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DATA EVALUATION REPORT

ZIRAM

Study Type: CHRONIC FEEDING/ONCOGENICITY RAT (83-5)

8/2/2000

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding/Oncogenicity-Rat (83-5)

TOX. CHEM. NO: 931

P.C.CODE.: 034805

MRID NO.: 43404201

TEST MATERIAL: Ziram (98.7% a.i.)

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

STUDY NUMBER: ZIR 9/942098

SPONSOR: Ziram Task Force, c/o NPC Inc., 22636 Glenn Drive, Suite 304, Sterling, VA 20164

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

TITLE OF REPORT: Combined chronic toxicity and oncogenicity of Ziram (Technical) administered in the diet to rats

AUTHORS: Lindsey A.J. Powell, Sarah M. Bottomley, David Crook, Richard L. Gregson, John M. Offer, William A. Gibson, Alan Anderson

REPORT ISSUED: September 27, 1994 (study completion date)

EXECUTIVE SUMMARY: Male and female CD(SD)BR rats, 50/sex/dose in the main group, 20/sex/dose in the satellite group were treated with Ziram (98.7%, Lot# 8331 AA) at 0, 60, 180, and 540 ppm for 104 weeks, MRID No. 43404201. These doses corresponded to achieved intakes of 0, 2.5, 7.7, and 23.7 mg/kg/day for males in the main group and 0, 3.4, 10.2, 34.6 mg/kg/day for females in the main group.

There was no excess mortality in any of the treated groups relative to controls. Group mean body weight gains were decreased for males (86% of control, $p<0.01$ and females (74% of control, $p<0.01$) in the high dose group (540 ppm). Food consumption was decreased compared to controls for males (540 ppm: 91%, $p<0.01$) and females (180 ppm: 92%, $p<0.05$; 540 ppm: 94%, $p<0.05$). Hematology parameters (RBC, HGB, and PCV) were decreased relative to controls for females in the 540 ppm (weeks 26-104, $p<0.05$, $p<0.01$) and 180 ppm (weeks 26-52, $p<0.05$, $p<0.01$)) dose groups. There were statistically significant decreases ($p<0.05$, $p<0.01$) in clinical chemistry parameters (calcium, total protein, albumin, calcium and SGPT) during weeks 13-52 for females. For males (540 ppm, week 104) organ weight for the adrenals was decreased (absolute, 59% of control, $p<0.01$; relative, 67% of control, $p<0.05$). There were macroscopic pathological findings (not statistically significant) for animals in the 180 and 540 ppm dose

groups for the stomach and skeletal muscle (males and females), and the adrenals (females only). There were microscopic pathological findings for males and females in the 180 and 540 ppm dose groups for spleen ($p<0.01$), liver ($p<0.01$, $p<0.05$), stomach ($p<0.05$, $p<0.01$), thyroid ($p<0.01$, $p<0.05$), skeletal muscle ($p<0.01$), spinal cord (males only, $p<0.05$), sciatic nerve (females only, $p<0.01$), and adrenal cortex ($p<0.05$, $p<0.01$). As there were histopathological findings for males in the 60 ppm dose group for spleen ($p<0.01$), stomach ($p<0.01$, $p<0.05$), skeletal muscle ($p<0.05$), and adrenal cortex ($p<0.05$), a NOAEL for males could not be identified. For females, there was an increase in prominent ultimobranchial cysts in the thyroid in all dose groups (Controls: 3/50; 60 ppm: 12/50, $p<0.05$; 180 ppm: 22/50, $p<0.01$;, 540 ppm: 27/50, $p<0.01$), precluding the identification of a NOAEL for females. **The NOAEL could not be identified for either males or females, due to histopathological findings for animals in the low dose group (60 ppm).**

Carcinogenic potential was evidenced by the finding of treatment-related tumors (benign hemangioma) in mesenteric lymph nodes (5/50, $p<0.05$) and in spleen (1/50) in males in the 540 ppm dose group. There were no treatment-related tumors identified in males in the 180 or 60 ppm dose groups, or in females in any dose group. There were no treatment-related malignant tumors in either sex. The dosing is adequate. Treatment of males with Ziram for 104 weeks at the MTD resulted in neoplastic changes.

