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

009433

APR 16 1992

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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metiram® Pre-mix 95% - 52-Week Dog Study

TO: Terri Stowe
Product Manager (71)
Reregistration Branch, SRRD (H7508C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 4/13/92
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 4/14/92
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia Gemert 4/15/92
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®
Project No.: 2-0910
Caswell No.: 041A
Record No.: none. Case: 818620; Submission: S409215
Identifying No.: 014601
MRID No.: 421331-01
Action Requested: Please review maneb (sic) data for GLN 83-1B Chronic feeding tox. dog study.

Comment: The Registrant has submitted the final report of the 52-week dog study on Metiram, in response to a letter from the Agency dated 11/22/91. The study has been reviewed, and the DER is attached.

Under the conditions of the study, exposure to Metiram via the diet at dose levels of 30, 80, 1000, and 3000 ppm for one year resulted in decreased body weight/body weight gain and food consumption in

the 3000 ppm dogs of both sexes and in the 1000 ppm female dogs. Several hematology parameters were affected by treatment at the highest dose levels, suggestive of a hemolytic anemia. Additionally, several clinical chemistry parameters were altered, which are consistent with hypothyroidism. None of the neurological parameters examined were affected by treatment. Thyroid (absolute and relative to body/brain) weight was increased in both sexes at the 1000 and 3000 ppm dose levels, but statistical significance was attained only at the highest dose level. Brain weight (absolute) was significantly decreased in the 3000 ppm males. Follicular hyperplasia (thyroid) was displayed in all dogs (both sexes) at the two highest dose levels, with the severity increasing with dose. A tentative NOEL can be set at 80 ppm (2.49 ♂/2.69 ♀ mg/kg), the LEL at 1000 ppm (29.8 ♂/29.9 ♀ mg/kg), based on decreased body-weight gain/food consumption, changes in hematology/clinical chemistry parameters indicative of thyroid toxicity/anemia, increased thyroid weight, and follicular (thyroid) hyperplasia. Although no signs of neurotoxicity were observed in any of the clinical/physical parameters examined in any of the dogs, neuropathological examinations should be performed, in light of the fact that microscopic lesions can occur in the absence of any clinical signs.

Classification: core-supplementary, pending submission of the neuropathological examination of the sciatic nerve. This study does not satisfy the guideline requirement (83-1) for a chronic toxicity study in a non-rodent, but it may be upgraded with the submission of the required data.

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 4/13/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 4/14/92
Section II Head, Tox. Branch II (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: Chronic - dog TOX. CHEM NO: 041A

MRID NO.: 421331-01

TEST MATERIAL: Metiram Premix 95%

SYNONYMS: Polyram

CHEMICAL NAME: Zinc ammoniate ethylene bisdithiocarbamate poly [ethylen(thiuram disulfide)]

STUDY NUMBER: RCC Project 206627; BASF Project ZST 33 DO 636/899005

SPONSOR: BASF Aktiengesellschaft

TESTING FACILITY: a) RCC, Research & Consulting Co. Ltd/Switzerland
b) RCC Umweltchemie AG/Switzerland
c) Experimental Pathology Services (UK) Ltd. & RCC (UK) Ltd/England
d) The Department of Oral Pathology, Dental Institute/England

TITLE OF REPORT: 52-Week Oral Toxicity (Feeding) Study with METIRAM PREMIX 95% in the Dog

AUTHOR(S): SJ Corney, TR Allen, Th. Frei, H Luetkemkier, K Biedermann, Dr. O Vogel, Dr. C Springall

REPORT ISSUED: August 22, 1991

CONCLUSION: Under the conditions of the study, exposure to Metiram via the diet at dose levels of 30, 80, 1000, and 3000 ppm for one year resulted in decreased body weight/body weight gain and food consumption in the 3000 ppm dogs of both sexes and in the 1000 ppm female dogs. Several hematology parameters were affected by treatment at the highest dose levels, suggestive of a hemolytic anemia. Additionally, several clinical chemistry parameters were altered, which are consistent with hypothyroidism. None of the neurological parameters examined was affected by treatment. Thyroid (absolute and relative to body/brain) weight was increased in both sexes at the 1000 and 3000 ppm dose levels, but statistical significance was attained only at the highest dose level. Brain weight (absolute) was significantly decreased in the 3000 ppm males. Follicular hyperplasia (thyroid) was displayed in all dogs (both sexes) at the two highest dose levels, with the severity increasing with dose. The NOEL for effects other than

neuropathological effects was 80 ppm (2.49 ♂/2.69 ♀ mg/kg) and the LEL was 1000 ppm (29.8 ♂/29.9 ♀ mg/kg), based on decreased body-weight gain/food consumption, changes in hematology/clinical chemistry parameters indicative of thyroid toxicity/anemia, increased thyroid weight, and follicular (thyroid) hyperplasia. Although none of the neurological parameters examined was affected by treatment, microscopic lesions can occur in the absence of clinical signs. Therefore, neuropathological examination of the sciatic nerve is required before a final determination regarding the NOEL can be made.

Classification: core-supplementary, pending submission of the results of the neuropathological examination of the sciatic nerve. This study does not satisfy the guideline requirement (83-1) for a chronic toxicity study in a non-rodent, but it may be upgraded.

