041A

CASWELL FILE

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

010071

MAR 10 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metiram® Premix 95% - 3 Month Feeding Study in Mice

TO: Terri Stowe
PM Team Reviewer (71)
Reregistration Branch, SRRD (H7508W)

FROM: Linda L. Taylor, Ph.D Linda Lee Taylor 3/8/93
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 3/8/93
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 3/8/93
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant: BASF
Chemical: mixture of ammoniates of [ethylenebis (dithiocarbamate)]zinc with ethylenebis [dithiocarbamic acid], bimolecular and trimolecular cyclic anhydrosulfides and disulfides
Synonyms: Metiram®, Polyram®, Polram-Comi®, Carbatene® Zinc Metiram®
Submission No.: S432998
Caswell No.: 041A
DP Barcode: D186334
Identifying No.: 014601
MRID No.: 425950-01
Action Requested: Please review the metiram oral Tox. mice study and give an updated status of the metiram and ETU tox. data requirements. An oral tox. rat study was sent to review earlier on 12/16/92 (D185804). Please send a copy of the review and update.

Comment: The Registrant has submitted a study entitled: "Toxicology Study; Study of the Oral Toxicity of Metiram Premix 95% in the B6C3F1 mice - Administration in the Diet for 3 Months". The stated objective of the study was to determine the toxicological profile of Metiram Premix 95% after dietary administration for 3 months,

including the target organs and the "no adverse effect level". This subchronic study has been reviewed, and the DER is appended.

Under the conditions of the study, oral administration of Metiram Premix 95% to B6C3F1 mice for 3 months at dose levels of 300 ppm (84 ♂/133 ♀ mg/kg), 1000 [302 ♂/465 ♀ mg/kg], 3000 ppm [853 ♂/1448 ♀ mg/kg], and 7500 ppm [2367 ♂/3565 ♀ mg/kg] resulted in decreased body weight/ gain in both sexes at the high-dose levels and in females at the next two lower dose levels also. There was a decrease in total serum T_4 concentrations in both sexes at the 1000, 3000, and 7500 ppm dose levels (dose-related), and an increase in total serum T_3 concentration in the high-dose males. The absolute and relative liver weights were increased in both sexes at the high-dose level, and the absolute and relative weights of the adrenal glands were increased (dose-related) in females at the 3000 and 7500 ppm dose levels, with an increase in the severity of fatty degeneration of the "X" zone noted in these mice. In the thyroid, there was a dose-related increase in the incidence of minimal or slight hypertrophy and vacuolation of the thyroid follicular epithelium in both sexes at the 3000 and 7500 ppm dose levels. The NOEL can be set at 300 ppm (84 ♂/133 ♀ mg/kg), the LOEL at 1000 ppm (302 ♂/465 ♀ mg/kg), based on the decrease in total serum T_4 concentrations in both sexes.

This study is classified Core Minimum, and it satisfies the guideline requirement (82-1) for a subchronic feeding study in rodents.

In the mouse carcinogenicity study on Metiram (Accession # 242192), no significant effects were observed at any dose level (0, 100, 300, 1000 ppm). Based on the data from the current 90-day study and the known effect of Metiram on the thyroid, it is concluded that the carcinogenicity study may be upgraded to Core Minimum, based on the dose-related decrease in T_4 levels observed in both sexes at dose levels of 1000 ppm and above.

The data available on Metiram are listed below.

A. Acute oral LD_{50} - rat	LD_{50} > 10 ♂/8 ♀ g/kg Tox.Cat.IV
B. Acute dermal LD_{50} -rabbit	LD_{50} > 2000 mg/kg Tox.Cat.III
C. Acute inhalation LD_{50} - rat	LC_{50} > 5.7 mg/L Tox.Cat. IV
D. Primary eye irritation - rabbit	nonirritating; Tox.Cat. III
E. Primary dermal irritation - rabbit	nonirritating; Tox.Cat. IV
F. Dermal sensitization - guinea pig	strong-to-severe sensitizer
G. 21-Day dermal - rabbit	Supplementary
H. 90-day feeding - rat (bridging)	Minimum
90-day feeding - mouse	Minimum
90-day inhalation - rat	Minimum
I. 13-week subchronic - dog	not required
J. Developmental toxicity - rat	Minimum
K. Developmental toxicity - rabbit	Supplementary
L. Chronic toxicity - dog	Minimum
M. 2-Generation reproduction - rat	Minimum

N. Chronic tox/carcinogenicity - rat		Minimum
O. Carcinogenicity - mouse		Minimum
P. Mutagenicity -	Category I	Acceptable
	Category II	Acceptable
	Category III	Acceptable
Q. Metabolism - rat		Minimum

With regard to ETU data requirements, ETU is not one of this reviewer's chemicals. The latest information on ETU of which I am aware may be found in the FEDERAL REGISTER document, Volume 57, # 41, Monday, March 2, 1992, Notice of Intent to Cancel.

