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CASWELL FILE

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

010069

MEMORANDUM

MAR 9 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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

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

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

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

and

Marcia van Gemert, Ph.D. 3/4/93
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.: S432082
Caswell No.: 041A
DP Barcode: D185804
Identifying No.: 014601
MRID No.: 425391-01

Action Requested: Please review the Oral Toxicology of Metiram Premix 95% in the Wistar Rat - Admin. in the diet for 3 Months including the Exam of Neurotox. DCIs for the EBDCs/ETU are currently being prepared in RB/SRRD. Please give the status if this bridging study will fulfill the Chronic Tox. - rat study and 90-day feeding study. Also, please indicate if there are any outstanding or new data gaps, i.e. neurotox. battery.

Comment: The Registrant has submitted a study entitled: "Study of the Oral Toxicity of Metiram Premix 95% in the Wistar Rat Administration in the Diet for 3 Months Including the Examination

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of Neurotoxicology". The stated objective of the study was to investigate the toxicological profile and possible neurotoxic effects of Metiram Premix 95% in a 3 months feeding study in rats. This subchronic study has been reviewed, and the DER is appended.

Under the conditions of the study, oral administration of Metiram Premix 95% to Wistar rats for 3 months at dose levels of 5 ppm (0.4 mg/kg), 80 [6.3 mg/kg (5.8 ♂/6.7 ♀)], 320 ppm [25.4 mg/kg (23.5 ♂/27.3 ♀)], and 960 ppm [81.4 mg/kg (73.9 ♂/88.8 ♀)] resulted in decreased body weight/gain and serum T_4 concentrations and increased liver and thyroid weights in both sexes at the high-dose (960 ppm) level, ataxia and slightly decreased areas of myelinated axons in females at the high-dose level, reduced hindlimb grip strength in both sexes at the high-dose level, changes in hematology and clinico-chemistry parameters at the 320 and 960 ppm dose levels, reduced forelimb grip strength in females at the 80, 320, and 960 ppm dose levels, and increased TSH concentrations at all dose levels in both sexes ($p<0.05$ not attained). The results demonstrate that Metiram Premix 95% affects the thyroid and, directly or indirectly, may have neuro-toxicological properties and/or affect skeletal muscle. A definitive conclusion as to a lack of neurological involvement of Metiram is not possible from these data. The NOEL can be set at 5 ppm, the LEL at 80 ppm, based on reduced forelimb grip strength in females. This study is classified Core minimum, although the brain (a possible target organ) was not weighed and microscopic lesions (muscle atrophy and thyroid hyperplasia) noted in 2 previous 13-week studies and a 4-week study were not exhibited in this bridging study. This study satisfies the guideline requirement (82-1) for a subchronic feeding study in rodents. While it appears that the current study adequately satisfies the data requirement for a subchronic neurotoxicity study, a final determination will be made after an acute neurotoxicity study of Metiram has been evaluated.

With regard to the long-term rat study (classified Core Supplementary for chronic toxicity because a NOEL for systemic toxicity could not be established due to the fluctuations observed in T_3, T_4, and [^{131}I] uptake values), based on the findings in the current 90-day study, the chronic toxicity phase of the long-term study may be upgraded to Core Minimum, and the NOEL for systemic toxicity can be set at 5 ppm, the LEL at 80 ppm, based on reduced forelimb grip strength in females.

The data available on Metiram are listed below.

DATA AVAILABLE

A. Acute oral LD_{50} - rat	LD_{50} > 10000 ♂/8000 ♀ mg/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 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

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/9/93
Tox. Branch II, Section II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 3/9/93
Tox. Branch II, Head Section II (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: 90-Day Oral - rat

TOX. CHEM. NO.: 041A

MRID NO.: 425391-01

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: 99C0331/90037

SPONSOR: BASF Corporation/Agricultural Products

TESTING FACILITY: BASF Aktiengesellschaft/Crop Protection, Product Safety/Department of Toxicology

TITLE OF REPORT: Study of the Oral Toxicity of Metiram Premix 95% in the Wistar Rat - Administration in the Diet for 3 Months Including the Examination of Neurotoxicology