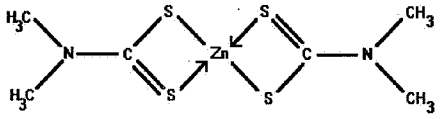
This study is classified as Acceptable and satisfies the guideline requirements for a chronic/oncogenicity study (§83-5). This study did not establish a NOAEL.

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test material: Ziram (Technical)

Description: white powder
Lot/Batch No.: 8331 AA
Purity: 98.7% a.i.
Stability of compound: stable for at least 2 years when stored at ambient temperature and protected from light
CAS No.: 137-30-4
Structure: (MRID No. 434042-01, p. 2247)



2. Vehicle and/or positive control

Negative controls received untreated diet. There was no positive control group described.

3. Test animals

Species: rat
Strain: Crl: CD(SD)BR
Age and weight at study initiation: ~6 weeks, males: 157-192 g, females: 114-175 g
Source: Charles River Breeding Laboratories, Portage, MI
Housing: 5 rats/sex/cage in suspended cages with wire mesh floors
Environmental conditions:
- Temperature: 21±2°C
- Humidity: 55+10%
- Air changes: Not described in the study report
- Photoperiod: 12 hour light/dark cycle

Acclimation period: 13 days

B. STUDY DESIGN

1. Animal assignment

Animals (50/sex/dose) were assigned to cages such that mean cage weights were approximately equal. Cages of animals were then assigned to the test groups in Table 1. For each sex and dose group, there was a satellite group of 20 animals. Five animals of each sex were inspected for health problems prior to assignment of animals to dose groups.

Dose selection rationale: The dosages for the current study were selected on the basis of a preliminary 13-week feeding study, MRID No. 42450301 (see Appendix). There were minor hematologic and clinical chemistry changes for the low dose group (100 ppm). There were effects on weight gain and food intake and minor hematological, clinical chemistry, organ weight and pathological changes for the medium and high dose groups (300 and 1000 ppm, respectively). The low dose for the current study was chosen to be 60 ppm, in an attempt to provide a NOEL. The medium and high doses were set at 3 and 9 times this dose (180 and 540 ppm, respectively).

TABLE 1. STUDY DESIGN

Dose Group	Doses (mg/kg/day)			No. Animals	
	Target Dose Both Sexes	Dosage Achieved (Mean[a])		Male	Female
		Males	Females		
1 Control[b]	0 ppm	0	0	50	50
2 Low (LDT)[b]	60 ppm[c]	2.5	3.4	50	50
3 Mid (MDT)[b]	180 ppm	7.7	10.2	50	50
4 High (HDT)[b]	540 ppm	23.7	34.6	50	50
5 Satellite Control[d]	0 ppm	0	0	20	20
6 Satellite (LDT)[d]	60 ppm[c]	3.0	3.9	20	20
7 Satellite (MDT)[d]	180 ppm	9.1	11.7	20	20
8 Satellite (HDT)[d]	540 ppm	27.3	37.5	20	20

Data obtained from summary table on p. 43, MRID No. 43404201.
[a]Compound intake was calculated by the study authors on a weekly basis. The mean of the weekly values over the 104 week test period is presented.
[b]Data for weeks 1-104: includes combined data for main and satellite groups during weeks 1-52 and data for main group during weeks 53-104.
[c]Diet prepared contained 66 ppm.
[d]Achieved dose for satellite groups alone was not presented in the study report (MRID No. 434042-01). Data was combined by the study authors for satellite and main groups for weeks 1-52