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/8/93
Tox. Branch II, Section II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 3/8/93
Tox. Branch II, Head Section II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Oral - mouse TOX. CHEM. NO.: 041A

MRID NO.: 425950-01 Schaughnessy No.:014601

TEST MATERIAL: Metiram Premix 95%

SYNONYMS: mixture of ammoniates of [ethylenebis(dithiocarbamate)] zinc with ethylenebis [dithiocarbamic acid], bimolecular and trimolecular cyclic anhydrosulfides and disulfides

STUDY NUMBER: 35C0331/90036; Document # 92/11223

SPONSOR: BASF Corporation/Agricultural Products Group, Research Triangle Park, NC

TESTING FACILITY: Department of Toxicology/BASF Aktiengesellschaft, D-W6700 Ludwigshafen/Rhein, FRG

TITLE OF REPORT: Study of the Oral Toxicology of Metiram Premix 95% in the B6C3F1 Mice - Administration in the Diet for 3 Months

AUTHOR: W. Mellert

REPORT ISSUED: October 16, 1992

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, oral administration of Metiram Premix 95% to B6C3F1 mice for 3 months at dose levels of 300 ppm (84 ♂/133 ♀ mg/kg), 1000 [302 ♂/465 ♀ mg/kg], 3000 ppm [853 ♂/1448 ♀ mg/kg], and 7500 ppm [2367/3565 mg/kg] resulted in decreased body weight/gain in both sexes at the high-dose levels and in females at the next two lower dose levels also. There was a decrease in total serum T_4 concentrations in both sexes at the 1000, 3000, and 7500 ppm dose levels (dose-related), and an increase in total serum T_3 concentration in the high-dose males. The absolute and relative liver weights were increased in both sexes at the high-dose level, and the absolute and relative weights of the adrenal glands were increased (dose-related) in females at the 3000 and 7500 ppm dose levels, with an increase in the severity of fatty degeneration of the "X" zone noted in these mice. In the thyroid, there was a dose-related increase in the incidence of minimal or slight hypertrophy and vacuolation of the thyroid follicular epithelium in both sexes at the 3000 and 7500 ppm dose levels. The NOEL can be set at 300 ppm (84 ♂/133 ♀ mg/kg), the LOEL

at 1000 ppm (302 ♂/465 ♀ mg/kg), based on the decrease in total serum T_4 concentrations in both sexes.

Classification: Core Minimum. This study satisfies the guideline requirement (82-1) for a subchronic feeding study in rodents.

A. MATERIALS:

1. Test Compound: Metiram Premix 95%; Description: solid/pale yellow powder; Batch #: WF 5103/Test substance #: 90/331-1; Purity: 94.8% [data from rat study (MRID # 425391-01)].

2. Test Animals: Species: mouse; Strain: B6C3F1; Age: 49 days old at study start; Weight: males 22-25 g, females 17-21 g; Source: Charles River Wiga GmbH, D-W8741 Sulzfeld, FRG.

3. Statistics: Means and standard deviations were calculated for the variables: food consumption, body weight, body-weight change, and test material intake: with the exception of test material intake, statistical significance was determined by analysis of variance (ANOVA) followed by a Dunnett's test; clinical chemistry, hematology, and hormones: with the exception of the differential blood count, a statistical one-way analysis of variance was done via the KRUSKAL-WALLIS-h-test. If the resulting p-value was ≤ 0.05, a comparison of each dose group with the control group was carried out. This comparison was done using the MANN-WHITNEY-U-test for the hypothesis of equal medians; terminal body weight, absolute, and relative organ weights: statistical evaluation was determined using the DUNNETT test for simultaneous comparison of several dose groups with a control group.