A. MATERIALS

1. Test Compound: Metiram Premix 95%; Description: solid, light yellow; Batch #: WF 5103; Purity: 93.6 %.

2. Test Animals: Species: dog, Strain: pure-bred beagle; Age: 6-7 months; Weight: 5.0-9.5 kg; Source: KFM Kleintierfarm Madörin AG/Switzerland; Breeder: Hazleton-LRE/Kalamazoo, MI, USA.

3. Statistics: Body weights, clinical laboratory data, and organ weights-Univariate one-way analysis of variance to assess the significance of intergroup differences; if the variables assumed to follow a normal distribution, the Dunnett-Test (many to one t-test)based on a pooled variance estimate was applied for comparison between treated and control groups; if a normal distribution was not assumed, the Steel-test (many-one rank test) was applied. Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables.

B. STUDY DESIGN

1. Animal Assignment: Dogs were acclimated for at least 14 weeks (1 dog for 11 weeks) prior to study initiation. Each was dewormed, vaccinated (distemper, leptospirosis, contagious hepatitis, parvovirus, parainfluenza, bordetella, and rabies), and treated with Telmin KH shortly after arrival to remove gastrointestinal parasites. During the acclimatization period, 5 dogs per sex were randomly selected for parasitological and bacteriological investigations as an additional health control. Dogs were assigned to groups by a computer-generated algorithm. The animals in each treatment group were housed in adjacent kennels (minimum of 2.0 square meters floor space each) until the middle of week 4 of treatment. Thereafter, four animals from each group were housed, in adjacent kennels, in Room UO9; the remaining dog from each group was housed in Room UO2 (no explanation was provided).

   Five dogs per sex were assigned to each group (control, 30, 80, 1000, and 3000 ppm) and the test material was administered via the diet for 52 weeks. The dogs were provided with 300 grams of granular standard Kliba 335 dog maintenance diet (Kliba Klingentalmühle AG/Switzerland), which was presented at ≈ 10 a.m. daily and removed at 1 p.m. and water ad libitum.

2. Diet Preparation: Feed admixture was prepared every 2 weeks and stored at room temperature. METIRAM PREMIX 95% was mixed with microgranulated feed (not further described). Samples of treated feed were analyzed for homogeneity and concentration at three-month intervals. Prior to study initiation, a trial mix was performed for the determination of stability,

homogeneity, and content of the test material in the feed.

RESULTS

After study termination, the stability of the test material was assessed. Active ingredient content decreased from an initial value of 93.6% (determined 8/89) to a value of 89.2% (determined 4/91). NOTE: In the section of the report that describes the test material, it states that the Expiration Date of the test material was August 31, 1990. The study dates were from 9/4/89 to 9/3-7/90.

C. METHODS AND RESULTS

1. Observations

Animals were inspected twice daily for viability and changes in behavior.

Toxicity/Mortality (survival)

There were no deaths during the study. Diarrhea (slight to marked) was observed in males at 80 ppm and in both sexes at 1000 and 3000 ppm; the mean severity increased with dose. Examination of the estrus cycle records indicated that 2/5, 5/5, 3/5, and 3/5 dogs at 30, 80, 1000, and 3000 ppm, respectively, displayed estrus changes compared to all control dogs; however, this was not considered treatment-related by the authors due to a lack of a dose relationship and the fact that a wide variation in the age of onset of estrus occurs in beagle dogs. No other clinical signs were reported.

2. Bodyweight

Animals were weighed weekly, including the first and last complete day of dosing and before necropsy.

RESULTS

In general, males at the highest dose level displayed the lowest body weights during the study, from the first week of dosing on, although the decrease was never more than 10% less than the control value. In females, the two highest dose levels displayed lower body weights compared to the control values. It is to be noted that the starting body weights of the females at the 1000 ppm level were the lowest prior to treatment, and this relationship to the other groups persisted throughout most of the study.

Body Weight (% of Control)

Week/Dose	30 ppm	80 ppm	1000 ppm	3000 ppm
MALES				
Pre-test	105	100	102	104
1	105	100	102	102
2	105	102	103	95
3	105	100	102	93
4	105	101	106	98
5	103	100	103	95
6	104	101	103	92
7	104	100	103	93
8	105	101	107	95
9	104	101	107	95
10	104	102	108	93
11	104	102	106	93
12	105	102	107	93
13	103	101	105	91
26	102	100	04	96
40	101	98	101	97
50	102	95	102	97
52/52	103/104	96/96	102/102	95/97
FEMALES				
Pre-test	100	99	95	100
1	99	99	93	99
2	99	99	91	95
3	99	100	91	95
4	99	99	90	95
5	96	98	89	93
6	99	101	90	90
7	98	99	87	89
8	99	100	91	93
9	99	100	93	95
10	96	99	89	90
11	95	98	89	87
12	95	97	89	90
13	97	100	91	92
26	91	97	89	88
40	92	93	91	93
50	96	98	92	96
52/52	94/92	99/96	92/88	91/90

The authors presented the body-weight gain data on a % basis; the 3000 ppm males showed a statistically significant decrease from week 1 through 18 of the study. The 1000 and 3000 ppm females also showed a decrease in body-weight gain on a % basis, but statistical significance was not attained. NOTE: TB II was unable to verify the data presented as a %, apparently due to the rounding of the numbers in the computer-generated tables.