2. Diet preparation and analysis

Diet was prepared weekly. A concentrate was prepared by grinding appropriate amounts of Ziram with untreated sieved basal diet and mixing in a Turbula mixer for at least 2 minutes. The concentrated diet was diluted with appropriate quantities of untreated diet and homogeneity was achieved by mixing in a double-cone blender for at least 7 minutes. The total volume of diet required was large, such that the diets for each dose were prepared in 2 batches. Trial studies evaluating the stability of Ziram in rodent diet formulations showed that the compound was not stable at low doses (70 ppm) when stored at ambient temperature, even for a couple days, but when low dose diets (100 and 300 ppm) were stored at 4°C for 7 days followed by ambient temperature storage in the animal rooms for 1 day, no significant change in concentration occurred (-9 to -10%). The low dose diet (60 ppm) was prepared at 10% above nominal levels (66 ppm) in order to compensate for losses during preparation and storage. Diets were stored at 4°C. Homogeneity and stability were

tested prior to commencement of the trial. During the study, samples of treated food from weeks 1, 3, 13, 26, 39, 41, 52, 65, 78, 91, and 104 were analyzed for concentration. For homogeneity analysis, duplicate samples at 25 and 50 ppm (nominal) were obtained from the top, middle, and bottom of the discharge from the blender. Duplicate subsamples from each of the samples were subjected to analysis for homogeneity. For stability analysis, four samples of freshly prepared diets at 25 and 50 ppm (nominal) inclusion levels were obtained. The samples were either analyzed immediately, after 24 hours at room temperature, after 7 days at 4°C, or after storage for 7 days at 4°C and 24 hours at room temperature. For each sample, duplicate subsamples were analyzed. For concentration analysis, samples of diet were obtained from freshly prepared diet (day 0) and from stored diet (day 8: 7 days at 4°C, followed by 24 hours at room temperature). Diet was sample at weeks 1, 3, 13, 26, 39, 41, 52, 65, 78, 91, and 104. Two determinations were performed for each sample.

Results

a. Homogeneity analysis The recoveries of Ziram from each sampled region were similar. The overall mean recoveries of Ziram for the 25 and 50 ppm diets were 24.3 ppm (97.2%) and 53 ppm (106%).

b. Stability analysis Storage of diet formulations resulted in losses in Ziram concentrations. Exposure to room temperature for 24 hours resulted in 17.1% and 11.6% losses for the 25 and 50 ppm diets, respectively. For the samples stored for 8 days, the losses were 22.6% (25 ppm) and 13.1% (50 ppm).

c. Concentration analysis Results for day 8 of week 1 were not reported. Mean results for each dose group were between +14.5% and -8.3% of nominal concentrations for day 0 samples and between +6.0% and -16.3% for day 8 samples.

3. Diet

Animals received food (ground SDS Rat and Mouse No. 1 modified maintenance diet) and tap water *ad libitum.*

4. Statistics

Statistical analyses were performed for males and females separately. Food and water consumption were analyzed on a per-cage basis. Totals were recorded over selected time periods and expressed on a weekly basis. All other parameters were analyzed with individual animals as the basic unit for comparison. The data for food and water consumption, body weight gain, clinical pathology and organ weight data were analyzed statistically with the following sequence. Invalid values (documented by machine fault, organ loss or damage) are excluded. If the relative frequency of the mode is greater than 75%, the proportion of animals with values different from the mode were analyzed. Otherwise, Bartlett's test was used for analysis of heterogeneity of variance between treatments. If there was significant (1% level) heterogeneity, the data were transformed logarithmically. If no significant heterogeneity was found, or if the data transformation was satisfactory, a one-way analysis of variance was performed. If significant heterogeneity of variance was present and could not be removed by logarithmic transformation, the Kruskal-Wallis' analysis of ranks was used. Analyses of variance were followed by Student's *t*-test and Williams' test for a dose-related response. Kruskal-Wallis' test was followed by non-parametric equivalents of the *t*-test and Williams' test (Shirley's test). Where appropriate, an analysis of covariance was used in place of an analysis of variance. For organ weight data, an analysis of variance was performed using terminal body weight as a covariate when the within-group relationship between organ weight and body weight was significant at the 10% level. Mortality was analyzed using log rank methods. Incidence of tumors was

analyzed according to the context of the observation as interpreted by the pathologist. Trend tests were used based upon nominal dose levels.