B. STUDY DESIGN

1. Methodology: Fifty males and 50 females [acclimated for 8 days] were assigned (distributed according to body weight among groups/sex; computer-generated randomization list) to one of five groups, each composed of 10 mice/sex, and administered the test material [0, 300, 1000, 3000, or 7500 ppm] via the diet for 90 consecutive days. The mice were housed individually and were provided with food [Kliba rat/mouse/ hamster maintenance diet, 343 meal, Klingentalmühle AG, CH-4303 Kaiseraugst, Switzerland] and water ad libitum. The dose levels selected were based on the results of a 4-week study (1976) and a carcinogenicity (1979) study [references do not list MRID/Accession #'s for either study].

   Dose preparation: The test material was sieved (500/250 μm) and then weighed out and mixed thoroughly with a small amount of food. Subsequently, additional food was added to this premix and mixed to obtain the desired concentration. The test diets were prepared twice a week. Prior to study start and after termination, investigations were performed to characterize the test material (purity, homogeneity, and stability). Stability of the test material in the food was determined analytically for 4- and 35-day intervals for the 300 and 7500 ppm dose levels. The concentrations attained were measured (using gas chromatography) prior to and at the

end of the study. Homogeneity of the test material in food was verified at the high- and low-dose levels in the parallel-running rat study (MRID # 425391-01).

RESULTS

Data from the parallel-running rat study were referenced for test material purity (94.8%) and homogeneity. At study end, reanalysis showed a degree of purity of 88.2%, which is said to be in agreement with the known stability of Metiram Premix 95%. It was concluded that the stability of the test material in the diet was acceptable over a 4-day period (test diets prepared twice a week). The concentration analyses at the start and end of the study indicate that the animals received the proposed amounts of test material (Table 1).

Table 1. Concentration of Test Material Achieved (=%)

Measurement/Dose	300 ppm	1000 ppm	3000 ppm	7500 ppm
Start	105%	107%	107%	96%
End	94%	94%	97%	92%

2. Clinical Observations/Body Weight/Food/Compound Consumption: The animals were observed daily (twice on weekdays, once weekends) for mortality/moribundity and general state of health. An additional inspection with palpation was performed once a week. Individual body weights were determined for the randomization procedure (Day 0) and weekly thereafter. Food consumption was determined once a week (one-day measure). Test material intake was calculated using the formula:

$$\frac{FC \times D}{BW_x} = \text{substance intake in mg/kg body weight}$$

FC = food consumption (grams) from Day x-1 to Day x
D = dose in ppm
BW_x = body weight (grams) on Day x of study

RESULTS

Survival and Clinical Observations

There were no deaths during the study, and no treatment-related clinical symptoms were observed.

Body Weight and Food Consumption

Although there were several time points where a statistically significant decrease in body weight was observed at the high-dose level (7500 ppm) for both sexes and at the next two lower dose levels (3000 and 1000 ppm) in females compared to the control groups, the magnitude of the decreases was small and not always dose-related or consistent with a 75-fold difference in dose. Body-weight gain was reduced in males

(dose-related from Day 14 on), with the high-dose displaying a statistically significant decrease from Day 14 to 28 (Table 3). In females, a similar (not always dose-related) decrease in body-weight gain was observed from Day 14 on, with statistical significance being attained at the 1000, 3000, and 7500 ppm dose levels. On a percent basis, body-weight change was reduced in both sexes (dose-related in males from Day 14 on and in females at Days 14 and 20 only) at the high-dose level throughout most of the study (Table 4, calculated by this reviewer, statistical analysis was not performed).

Table 2. Mean Body Weight (% of control)

Day/Dose	300 ppm	1000 ppm	3000 ppm	7500 ppm
MALES				
0	101	100	100	99
7	101	99	99	97
14	101	98	97	95*
21	103	99	98	95*
28	102	98	96	93**
35	103	100	98	95
42	102	99	98	95
49	103	101	99	97
56	104	101	99	97
63	104	101	99	97
70	105	101	99	95
77	102	99	97	94
84	103	99	97	95
91	103	100	96	96
FEMALES				
0	101	101	99	102
7	98	97	97	96
14	99	95*	94**	94**
21	99	95*	95**	95**
28	99	95*	97	95*
35	98	94**	94**	92**
42	99	95**	94**	94**
49	100	95*	94**	95*
56	99	96	96	95
63	99	95*	95*	95*
70	99	95*	93**	93**
77	100	96	95	96
84	98	94*	94*	95
91	96	93*	94*	93*

