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DATA EVALUATION REPORT

ZIRAM

Study Type: CHRONIC FEEDING  DOG (83-1b)

8/2/2000

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
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[ZIRAM]

Chronic Oral Study (83-1b)

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DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding (52 week) Study ☒ Dog (83-1b)

TOX. CHEM. NO.: 931

P.C.CODE.: 034805

MRID NO.: 42823901 (Main Study), 42375101 (Range-finding study)

TEST MATERIAL: ZIRAM (98.5% a.i.)

SYNONYMS: Dithiocarbamate pesticide, methyl cymate, Methasan, Zimate, Zirberk, Karbam White, Corozate, Zerlate, Chemical Names: bis(dimethyldithiocarbamate) zinc, bis(dimethylcarbomodithioato-S,S') zinc

STUDY NUMBER: ZIR 10/920533 (Main study), ZIR 8/901813 (Range-finding study)

SPONSOR: Ziram Task Force (Consortium No. 62405), c/o UCB Chemicals Corporation, 5505-A Robin Hood Road, Norfolk, VA 23513

TESTING FACILITY: Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridge-shire, PE18 6ES, England

TITLE OF REPORT: Ziram toxicity to dogs by repeated dietary administration for 52 weeks (2 volumes)

AUTHORS: Thomas G. Smith, David P. Buist, David Crook, Judith Morrow, Chirukandath Gopinath

REPORT ISSUED: June 8, 1993 (Study completion date)

EXECUTIVE SUMMARY: In a chronic feeding study (MRID No. 428239-01), Ziram (98.5%; Lot No. 8331 AA) was administered for 52 weeks in the diet to four male and four female beagle dogs per dose at concentrations of 0, 50, 185, and 700 ppm (700 ppm dose reduced to 500 ppm at day 3 of week 12), equivalent to doses of 0, 1.6, 6.6, 17.4 mg/kg/day for males and 1.9, 6.7, and 20.6 mg/kg/day for females, respectively.

There was a treatment-related convulsive episode at week 11 for a female in the 700/500 ppm dose group that required the animal to be euthanized. In addition to the convulsive episode, the findings for the 700/500 ppm dose group include: 1) decreased body weight gain (↓81%) in females over the treatment period and 2) histologic findings for livers (aggregates of Kupffer cells and macrophages, increased foci of degenerate hepatocytes, infiltration of inflammatory cells around central veins and branches of the hepatic vein and portal areas, and increased centrilobular fibrocytes in males). The findings for the 185 ppm dose group include decreased body weight gain (↓69%) in females during the treatment period. **The NOAEL is**

50 ppm based on the lack of significant toxicological effects. The LOAEL is 185 ppm based on decreased body weight gain in females.

Classification: This study is classified as **acceptable**. The study satisfies most of the guideline requirements for a chronic feeding study in beagle dogs (§83-1).

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test material: Ziram (technical)

Description: white powder

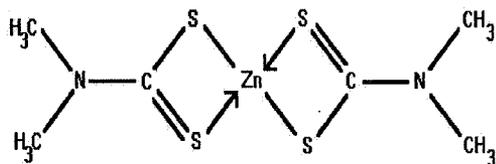
Lot/Batch No.: 8331 AA

Purity: 98.5% ai.

Stability of compound: active ingredient content stable for 2 years

CAS No.: 137-30-4

Structure:



2. Vehicle and/or positive control

Test material was mixed with diet. Negative control group was fed diet only. No positive control was described.

3. Test animals

Species: dog

Strain: Purebred beagle

Age and weight at study initiation: 30-34 weeks, males: 9.5-12.0 kg, females: 8.7-11.0 kg

Source: Interfauna UK Limited, Abbots Ripton Road, Wyton, Huntingdon, PE17 2DT England

Housing: The animals were housed in pairs (same sex and dosage group) in a block of several adjacent kennels and each kennel identified by a colored label displaying identification numbers, dosage level and study schedule number. Each kennel was 4.5 square meters. Graded whitewood sawdust (Lignocel, R.S. Supplies) was used as litter and changed daily.

Environmental conditions:

Temperature: 15-24°C

Humidity: Not reported

Air changes: 12 air changes/hour

Photoperiod: 12 hours light/dark cycle per 24 hour period

Acclimation period: 4 weeks prior to treatment initiation for all dogs, except the replacement female (112) in the 500 ppm group (acclimated for 0 weeks).

B. STUDY DESIGN1. Animal assignment

Thirty-two dogs were assigned in a pseudo-random manner to the test groups in Table 1, to give groups with approximately equal mean body weights while avoiding placing litter mates in the same group.

Dose selection rationale: Doses were selected on the basis of two preliminary studies, a 4-week feeding in dogs (HRC Report # ZIR 1/89637 and a 13-week feeding study in beagles (MRID # 423751-01, see Appendix to this report). The maximum tolerated dose for long-term administration to beagles based on these studies was considered to be <1000 ppm and the NOAEL to be <100 ppm. With Ziram treatment, minor pathologic changes in livers were seen at 300 ppm and minor increases in liver weight were seen at 100 ppm. Thus, the high dose treatment level was selected to be 700 ppm and the 50 ppm chosen as the NOAEL. However after one female in the 700 ppm group suffered convulsions thought to be related to Ziram treatment, the dosage was lowered to 500 ppm at day 3 of week 12 for all dogs (male and female) in the high dose group.

TABLE 1. STUDY DESIGN					
Dose Group	Conc. in Diet (ppm)	Dose (mg/kg/day)		No. of Animals	
		Male	Female	Male	Female

1 Control	0	0.0	0.0	4	4
2 Low (LDT)	50	1.6	1.9	4	4
3 Mid (MDT)	185	6.6	6.7	4	4
4 High (HDT)	700/500	17.4	20.6	4	4/3 ^a

Data taken from Table 4, pp. 63-64, MRID No. 428239-01.