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 Wistar rats for 3 months at dose levels of 5 ppm (0.4 mg/kg), 80 ppm [6.3 mg/kg (5.8 ♂/6.7 ♀)], 320 ppm [25.4 mg/kg (23.5 ♂/27.3 ♀)], and 960 ppm [81.4 mg/kg (73.9 ♂/88.8 ♀)] resulted in decreased body weight/gain and serum T_4 concentrations and increased liver and thyroid weights in both sexes at the high-dose (960 ppm) level, ataxia and slightly decreased areas of myelinated axons in females at the high-dose level, reduced hindlimb grip strength in both sexes at the high-dose level, changes in hematology and clinicochemistry parameters at the 320 and 960 ppm dose levels, reduced forelimb grip strength in females at the 80, 320, and 960 ppm dose levels, and increased TSH concentrations at all dose levels in both sexes ($p<0.05$ not attained). The results demonstrate that Metiram Premix 95% affects the thyroid and, directly or indirectly, may have neurotoxicological properties and/or affect skeletal muscle. A definitive conclusion as to a lack of neurological involvement of Metiram is not possible from these data. The NOEL can be set at 5

ppm, the LEL at 80 ppm, based on reduced forelimb grip strength in females.

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/ZST test substance #: 90/331-1; Purity: 94.8% at study initiation.

2. Test Animals: Species: rat; Strain: Wistar [Chbb:THOM (SPF)]; Age: 42 days old at study start; Weight: males 177-204 g, females 136-156 g; Source: Dr. Karl Thomae GmbH, D-W7950 Biberach/Riss, FRG.

3. Statistics: Means and standard deviations were calculated for the variables: food consumption, body weight, body-weight change, test material intake, grip strength (fore- and hindlimb), hot-plate test, clinical chemistry and hematology parameters; with the exception of test material intake and differential blood count, statistical significance was determined by analysis of variance (ANOVA) followed by a Dunnett's test. Urinalyses parameters: a pairwise comparison of each dose group with the control was carried out using Fisher's exact test for the hypothesis of equal proportions. Hormones: a comparison of each dose group with the control group was carried out using the Dunnett's test for the hypothesis of equal means. Perfused rat data: group means and standard deviations were calculated for the area means of myelinated axons of peripheral nerves (in square micrometers) of all control and high-dose rats; Student's t-test was used of comparison of dose group to control values. Group means and standard deviations were calculated for the variables of body weight and absolute/relative organ weight of the immersion fixed rats in each test group and tabulated together with the individual values; Dunnett's test was used for a simultaneous comparison of several dose groups with a control group.

B. STUDY DESIGN

1. Methodology: Sixty-five males and 65 females [acclimated for 10 (♂♂)/8 (♀♀) days] were assigned (distributed according to weight among groups; computer-generated randomization list) to one of five groups, each composed of 13 rats/sex, and administered the test material [0, 5, 80, 320, or 960 ppm] via the diet for 90 consecutive days. The rats 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 and 2 13-week studies, each with withdrawal phases and a chronic toxicity/carcinogenicity 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

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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 at 5-, 19-, and 34-day intervals for the 5 and 80 ppm dose levels. Homogeneity of the test material in food was verified at the high- and low dose levels at the start of the study, and concentrations attained were measured (using gas chromatography) prior to and at the end of the study.

RESULTS

At study start, characterization of the test material indicated a degree of purity of 94.8% and a homogeneous mixture. 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 5-day period (test diets prepared twice a week). Homogeneity of the test material in the diet was verified in subsequent preparations. 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	5 ppm,	80 ppm	320 ppm	960 ppm
Start	117%	99.8%	116%	95.5%
End	88.8%	103.3%	91.9%	92.3%

2. Clinical Observations: The animals were observed daily (twice on weekdays, once weekends) for mortality/moribundity and evidence of any systemic reaction to treatment or ill-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. The only treatment-related clinical symptoms were displayed in the high-dose female group, which consisted of a reduced general state in 3

females and ataxia in 3 females (2 females displayed both symptoms and 2 others each displayed one of the symptoms).

Body Weight and Food Consumption

Body weight was decreased at the high-dose level for both sexes compared to the control group, with the females displaying a greater decrease than the males. The high-dose males showed a minimal (94-95% of control value) decrease from Day 21 through Day 70, while the decrease (85-88% of control value) in the high-dose females was observed from Day 14 until study termination (Table 2). A decrease in body-weight gain was evident for both sexes at this dose level from Day 14 on, with the females being the more severely affected (Table 3). On a percent basis, body-weight change was reduced in both sexes at the high-dose level throughout most of the study (Table 4, statistical analysis was not performed).