Body-Weight Change (%)

Week/Group	0 ppm	30 ppm	80 ppm	1000 ppm	3000 ppm
MALES					
2	2.0	1.5	3.0	1.3	-5.4**
3	3.7	3.2	3.1	2.8	-5.9**
4	4.5	3.8	5.0	6.1	-1.5*
5	7.2	5.8	6.6	7.2	-1.5**
13	13.0	11.7	14.3	15.9	0.4**
16	13.0	12.1	14.3	16.4	0.3*
18	13.5	11.6	13.1	15.3	1.4*
26	13.6	9.9	13.1	14.9	3.9
52	18.8	17.3	13.5	18.0	10.8
FEMALES					
2	1.4	1.6	1.1	-1.5	-2.2
3	2.7	3.1	2.9	-0.4	-0.6
4	5.5	5.0	5.1	0.5	2.0
5	6.1	4.7	5.0	0.7	0.0
13	14.1	11.1	13.5	10.2	6.2
16	13.7	10.8	13.0	12.8	7.6
18	13.7	11.7	14.5	13.7	7.0
26	18.4	9.8	15.5	12.7	5.8
52	16.6	13.5	16.4	16.3	8.7

TB II calculated the body-weight gain/week for each group for the first 13 weeks, as well as the overall body-weight gain for this time period (see table below), but no statistical analysis was performed.

Mean Body-Weight Change (kg)

Interval/Group	0 ppm	30 ppm	80 ppm	1000 ppm	3000 ppm
MALES					
Pre-test	0.16	0.08	0.16	0.22	0.10
1-8	0.16	0.16	0.24	0.08	-0.42
8-15	0.14	0.14	0.04	0.14	-0.02
15-22	0.08	0.06	0.12	0.28	0.34
22-29	0.2	0.16	0.16	0.46	0.04
29-36	0	0.1	0.12	0.06	-0.16
36-43	0.2	0.08	0	0.04	0.18
43-50	-0.1	-0.02	0.04	0.16	0.04
50-57	0.14	0.06	0.1	0.22	0.2
57-64	0.04	-0.08	0.08	0.2	-0.12
64-71	0.12	0.24	0/16	0.12	0.06
71-78	0.06	0.06	0.06	0.12	-0.06
78-85	0.14	0.04	0.12	0.04	0.08
85-92	0.2	-0.08	-0.04	-0.075	-0.14
1-92	0.90	0.98	1.16	1.30	-0.08
FEMALES					
Pre-test	0.10	0.12	0.12	0.10	0.16
1-8	0.10	0.14	0.08	-0.08	0.18
8-15	0.08	0.08	0.14	0.08	0.14
15-22	0.18	0.16	0.18	0.04	0.22
22-29	0.12	-0.02	-0.02	0	-0.18
29-36	0	0.16	0.22	0.14	-0.12
36-43	0.30	0.22	0.16	0.08	0.12
43-50	-0.34	-0.18	-0.16	0.06	0.04
50-57	0.16	0.06	0.02	0.22	0.32
57-64	0.16	0	0.12	-0.14	-0.22
64-71	0.28	0.2	0.10	0.18	-0.04
71-78	0	0.02	-0.02	0.04	0.26
78-85	-0.02	0.02	0.14	0.1	0.12
85-92	-0.08	-0.08	-0.20	-0.12	-0.18
1-92	0.94	0.74	0.94	0.60	0.30

3. Food Consumption and Compound Intake

Food consumption was recorded daily for each dog. Test material intake was calculated by multiplying the nominal test material concentration (mg/g diet) by the average daily food consumption over one week (g/dog/day) and then dividing by body weight (kg).

RESULTS

Group mean food intake was decreased in males at the 3000 ppm dose level throughout the study and in females at the 1000 and 3000 ppm dose levels. Although the decrease at the 1000 ppm dose level in females was usually greater than the decrease at 3000 ppm, the pre-test values for the 1000 ppm group were the lowest and this relationship did not alter. With regard to the male groups, the 30, 80, and 1000 ppm group dogs consistently consumed nearly all of the 300 grams provided per day, while the control dogs fluctuated up and down; as a result, the 3000 ppm males appear to attain comparable consumption values at some time points.