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

C. METHODS AND RESULTS

1. Observations

Animals were palpated and inspected daily during the first 4 weeks and once a week thereafter for signs of behavioral changes, reactions to treatment, and ill health. Checks for dead and/or moribund animals were performed twice daily.

Results There were no clinical signs indicative of a response to treatment. The mortality distribution did not indicate an adverse effect of Ziram treatment (Table 2). Mortality for females in the 180 (p=0.035) and 540 (p=0.029) ppm dose groups was statistically significantly decreased relative to controls. This was likely due to decreased survival for the control group (32%, expected 50% at 104 weeks). The overall test for trend was not significant.

TABLE 2. MORTALITY

Week of Study	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	1 (0)	2 (60 [a])	3 (180)	4 (540)	1 (0)	2 (60 [a])	3 (180)	4 (540)
Main Group, weeks 1-104	18 (19)[b]	22	27	19	34	28 (29)[b]	25*	26*
% Mortality	36%	44%	54%	38%	68%	56%	50%	52%
Satellite Group, weeks 1-52	2	1	1	3	0	1	1	0

Data taken from Mortality table on p. 38, MRID No. 43404201. There were 50 main group animals/sex/dose and
20 satellite group animals/sex/dose at treatment commencement.
[a]Diet prepared to contain 66 ppm.
[b]() includes animals found dead during the 10-day post-terminal period.
*Significantly decreased relative to control, $p<0.05$. For females treated with Ziram at 180 ppm and 540 ppm, $p=0.035$, and $p=0.029$, respectively.

2. Body weight

Animals were weighed at the time of allocation to test groups, on the first day of treatment and weekly thereafter.

Results ▩ There was a statistically significant ($p<0.01$) decrease in body weight gain for males (86% of control) and females (74% of control) treated with Ziram at 540 ppm (Table 3). Body weight gain for animals in the 540 ppm dose group was substantially decreased during the first week of treatment and remained depressed for the remainder of the treatment period. It is of interest to note that, for males in the 540 ppm dose group, if the decrease in body weight gain relative to controls for weeks 0-1 is discarded, body weight gain for the remainder of the treatment period (weeks 1-104) is similar to controls (92% of control). Body weight gain for males in the 180 ppm dose group was 70% of controls at week 1 ($p<0.01$), but was similar to controls for the 104 week treatment period. For females in the 180 ppm dose group, body weight gain was statistically significantly decreased for weeks 0-1 (80% of controls, $p<0.01$) and for weeks 0-52 (85% of control, $p<0.01$), but was greater than controls for the second half of the treatment period. Thus, body weight gain for animals in the 180 ppm dose group was similar to controls over the entire treatment period. There was no adverse effect of treatment on animals treated with Ziram at 60 ppm.

TABLE 3. GROUP MEAN BODY WEIGHTS (G/RAT) AT SELECTED WEEKS & GROUP MEAN BODY WEIGHT CHANGES (G/RAT) AT SELECTED WEEKLY INTERVALS

Week of Study	Males				Females			
	0	60[a]	180	540	0	60[a]	180	540
0	174	173	174	174	142	142	142	144
1	222	219	208	186	168	168	163	150
4	332	331	318	285	220	220	214	193
13	485	490	477	428	282	283	275	252
26	597	607	584	522	332	333	311	287
52	707	730	694	620	426	424	383	338
104	752	740	697	671	470	466	465	388
Weeks 0-1	48.4	45.9 (95%[b])	34.1** (70%)	12.2** (25%)	25.6	26.0 (102%)	20.6** (80%)	5.8** (23%)
Weeks 0-52	534	557 (104%)	520 (97%)	446** (84%)	284	282 (99%)	241** (85%)	194** (68%)
Weeks 1-104	528	520 (98%)	489 (93%)	486 (92%)	304	303 (100%)	302 (99%)	238* (78%)
Weeks 0-104	576	565 (98%)	523 (91%)	496** (86%)	329	326 (99%)	323 (98%)	245** (74%)