Table 2. Mean Body Weight (% of control)

Day/Dose	5 ppm	80 ppm	320 ppm	960 ppm
MALES				
0	101	101	101	100
7	101	100	99	99
14	101	100	99	96
21	101	99	98	94*
28	100	99	98	95*
35	101	100	98	95*
42	101	100	98	95*
49	101	99	98	94*
56	101	99	97	94*
63	101	100	98	94
70	101	100	98	94*
77	101	100	97	94
84	101	100	97	94
91	102	101	98	94
FEMALES				
0	100	100	100	100
7	100	99	98	97
14	101	100	96	88**
21	102	100	95	87**
28	101	99	94	86**
35	101	99	94	88**
42	101	99	94	88**
49	102	98	94	87**
56	102	99	94	87**
63	101	99	94	86**
70	101	99	94	86**
77	101	98	94	85**
84	101	98	93	85**
91	102	99	95	87**

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

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

Interval/Group	0 ppm	5 ppm	80 ppm	320 ppm	960 ppm
MALES					
From Day 0 to:					
7	50.0	50.7	48.4	47.2	47.7
14	90.1	90.1	87.1	86.2	79.4**(88)
21	122.0	122.7	118.0	115.6	103.9**(85)
28	148.1	146.6	143.2	141.5	129.1**(87)
35	159.1	160.8	155.6	152.4	141.2**(89)
42	187.8	188.5	184.6	178.5	167.5**(89)
49	208.3	209.4	202.7	198.9	185.4**(89)
56	223.8	227.0	217.7	212.1	198.3**(89)
63	236.0	238.6	233.7	225.8	211.7**990)
70	251.0	254.6	247.4	238.8	224.5**(89)
77	262.9	267.3	260.6	249.1	236.0**(90)
84	266.4	269.7	262.8	253.2	239.3**(90)
91	265.6	272.9	266.0	254.2	238.8**(90)
FEMALES					
From Day 0 to:					
7	22.6	21.9	20.4	19.9	17.4(77)
14	40.9	42.4	39.4	33.4	18.1**(44)
21	51.3	54.2	50.4	42.6	25.1**(49)
28	64.2	65.2	61.0	52.2	33.7**(52)
35	74.5	76.4	71.9	61.9	48.5**(65)
42	88.4	91.1	84.8	74.1	58.8**(67)
49	99.9	103.4	94.1	84.6	66.1**(66)
56	105.8	109.9	102.2	91.6	72.8**(69)
63	112.6	115.0	108.6	97.9	76.5**(68)
70	119.3	122.4	114.9	104.9	82.5**(69)
77	125.5	129.1	119.5	110.0	85.5**(68)
84	128.5	131.2	122.1	110.4	87.8**(68)
91	123.7	128.5	119.2	110.7	88.1**(71)

* p<0.05; ** p<0.01;

Table 4. Body-Weight Change (%)

Interval/Group	0 ppm	5 ppm	80 ppm	320 ppm	960 ppm
MALES					
From Day 0 to:					
7	26	26	25	25	25
14	47	47	45	45	42
21	64	64	61	60	54
28	78	76	74	74	68
56	118	118	113	111	104
91	140	142	138	133	125
FEMALES					
From Day 0 to:					
7	16	15	14	14	12
14	28	29	27	23	12
21	35	37	35	29	17
28	44	45	42	36	23
35	51	52	49	43	33
56	73	75	70	63	50
91	85	88	82	76	60

Food consumption was comparable among the groups of both sexes throughout the study, except for Day 49 when females in the 80 and 960 ppm groups displayed decreased (88% of control) food intake. This decrease is considered to be incidental.

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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
5	0.4	0.4
80	5.8	6.7
320	23.5	27.3
960	73.9	88.8

3. Blood Analyses

Hematology: Blood samples were obtained from the retroorbital venous plexus from all rats (non-fasted, unanesthetized, a.m.). The sampling [days 29 and 84(♂♂)/85(♀♀)] and analyses were performed in a random sequence. The CHECKED (X) parameters were examined.