Mean Food Consumption (g/dog/day; % of control)

Week/Group	30 ppm	80 ppm	1000 ppm	3000 ppm
MALES				
Pre-test	107	105	102	101
1	103	103	97	63
2	102	103	100	56
3	103	103	99	64
4	103	101	101	69
5	102	102	96	55
6	101	101	99	59
7	101	101	100	63
8	106	106	106	68
9	100	100	100	63
10	101	101	101	64
11	103	103	103	74
12	104	104	102	74
13	106	105	99	74
16	105	105	105	80
23	107	106	106	90
35	108	108	108	100
42	105	103	105	94
52	106	102	106	89
1-52	105	103	103	82

Week/Group	30 ppm	80 ppm	1000 ppm	3000 ppm
FEMALES				
Pre-test	101	94	88	103
1	99	97	74	84
2	108	104	80	80
3	104	102	77	74
4	102	96	77	74
5	102	104	81	67
6	102	102	82	75
7	103	103	81	79
8	111	111	93	94
9	98	100	80	77
10	98	99	82	74
11	99	100	83	84
12	102	104	85	89
13	99	108	84	86
15	105	101	89	96
18	100	104	88	90
20	100	101	80	88
29	104	99	76	93
31	110	101	97	100
39	108	95	91	94
40	100	88	76	87
48	103	107	84	97
52	94	95	81	88
1-52	101	101	84	88

Test material intake is listed below for each sex and dose level.

Dose Level (ppm)	Test Material Intake (mg/kg/day)	
	MALES	FEMALES
30	0.91	1.05
80	2.49	2.69
1000	29.84	29.89
3000	76.85	92.69

4. Ophthalmological examination

Each dog was examined for abnormalities of the eyes ≈ 20 minutes after the instillation of 0.5% tropicamide solution (Mydriaticum, Dispersa AG) using a binocular indirect ophthalmoscope (all pupil model, Keeler Instruments, Inc., USA). The observation area included the cornea, lens, conjunctiva, sclera, iris, and fundus. Examinations were performed pre-test, and at weeks 13, 26, and 52.

RESULTS

There were no adverse effects noted at any dose level in either sex throughout the study.

5. Clinical Laboratory Investigations

Blood and urine samples were collected from all dogs (fasted overnight, but allowed access to water ad libitum. In order to

reduce biological variation caused by circadian rhythms, the samples were collected between 7 and 9:30 a.m.. Blood samples were drawn from the jugular vein, and urine was collected using a catheter. Blood and urine were collected twice pre-test and at weeks 13, 26, and 52.

a. Hematology: 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	X	Reticulocyte count
	Blood clotting measurements	X	Red cell morphology
X	(Thromboplastin time)		
X	(Activated partial thromboplastin time)		
X	Nucleated erythrocytes normoblasts		

RESULTS

Mean erythrocyte count, hemoglobin concentration, and hematocrit (occasionally) were decreased, with increased mean cell volume and decreased mean cell hemoglobin were displayed by both sexes at the 1000 and 3000 ppm dose levels at each time point. Slight reticulocytosis (statistically significant) was observed in females at the 1000 ppm dose level and in both sexes at the 3000 ppm dose level at weeks 26 and 52. At week 52, slight polychromasia was observed in 3 females at 1000 ppm and in 2 males and 3 females at 3000 ppm. The latter finding was observed in one 1000 ppm male and 1 control male also. No other changes were reported.

Hematology Findings (males)♥

Parameter/Dose	0 ppm	30 ppm	80 ppm	1000 ppm	3000 ppm
Erythrocytes					
pre-test	5.77	5.94	5.79	5.65	6.11
pre-test	5.67	5.81	5.69	5.49	6.06
13	6.26	6.12	5.84	5.46*	5.58
26	6.86	6.36	6.31	5.87**	5.96*
52	6.82	6.53	6.64	5.95*	6.10
Hemoglobin					
pre-test	8.1	8.2	8.0	8.0	8.3
pre-test	7.9	8.1	7.9	8.0	8.4
13	9.0	8.7	8.3	8.2	8.2
26	9.8	8.9	9.0	8.8*	8.6*
52	9.8	9.3	9.4	9.0	8.9
Hematocrit					
pre-test	0.39	0.40	0.39	0.39	0.41
pre-test	0.39	0.40	0.38	0.39	0.41
13	0.41	0.40	0.38	0.39	0.39
26	0.46	0.42	0.42	0.42	0.42
52	0.47	0.44	0.45	0.43	0.44

Parameter/Dose	0 ppm	30 ppm	80 ppm	1000 ppm	3000 ppm
MCV					
pre-test	68.4	67.5	66.9	69.4	67.9
pre-test	69.5	68.0	67.4	70.3	68.4
13	66.1	65.6	65.5	70.8**	70.8**
26	66.9	66.0	66.2	70.7**	70.3*
52	69.0	67.9	67.9	72.6*	72.6*
MCHC					
pre-test	20.5	20.5	20.6	20.4	20.1
pre-test	20.2	20.5	20.5	20.7	20.2
13	21.8	21.7	21.8	21.1**	20.9**
26	21.5	21.3	21.7	21.1	20.6**
52	20.8	20.9	21.0	20.9	20.1**
Reticulocytes					
pre-test	0.002	0.002	0.002	0.001	0.001
pre-test	0.008	0.005	0.007	0.008	0.008
13	0.002	0.002	0.001	0.003	0.006**
26	0.014	0.009	0.009	0.009	0.017
52	0.005	0.005	0.008	0.010	0.016*