Data adapted from summary tables, pp. 39-40 and Table 2, pp. 72-75, Appendix 2, pp. 303-446, MRID No. 42450301.
[a]Diet prepared to contain 66 ppm.
[b]% of control
*p<0.05, **p<0.01, Data for weeks 0-52 included both main and satellite group animals. For statistical analysis data for females for weeks 0-52 were log-transformed. For weeks 0-1, 1-104, and 0-104 (males), Kruskal-Wallis analysis of mean ranks was applied.

3. Food consumption and compound intake

Food consumption for each cage was determined daily and reported as food intake (g) per rat per week, based upon the number of surviving animals in the cage. Mean daily diet consumption was calculated by the reviewer as g food/kg body weight/day. Food efficiency (body weight gain, kg/food consumption, kg per unit time X 100) was calculated by the reviewer. Compound intake (mg/kg/day) values were calculated as time-weighted averages from the food consumption and mid-week body weight data.

Results

a. Food consumption Food consumption (Table 4) was statistically signficantly decreased for males in the 540 ppm dose group (91% of control, $p<0.01$) and females in the 180 (92% of control, $p<0.05$) and 540 ppm dose groups (94% of control, $p<0.05$). Food consumption for rats treated with Ziram at 60 ppm was similar to controls.

b. Compound consumption (time-weighted average) Overall compound intakes are presented in Table 1.

c. Food efficiency ▒ Food efficiency was calculated by the reviewer (the study authors calculated a food utilization ratio with the data from the first 26 weeks of the study). Body weight gain and food consumption were both decreased for rats in the 180 and 540 ppm dose groups, resulting in a food efficiency for these dose groups that was similar to controls (Table 4).

TABLE 4. GROUP MEAN FOOD CONSUMPTION AND FOOD EFFICIENCY

Parameter	Exposure Level (ppm)							
	Males				Females			
	0	60[a]	180	540	0	60[a]	180	540
Mean Total Food Consumption (g/rat), Weeks 1-104	19279	19163 (99%)[b]	18885 (98%)	17615** (91%)	15975	15664 (98%)	14697* (92%)	15065* (94%)
Mean Daily Food Consumption[c] (g/rat/day)	26.5	26.3	25.9	24.2	21.9	21.5	20.2	20.7
Food Efficiency[d] [((bodyweight gain(g))/(total food consumed (g))]x100	2.99	2.95	2.77	2.82	2.06	2.08	2.20	1.63

Data adapted from summary table, p. 42, MRID No. 43404201.
[a]Diet prepared to contain 66 ppm.
[b]% of control
[c]Calculated by reviewer, based upon 728-day (104 week) treatment period.
[d]Calculated by reviewer, using body weight gains for weeks 0-104 (see Table 3).
*$p<0.05$
**$p<0.01$

4. Ophthalmoscopic examination

The eyes of all surviving animals were examined using a Keeler indirect ophthalmoscope. Examinations were performed prior to commencement of treatment, at week 52 for the main and satellite groups and at week 104 for the main group. Pupils were dilated using a Tropicamide ophthalmoscopic solution.

Results ▒ Five animals were excluded from the study due to pre-treatment evaluation. Of the animals in the study, there were no lesions found considered to be related to treatment with Ziram. All lesions were typical of age and strain of rat used for the study.