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
X	Blood clotting measurements (Prothrombin time)		Red cell morphology
X	(Thromboplastin time)		
	Nucleated erythrocytes normoblasts		

RESULTS

There were no statistically significant differences noted in any of the parameters on Day 29 of the study in either sex. On Day 84 (♂♂)/85 (♀♀), there was a dose-related decrease in red blood cells in both sexes, with statistical significance being attained at the 320 and 960 ppm dose levels. The females of these same two dose groups also displayed decreases in hemoglobin and hematocrit values (dose-related) on Day 85 of the study. It is noted that the magnitude of the decreases in each case is small.

Table 6. Hematology Values (% of control)

Parameter/Dose	5 ppm	80 ppm	320 ppm	960 ppm
MALES				
RBC (TERA/L)	99	102	95*	95*
FEMALES				
RBC (TERA/L)	99	96	95*	93**
HGB (MMOL/L)	99	97	96*	94**
HCT (L/L)	99	97	95*	93**

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

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		
X	Thyroid stimulating hormone (TSH)		

RESULTS

Enzyme activities: On Day 85 of the study, females at the 80 and 960 ppm dose levels displayed decreased values for alanine aminotransferase compared to the control value, and the high-dose (960 ppm) females also displayed decreased alkaline phosphatase (ALP) values compared to the control value. Although the latter finding is consistent with fasting/ starvation and this dose group displayed lower body weights than the control, food consumption was comparable to the control value. The decrease in ALP may be treatment-related.

Blood chemistry: Electrolyte levels were decreased slightly in both sexes at the high-dose level at one or both time points measured, which is consistent with starvation/malnutrition, but there was no drastic decrease in food intake by either sex, although there was a decrease in body-weight gain in both sexes. There was no associated pathology noted in the kidney, bone, or other organ.

Parameter Dose	Table 7. FEMALE Blood Chemistry Values (% of control) 5 ppm	80 ppm	320 ppm	960 ppm
Blood urea nitrogen				
Day 29	96	92	97	92
Day 85	100	96	94	95
Potassium				
Day 29	102	99	101	95
Day 85	95	92	93	87**
Creatinine				
Day 29	102	101	101	90*
Day 85	92	89*	92	79**
Inorganic phosphate				
Day 29	103	94	100	102
Day 85	98	94	95	92
Calcium				
Day 29	102	99	100	100
Day 85	100	100	100	97*
Magnesium				
Day 29	103	99	102	100
Day 85	92*	93	94	90**

Parameter Dose	Table 8. MALE Blood Chemistry Values (% of control) 5 ppm	80 ppm	320 ppm	960 ppm
Blood urea nitrogen				
Day 29	99	98	96	89**
Day 84	94	95	101	89*
Potassium				
Day 29	99	102	102	96
Day 84	98	98	99	92*
Creatinine				
Day 29	99	104	98	91*
Day 84	96	101	103	95
Inorganic phosphate				
Day 29	99	101	100	96
Day 84	96	91	85**	84**
Calcium				
Day 29	102	101	102	100
Day 84	100	100	100	97*
Magnesium				
Day 29	98	102	97	93
Day 84	92*	95	95	89**

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

Hormones: **Serum TSH Concentration** - At study termination, there was a significant increase in TSH at all dose levels in both sexes, although no dose response was noted.

Dose Level (ppm)	Table 9. TSH Concentration (μU/mL)	
	MALES	FEMALES
0	134	90
5	229	129
80	287	146
320	260	136
960	232	147

Serum T_4 Concentration - There was a significant decrease in T4 at the highest dose level in both sexes at study termination.

Dose Level (ppm)	Table 10. Serum T_4 Concentrations (μg/dL)	
	MALES	FEMALES
0	5.1	3.8
5	5.9	3.5
80	5.2	4.1
320	5.2	3.0
960	3.3**	1.9**

* p<0.05; ** p<0.01

Serum T_3 Concentrations - There was a statistically significant increase in T_3 concentration in the males at the 320 and 960 ppm dose levels, although the level at the 320 ppm level was greater than that at the high dose. Female values were comparable among the groups.