^aOne female was sacrificed during week 11, after a convulsive episode. A second female was removed due to suspicion of polyarteritis during week 6, and a replacement female entered into the study at week 8.

2. Diet preparation and analysis

Diet was prepared weekly by mixing appropriate amounts of Ziram with ground standard dry diet (Diet A from Special Diets Services Ltd.) and was stored in daily aliquots, protected from light, at -20°C. Daily portions were fed and then discarded after 8 hours. Concentrated premixes were prepared by grinding appropriate amounts of Ziram and untreated basal diet in a turbula mixer for at least 2 minutes. The premixes were diluted to the appropriate concentrations by addition of basal diet and blending in a Gardner double-cone blender for at least 7 minutes. Diets for the 50 ppm group were prepared at 57.5 ppm in order to compensate for losses occurring during preparation (as determined by pre-study analyses). There were no significant losses for the diets prepared for the 185 or 700/500 ppm groups. Homogeneity and stability were tested at 5000, 100 and 50 ppm at room temperature. During the study, samples of treated food were analyzed at weeks 1, 13, 26, 39, and 52 for stability and concentration.

Results ❖

- a. Homogeneity analysis ❖ Diet was prepared with Ziram at concentrations of 50, 100, and 5000 ppm. Two samples from the top, middle, and bottom regions of each preparation were analyzed and were essentially homogeneous with respect to Ziram concentration.
- b. Stability analysis ❖ The stability of Ziram in diet preparations was assessed during four trials (Addendum 1, p. 287, MRID No. 428239-01). In the first two trials, stability at room temperature was assessed at the 100 and 5000 ppm inclusion levels. At 5000 ppm, Ziram mixed with diet was stable for 14 days (recovery of approximately 98%). However, at the 100 ppm inclusion level, losses occurred during storage at room temperature: ~16% after 24 hours, ~30% after 8 or 14 days. These losses were considered significant by the study authors. In trials three and four, the losses of Ziram in diet formulations at 100 ppm and 50 ppm were ~6% and ~16%, respectively during 8 hours at room temperature. These losses were not considered significant by the study authors.
- c. Concentration analysis ❖ Samples of diet for the 50, 185, and 700/500 ppm inclusion levels were analyzed at weeks 1, 13, 26, 39, and 52 of the study. Mean concentrations of Ziram were determined and varied between +10% and -14% of inclusion levels.

3. Diet

Animals were fed 400 g of diet between 1000 and 1100 hours (earlier on weekends). Any remaining food was removed by 1700 hours and weighed. Drinking water was available

ad libitum. Drinking water was subjected routinely to chemical analysis (for details, see Appendix 2 of Addendum 4, p. 494, MRID No. 428239-01).

4. Statistics

Food consumption was analyzed as totals over selected time periods and expressed on a weekly basis. Body weight data were analyzed using weight gains. The following statistical tests were used in sequence: Analysis of frequency of the mode, if greater than 75% the proportion of animals with values different from the mode was analyzed. Bartlett's test was used to test heterogeneity of variance between treatments. If significant heterogeneity of variance was found and could not be removed by logarithmic transformation, Kruskal-Wallis analysis of ranks was used. For pre-dose data, analysis of variance was followed by Student's *t*-test. For data during dosing, ANOVA was followed by Williams' test for a dose-related response. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of the *t*-test and Williams' test (Shirley's test). Selected histopathological findings were analyzed using Fisher's exact and Mantel's tests. Also, the final bodyweight was a covariate with the organ weight data if both were significantly different from controls at the 10% level.

5. Signed and dated GLP and quality assurance statements were present.

C. METHODS AND RESULTS

1. Observations

Animals were inspected regularly throughout the day (unspecified number of times) between 0900 and 1700 on weekdays and between 0900 and 1200 and again at 1700 on weekends and holidays for signs of toxicity and mortality. Summary of clinical signs and data for individual animals are found in Table 1, p. 58 Appendix 6, pp. 126-160.

Results ❖ One female (#368, 700 ppm dose group) was euthanized on day 7 of week 11, after a suspected convulsion thought to be related to Ziram treatment (clinical signs: collapsed and salivating with trembling, champing of the jaws and jerking limbs). Dosage was decreased for all animals in the 700 ppm group to 500 ppm at day 3 of week 12. One other female (No. 372) in the 700 ppm dose group was sacrificed during week 6 due to a suspicion of polyarteritis. This suspicion was not confirmed upon pathological examination although a mild synovitis was discovered that likely contributed somewhat to the clinical signs. This animal was replaced by another female (No. 112) at week 8.

2. Body weight

Animals were weighed, before feeding, once each week for the 4 weeks prior to and for the 52 weeks of the treatment period.

Results ❖ For males, there was no effect of Ziram treatment on weight gain (Table 2). For females treated with Ziram, there was a dose-dependent reduction in mean group weight gain and mean group body weights relative to controls (Table 2). From 0 to 52 weeks, mean weight gain for females treated with Ziram at 185 and 700/500 ppm was statistically significantly different from controls ($p < 0.05$ and $p < 0.01$, respectively) with body weight gain decreased by 69% at 185 ppm and decreased by 81% at 700/500 ppm as compared to the controls. During the first 39 weeks of treatment, control and Ziram-treated female dogs gained weight at similar rates. From 39-52 weeks, mean weight gain for female dogs treated with Ziram at 185 and 700/500 ppm was statistically significantly

($p < 0.05$) less than controls and was, in fact, weight loss while the controls still gained weight. At 52 weeks, mean group body weights for females were reduced, dose-dependently, compared to controls.