5. Blood was collected during weeks 13, 26, 52, 78, and at termination under ether anesthesia and after overnight fast from the orbital sinus of 10 male and 10 female rats from each dose group for hematology and clinical analysis. Rats from the satellite group were used for samples obtained in weeks 13, 26, and 52. Rats from the main group were used for the week 78 and termination samples. Where possible, the same rats were used for drawing blood. In week 4, blood samples were withdrawn from the orbital sinus of 10 male and 10 female rats for T3, T4, and TSH measurements. Blood samples were collected and mixed with EDTA for hematology, citrate for blood coagulation tests, or heparin for clinical chemistry evaluations. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpusc. HGB conc. (MCHC)
X	Leukocyte count (WBC)*	X	Mean corpusc. volume (MCV)
X	Erythrocyte count (RBC)*		Reticulocyte count
X	Platelet count*		
X	Blood clotting measurements		
	(Thromboplastin time)		
X	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies.

Results ▒ There were changes in the hematological parameters RBC, HCT, HGB, MCHC, MCV, and clotting time that were statistically significantly different from control values (Table 5, $p<0.05$ and $p<0.01$). The most consistent change was decreased RBC levels relative to controls. The decreases in RBC were evident for males (180, 540 ppm) at week 13 and for females treated with Ziram at 180 ppm at weeks 26 and 52 and at 540 ppm from week 26 to 104. HGB and PCV values were correspondingly decreased at most of these timepoints and the decreases were generally statistically significant ($p<0.05$, $p<0.01$). The values for all of the hematological parameters fell within the historical control range established by the testing facility. The only statistically significant change for animals treated with Ziram at 60 ppm was a decreased blood clotting time for males at week 26 ($p<0.05$).

TABLE 5. SUMMARY OF STATISTICALLY SIGNIFICANT CHANGES IN HEMATOLOGY PARAMETERS IN RATS ADMINISTERED ZIRAM FOR 104 WEEKS.

Parameter	Week of Study	Exposure Level (ppm)							
		Males (N=10)				Females (N=10)			
		0	60[a]	180	540	0	60[a]	180	540
RBC	13	7.6	7.3	7.2*	7.1**	6.9	6.8	6.7	6.7
	26	7.1	7.0	7.1	7.1	6.7	6.6	6.2**	6.3**
	52	6.7	6.8	6.8	6.9	6.3	6.2	5.8**	5.5**
	78	7.2	7.2	7.2	7.5	6.6	6.7	6.5	6.0**
	104	7.1	6.9	7.0	7.4	7.0	6.7	6.5	6.0**
HGB	13	15.3	14.9	14.9	14.6*	13.8	14.3	14.2	14.7
	26	14.5	14.3	14.7	14.3	14.2	14.1	13.4*	13.9*
	52	14.4	15.0	15.2	14.8	14.5	14.4	13.9	13.6**
	78	14.9	14.7	14.6	14.9	14.2	14.5	14.2	13.2**
	104	13.8	13.5	13.3	14.2	14.4	13.9	13.8	12.7**
PCV	13	50	50	50	49	48	48	47	49
	26	50	50	51	50	49	48	46**	46**
	52	50	52	52	51	49	48	46**	45**
	78	55	54	54	55	51	52	50	47**
	104	50	48	48	50	49	47	46	42**
MCHC	13	30.3	29.8	29.9	29.9	28.7	29.9	30.2*	30.3**
	26	28.7	28.5	29.0	28.5	29.1	29.4	29.5	30.1**
	52	28.8	29.1	29.4	28.9	29.6	29.7	30.1	30.5**
	78	27.3	27.3	27.2	27.1	27.8	28.1	28.2	28.2
	104	27.7	28.3	27.9	28.6**	29.4	29.7	30.2	29.9
MCV	13	67	68	69**	69**	70	71	71	72**
	26	71	72	72	71	73	73	73	73
	52	75	76	76	74	78	79	80*	81**
	78	75	75	75	73*	78	77	78	79
	104	70	69	68	67*	69	70	71	71
TT[b]	13	27	25	23**	23**	20	19	19	19*
	26	27	25*	24**	23**	19	19	19	20
	52	24	26	23	23	19	18	19	18
	78	24	23	23	24	19	19	19	19
	104	22	22	21	23	19	18	18	18