Dose (ppm	Table 11. Serum T_3 Concentrations (ng/dL)	
	MALES	FEMALES
0	64	82
5	72	79
80	76	94
320	89**	93
960	80*	82

* p<0.05; ** p<0.01

4. Urinalysis: Urine samples were collected from each animal [days 33(♂♂)/31(♀♀) and 89(♂♂)/87(♀♀)]; housed in individual metabolic cages overnight (food and water withheld). The CHECKED (X) parameters were examined.

X		X	
	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen
	Osmolality	X	Color
	Sodium	X	Chloride
	Potassium	X	Nitrite
X	Turbidity		

RESULTS

There were no consistent effects noted in any of the parameters monitored.

6. Ophthalmoscopy: Prior to study initiation and at termination, the eyes of all rats of the control and high-dose groups were examined for pathological changes using an ophthalmoscope.

RESULTS

There were no treatment-related effects.

7. Neurofunctional Test: A neural function examination was performed on all rats one day prior to the start of dosing and on days 1, 7, 14(♂♂)/ 15(♀♀), 26(♂♂)/27(♀♀), 56, and 90 using a "functional observational battery", which includes an assessment of the following parameters of sensoric and motoric functions.

general state	tremors	convulsions	piloerection
salivation	pupil size	lacrimation/secretion of	pigmented tears
feces	vocalization	paresis	paralysis
ataxia	body tone	posture	appearance (body)
activity	respiration	urine	skin color
righting response	behavior	grip strength	pupillary reflex
winking reflex	audition	olfaction	sensitivity of body surface
pain perception	tail pinch	toe pinch	visual placing response

RESULTS

On Day 27, three high-dose females exhibited ataxia during the neurofunctional tests. None of the males in any group and no females of the other test groups displayed any neurological signs. With regard to grip strength, females of the high-dose group displayed a reduction in forelimb grip strength from Day 15 on. Additionally, females in the 80 and 320 ppm dose groups displayed reduced forelimb grip strength (dose-related) on Day 90, although the author concluded that since the magnitude of the difference in the reductions at these dose levels was small (6.2 vs 6.0) compared to the dose range (80 vs 320 ppm), the reductions were not assigned any biological significance. TB II notes that the ranges for these groups tended to be slightly lower than the control range, especially at the later time points; therefore, it is possible that with continued exposure, the reduction in forelimb grip strength at these two lower dose levels would increase in magnitude. The males showed comparable forelimb grip strength among the groups. With regard to hindlimb grip strength, females at the high-dose level displayed a reduction from Day 7 on, with the largest reduction occurring on Day 15. Males at the high-dose level also displayed a reduction at Day 90.

Day/ Sex/ Dose (ppm)	Table 12. FORELIMB GRIP STRENGTH (Newton)				
	0	5	80	320	960
MALES					
-1	4.2	4.1	4.2	4.2	4.4
1	4.4	4.5	4.7	4.4	4.5
7	5.0	5.1	5.0	5.0	5.0
14	5.1	5.2	5.1	5.1	5.1
26	5.6	5.5	5.6	5.4	5.5
56	6.5	6.7	6.5	6.4	6.5
90	7.7	7.5	7.2	7.1	7.1
FEMALES					
-1	4.0	4.0	4.2	3.9	4.1
1	4.4	4.4	4.5	4.5	4.4
7	4.6	4.6	4.7	4.4	4.5
15	4.9	5.1	4.9	4.6	3.7** (76)
27	5.0	5.2	5.2	5.0	4.1** (82)
56	5.3	5.4	5.1	5.0	4.2** (79)
90	6.9	6.3	6.2* (90)♦	6.0** (87)	5.1** (74)

Day/ Sex/ Dose (ppm)	Table 13. HINDLIMB GRIP STRENGTH (Newton)				
	0	5	80	320	960
MALES					
-1	2.3	2.2	2.2	2.3	2.3
1	2.3	2.4	2.4	2.4	2.5
7	3.7	3.5	3.8	3.8	3.7
14	3.9	4.1	4.0	4.0	3.9
26	3.4	3.5	3.5	3.4	3.3
56	5.2	5.3	5.2	5.4	5.2
90	5.7	5.6	5.4	5.5	5.0* (88)
FEMALES					
-1	2.7	2.6	2.7	2.5	2.7
1	2.9	3.0	3.1	2.9	2.9
7	3.2	3.2	3.2	3.2	2.5** (78)
15	2.3	2.3	2.4	2.2	1.1** (48)
27	2.3	2.1	2.2	2.1	1.8 (78)
56	4.1	3.9	3.9	3.8	3.3** (80)
90	5.0	5.0	4.9	4.7	4.4* (88)

* $p<0.05$; ** $p<0.01$; ♦ (% of control)

8. Gross Pathology: All animals (except those designated for perfusion) were subjected to a full macroscopic examination at sacrifice (fasted 16-20 hours). The following organs were weighed: kidneys, liver, testes, adrenal glands, and thyroid glands.