TABLE 2. GROUP MEAN BODY WEIGHTS (KG) AT SELECTED WEEKLY INTERVALS								
Week of Study	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	50	185	700/ 500	0	50	185	700/ 500
0	11.0	11.3	10.5	11.1	9.7	9.6	10.1	9.6
26	12.0	12.8	11.4	12.8	10.8	10.8	11.2	10.3
39	12.5	13.4	11.8	13.4	11.1	11.1	11.3	10.4
52	12.4	13.3	11.4	13.3	11.3	10.8	10.6	9.9
Weight Gain (kg)								
Weeks 0-26	1.0	1.6	0.9	1.7	1.1	1.2	1.1	0.7
Weeks 26-39	0.5	0.6	0.4	0.6	0.3	0.3	0.1	0.1
Weeks 39-52	-0.1	-0.1	-0.4	-0.2	0.3	-0.3	-0.7*	-0.5*
Weeks 0-52	1.4	2.0	0.9	2.2	1.6	1.2	0.5*	0.3**

Data adapted from Table 2 (pp.59-60) and Appendix 7 (pp.161-168), MRID No. 428239-01.

*p<0.05, Williams' test

**p<0.01, Williams' test

3. Food consumption and compound intake

Food consumption for each animal was determined daily and results presented as total grams consumed per week. Mean daily diet consumption (g food/kg body weight/day) was not presented by the study authors. Food efficiency (body weight gain, kg/food consumption, kg per unit time X 100) was not calculated by the study authors. Compound intake (mg/kg/day) values were expressed as $\frac{\text{achieved intake of Ziram}}{\text{weekly food consumption (g) x ppm (nominal)}} \times \text{mid-week bodyweight (g) x 7}$.

Results

- a. Food consumption The total mean food consumption for males in the 50, 185, and 700/500 ppm treatment groups was 100% of controls (Table 3). The total mean food consumption for females in the 50, 185, and 700/500 ppm treatment groups was 100%, 98% and 96% of control levels. Thus, the decreased bodyweight in females treated with Ziram at 185 and 700/500 ppm was not due to greatly decreased food consumption. No historical data on the average amount of food consumed by beagles was provided by the study authors.
- b. Compound consumption (time-weighted average) Mid-week body weights were calculated by the study authors for each animal for use in the determination of

TABLE 3. GROUP MEAN FOOD CONSUMPTION, FOOD EFFICIENCY, AND COMPOUND INTAKE								
Parameter	Exposure Level (ppm)							
	Males				Females			
	0	50	185	700/ 500	0	50	185	700/ 500
Food Consumption ^a (g/dog/day)	400	400	400	399.5	398.5	400	392.2	384.1
Total Food Consumption ^a (kg/dog)	145.6	145.6	145.6	145.4	145.1	145.6	142.8	139.8
Average Compound Intake (mg/kg/day)	---	1.6	6.6	17.4 ^b	---	1.9	6.7	20.6 ^b
Food Efficiency ^a	0.96	1.37	0.62	1.51	1.1	0.82	0.35	0.21

Data for calculation of food consumption taken from Table 3 (pp. 61-62), data for bodyweight gain taken from Table 2, pp. 59-60, data for average compound intake taken from Table 4 (pp.63-64), MRID No. 428239-01.

^a Calculated by reviewer.

^b Data reflect the initial dose of Ziram at 700 ppm, and the reduction to 500 ppm during week 12.

compound consumption (Table 3). Achieved intakes reflect the consumption of Ziram at the 700 ppm level and the reduction to 500 ppm at day 3 of week 12.

- c. Food efficiency ❖ Food efficiency was not calculated by the study authors. Using the data from Appendix 7 (Body weights, pp.161-168) and Appendix 8 (Food Consumption, pp.169-176), mean group food efficiency [(kg of weight gained per kg food consumed)x100] was calculated by the reviewer (Table 3). For males, food efficiency was quite variable, but the variations were not dose-related. For females, food efficiency decreased with increasing dose of Ziram.

4. Ophthalmoscopic examination

Prior to commencement of treatment, and at weeks 13, 26, and 52, the eyes of all animals were examined by means of a Keeler indirect ophthalmoscope. Pupils were dilated using Tropicamide ophthalmic solution (❖Mydricyl,❖ Alcon Laboratories, Inc.)

Results ❖ No results attributable to treatment with Ziram. All findings considered typical of the age and strain of animals. One dog (male 361) developed a corneal ulcer with keratitis and associated conjunctivitis in week 32 due to a sawdust particle lodging in the eye. Surgery was performed under anesthesia and by week 37 no further treatment was necessary.

5. Blood was collected for hematology and clinical analysis from all animals after an overnight fast. Samples of blood were withdrawn from the jugular or cephalic vein at weeks -2 (or for replacement dog 112, week 0), 13, 26, and 52. Blood was divided into 4 tubes: EDTA anticoagulant (hematology), citrate anticoagulant (coagulation tests), fluoride anticoagulant (plasma glucose), no anticoagulant (all other biochemical tests). The CHECKED (X) parameters were examined.

a. Hematology

<u>X</u>		<u>X</u>	
x	Hematocrit(HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular Hb conc. (MCHC)
x	Leukocyte count (WBC)*	x	Mean corpuscular volume (MCV)
x	Erythrocyte count (RBC)*	x	Reticulocyte count
x	Platelet count (PLTS)*		
	Blood clotting measurements		
x	(Thromboplastin time, APTT)		
	(Clotting time)		
x	(Prothrombin time, PT)		

*Required for chronic studies.