Hematology Findings (females)♥

Parameter/Dose	0 ppm	30 ppm	80 ppm	1000 ppm	3000 ppm
Erythrocytes					
pre-test	6.17	6.38	6.40	6.27	6.09
pre-test	6.13	6.24	6.29	6.41	6.09
13	6.91	6.83	6.60	6.19	5.77*
26	7.05	7.03	6.71	6.33	6.00
52	7.23	6.84	6.71	6.34	5.80*
Hemoglobin					
pre-test	8.6	8.9	8.8	8.6	8.4
pre-test	8.8	8.8	8.8	8.8	8.4
13	9.8	9.6	9.4	9.2	8.4*
26	10.0	10.0	9.6	9.3	8.8
52	10.3	9.7	9.6	9.3	8.5
Hematocrit					
pre-test	0.43	0.44	0.43	0.43	0.42
pre-test	0.43	0.43	0.43	0.45	0.42
13	0.46	0.45	0.43	0.43	0.41
26	0.47	0.47	0.45	0.45	0.42
52	0.50	0.47	0.46	0.46	0.43
MCV					
pre-test	69.0	68.6	67.5	69.1	68.8
pre-test	69.2	69.2	67.9	69.5	69.2
13	66.0	65.7	65.6	70.0*	71.0**
26	66.6	66.5	66.4	71.0**	70.2*
52	69.6	69.0	68.1	72.6	73.5*
MCHC					
pre-test	20.3	20.4	20.5	19.9	20.1
pre-test	20.4	20.3	20.6	19.9*	20.1
13	21.6	21.5	21.7	21.1	20.5**
26	21.4	21.4	21.5	20.7	20.8
52	20.4	20.7	21.0*	20.2	19.8*
Reticulocytes					
pre-test	0.002	0.002	0.003	0.003	0.002
pre-test	0.014	0.011	0.012	0.016	0.012
13	0.001	0.003	0.001	0.004	0.005*
26	0.010	0.009	0.005	0.020**	0.016
52	0.008	0.006	0.003	0.014	0.018*

♥ units: RBC-T/l; HB-mmol/l; HCT-l/l; MCV-fl; MCHC-mmol/l; ret-l

b. Clinical Chemistry: The CHECKED (X) parameters were examined.

X	Electrolytes:
X	Calcium
X	Chloride
X	Magnesium
X	Phosphorous
X	Potassium
X	Sodium
X	Iron
	Enzymes
X	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
X	Creatine kinase (CK)
X	Lactate dehydrogenase (LAD)
X	Serum alanine aminotransferase
X	Serum aspartate aminotransferase
X	Gamma glutamyl transferase (GGT)
X	Glutamate dehydrogenase (GLDH)
X	Ornithine carbamyltransferase (OCT)
X	Serum protein electrophoresis*
X	Thyroxine, total T4

X	Other:
X	Albumin
X	Blood creatinine
X	Blood urea nitrogen
X	Cholesterol
X	Globulins
X	Glucose
X	Phospholipids
X	Total bilirubin
X	Total serum Protein (TP)
X	Triglycerides
X	Lipids, total
X	Triiodothyronine, total T3

* Electrophoretic fractions: Albumin, α1-globulin, α2-globulin, b1-globulin, b2-globulin, sb (sum of beta globulins), c-globulin

RESULTS

Group mean glucose concentration was decreased slightly at 3000 ppm in both sexes throughout the study, but statistical significance was attained in both sexes only at week 52. In females, the decrease at week 52 was observed at all dose levels, but the low (30 ppm) dose displayed the lowest value and no clear dose response was observed. Increased group mean values for total lipids, cholesterol, and triglycerides were observed in males at the 1000 and 3000 ppm dose levels (dose-related), and an increase in phospholipids was displayed in the 3000 ppm males. Similar changes were displayed in the females at the highest dose level, although statistical significance was not always attained. Thyroxine (T_4) values were decreased in both sexes at the 3000 ppm dose level and in males at the 1000 ppm dose level throughout the study, although statistical significance was not always attained.

MALE CLINICAL BIOCHEMISTRY FINDINGS♥

Parameter/Dose	0 ppm	30 ppm	80 ppm	1000 ppm	3000 ppm
Glucose					
pre-test	6.40	6.34	6.22	6.37	6.43
pre-test	6.22	5.98	5.96	6.09	6.12
13	6.06	5.54*	5.67	5.58*	5.52*
26	5.62	5.46	5.26	5.53	5.25
52	6.26	5.67	5.66	5.96	5.37**
Total Lipids					
pre-test	2.6	2.8	2.9	2.8	3.0
pre-test	2.7	3.1	3.1	3.1	3.2
13	2.9	3.0	3.2	3.9	6.4*
26	2.6	3.1	3.2	3.7	4.9**
52	2.6	3.3	3.3	4.0*	5.4**
Cholesterol					
pre-test	3.45	3.85	4.14	3.80	3.94
pre-test	3.09	3.62	4.09	3.74	3.87
13	2.92	3.50	3.87	4.41	6.29**
26	2.72	3.37	3.73	4.43*	5.85**
52	3.13	3.86	3.55	4.63*	6.41**
Phospholipids					
pre-test	3.65	3.99	4.22	3.96	4.02
pre-test	3.37	3.84	3.84	3.97	3.93
13	3.36	3.88	3.92	4.33	5.23**
26	2.92	3.43	3.65	4.09*	4.94**
52	3.32	4.02	3.75	4.33	5.36**
Triglycerides					
pre-test	0.45	0.45	0.47	0.45	0.43
pre-test	0.41	0.45	0.43	0.45	0.46
13	0.45	0.41	0.40	0.55	0.84*
26	0.43	0.49	0.48	0.55	0.62
52	0.45	0.55	0.60	0.68*	0.71*
Thyroxine (T_4)					
pre-test	60.2	58.1	58.1	56.6	57.3
pre-test	58.5	55.5	58.2	50.5	50.8
13	41.3	36.3	36.7	28.3*	17.0**
26	43.3	40.2	44.4	36.6	31.6**
52	53.9	48.7	50.5	39.9*	31.3**
Alkaline Ph.					
pre-test	5.50	4.48	3.97	5.53	4.84
pretest	5.17	4.04	3.78	5.23	4.16
13	3.45	2.94	2.68	4.47	4.02
26	2.62	2.50	2.09	3.36	3.91*
52	2.25	2.13	1.79	2.87	2.90