RESULTS

There were very few findings at necropsy in all groups, and no treatment-related effects were observed. **Absolute organ weights:** The absolute thyroid weight was increased at the highest dose level in both sexes, although only the male value reached statistical significance. The adrenal glands were decreased to the same extent at the 320 and 960 ppm dose levels in males compared to the control value, although statistical significance was not attained. **Relative organ**

weight: Relative liver and kidney weights were increased at the highest dose level in both sexes compared to the control values, which in the case of the females may be attributed to the statistically significant decrease in body weight at termination. The terminal body weight of the high-dose males was slightly (6%) lower ($p < 0.05$ not attained) than the control value, suggesting that the decreases may also be due to body weight and not directly to treatment. High-dose males also displayed a statistically significant increase in relative testes weight compared to the control value. With regard to thyroid weight, there was a dose-related increase in females (statistical significance attained only at the high dose), and the high-dose males displayed a significant increase, which appears to be treatment-related also. NOTE: It is to be noted that the brain, which is a possible target organ for Metiram, was not weighed.

Table 14. ABSOLUTE ORGAN WEIGHT (grams)

Dose (ppm)/ Organ	0	5	80	320	960
MALES					
liver	13.29	13.66	13.79	13.10	13.87
kidney	2.84	2.98	2.80	2.77	2.87
thyroid	27.0	29.1	26.2	26.9	34.0**
adrenal	83.2	83.5	79.9	73.8	74.2
testes	3.53	3.65	3.61	3.68	3.77
FEMALES					
liver	7.37	7.15	6.97	6.80	7.85
kidney	1.97	1.88	1.82	1.78*	2.06
thyroid	20.5	20.9	20.6	23.6	24.3
adrenal	93.4	95.6	99.5	97.5	91.6

Table 15. RELATIVE ORGAN WEIGHT (g or mg)

Dose (ppm)/ Organ	0	5	80	320	960
MALES					
liver	3.056	3.070	3.152	3.115	3.400**
kidney	0.655	0.673	0.640	0.658	0.705*
thyroid	0.006	0.007	0.006	0.006	0.008**
adrenal	0.019	0.019	0.018	0.018	0.018
testes	0.822	0.826	0.828	0.876	0.927*
FEMALES					
liver	2.929	2.802	2.905	2.948	3.510**
kidney	0.782	0.738	0.761	0.776	0.925**
thyroid	0.008	0.008	0.009	0.010	0.011*
adrenal	0.037	0.038	0.042	0.043	0.041

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

7. Histopathology: The following organs/tissues (CHECKED (X)) were preserved from all rats noted above. Microscopic examinations were performed of all organs/tissues of the control and highest-dose group, and the thyroids, lungs, liver, kidneys, skeletal muscle, and sciatic nerve were examined of all groups. Additionally, all gross lesions were examined.

X	Digestive system	X	Cardiovasc./Hemat.	X	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		Epididymides	X	Thyroids
	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		Coagulating gland		
	Pharynx				
	Larynx				

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

RESULTS

There were no findings that could be related to treatment.

8. Neuropathology: The 3 rats/sex and test group showing the most explicit neurotoxic symptoms were deeply anesthetized at study termination and sacrificed by perfusion fixation. The first 3 rats of the control and test groups without any neurotoxic clinical signs were fixed by perfusion [SOERENSEN's phosphate buffer was rinsing solution and KARNOVSKY fixative was fixative solution]. The sacrificed rats were necropsied, and the visible organs were assessed by gross pathology.

PART 1. **Epoxy resin embedding** - The following organ samples from all 3 rats/sex/group sacrificed by perfusion fixation were removed after perfusion, processed histotechnically [epoxy resin embedding, semithin sectioning (cross and longitudinal sections), and staining with toluidinblue], and evaluated histopathologically.