Results

- a. Males There were statistically significant changes ($p < 0.05$ or $p < 0.01$) in the hematologic parameters of dogs treated with Ziram at 700/500 ppm, relative to controls: increases in MCV, total WBC, lymphocytes and APTT at week 13; increased MCV at week 26; and decreased MCHC and increased MCV and lymphocytes at week 52 (Table 4). There were no statistically significant changes from control at weeks 13, 26, or 52 in PCV (packed cell volume, or hematocrit), Hb (HGB), RBC, neutrophils, eosinophils, basophils, monocytes, PLTS or PT with Ziram treatment. Reticulocyte counts were increased relative to controls at week 26 for 1 dog treated with Ziram at 700/500 ppm and at week 52 for 5 dogs (1 at 50 ppm, 2 at 185 ppm, and 3 at 700/500 ppm). Additional findings included elevated (peak at week 13) total WBC, lymphocyte, eosinophil and monocyte counts in all treatment groups at all time points examined during treatment. These elevations did not reach statistical significance. The toxicological importance of this finding is unclear. The study authors state that all the values determined in the hematologic tests are within the normal range for beagles (normal range data not provided).
- b. Females There were statistically significant changes ($p < 0.05$ or $p < 0.01$) in the hematologic parameters of dogs treated with Ziram at 185 and/or 700/500 ppm, relative to controls. The findings included increased platelet counts and APTT at week 13 and decreased RBC and increased MCV, monocytes and platelets at week 26 (Table 4). There were no statistically significant changes in PCV (HCT), Hb

TABLE 4. HEMATOLOGY, WEEKS 13-52, MEANS OF SELECTED PARAMETERS									
Males		RBC	MCHC	MCV	Total WBC	Lymph h.	Mono .	Plts	APTT
Week	Dose								
13	0	5.9	29.9	79	9.5	2.61	0.09	385	12.5
	50	5.9	29.4	81	13.6	4.12	0.00	366	12.5
	185	5.5	29.9	78	11.3	3.51	0.16	392	13.4
	500	5.4	30.0	83**	13.5*	4.56*	0.37	374	14.3*
26	0	6.2	29.6	76	8.0	2.15	0.09	403	11.5
	50	6.6	29.1	76	8.0	2.62	0.06	440	12.2
	185	6.3	29.1	78	10.3	3.37	0.23	460	12.4
	500	5.9	28.8	80**	8.4	2.77	0.19	365	12.4
52	0	6.1	31.0	83	7.9	2.15	0.21	421	11.8
	50	6.7	30.7	82	9.6	2.36	0.30	394	11.9
	185	5.8	30.5	81	9.2	2.56	0.22	442	12.5
	500	5.9	29.7**	87*	10.0	3.47*	0.23	483	12.8
Females		RBC	MCHC	MCV	Total WBC	Lymph h.	Mono .	Plts	APTT
13	0	6.4	29.4	80	9.8	3.02	0.27	328	13.0
	50	6.3	29.6	78	12.1	4.29	0.10	357	13.1
	185	5.9	29.7	80	16.3	5.24	0.11	444*	14.1
	500	5.8	28.3	85	12.1	4.35	0.27	480*	15.2**
26	0	6.7	29.2	77	8.2	2.45	0.11	365	12.7
	50	6.7	29.3	77	8.3	2.45	0.17	450	13.1
	185	5.8*	29.3	78	8.2	2.08	0.04	392	13.1
	500	6.0*	29.2	81*	10.3	2.90	0.34*	492*	15.8
52	0	6.7	30.3	83	10.4	2.72	0.35	446	13.4
	50	6.4	30.0	83	11.3	2.58	0.30	532	13.3
	185	6.5	30.3	84	11.7	3.01	0.25	501	13.7
	500	6.4	29.3	88	11.1	4.25	0.58	436	14.3

Data adapted from Table 7 (pp.72-74), MRID No. 428239-01. Measurements: RBC ($\times 10^6/\text{mm}^3$), MCHC (%), MCV (fL), WBC (Total, L, and M: $\times 10^3/\text{mm}^3$), Plts ($\times 10^3/\text{mm}^3$), APTT (seconds).

* $p < 0.05$, ** $p < 0.01$, Williams' test

(HGB), MCHC, total WBC, neutrophils, lymphocytes, eosinophils, basophils or PT. There were increased reticulocyte counts for 2 dogs at week 52 (1 at 50 ppm, 1 at 500 ppm). There were other findings that, although not always statistically significant, are of interest relative to the results for males. MCV values for dogs treated with Ziram at 700/500 ppm were consistently elevated relative to controls. Total WBC counts were generally greater than controls at all time points, and for all dose groups. These increases were reflected in increases in neutrophils and lymphocytes.

b. Clinical chemistry

<u>X</u>		<u>X</u>	
Electrolytes		Other	
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
Enzymes		x	Total Serum protein (TP)*
x	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Triglycerides
x	Creatinine phosphokinase*	x	Bilirubin*
	Lactic acid dehydrogenase (LDH)*	x	T3 (tri-iodothyronine)
x	Serum alanine aminotransferase (also SGPT)*	x	T4 (thyroxine)
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
x	α -Hydroxybutrate dehydrogenase (α -HBDH)		
x	Ornithine carbamoyltransferase (OCT)		

* Required for subchronic studies.