* $p<0.05$; ** $p<0.01$

Table 3. Mean Body-Weight Change [grams (% of control value)]

Interval/Group	0 ppm	300 ppm	1000 ppm	3000 ppm	7500 ppm
MALES					
From Day 0 to:					
7	1.3	1.3	0.9(69)	0.7*(54)	0.8(62)
14	2.1	2.1	1.6(76)	1.5(71)	1.0**(48)
21	2.5	2.9	2.2(88)	2.1(84)	1.3**(52)
28	3.6	3.8	3.0(83)	2.7(75)	2.1**(58)
35	3.9	4.6	4.0	3.6(92)	2.9(74)
42	4.3	4.7	4.1(95)	3.8(88)	3.2(74)
49	4.8	5.5	4.9	4.6(96)	4.1(85)
56	5.0	5.8	5.1	4.9(98)	4.4(88)
63	5.2	6.2	5.3	4.9(94)	4.6(88)
70	6.2	7.3	6.3	5.9(95)	5.0(81)
77	6.7	7.3	6.3(94)	5.9(88)	5.3(79)
84	7.2	8.0	6.9(96)	6.5(90)	5.9(82)
91	7.5	8.3	7.3(97)	7.2(96)	6.4(85)

Interval/Group	0 ppm	300 ppm	1000 ppm	3000 ppm	7500 ppm
FEMALES					
From Day 0 to:					
7	1.5	0.9(60)	0.8(53)	0.9(60)	0.4(27)
14	2.9	2.4(83)	1.8*(62)	1.5**(52)	1.2**(41)
21	3.5	3.1(89)	2.5*(71)	2.4**(69)	2.0**(57)
28	4.4	4.0(91)	3.4*(77)	3.8(86)	3.0**(68)
35	5.5	4.8(87)	4.0**(73)	4.1**(75)	3.3**(60)
42	5.5	4.9(89)	4.2**(76)	4.1**(75)	3.8**(69)
49	5.8	5.6(97)	4.6**(79)	4.6**(79)	4.5**(78)
56	5.9	5.5(93)	4.7*(80)	4.8(81)	4.4**(75)
63	6.6	6.2(94)	5.3**(80)	5.5*(83)	5.9**(76)
70	7.4	6.9(93)	6.0**(81)	5.7**(77)	5.2**(70)
77	7.4	7.1(96)	6.2(84)	6.1*(82)	6.1*(82)
84	7.8	7.2(92)	6.2**(79)	6.3*(81)	6.1**(78)
91	8.1	6.9(85)	6.3**(78)	6.7*(83)	6.0**(74)

* $p<0.05$; ** $p<0.01$;

Table 4. Body-Weight Change (%)

Interval/Group	0 ppm	300 ppm	1000 ppm	3000 ppm	7500 ppm
MALES					
From Day 0 to:					
7	5.4	5.4	3.8	2.9	3.4
14	8.8	8.7	6.7	6.3	4.2
21	10.5	12.0	9.2	8.8	5.5
28	15.1	15.8	12.5	11.3	8.9
56	20.9	24.1	21.3	20.6	18.6
91	31.4	34.4	30.4	30.3	27.0
FEMALES					
From Day 0 to:					
7	8.0	4.8	4.3	4.8	2.1
14	15.5	12.7	9.6	8.1	6.3
21	18.7	16.4	13.3	12.9	10.5
28	23.5	21.2	18.1	20.4	15.8
35	29.4	25.4	21.3	22.0	17.4
56	31.6	29.1	25.0	25.8	23.2
91	43.3	36.5	33.5	36.0	31.6

Food consumption was comparable among the groups of both sexes throughout the study, although there was wide variability and the differences are considered incidental (spillage of food).

Test Material Intake: The mean daily intake of test material for each group is listed below.