♥ units: GLU/CHOL/PL/TG-mmol/l; TL-g/l; T_4-nmol/l; AlkP-ukat/l

FEMALE CLINICAL BIOCHEMISTRY VALUES♥

Parameter/Dose	0 ppm	30 ppm	80 ppm	1000 ppm	3000 ppm
Glucose					
pre-test	6.26	6.34	6.30	6.64	6.56
pre-test	6.37	6.15	5.97	6.11	6.59
13	6.17	5.77	5.79	5.71	5.67
26	5.81	5.37	5.33	5.28	5.08**
52	6.61	5.55**	5.78*	5.74**	5.56**
Total Lipids					
pre-test	2.8	2.8	3.3	2.6	3.0
pre-test	3.0	3.3	3.3	2.9	2.9
13	3.1	2.8	3.6	3.8	5.2**
26	3.3	2.9	4.9	4.6	5.0
52	3.8	3.1	5.8	5.2	6.8
Cholesterol					
pre-test	3.63	3.62	4.34	3.59	4.02
pre-test	3.55	3.57	4.27	3.54	3.68
13	3.55	3.20	4.42	4.50	5.79**
26	3.84	3.24	5.19	5.09	5.52
52	3.86	4.07	6.38*	5.69	6.88*
Phospholipids					
pre-test	3.72	3.70	4.36	3.65	3.88
pre-test	3.88	3.76	4.41	3.77	3.76
13	3.74	3.52	4.46	4.31	5.02**
26	3.90	3.38	4.82	4.61	4.77
52	4.05	4.19	5.57*	5.07	5.63*
Triglycerides					
pre-test	0.47	0.46	0.49	0.41	0.46
pre-test	0.44	0.52	0.46	0.42	0.42
13	0.51	0.41	0.47	0.51	0.70
26	0.48	0.49	0.71	0.59	0.68
52	0.71	0.47	0.76	0.68	0.82
Thyroxine(T_4)					
pre-test	58.6	58.0	60.0	56.1	50.5
pre-test	53.7	56.4	52.8	57.8	56.4
13	39.0	39.9	41.4	35.0	20.3**
26	44.6	41.9	44.9	39.2	31.1*
52	37.9	45.3	55.5**	38.2	29.4
Alkaline Ph.					
pre-test	3.87	5.80**	4.53	4.62	5.83**
pre-test	3.56	5.14*	4.31	3.99	4.92
13	2.72	4.23	3.47	4.19	5.52**
26	2.55	3.76	3.17	4.61	4.57
52	2.44	3.67	2.93	5.07	4.14*

♥ units: GLU/CHOL/PL/TG-mmol/l; TL-g/l; T_4-nmol/l; AlkP-ukat/l

6. Urinalysis: The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen
X	Osmolality	X	Color

RESULTS

The authors stated that none of the parameters were affected by treatment, but TB II notes that the 3000 ppm animals (both sexes) displayed slightly increased blood scores and RBC scores compared to the control and other treated groups.

7. Neurological Examinations

Neurological examinations, including examination of gait, postural reactions, spinal reflexes, and cranial nerves, were performed on each dog pre-test and at weeks 14, 26, 40, 46, and 51.

(a) **Postural Reactions:**
(1) Wheelbarrowing, wheelbarrowing with extended neck;
(2) Hopping - thoracic and pelvic limbs;
(3) Extensor postural thrust;
(4) Hemistanding and hemiwalking;
(5) Placing thoracic limbs - visual and tactile;
(6) Tonic neck reaction;
(7) Proprioceptive positioning.

(b) **Spinal Reflexes**
(1) Muscle tone;
(2) Patellar reflexes;
(3) Biceps and triceps reflex;
(4) Flexor reflexes - pelvic and thoracic limbs;
(5) Pain perception;
(6) Crossed extensor reflex
(7) Perineal reflex;
(8) Panniculus reflex.