Results ❖ Blood samples were analyzed at weeks -2, 13, 26 and 52 for clinical chemistry parameters. Two different automated analyzers were used during the study to assess glucose, SGPT, SGOT and GGT levels (comparisons of machines were performed for validation): for weeks -2, 13, and 26 a Roche Cobas Centrifugal analyzer was used and for week 52, a Hitachi 737 Clinical Chemistry analyzer was used. At various weeks of the study, there were changes in several clinical chemistry parameters in males or females that reached statistical significance ($p < 0.05$ or $p < 0.01$) relative to controls, including: ALB, GGT, globulin, CPK, total protein, α -HBDH, K, Cl, OCT, P, cholesterol, Ca, Na, bilirubin, T3 and T4 levels. The mean values for parameters with patterns of statistically significant changes are presented in Table 5. In addition to the statistically significant elevations, there were changes in other parameters that may appear to be of toxicological importance, but after careful evaluation were not considered treatment related. For males, SGPT (GPT) and ALK (AP) levels were elevated at weeks 26 and 52 for the

TABLE 5. MEANS OF SELECTED CLINICAL CHEMISTRY PARAMETERS FOR WEEKS 13-52

Males		Prot.	ALB	Glob.	GGT	OTC	AP	GPT	CPK	Phosph.	Chol.	Bili.
Week	Dose											
13	0.00	5.5	2.7	2.8	2	4.1	126	23	98	3.4	140	0.1
	50	5.5	2.7	2.8	2	5.1	107	20	100	3.8	143	0.2
	185	5.6	2.7	2.9	2	2.7	133	21	115	3.6	150	0.1
	500	5.6	2.6	3.0	1**	2.6	161	21	102	4.0	205*	0.1
26	0.00	5.5	2.7	2.8	3	4.2	104	27	67	2.8	141	0.2
	50	5.5	2.8	2.8	2	5.2	84	33	75	3.7**	146	0.2
	185	5.5	2.7	2.8	2	6.0	143	70	82	3.2**	144	0.2
	500	5.4	2.4*	3.0	2*	6.1	148	86	101	3.7**	211*	0.2
52	0.00	5.7	3.0	2.8	3	2.9	99	29	55	2.6	142	0.1
	50	5.7	3.0	2.7	<2	3.3	81	23	62	3.0	156	0.1
	185	5.6	2.9	2.7	2	4.9	175	69	104*	2.9	140	0.1
	500	5.6	2.6*	2.9	<2	4.1	160	51	75*	3.0	214*	0.1
Females		Prot.	ALB	Glob.	GGT	OTC	AP	GPT	CPK	Phosph.	Chol.	Bili.
13	0.00	5.8	3.0	2.8	2	4.2	135	22	155	3.1	141	0.2
	50	5.4	2.7	2.6	1	4.8	113	21	135	3.3	125	0.2
	185	5.4	2.8	2.6	1	2.7	139	19	75	3.5	146	0.2
	500	5.2	2.6*	2.7	<1	3.4	175	21	115	3.7	162	0.1
26	0.00	5.7	2.9	2.8	2	4.2	120	25	104	2.6	152	0.2
	50	5.2	2.6	2.6	3	4.1	96	20	74	3.1	126	0.2
	185	5.4	2.6	2.8	2	5.8	125	18	67	2.9	171	0.2
	500	5.2*	2.4*	2.7	2	3.8	144	19	91	3.4*	174	0.2
52	0.00	6.0	3.2	2.8	<2	2.2	119	20	53	2.6	189	0.1
	50	5.4*	2.8	2.6*	<2	2.9	100	20	69	2.7	125	0.2
	185	5.2*	2.7*	2.5*	3	5.3*	109	21	64	3.3*	153	0.2*
	500	5.2*	2.7*	2.5*	1	4.8*	151	19	57	3.2*	183	0.2*

Data adapted from Table 8 (pp. 75-78), MRID No. 428239-01. Units: Total Protein (Prot.), ALB, and Globulin fraction (Glob.) were measured in g/dL, GGT, OTC, AP, GPT and CPK were measured in (mU/mL), P (mEq/L), and Cholesterol levels (Chol.) were measured in mg/dL. *p<0.05, Williams' test. **p<0.01, Williams' test.

185 and 700/500 ppm groups, compared to controls. This is of interest as AP levels generally decrease as animals age. However, the increases were not dose-related at week 52 and the high-dose levels were actually within normal parameters. Other findings were not considered to be treatment related since there was either no dose-related effect or the levels measured were well within normal parameters. Clinical chemistry findings for females (Table 5) included statistically significant ($p < 0.05$ or $p < 0.01$) decreases in total protein, albumin and globulin (weeks 26 and 52), and statistically significant ($p < 0.05$) increases in OCT and bilirubin (week 52) were within normal parameters, not treatment related, or not sustained during the treatment period.

6. Urinalysis

Urine samples were collected from animals by an unspecified method on weeks -2, 13, 26, and 52 of treatment. Water was withheld from animals for approximately 5 hours prior to collection. Urine was collected over a period of 16 hours (overnight). As food was removed from pens at 1700 hours each day, there was no food present during urine collection. Analysis of urine was performed as quantitative tests and qualitative tests (Clintest for reducing substances and Multistix for glucose, ketones and pigments-test kits were obtained from Ames company, Stoke Poges, England). Qualitative tests for glucose, ketones, bile pigments and urobilinogen were reported as negative (0), trace (tr), or small, moderate, or large amounts (+, ++, or +++, respectively). Qualitative tests for heme pigments (blood) were reported as positive or negative only. Microscopic examination of urine for cellular material (epithelial cells, polymorphonuclear leukocytes, mononuclear leukocytes, erythrocytes, organisms, renal tubule casts, other abnormal constituents) was performed on sediment obtained by centrifugation for 10 minutes at 1500 x g. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity*		Bilirubin*
x	Sediment (microscopic)*	x	Blood*
x	Protein*		Nitrate
		x	Urobilinogen*

* Required for chronic studies.

Results ❖ There were no changes in treated animals relative to controls at either 13, 26, or 52 weeks considered indicative of a response to Ziram treatment.

