	1.08	441
10	ETU	0.0041	1.69	1.11	455
100	ETU	0.0041	1.68	1.23	499*
1000	ETU	0.0092**	3.76**	1.30	529**

* $p<0.05$ when compared to combined control groups
** $p<0.01$ when compared to combined control groups

Although not shown in the above table, there was also a significant increase in relative kidney weights at 10,000 ppm in both sexes of mice treated with Dithane M-45. No other treatment related effects on organ weights were observed.

Necropsy and Histopathology:

The report states that special attention was given to the examination of thyroids and liver since ETU is known to have adverse effects on these tissues. Three different pathologists examined slides. Their diagnoses will be given in separate tables.

Results of the Original Pathologist: Roger A. Ball, D.V.M. Ph.D.

005038

Incidence of Pertinent Lesions
Dithane M-45

Group (ppm)	0	0	10	100	1000	10000
			Males			
Thyroid (N)	15	15	15	15	15	15
follicular hyperplasia	2	3	2	3	2	15
decreased colloid density	2	0	1	4	1	14
congestion	0	1	1	0	1	13
follicular epithelium cytopl. vacuoles	0	0	1	1	1	11
follicular colloid granular	0	0	0	0	0	1
papillary hyperplasia	0	0	0	0	0	1
Liver (N)	15	15	15	15	15	15
centrilobular hypertrophy inc. cytopl. eosino.	0	0	1	1	0	0
hepatocyte pleomorphism	5	5	7	7	6	7
inc. intranuclear inclusions	2	0	0	0	1	4
diffuse cytoplasmic vacuolation	8	8	7	11	11	3
			Females			
Adrenal Glands (N)	15	15	15	15	15	15
increased pigment	1	0	1	0	0	7
Thyroid (N)	15	15	15	15	15	15
follicular hyperplasia	0	1	2	5	6	15
decreased colloid density	0	2	1	2	3	14
congestion	0	0	0	1	3	14
follicular epithelium cytopl. vacuoles	0	0	1	0	1	11
follicular colloid granular	0	0	0	0	0	1
Liver (N)	15	15	15	15	15	15
centrilobular hypertrophy inc. cytopl. eosinoph.	0	0	0	0	0	1
hepatic pleomorphism	3	1	5	4	5	10
focal/multifocal hepatic necrosis	1	1	1	0	6	3

Dimethane M-45 treatment resulted in an increased incidence of hepatic pleomorphism in the 10000 ppm female group and of follicular hyperplasia, decreased colloid density, congestion and follicular epithelial cytoplasmic

vacuoles in the thyroids of the 10000 ppm male and female groups. In females there was a dose related increase in thyroid hyperplasia. The no-observed-effect level appeared to be 10 ppm for this effect.

Incidence of Pertinent Lesions
ETU

Group (ppm)	0	0	1	10	100	1000
			Males			
Thyroid (N)	15	15	15	14	15	15
follicular hyperplasia	2	3	1	0	5	15
decreased colloid density	2	0	0	0	5	15
congestion	0	1	0	0	0	14
follicular epithelium cytopls. vacuoles	0	0	1	0	0	14
follicular colloid granular	0	0	0	0	0	5
Liver (N)	15	15	15	15	15	15
centrilobular hypertrophy inc. cytopl. eosinoph.	0	0	0	1	0	11
inc. cytopl. basophil.	0	0	0	0	0	4
hepatocytic pleomorphism	5	5	6	5	5	13
inc. intranuclear inclusions	2	0	0	0	0	7
			Female			
Thyroid (N)	15	15	15	15	15	15
follicular hyperplasia	0	1	1	2	4	15
decreased colloid density	0	2	0	2	3	13
congestion	0	0	1	2	2	8
follicular epithelium cytoplas. vacuoles	0	0	0	1	1	10
follicular colloid granular	0	0	0	0	0	5
Liver (N)	15	15	15	15	15	15
centrilobular hypertrophy inc. cytopl. eosinoph.	0	0	0	0	0	7
hepatocytic pleomorphism	3	1	4	4	4	12

ETU administration resulted in an increased incidence of:

Liver: 1. centrilobular hypertrophy, hepatic pleomorphism and increased intranuclear inclusions in the high dose male mice; and

2. centrilobular hypertrophy and hepatocytic pleomorphism in the high dose female mice.

005038

Thyroid: 1. follicular hyperplasia and decreased colloid density in the 100 and 1000 ppm male and female mice; and

2. congestion, cytoplasmic vacuoles of the follicular epithelium and granular follicular colloid in the high dose male and female mice.

Results of the Second Pathologist: Dr. W. R. Brown

Dr. Brown examined only thyroid and liver slides of the Dithane M-45 treated groups and ~~thyroid~~ slides of the ETU treated groups.