(c) **Cranial Nerves**
The cranial nerves examined are in brackets ( ). The head was observed for evidence of abnormal posture (vestibular VIII), facial muscle weakness or contracture (VII), or atrophy of the muscles of mastication (Motor V). With one eye covered, the other eye was menaced (II-VII). If the response was absent, the eyelids were checked for their ability to close (VII). The symmetry of the pupils and their reaction of light were observed (II-III). The eyes were checked for evidence of abnormal posture, strabismus (III, VI, vestibular VIII), or abnormal nystagmus (vestibular VIII). The corneal and palpebral reflexes (sensory V-VII), ear movement, (VII)< and the position of the philtrum (VII) were tested. The commissure of the lips was checked for hypertonia (VII). The skin sensation was checked over the entire head (sensory V). The jaws were observed for normal closure (motor V). The resistance to opening of the mouth was tested for normality (motor V). The position of the tongue, its movements, and size (atrophy) and strength were tested (XII). The gag reflex was checked (IX, X).

RESULTS

There were no treatment-related effects on any of the neurological parameters examined.

8. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The following organs were weighed: adrenal glands, brain (including brainstem), heart, kidneys, liver, pituitary, testes with epididymides, thyroid gland with parathyroid.

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

♥femur & sternum; ♦medulla/pons, cerebellar & cerebral cortex; ♣mesenteric & retropharyngeal; ♠parotid & mandibular; ■cervical, midthoracic, & lumbar

Bone marrow smears from one sternebrum from all dogs were taken for possible further investigation. Samples of sciatic nerve, after perfusion fixation with glutaraldehyde, were embedded in epoxy resin blocks for possible future examination.

a. Organ Weight: The weight (absolute, relative to body and brain weight) of the thyroid gland was increased in both sexes at the 3000 ppm dose level. Additionally, the thyroid weight was increased in both sexes at the 1000 ppm dose level, but statistical significance was not attained. There was a dose-related decrease in absolute brain weight in males, but

statistical significance was attained only at the 3000 ppm dose level.

Organ-Weight Data (grams)

Group/Organ	0 ppm	30 ppm	80 ppm	1000 ppm	3000 ppm
MALES					
Thyroid (absolute)					
left	0.394	0.380	0.448	0.543	1.553**
right	0.323	0.361	0.295	0.615	1.345**
total	0.717	0.741	0.743	1.158	2.898**
(relative) to: body wt.					
left	0.0042	0.0039	0.0051	0.0058	0.0171**
right	0.0035	0.0037	0.0033	0.0065	0.144**
brain wt.					
left	0.4658	0.4655	0.5580	0.6832	1.9959**
right	0.3839	0.4419	0.3667	0.7723	1.7283**
Brain					
(absolute)	85.4	81.5	80.2	79.9	77.6*
(relative)	0.9	0.8	0.9	0.8	0.9
FEMALES					
Thyroid (absolute)					
left	0.403	0.334	0.409	0.488	1.296**
right	0.397	0.347	0.448	0.490	1.245**
total	0.800	0.681	0.857	0.978	2.541**
(relative to): body wt.					
left	0.0049	0.0045	0.0052	0.0065	0.0179**
right	0.0048	0.0046	0.0057	0.0066	0.0173**
brain wt.					
left	0.5402	0.4462	0.5743	0.6489	1.7462**
right	0.5264	0.4622	0.6288	0.6508	1.6755**
Brain					
(absolute)	74.8	75.8	71.3	75.3	74.0
(relative)	0.9	1.0	0.9	1.0	1.0

** $p<0.01$

b. Gross Pathology: All 3000 ppm dogs displayed thyroid enlargement and thickening macroscopically. Thickening alone was observed at all other dose levels (two ♀♀ at 30 ppm, one ♂ & two ♀♀ at 80 ppm, 2 ♂♂ & 4 ♀♀ at 1000 ppm), and the incidence was dose-related.

c. Microscopic Pathology: Follicular hyperplasia (thyroid) was displayed in both sexes at the two highest dose levels (all dogs), with severity increasing with dose.

Severity of Follicular Hyperplasia in the Thyroid

Group (PPM) Parameter	MALES					FEMALES				
	0	30	80	1000	3000	0	30	80	1000	3000
THYROID n=	5	5	5	5	5	5	5	5	5	5
Follicular hyperplasia										
Grade 1	0	0	0	1	0	0	0	0	1	0
Grade 2	0	0	0	4	0	0	0	0	4	0
Grade 3	0	0	0	0	0	0	0	0	0	1
Grade 4	0	0	0	0	2	0	0	0	0	2
Grade 5	0	0	0	0	3	0	0	0	0	2

The incidence of focal hepatic pigment deposition increased with dose. Lipofuscin was identified as the pigment in 2 dogs (Schmoris stain), and it was assumed that lipofuscin was the pigment in the others as well.

Incidence and Severity of Pigment Deposition

Group (PPM) Parameter	MALES					FEMALES				
	0	30	80	1000	3000	0	30	80	1000	3000
LIVER n=	5	5	5	5	5	5	5	5	5	5
Pigment deposit.										
Grade 1	0	0	0	0	1	0	1	0	1	1
Grade 2	1	0	0	1	1	0	0	0	0	2
Grade 3	0	0	0	1	2	0	0	0	1	2

D. DISCUSSION

The thyroid is the target organ for Metiram. Effects on the thyroid in the current study include increased organ weight, enlargement and thickening (macroscopically), and follicular hyperplasia (microscopically). There was a slight, but consistent, decrease in plasma thyroxine concentration, which the authors contend may have lead to an increased release of thyroid stimulating hormone from the pituitary gland, via a feedback mechanism, and the increase in the size of the thyroid gland. The authors stated that the decrease in glucose concentration and increase in protein and lipid levels are indicative of metabolic adaptation following thyroid disturbances. TB II notes that the changes observed in the clinical chemistry parameters (increased triglycerides, cholesterol, decreased glucose) are consistent with decreased thyroid function.