Table 5. Test Material Intake

Dose level (ppm)	Mean Daily Test Material Intake (mg/kg)		
	MALES	**FEMALES**	**ALL MICE**
300	≈ 84	≈ 133	≈ 100
1000	≈ 302	≈ 465	≈ 400
3000	≈ 853	≈ 1448	≈ 1200
7500	≈ 2367	≈ 3565	≈ 3000

3. Blood Analyses

Hematology: Blood samples were obtained from the retroorbital venous plexus and after decapitation in the morning from

fasted (16-20 hours) mice [due to the limited amount of blood available from each mouse, the first 5 survivors/sex/group were used for hormone determination, and the remaining mice/sex/group were used for the determination of hematology, enzymes, and blood chemistry]. The sampling [Day 97] and analyses were performed in a random sequence. The CHECKED (X) parameters were examined.

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements (Prothrombin time) (Thromboplastin time)		Red cell morphology
	Nucleated erythrocytes normoblasts		

RESULTS

There was a dose-related, statistically significant decrease in RBCs noted in the females, although the magnitude of the difference among the three highest dose levels was small. Additionally, there was a statistically significant decrease in HGB in females at the 1000 and 7500 ppm dose levels. Although these changes are minimal, similar decreases were noted in the female rats. No statistically significant or dose-related changes were observed in the males.

Table 6. Hematology Values (% of control)

Parameter/Dose	300 ppm	1000 ppm	3000 ppm	7500 ppm
FEMALES				
RBC (TERA/L)	97	96**	96**	95**
HGB (MMOL/L)	98	97**	98	95**

* p<0.05; ** p<0.02

Clinical Chemistry: Blood samples were obtained as stated above. The CHECKED (X) parameters were examined.

X	Electrolytes:	X	Other:
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
X	Magnesium	X	Blood urea nitrogen
X	Phosphorous	X	Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	Iron		Phospholipids
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine kinase (CK)		Lipids, total
	Lactate dehydrogenase (LAD)	X	Triiodothyronine, total T3
X	Serum alanine aminotransferase		
X	Serum aspartate aminotransferase		
X	Gamma glutamyl transferase (GGT)		
	Gamma glutamyl transpeptidase (GTP)		
	Glutamate dehydrogenase (GLDH)		

	Ornithine carbamyltransferase (OCT)
	Serum protein electrophoresis*
X	Thyroxine, total T4
	Thyroid stimulating hormone (TSH)

RESULTS

Enzyme activities: There were no significant differences observed in any of the enzyme activities monitored in either sex.

Blood chemistry: There were no treatment-related changes in any of the parameters monitored in either sex.

Hormones: **Serum T_4 Concentration** - There was a dose-related decrease in T4 at the three highest dose levels in both sexes at study termination.

Table 10. **Serum T_4 Concentrations** (µg/dL)

Dose Level (ppm)	MALES	FEMALES
0	5.0	3.8
300	3.9	2.9
1000	3.2*	2.0**
3000	2.4**	1.3**
7500	1.3**	1.2**

* $p<0.05$; ** $p<0.01$

Serum T_3 Concentrations - There was a dose-related increase in T_3 concentration in the males, but statistical significance was attained only at the high-dose (7500 ppm) level. In females, an increase was observed at the high-dose level, but statistical significance was not attained.

Table 11. **Serum T_3 Concentrations** [ng/dL]

Dose (ppm)	MALES	FEMALES
0	68	64
300	63 (93)♦	54 (84)
1000	77 (113)	53 (83)
3000	100 (147)	65 (102)
7500	126 (185)	85 (133)

♦ (% of control)

4. Urinalysis: No samples were collected.

6. Ophthalmoscopy: The eyes were not examined for pathological changes.

7. Gross Pathology: All animals were subjected to a full macroscopic examination at sacrifice (not clear if mice were fasted). The following organs were weighed: kidneys, liver, testes (♂), and adrenal glands. The body weight of each

anesthetized mouse was recorded.

RESULTS

There were very few findings at necropsy in all groups, and no treatment-related effects were observed. **Organ Weights:** LIVER:The absolute liver weight was significantly increased at the highest dose level in males, and the relative liver of both sexes at the high-dose and of males at the next lower dose level was significantly increased relative to the control values. KIDNEY: There was a dose-related increase in relative kidney weight in both sexes, with statistical significance being attained only at the high dose in males and at all dose levels in females. ADRENAL GLAND: There was a dose-related increase in both the absolute and relative weight of the adrenal glands in females at the 3000 and 7500 ppm dose levels compared to the control values. TESTES: The absolute weight of the testes was increased at the high-dose level and the relative weight was increased (dose-related) at the 3000 and 7500 ppm dose levels relative to the control values. Although there was a small (93% of control value) decrease (dose-related) in terminal body weight in the males, statistical significance was not attained. In females, the two highest dose groups displayed comparable (statistically significant) decreases, which were also small (93% of control) relative to the control value. NOTE: It is to be noted that the thyroid (target organ for Metiram) and brain (a possible target organ for Metiram) were not weighed.