7. Sacrifice and pathology

All animals were sacrificed after an overnight fast by exsanguination under anesthesia (Expiral [pentobarbitone sodium]). The macroscopic appearance of tissues was noted. Parathyroids and thyroids were weighed together as were testes and epididymides. Certain tissues were not taken from female dogs 368 and 372 (700/500 ppm group, sacrificed in week 11 and 6, respectively): median nerves, sciatic nerves (proximal), semilunar ganglia, spinal cord (C6-67, T5-T8, ventral roots and dorsal root ganglia, and tibial nerves. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

<u>X</u>		<u>X</u>		<u>X</u>	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta*	XX	Brain**
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen	X	eye (optic n.)*
X	Jejunum*	XX	Thymus*		Glandular
X	Ileum*		Urogenital	XX	Adrenal gland*
X	Cecum*	XX	Kidneys**		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes**	XX	Parathyroids**
XX	Liver**	XX	Epididymides	XX	Thyroids**
X	Gall Bladder*	XX	Prostate		Other
XX	Pancreas*		Seminal vesicle	X	Bone*
	Respiratory	XX	Ovaries**	X	Skeletal muscle*
X	Trachea*	XX	Uterus*	X	Skin*
XX	Lung*				All gross lesions and masses*
	Nose				
	Pharynx				
	Larynx				

* Required for subchronic and chronic studies.

* Organ weight required in subchronic and chronic studies.

**Organ weight required for non-rodent studies.

Results ❖

- a. Organ weight ❖ The mean group liver weight for males treated with Ziram at 700/500 ppm was significantly increased relative to controls ($p < 0.05$, Table 6), due mainly to increased liver weights for males 365 and 367. Liver weights for these dogs equaled or exceeded the expected upper limit of liver weight of 4% of

TABLE 6. GROUP MEAN ORGAN WEIGHTS (g)							
Dose (ppm)	Males			Females			
	Heart	Liver	Prostate	Heart	Liver	Uterus	Ovaries
0	115.2	394.9	10.56	97.0	372.5	28.52	2.06
50	116.6	394.9	9.38	98.4	348.0	9.39	1.09**
185	102.6	408.7	8.27	94.3	371.3	5.81	0.85**
700/500	107.0	459.1*	5.89	81.9	404.7	7.74	0.79**
Values adjusted for bodyweight ^d							
0	116.4					27.55	
50	110.7					9.39**	
185	112.9					5.82**	
700/500	101.4					9.01**	

Data adapted from Table 10 (pp. 84-86), MRID No. 428239-01.

^dBody weight used as a covariate when the within-group relationship between bodyweight and organ weight is significant at the 10% level.

*p < 0.05, Williams' test

**p < 0.01, Williams' test.

terminal bodyweight. Group mean heart weights for males and females were decreased (not statistically significant). Group mean prostate weights were decreased (not statistically significant) in a dose-related fashion compared with controls. Group mean uterus and ovary weights were statistically significantly decreased in treated animals relative to controls. Organ weights for all other organs including kidneys were considered to be within normal range.

- b. Gross pathology ❖ Multiple red linear regions of the mucosal surface of the stomach (later histologically confirmed as focal erosion of the superficial gastric mucosa) was found for female 364 (185 ppm). Single oval, flat choleliths, firm but friable, were present in the gall bladders of males 359 and 363 (185 ppm).
- c. Microscopic pathology ❖
- 1) Non-neoplastic ❖ The pathological findings identified the liver and spleen as the target organs (Table 7). There were no histological changes noted for prostates, hearts, uterus, or ovaries to account for the decreased organ weights in treated animals relative to controls. All bone marrow smears were considered normal for morphology and distribution of cell types. There were no significant pathological findings for kidneys. The results for females #368 and #372 (700/500 ppm dose group) are not included. Female #368 suffered an apparent convulsion during week 11 and the pathology results reflected the collapsed state of the animal.

TABLE 7. DEGREE OF MICROSCOPIC PATHOLOGY-INDIVIDUAL ANIMALS

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	50	185	700/ 500	0	50	185	700/ 500
LIVER (1) Foci of degenerate hepat. Incidence/Total # of animals	1/4	2/4	2/4	3/4	3/4	0/4	3/4	2/3
Individual animals: Moderate	---	---	---	371	---	---	358	112, 366
Minimal Trace	345	349 353	359, 363	369 367	342, 344 346	---	360, 362	---
(2) Infiltration of inflammatory cells: (a) around central veins Incidence/Total No. of animals	0/4	0/4	0/4	1/4	0/4	1/4	2/4	1/3
Individual animals: Minimal	---	---	---	367	---	354	358, 360	366
(b) around central veins and branches of the hepatic vein Incidence/Total No. of animals	0/4	0/4	2/4	3/4	0/4	0/4	0/4	1/3
Individual animals: Moderate Minimal	---	---	---	371	---	---	---	---
	---	---	357, 359	365, 369	---	---	---	370
(c) around portal areas Incidence/Total No. of animals	1/4	0/4	1/4	3/4	0/4	0/4	0/4	1/3
Individual animals: Minimal	343 (foci)	---	359	367, 369, 371	---	---	---	370
(3) Single cell necrosis Incidence/Total No. of animals	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/3
Individual animals: Minimal	---	---	---	371	---	---	---	---
(4) Increase in centrilobular fibrocytes Incidence/Total No. of animals	0/4	0/4	1/4	3/4	0/4	0/4	0/4	0/3
Individual animals (Not graded)	---	---	359	367, 369, 371	---	---	---	---
(5) Aggregates of pigmented Kupffer cells and macrophages Incidence/Total No. of animals	0/4	1/4	2/4	4*/4	0/4	1/4	4*/4	2/3

Table 7. Continued								
Individual animals: Moderate Minimal	---	---	357, 359	365, 367, 369, 371	---	---	358, 362	112, 366
	---	353	---	---	---	354	360, 364	---
(6) Pigmented Kupffer cells Incidence/Total No. of animals	3/4	2/4	1/4	0/4	2/4	2/4	0/4	1/3
Individual animals: Moderate Minimal	---	---	---	---	---	---	---	370
	341, 345, 347	351, 355	363	---	342, 344	352, 356	---	---
SPLEEN Pigmented macrophages Incidence/Total No. of animals	1/4	1/4	3/4	3/4	2/4	2/4	2/4	3/3
Individual animals: Moderate Minimal Trace	---	---	363	367, 371	---	---	---	---
	341	353	357,359	369	348	---	360,362	366, 370
	---	---	---	---	344	350, 354	---	---

Data adapted from Tables A, B, and C (pp.45-46), Table 12 (pp. 93-105), and Appendix 16 (pp.216-278), MRID No. 428239-01.
*p<0.05, Fisher's exact test.