The treated dogs displayed anemia, which the authors contend had the characteristics of a hemolytic type anemia; i.e., a reduction of red cell values followed by compensatory hemopoiesis, which was indicated by the increased reticulocyte count and an increase in red cell size. The authors also stated that the increase in polychromasia indicates a

generalized bone marrow response, although TB II notes that no effects were reported in the bone marrow. In the Methods' section of the report, it is stated that bone marrow smears from one sternebrum from all dogs were taken for possible further investigation, but no data were provided.

Although none of the neurological examinations (performed to assess gait, postural reactions, spinal reflexes, and cranial nerves) was affected by treatment, no microscopic examination of the sciatic nerve was performed, other than routine histological examination. Since microscopic lesions can occur in the absence of any clinical signs, neuropathological examination of the sciatic nerve should be performed and the results submitted.

E. CONCLUSION

Under the conditions of the study, exposure to Metiram via the diet at dose levels of 30, 80, 1000, and 3000 ppm for one year resulted in decreased body weight/body weight gain and food consumption in the 3000 ppm dogs of both sexes and in the 1000 ppm female dogs. Several hematology parameters were affected by treatment at the highest dose levels, suggestive of a hemolytic anemia. Additionally, several clinical chemistry parameters were altered, which are consistent with hypothyroidism. Thyroid (absolute and relative to body/brain) weight was increased in both sexes at the 1000 and 3000 ppm dose levels, but statistical significance was attained only at the highest dose level. Brain weight (absolute) was significantly decreased in the 3000 ppm males. Follicular hyperplasia was displayed in all dogs (both sexes) at the two highest dose levels, with the severity increasing with dose. The NOEL for effects other than neuropathological effects was 80 ppm (2.49 ♂/2.69 ♀ mg/kg) and the LEL was 1000 ppm (29.8 ♂/29.9 ♀ mg/kg), based on decreased body-weight gain/food consumption, changes in hematology/clinical chemistry parameters indicative of thyroid toxicity/anemia, increased thyroid weight, and follicular (thyroid) hyperplasia. Although none of the neurological parameters examined was affected by treatment, microscopic lesions can occur in the absence of clinical signs. Therefore, neuropathological examination of the sciatic nerve is required before a final determination regarding the NOEL can be made.

This study is classified core supplementary, pending submission of the neuropathological examination of the sciatic nerve.

Tox Chem No. 041A Metiram File Last Updated _____ Current Date 4/13/92

STUDY/LAB/STUDY #/DATE	MATERIAL	EPA MRID NO.	RESULTS: LD50, LC50, PIS, NOEL, LEL	TOX CATEGORY	CORE GRADE/DOC. #
Chronic toxicity - dog RCC Project # 206627; BASF ZST 33 DO 636/899005; RCC Research & Consult. Co. Ltd/Switz.; Experimental Path. Services Ltd./ England; The Depart. Oral Path. England; 8/22/91	Metiram Premix 95%	421331-01	Under the conditions of the study, exposure to Metiram via the diet at dose levels of 30, 80, 1000, and 3000 ppm for one year resulted in decreased body weight/body weight gain and food consumption in the 3000 ppm dogs of both sexes and in the 1000 ppm female dogs. Several hematology parameters were affected by treatment at the highest dose levels, suggestive of a hemolytic anemia. Additionally, several clinical chemistry parameters were altered, which are consistent with hypothyroidism. None of the neurological parameters examined was affected by treatment. Thyroid (absolute and relative to body/brain) weight was increased in both sexes at the 1000 and 3000 ppm dose levels, but statistical significance was attained only at the highest dose level. Brain weight (absolute) was significantly decreased in the 3000 ppm males. Follicular hyperplasia (thyroid) was displayed in all dogs (both sexes) at the two highest dose levels, with the severity increasing with dose. The NOEL for effects other than neuropathological effects was 80 ppm (2.49 ♂/2.69 ♀ mg/kg) and the LEL was 1000 ppm (29.8 ♂/29.9 ♀ mg/kg), based on decreased body-weight gain/food consumption, changes in hematology/clinical chemistry parameters indicative of thyroid toxicity/anemia, increased thyroid weight, and follicular (thyroid) hyperplasia. Although none of the neurological parameters examined was affected by treatment, microscopic lesions can occur in the absence of clinical signs. Therefore, neuropathological examination of the sciatic nerve is required before a final determination regarding the NOEL can be made. Classification: core-supplementary, pending submission of the results of the neuropathological examination of the sciatic nerve. This study does not satisfy the guideline requirement (83-1) for a chronic toxicity study in a non-rodent, but it may be upgraded.		Supplementary

00133