Table 14. ABSOLUTE ORGAN WEIGHT (grams)

Dose (ppm)/ Organ	0	300	1000	3000	7500
MALES					
liver	1.071	1.107	1.088	1.139	1.177*
kidney	0.462	0.480	0.472	0.470	0.471
adrenal	5.2	4.9	5.8	5.2	5.2
testes	0.216	0.235	0.232	0.236	0.240*
FEMALES					
liver	1.042	1.003	0.977	1.015	1.057
kidney	0.347	0.359	0.351	0.371	0.373
adrenal	9.4	10.5	10.4	11.3*	12.7**

Table 15. RELATIVE ORGAN WEIGHT (g or mg)

Dose (ppm)/ Organ	0	300	1000	3000	7500
MALES					
liver	4.026	4.107	4.149	4.453**	4.733**
kidney	1.737	1.780	1.806	1.835	1.895**
adrenal	0.02	0.018	0.022	0.021	0.021
testes	0.812	0.872	0.890	0.923**	0.968**

	Table 15. RELATIVE ORGAN WEIGHT (g or mg)				
Dose (ppm)/ FEMALES					
liver	4.748	4.706	4.723	4.988	5.187**
kidney	1.581	1.682*	1.702**	1.820**	1.830**
adrenal	0.043	0.049	0.05	0.056**	0.063**

* p<0.05; ** p<0.01

7. Histopathology: The following organs/tissues (CHECKED (X)) were preserved from all mice noted above. Microscopic examinations were performed of all organs/tissues of the control and highest-dose group, and the thyroids, lungs, liver, gall bladder, kidneys, and urinary bladder were examined of all groups/both sexes. Additionally, all gross lesions were examined, and the adrenal glands of all females were examined.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue	X	Aorta	X	Brain
X	Salivary glands♦	X	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow♥	X	Spinal cord♣
X	Stomach	X	Lymph nodes♣	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes
X	Jejunum	X	Thymus	Glandular	
X	Ileum	Urogenital		X	Adrenal gland
X	Cecum	X	Kidneys	X	Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland ♀
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
X	Gall bladder	X	Prostate	Other	
X	Pancreas	X	Seminal vesicle	X	Bone♥/knee joint/sternum
Respiratory		X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung	X	Vagina	X	All gross lesions
	Nose	X	Sternum with marrow		
	Pharynx	X	Coagulating glands		
	Larynx				

♥femur; ♦ mandibular & sublingual; ♣ mandibular & mesenteric; ♠ cervical, thoracic & lumbar cord

RESULTS

There were histopathological changes noted in the thyroid and urinary bladder of both sexes and in the adrenal glands of the females, which may be related to treatment. In the thyroid, there was minimal or slight hypertrophy and vacuolation of the thyroid follicular epithelium. Fatty degeneration of the 'X' zone in the adrenal glands was more severe in females at the 3000 and 7500 ppm dose levels compared to the control females. The superficial layer of urothelium in the urinary bladder contained a refractile, granular material in most of the treated mice, with the incidence and severity increasing with increasing dose. A material was noted in the controls also, but with special staining (Schmorl's or Ziehl-Neesen techniques), it was concluded that the material in the control mice was spontaneously-occurring lipofuscin pigment. The material in the treated mice was not identified, but was considered most likely to be the test material or a metabolite.

MALES

Lesion/Dose	0 ppm	300 ppm	1000 ppm	3000 ppm	7500 ppm
Thyroid N=10					
hypertrophy of follicular epithelium					
minimal	0	0	0	7	1
slight	0	0	0	3	9
vacuolation of follicular epithelium					
minimal	0	0	0	5	0
slight	0	0	0	5	10
Urinary Bladder N=10					
granular material in superficial cells					
minimal	2	8	2	0	0
slight	0	0	8	4	0
moderate	0	0	0	6	3
marked	0	0	0	0	7