Table 16. Epoxy Resin Embedding

Organ sample from:/Group
peripheral nervous system♦
sciatic nerve
tibial nerve
sural nerve

Morphometric analysis - The sciatic, tibial, and sural nerves of the control and high-dose (960 ppm) rats subjected to perfusion fixation were analyzed morphometrically. The areas of 200 myelinated axons were measured in cross sections of these nerves. It was stated that axons were not measured which showed a longitudinal cut surface or transitions to a

longitudinal section or myelin formations showing no axonal structure in the center. A semi-automatic analytical apparatus (Leitz ASM 68 K with microvideo imaging into the microscope) served as the measuring unit. To measure the object in the microscope, it was enlarged a thousandfold. The objects measured were marked electronically. As a field of measurement, central and peripheral nerve fiber zones in all nerve fiber cross sections were recorded by way of a systemic quantitative technique. The area mean was determined for all the measured 200 myelinated axons of the nerve fiber cross section.

PART 2. **Paraffin embedding** - After perfusion, the organs listed below were removed, asservated in 4% formaldehyde solution, processed histotechnically (as shown below), examined microscopically (light) and assessed.

Table 17. **Paraffin Embedding** - Samples Processed Microscopically

Organ sample/Dose group	0 ppm	5 ppm	80 ppm	320 ppm	960 ppm
BRAIN (cross section)					
Lobus frontalis	A3, R				A3, R
Lobus parietalis	A3, R				A3, R
Mesencephalon	A3, R				A3, R
Pons	A3, R				A3, R
Cerebellum	A3, R				A3, R
Medulla oblongata	A3, R				A3, R
SPINAL CORD (cross & long. section)					
Intumescentia cerv. (C3-C6)	A3, R				A3, R
Thoracic cord	A3, R				A3, R
Intumescentia lumb. (L1-L4)	A3, R				A3, R
ALL GROSS PATHOLOGICAL LESIONS	A2	A2	A2	A2	A2

Methods: A=Hematoxylin-eosin; R= preparation of 4 unstained reserve slides
Scope of examination: 2=all affected animals per group; 3=3 rats per group

RESULTS

There were no neuropathological findings reported that could be attributed to treatment. No axonal atrophy or decrease in nerve fiber diameter was reported, and no findings were observed in the CNS. TB II notes that the areas of myelinated axons in the high-dose females were slightly decreased compared with the control values, and the females examined for this parameter are those that displayed ataxia during the study. Although statistical significance was not attained (N=3), a treatment-related effect cannot be ruled out.

Nerve/ Sex/ Group	Table 18. Areas of Myelinated Axons (μm^2)			
	MALES		FEMALES	
	Control	High Dose	Control	High Dose
Sciatic nerve	53.71	48.85(91%)♦	59.19	46.57(79%)
Sural nerve	45.01	47.71(106%)	32.56	30.3(93%)
Tibial nerve	41.42	41.91(101%)	46.84	39.03(83%)

♦ (% of control value)

DISCUSSION

Three months of oral administration of Metiram Premix 95% to rats at dose levels of 5 ppm (0.4 mg/kg), 80 [6.3 mg/kg (5.8 ♂/6.7 ♀)], 320 ppm [25.4 mg/kg (23.5 ♂/27.3 ♀)], and 960 ppm [81.4 mg/kg (73.9 ♂/88.8 ♀)] resulted in decreased body weight and body-weight gain in the high-dose rats of both sexes, with the females displaying a greater response than the males. There were differences noted in hematological and clinicochemical parameters in both sexes at the two highest dose levels, but these were not related to any specific toxic effect [↓ HGB (♀), HCT (♀), RBC (♂&♀); ↓ electrolytes, ↓ creatinine (♀&♀) and urea nitrogen (♂). TB II notes that similar decreases in RBC, HCT, and HGB were observed in the chronic dog study on Metiram, suggesting that the decreases may be treatment related. Thyroid and liver weights were increased in both sexes at the highest dose level, but there were no accompanying histological changes observed in this subchronic study to account for these increases. With regard to the increased thyroid weight, the thyroid is a known target of the EBDC's, and the increased TSH values and the decreased T_4 values observed are consistent with a disturbance in the thyroid-pituitary feedback mechanism. Additionally, one cannot rule out a secondary effect on the thyroid resulting from an increased turnover of the thyroid hormones caused by the induction of various hepatic enzymes responsible for the catabolism of thyroxine.