Data obtained from summary table, p. 45, MRID No. 42434001. Parameter units were RBC ($x10^6/mm^3$), HGB (g/dL), HCT (%), MCHC (%), MCV(fL), TT (seconds)
[a]Diet prepared to contain 66 ppm.
[b]TT, Thrombotest
*$p \leq 0.05$
**$p \leq 0.01$

b. Clinical chemistry

X	
X	Calcium*
X	Chloride*
	Magnesium
X	Phosphorus*
X	Potassium*
X	Sodium*

Enzymes

X	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
	Creatinine phosphokinase
	Lactic acid dehydrogenase (LDH)
X	Serum alanine aminotransferase (also
X	Serum aspartate aminotransferase (also
X	Glutamic-oxaloacetic transaminase (GOT)
	Glutamate dehydrogenase

X	
X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
X	Globulins
X	Glucose*
X	Total bilirubin
X	Total serum protein (TP)*
	Triglycerides
	Serum protein electrophoresis
X	Tri-iodothyronine (T3)
X	Thyroxine (T4)
X	Thyroid Stimulating Hormone (TSH)

* Required for subchronic and chronic studies.

Results ▩ For males (Table 6), there were statistically significant decreases relative to controls in total serum protein (94-98.5% of control, $p \leq 0.05$), albumin (93-96.7% of control, $p \leq 0.05$, $p \leq 0.01$), SGPT (66.7-77% of control, $p \leq 0.05$, $p \leq 0.01$), calcium (96-98% of control, $p \leq 0.05$, $p \leq 0.01$), and T4 (75.7-85% of control, $p \leq 0.05$, $p \leq 0.01$) levels and increases relative to controls in urea (115-121% of control, $p \leq 0.05$) and ALK (125-142% of control, $p \leq 0.05$) levels. For females, there were statistically significant decreases relative to controls in total serum protein (93-95.7% of control, $p \leq 0.05$, $p \leq 0.01$), albumin (91-88.6% of control, $p \leq 0.05$, $p \leq 0.01$), SGPT (43-44% of control, $p \leq 0.05$, $p \leq 0.01$), SGOT (26% of control, $p \leq 0.05$), calcium (94-96% of control, $p \leq 0.05$, $p \leq 0.01$), and T4 (74% of control, $p \leq 0.05$) levels and increases in urea (142-143% of control, $p \leq 0.01$), chloride (101-102% of control, $p \leq 0.05$, $p \leq 0.01$) levels. The increases in ALK levels were dose-dependent for both males and females. There was a dose-dependent decrease in SGOT levels for females at weeks 26 and 52. SGOT levels for females in the control and 60 ppm dose groups were higher than males in these dose groups at weeks 26 and 52. Thus, SGOT levels for females in the 540 ppm dose group at week 26 were statistically significantly (26% of control, $p \leq 0.05$) lower than controls. The only statistically significant changes affecting rats in the 60 ppm dose group were a decrease in total protein levels for males at week 52, relative to control (97.1% of control, $p \leq 0.05$), and an increase in chloride levels for females at week 13, relative to control (101% of control, $p \leq 0.05$).

TABLE 6. SUMMARY OF STATISTICALLY SIGNIFICANT CHANGES IN CLINICAL CHEMISTRY PARAMETERS FOR RATS ADMINISTERED ZIRAM FOR 104 WEEKS