FEMALES

Lesion/Dose	0 ppm	300 ppm	1000 ppm	3000 ppm	7500 ppm
Thyroid N=10					
hypertrophy of follicular epithelium					
minimal	0	0	0	7	0
slight	0	0	0	0	9
moderate				0	1
vacuolation of follicular epithelium					
minimal	0	0	0	5	0
slight	0	0	0	5	10
depletion of colloid					
slight	0	0	0	0	1
Urinary Bladder N=10					
granular material in superficial cells					
minimal	3	8	0	0	0
slight	0	0	3	0	0
moderate	0	0	7	1	0
marked	0	0	0	9	10
Adrenal Gland					
subcapsular cell hyperplasia					
minimal	0	0	0	0	6
slight	10	9	10	10	3
moderate	0	1	0	0	0
fatty degeneration of the 'X' zone					
minimal	3	4	2	0	1
slight	3	4	4	1	0
moderate	4	2	3	5	2
marked	0	0	1	4	7

DISCUSSION

Three months of oral administration of Metiram Premix 95% to mice at dose levels of 300 ppm [84♂/133♀ mg/kg], 1000 [302♂/465♀ mg/kg], 3000 ppm [853♂/1448♀ mg/kg], and 7500 ppm [2367♂/3565♀ mg/kg] resulted in a decrease in body weight/gain in the high-dose mice (both sexes) and in females at the next two lower dose levels. Although a dose-response was not always evident in the females, the high-dose displayed the greatest decrease at each time point and the magnitude of the decrease at the 1000 and 3000 ppm dose levels was usually similar to but lower than the high-dose value. Because the magnitude of the decrease was usually greater than 20 %, it is difficult to

negate these decreases as incidental. The females displayed small decreases in RBCs and hemoglobin at the three highest dose levels, which were similar in magnitude across dose; although the significance of these decreases is not apparent, the females rats administered Metiram for 90 days also displayed these decreases. There was a dose-related decrease in total serum T_4 concentrations in both sexes, and an increase in total serum T_3 concentration in the high-dose males. The absolute and relative liver weights were increased in both sexes at the high-dose level, but there were no corresponding lesions to account for the increase. The absolute and relative weights of the adrenal glands were increased (dose-related) in females at the 3000 and 7500 ppm dose levels, and an increase in the severity of fatty degeneration of the "X" zone was noted in these mice. In the thyroid, minimal or slight hypertrophy and vacuolation of the thyroid follicular epithelium was observed in both sexes at the 3000 and 7500 ppm dose levels, which was dose-related.

Absolute kidney weight in the females at the 3000 and 7500 ppm dose levels was greater than the kidney weight displayed in 13 of the 14 historical control studies; the relative kidney weight at all dose levels in females was greater than those of all 14 historical control studies. Relative kidney weight of 7500 ppm males was greater than 13 of the 14 HC studies. There were no lesions reported in the kidneys to account for these apparent differences. Relative testes weight was greater than observed in 14 HC studies.

CONCLUSION

Under the conditions of the study, oral administration of Metiram Premix 95% to B6C3F1 mice for 3 months at dose levels of 300 ppm (84 ♂/133 ♀ mg/kg), 1000 [302 ♂/465 ♀ mg/kg], 3000 ppm [853 ♂/1448 ♀ mg/kg], and 7500 ppm [2367 ♂/3565 ♀ mg/kg] resulted in decreased body weight/gain in both sexes at the high-dose levels and in females at the next two lower dose levels. There was a decrease in total serum T_4 concentrations in both sexes at the 1000, 3000, and 7500 ppm dose levels (dose-related), and an increase in total serum T_3 concentration in the high-dose males. The absolute and relative liver weights were increased in both sexes at the high-dose level, and the absolute and relative weights of the adrenal glands were increased (dose-related) in females at the 3000 and 7500 ppm dose levels, with an increase in the severity of fatty degeneration of the "X" zone noted in these mice. In the thyroid, there was a dose-related increase in the incidence of minimal or slight hypertrophy and vacuolation of the thyroid follicular epithelium in both sexes at the 3000 and 7500 ppm dose levels. The NOEL can be set at 300 ppm (84 ♂/133 ♀ mg/kg), the LOEL at 1000 ppm (302 ♂/465 ♀ mg/kg), based on the decrease in total serum T_4 concentrations in both sexes.

This study is classified Core Minimum. This study satisfies the guideline requirement (82-1) for a subchronic feeding study in rodents.