With regard to neuropathology, no axonal atrophy or significant decrease in nerve fiber diameter was observed, and no findings were observed in the CNS. However, three of the high-dose females displayed hindlimb weakness (ataxia), and the areas of myelinated axons in these 3 rats were slightly decreased compared to the control values. Additionally, reduced grip strength was observed at the high-dose level. There were no microscopic lesions reported for skeletal muscle. Because Metiram is known to induce skeletal muscle atrophy, the authors suggested that these effects were due to an effect on muscle strength and body weight rather than being a specific effect on the nervous system. TB II notes that muscle weakness is observed in hyperthyroidism. Based on the available data, TB II concludes that neither can be ruled out,

since compounds that effect thyroid function are known to have effects on the nervous system as well as the muscles.

TB II notes that hindlimb paralysis and muscular atrophy of the hindlimb were observed at 3000 ppm in the 4-week study, as were increased thyroid and liver weights and thyroid hyperplasia. Thyroid hyperplasia was observed at the 1000 and 300 ppm dose levels in that study also. These effects were not observed following the withdrawl period. In the 13-week studies cited, hindlimb paralysis (900 ppm), skeletal muscle atrophy (300 and 900 ppm), and thyroid hyperplasia (900 ppm) were observed, but these effects were <u>not</u> observed in the current 3-month study.

Comparison of Studies

Parameter/study	current	4-week	13-week	chronic
hindlimb paralysis	-	3000 ppm ♂♀	900 ppm ♀	320 ppm ♂♀
hindlimb weakness	960 ppm ♀	-	-	-
skel. muscle atrophy	-	3000 ppm ♂♀	900/300 ppm ♂♀/♀	320 ppm ♂♀
↑ liver weight	960 ppm ♂♀	3000 ppm ♂♀	-	-
↑ thyroid weight	960 ppm ♂♀	3000 ppm ♂♀	-	-
thyroid hyperplasia	-	3000/1000/300 ppm ♂♀	900 ppm ♂	-
↓ T_4 levels	960 ppm ♂♀	-	300/900 ppm ♂♀	variable♣
↓ uptake of ^{131}I	NM♦	-	900/300/100/50 ppm	variable♣
↑ TSH levels	5/80/320/960 ppm	-	-	variable♣
↑ T_3 levels	320/960 ppm ♂	-	-	-

♦ NM = not measured; ♣ the same rats were not evaluated at each time point

CONCLUSION

Under the conditions of the study, oral administration of Metiram Premix 95% to Wistar rats for 3 months at dose levels of 5 ppm (0.4 mg/kg), 80 ppm [6.3 mg/kg (5.8 ♂/6.7 ♀)], 320 ppm [25.4 mg/kg (23.5 ♂/27.3 ♀)], and 960 ppm [81.4 mg/kg (73.9 ♂/88.8 ♀)] resulted in decreased body weight/gain and serum T_4 concentrations and increased liver and thyroid weights in both sexes at the high-dose (960 ppm) level, ataxia and slightly decreased areas of myelinated axons in females at the high-dose level, reduced hindlimb grip strength in both sexes at the high-dose level, changes in hematology and clinicochemistry parameters at the 320 and 960 ppm dose levels, reduced forelimb grip strength in females at the 80, 320, and 960 ppm dose levels, and increased TSH concentrations at all dose levels in both sexes ($p<0.05$ not attained). The results demonstrate that Metiram Premix 95% affects the thyroid and, directly or indirectly, may have neurotoxicological properties and/or affect skeletal muscle. A definitive conclusion as to a lack of neurological involvement of Metiram is not possible from these data. The NOEL can be set at 5 ppm, the LEL at 80 ppm, based on reduced forelimb grip strength in females. This study is classified Core minimum, although the brain (a possible target organ) was not weighed and microscopic lesions (muscle atrophy and thyroid hyperplasia) noted in 2 previous 13-week studies and a 4-week study were not exhibited in this bridging study. This study satisfies the guideline requirement (82-1) for a subchronic feeding study in rodents.