Female 372 was diagnosed with polyarteritis (although post-mortem pathological results did not confirm this diagnosis) and was sacrificed at week 6 to avoid complicating results within the high dose group; female 112 was acquired as a replacement animal.

700/500 ppm dose For liver, the major findings (Table 7) included moderate amounts of foci of degenerate hepatocytes (1♂, 2♀), single cell necrosis (1♂), infiltration of inflammatory cells around central veins and branches of the hepatic vein (4♂, 2♀), an increase in centrilobular fibrocytes (3♂), moderate amounts of aggregates of pigmented Kupffer cells and macrophages (4♂, 2♀), and moderate amounts of pigmented Kupffer cells (1♀). The incidence of aggregates of pigmented Kupffer cells and macrophages for males was statistically significantly increased compared to controls (p<0.05). For spleen, the major findings were moderate (2♂) to minimal (1♂, 2♀) amounts of pigmented macrophages. For the testes and the epididymides, male 369 had some degenerate tubules in the testes, multinucleate cells in some tubules of the testes and the epididymides, and stasis of sperm cells in the epididymides tubules. There were no histological changes seen in male thyroids to account for increased T3 levels seen in treatment groups, no changes in prostates to account for decreased organ weights, and no changes seen in the hearts of males or females to account for the decreased heart weights.

185 ppm dose The pathologic findings (Table 7) for the liver include moderate (1♀) to minimal (2♂, 2♀) amounts of focal hepatic degeneration, an increase in centrilobular fibrocytes (1♂), minimal infiltration of inflammatory cells around central veins and hepatic veins (2♂, 2♀), and moderate (2♂, 2♀) to minimal (2♀) amounts of aggregates of pigmented Kupffer cells and macrophages. The incidence of aggregates of pigmented Kupffer cells and macrophages for females was statistically significantly increased compared to controls (p<0.05). In addition,

female 358 showed a focus of hepatic necrosis. In the spleen, there were moderate (1♂) to minimal (2♂, 2♀) amounts of pigmented macrophages.

50 ppm dose ❖ There were few significant pathologic findings for this dose group (Table 7). For liver, there was minimal (1♂) or trace (1♂) amounts, or no detectable (2♂, 4♀) focal hepatic degeneration, and no single cell necrosis or increased incidence of centrilobular fibrocytes in males or females, minimal (1♂, 1♀) amounts of Kupffer cell and macrophage aggregates, minimal amounts (1♀) of inflammatory cell infiltration around central veins, minimal (2♂, 2♀) or zero amounts of pigmented Kupffer cells. There was minimal (1♂), trace (2♀), or zero amounts of pigmented macrophages in spleens of males and females in this dose group.

Controls ❖ For controls (Table 7), there were minimal (2♀) or trace (1♂, 1♀) amounts of focal hepatic degeneration in the liver, minimal amounts of inflammatory cell infiltration in the portal area of the liver (1♂), and minimal amounts of pigmented Kupffer cells (3♂, 2♀). The incidence of pigmented Kupffer cells was greater in controls than in treated animals. There were no single cell necrosis and no aggregates of pigmented Kupffer cells and macrophages. There were minimal (1♂, 1♀) or trace (1♀) amounts of pigmented macrophages found in spleen.

- 2) **Neoplastic** ❖ The study authors did not specifically state that they looked for neoplastic changes. There were no neoplastic changes recorded in the microscopic pathology report, MRID No. 428239-01

8. Neurological examination

Examinations were performed prior to beginning treatment and during weeks 34 and 50 to assess general physical condition, behavior, gait, cranial nerve function, spinal reflexes and postural reactions.

Results ❖ All responses were within the normal range and there was no evidence of neurological abnormality or deficit in any of the animals.

D. DISCUSSION

The high dose for the study was originally chosen as 700 ppm, based upon results from preliminary 4-week and 13-week (Appendix B) dietary toxicity studies and a previously published report. In the preliminary studies, it was determined that the MTD of Ziram for beagles (long-term administration) was <1000 ppm. The published toxicity study (Hodge, H., *et al.*, (1956) *J. Pharmacol. Exp. Ther.* **118**, 174-181) indicated convulsive-related toxicity in beagles with 5-9 month dietary intakes of Ziram ranging from 800-1000 ppm. The initial 700 ppm dose was decreased at week 12 to 500 ppm at sponsor's request due to a convulsive episode attributed to Ziram treatment. The LOAEL of 185 ppm was justified by comparisons of the results for the 700/500, 185, and 50 ppm dose groups. In addition to the convulsive episode for female 368, the findings for the 700/500 ppm dose group include: 1) decreased body weight gain in females over the treatment period and 2) histologic findings for livers (aggregates of Kupffer cells and macrophages, increased foci of degenerate hepatocytes, infiltration of inflammatory cells around central veins and branches of the hepatic vein and portal areas, and increased centrilobular fibrocytes in males). The findings for the 185 ppm dose group include decreased body weight gain in females during the treatment period. The increased infiltration of inflammatory cells around the central veins and branches of the hepatic

vein in males at 185 ppm is not considered of toxicological importance since it is not correlated with increases in serum levels of hepatic enzymes or other liver pathology. Kupffer cells are normal inhabitants of the liver and their presence cannot indicate liver pathology unless other microscopic findings suggest a correlation. Also, the lack of correlation between the decrease in body weight gain and liver pathology in both sexes suggest that the body weight decrements were not related to hepatic disease. For the 50 ppm dose group, there were no findings considered to be of toxicological significance.