Incidence of Liver and Thyroid Lesions in Dithane M-45 Treated Mice

Group (ppm)	0	0	10	100	1000	10000
			Males			
Thyroid (N)	15	15	15	15	15	15
follicular cell						
hypertrophy	2	1	1	3	7	14
hyperplasia	0	0	0	0	1	3
vacuolation	0	1	0	1	2	12
decreased colloid						
density	0	0	0	0	1	9
congestion	0	0	0	0	0	3
Liver (N)	15	15	15	15	15	15
centrilobular hepatocytic						
hypertrophy	1	5	1	3	0	1
hepatocytic vacuolation	12	10	10	12	10	4
			Females			
Thyroid (N)	15	15	15	15	14	15
follicular cell						
hypertrophy	1	3	2	4	7	15
hyperplasia	0	0	0	0	2	9
vacuolation	0	1	1	2	3	13
decreased colloid						
density	0	1	0	1	0	12
congestion	0	0	0	0	2	11
Liver (N)	15	15	15	15	15	15
centrilobular hepatocytic						
hypertrophy	0	0	1	0	1	0
hepatocytic vacuolation	4	10	7	12	7	11

005038

Incidence of Liver Lesions in ETU Treated Mice

Group (ppm)	0	0	1	10	100	1000
			Males			
Liver (N)	15	15	15	15	15	15
centrilobular hepatocytic hypertrophy	1	5	3	2	4	15
hepatocytic vacuolation	12	10	8	7	10	2
			Females			
Liver (N)	15	15	15	15	15	15
centrilobular hepatocytic hypertrophy	0	0	1	1	1	15
hepatocytic vacuolation	4	10	7	9	11	6

Dr. Brown did not find any treatment related lesions of the liver in the Dithane M-45 treated groups, but reported an increased incidence of centrilobular hepatocytic hypertrophy in male and female mice fed 1000 ppm ETU in the diet.

The no-observed-effect level for Dithane M-45 on the thyroid in both male and female mice was 100 ppm based upon follicular cell hypertrophy and hyperplasia seen at 1000 and 10000 ppm. This differed from Dr. Ball's report for female mice. He found a dose-related increase in thyroid hyperplasia in female mice with an apparent no-observed-effect level of 10 ppm.

Results of the Third Pathologist Gary Burger, D.V.M:

Dr. Burger examined the thyroid slides from all female mice in the study.

Incidence of Thyroid Lesions in Female Mice

Group (ppm)	0	0	10	100	1000	10000
			Dithane M-45 Treated			
Thyroid (N)	15	15	15	15	13	15
follicular cell hyperplasia	1	2	1	2	5	15
hypertropy	1	1	2	1	7	15
vacuolation	0	0	1	0	6	15
decreased colloid density	0	0	0	0	0	11

ETU Treated

Group (ppm)	0	0	1	10	100	1000
Thyroid (N)	15	15	15	14	15	14
follicular cell						
hyperplasia	1	2	3	3	6	14
hypertrophy	1	1	0	3	7	14
vacuolation	0	0	0	2	7	14
decreased colloid						
density	0	0	1	0	0	8

From Dr. Burger's reading of the thyroid slides, this reviewer concludes that the NOEL for thyroid effects is 100 ppm for Dithane M-45 and 10 ppm for ETU.

Discussion of the Three Pathologists Findings:

The two major areas of disagreement were thyroid hyperplasia in Dithane M-45 treated female mice and liver lesions in Dithane treated female mice. Dr. Ball reported an increased incidence of thyroid hyperplasia in the 100 ppm Dithane treated group which he described as slight. Drs. Brown and Burger did not report an increase of this lesion at 100 ppm.

Thyroid Hyperplasia in Dithane M-45 Treated Female Mice

Group (ppm)	0	0	10	100	1000	10000
Ball	0/15	1/15	2/15	5/15	6/15	15/15
Brown	0/15	0/15	0/15	0/15	2/14	9/15
Burger	1/15	2/15	1/15	2/15	5/13	15/15

This reviewer considers the NOEL for the effects of Dithane M-45 in female mice to be 100 ppm based upon the reports of all three pathologists. The effect described by Dr. Ball at 100 ppm was slight and not diagnosed by the other two pathologists.

Dr. Ball reported an NOEL of 1000 ppm Dithane M-45 for liver effects in female mice based upon an increase in hepatic pleomorphism. Dr. Brown did not report any treatment related effects due to Dithane M-45 on the liver of female mice.

Conclusions:

1. Administration of Dithane M-45 (mancozeb) to Charles River CD-1 mice for 90 days resulted in an increased incidence of thyroid follicular cell hyperplasia and hypertrophy in male and female mice and a decrease in hepatic aminopyrine N-demethylase in male mice at levels of 1000 ppm

and higher. The NOEL for these effects was 100 ppm. This level was equivalent to 18.13 mg/kg/day in male mice and 21.88mg/kg/day in female mice.

2. Administaration of ethylenethiourea to Charles River CD-1 mice for 90 days resulted in increased relative thyroid weights in female mice and an increased incidence of follicular cell hyperplasia in male and female mice at levels of 100 ppm and higher in the diet. The NOEL for these effects was 10 ppm. This level was equivalent to 1.71 mg/kg/day in male mice and 2.38 mg/kg/day in female mice.

Classification: This study is acceptable for the purposes of the DCI on ETU. ETU has been shown to induce liver tumors in mice. This study was designed to determine a NOEL for the effects of ETU on mouse liver. A NOEL of 100 ppm was established based upon centrilobular hepatocytic hypertrophy seen in mice at 1000 ppm ETU in the diet. However, the NOEL for the entire study was 10 ppm ETU based upon increased relative thyroid weights in female mice and an increased incidence of follicular cell hyperplasia in male and female mice at levels of 100 ppm and higher in the diet. Considering the sensitivity of the mouse thyroid to ETU and using hindsight, a determination of thyroid function in these animals would have been advantageous. This study was not part of the DCI on Dithane M-45 (mancozeb).