The NOAEL level for Ziram treatment was chosen as 50 ppm as the hematologic, clinical chemistry and histologic findings were not of toxicological significance.

E. STUDY DEFICIENCIES

There were minor omissions in the study, including assays for lactate dehydrogenase (LDH) and Mg^{2+} that were not performed in the clinical chemistry evaluations, and the appearance of urine and a test for bilirubin that were not performed in the urinalysis-assessments of each are required for chronic studies. The inclusion of these data would add little to the study report.

APPENDIX

Dose Selection Study in Dogs

MRID No.: 423751-01
Study Type: Subchronic Feeding-Dog (13-week, use for range-finding only), 82-1b
Test Material: Ziram
Study No.: ZIR 8/901813
Testing Facility: Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England
Study Title: 13-Week Dietary Toxicity Study in Beagle Dogs
Authors: Timothy A. McLean, Steven A. Horner, David P. Buist, David Crook, Rosemary M. Read, Chirukandath Gopinath, Alan Anderson, I. Suzanne Dawe, Steven F. Johnson, Sujit Patel
Study Completed: May 5, 1992

Methods:

Test Animals: Male and female beagle (pure-bred) dogs, 20-24 weeks, 6.2-10.0 kg
Group Size: 4 males, 4 females per dose group
Test material concentrations: Daily diet of Ziram at 0, 100, 300, or 1000 ppm.

Results:

Clinical signs: Convulsive episodes noted for 1 male (1000 ppm) for time periods up to 4 minutes during weeks 2 and 4. During week 5, this animal had a seizure and was euthanized. One female (1000 ppm) was trembling during week 8.

Mortality: 1 male (1000 ppm) euthanized, week 5

Bodyweight Gain: Slight reductions in bodyweight gain for 1 male and 1 female in the 1000 ppm group.

Clinical Pathology:

Hematology: Group mean APTT values for males were slightly but statistically significantly elevated for the 300 ppm group in week 13 ($p < 0.05$) and for the 1000 ppm group in both weeks 6 and 13 ($p < 0.01$). For females in the 1000 ppm group at weeks 6 and 13, there was a small, but not statistically significant increase in APTT.

Biochemistry: Group mean AP levels for females were increased statistically significantly ($p < 0.05$) relative to controls for animals in the 1000 ppm group at week 13. Group mean SGPT levels were decreased statistically significantly ($p < 0.05$) relative to controls for males in the 1000 ppm dose group at week 13. Cholesterol levels were significantly ($p < 0.01$) increased relative to controls for males and females in the 1000 ppm dose group during week 13, and for females only in week 6. Group mean albumin levels were significantly decreased relative to controls for males in the 1000 ppm group at week 6 ($p < 0.01$) and for males ($p < 0.01$) and females ($p < 0.05$) in all dose groups at week 13.

Urinalysis: With the exception of the 1 male that was euthanized, urinalysis parameters were within normal range for the remainder of the animals in the study.

Organ Weight Gain:

Liver: Group mean adjusted weights for liver for males and females at 1000 ppm were statistically significantly ($p < 0.05$) higher than control values.

Lungs and Heart: Statistically significant decreases in group mean adjusted heart weight for males at 1000 ppm ($p < 0.05$) and in group mean adjusted lung weights for females at 1000 ppm ($p < 0.01$) were considered by the study authors to be unrelated to treatment.

Macroscopic and Microscopic Pathology:

The liver from 1 female (1000 ppm) had multiple depressed pale areas on the liver, focal necrosis, loss of cells and dilated sinusoids (note: biochemical findings for this animal at week 6 included increased bilirubin, AP, GPT, GOT, OCT, and cholesterol, however these levels were unremarkable at week 13"). The liver from one male (300 ppm) also had focal necrosis. Minimal amounts of pigment in Kupffer cells from 2 males and 1 female (the female with liver necrosis described above) from the 1000 ppm group, and in Kupffer cells from 1 male and 1 female in the 300 ppm group. These changes were not seen in control dogs or dogs receiving 100 ppm.

Conclusions:

The toxicity data for the 13-week feeding study was used to set the highest dose level for the 52-week chronic dietary study at <1000 ppm-essentially based on the finding of convulsive episodes for 1 male during week 5 (1000 ppm group). Other findings supporting the chosen dose levels were the biochemical, hematologic, and liver weight data. The lack of findings for dogs in the 100 ppm group was used as evidence for <100 ppm as the NOAEL. On the basis of this study, levels of Ziram at 50, 185, and 700 ppm were chosen for use in the 52-week chronic feeding-dog study (MRID No. 428239-01).

Core Classification: Not applicable; dose range-finding study

There was a dose-related decrease in group mean body weight gain for females that was significant for the mid and high dose groups. Changes in clinical chemistry parameters included decreases in albumin (males and females) and total protein levels (females) and increases in SGPT (males) and alkaline phosphatase (males). Absolute weights for the liver were significantly increased for males in the high dose group. Significant dose-related decreases in absolute weight of the ovaries were observed in all dose groups. In males, there were microscopic pathologic findings (e.g., foci of degenerate hepatocytes, inflammatory cell infiltrations, and aggregates of macrophages and pigmented Kupffer cells) in the liver and pigmented macrophages in the spleen in the mid and high dose group.

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[ZIRAM]

Chronic Oral Study (83-1b)

SignOff Date:
DP Barcode:
HED DOC Number:
Toxicology Branch:

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