Parameter	Week of Study	Exposure Level (ppm)							
		Males (N=10)				Females (N=10)			
		0	60'	180	540	0	60'	180	540
Total Protein	13	6.9	6.7	6.5*	6.5*	7.0	7.1	6.9	6.7*
	26	7.0	7.0	7.0	6.8	7.3	7.5	7.3	6.8**
	52	7.0	6.8*	6.7*	6.9*	7.2	7.4	7.5	7.0
	78	7.4	7.2	7.0*	7.0*	7.7	7.5	7.5	7.4
	104	7.3	7.1	6.9	7.0	7.5	7.6	7.2	7.5
Albumin	13	3.0	3.0	2.9*	2.8**	3.5	3.4	3.3	3.1**
	26	2.9	2.9	2.9	2.8*	3.5	3.6	3.5	3.2**
	52	2.8	2.8	2.7	2.7	3.6	3.6	3.6	3.3*
	78	2.8	2.8	2.7	2.7	3.3	3.4	3.4	3.2
	104	2.6	2.7	2.5	2.6	3.2	3.3	3.2	3.2
Urea	13	14	16	16	17*	19	20	22	23
	26	13	13	15*	15*	18	20	22	21
	52	13	13	14	13	14	14	16	20**
	78	12	13	13	13	12	13	14	17**
	104	12	14	14	12	12	15	14	16
ALK	13	158	182	168	185	99	93	101	119
	26	118	124	143	148*	68	60	71	87
	52	105	116	119	128	49	45	52	64
	78	116	117	129	141	54	48	63	62
	104	100	108	121	142*	58	55	59	62
SGPT	13	30	29	23**	22**	29	29	29	22
	26	29	29	26	25	51	55	43	22**
	52	31	27	27	28	70	48	41	31*
	78	36	34	35	24*	44	54	42	29
	104	29	29	33	26	51	41	39	34

TABLE 6 (Continued)

Parameter	Week of Study	Exposure Level (ppm)							
		Males (N=10)				Females (N=10)			
		0	60[1]	180	540	0	60[1]	180	540
SGOT	13	54	54	51	52	82	62	57	53
	26	50	55	51	50	207	102	77	53*
	52	56	55	56	53	159	84	75	71
	78	53	61	59	45	77	73	90	63
	104	51	55	75	50	77	78	97	80
Calcium	13	5.4	5.5	5.4	5.2**	5.4	5.4	5.3*	5.1**
	26	5.5	5.5	5.5	5.4*	5.6	5.6	5.5	5.4**
	52	5.6	5.6	5.5	5.5	5.6	5.6	5.7	5.4**
	78	5.5	5.4	5.4**	5.4**	5.4	5.6	5.4	5.4
	104	5.4	5.4	5.3	5.3	5.4	5.6	5.3	5.3
Chloride	13	101	101	102	100	101	102*	103*	102*
	26	101	101	100	100	100	101	102**	102**
	52	100	102	101	101	99	100	100	101*
	78	102	103	102	102	99	100	100	101*
	104	100	100	100	101	98	96	97	98
T4	4	3.7	3.4	3.0**	2.8**	2.7	2.4	2.4	2.0*
	13	3.7	3.4	3.2	3.2	2.9	2.7	2.7	2.6
	26	3.4	3.3	3.1	2.9*	2.3	2.4	2.5	2.4

Data taken from summary table, p. 47, MRID No. 42434001. Parameter units are: Total serum protein (g/dL), Albumin (g/dL), Urea (blood urea nitrogen, mg/dL), ALK (mU/mL), SGPT (mU/mL), SGOT (mU/mL), Ca^{2+} (mEq/L), Cl^{-} (mEq/L), T4 (ug/dL).
[1]Diet prepared to contain 66 ppm.
*$p \leq 0.05$
**$p \leq 0.01$

6. Urinalysis

Urine was collected in an unspecified manner from 10 male and 10 female animals per dose group during weeks 13, 26, 52 (satellite group), 78, and at termination (main group). Food and water were removed during the overnight collection period (~16 hours). The CHECKED (X) parameters were examined.

X	
	Appearance*
X	Volume*
X	Specific gravity*
X	pH
X	Sediment (microscopic)*
X	Protein*

X	
X	Glucose*
X	Ketones*
X	Bilirubin*
X	Blood*
	Nitrate
X	Urobilinogen

* Required for chronic studies.

Results ▒ There were statistically significant increases in urinary *p*H for males treated with Ziram at 180 ppm at weeks 26 (110% of control, $p \leq 0.01$) and 52 (107% of control, $p \leq 0.05$) and at 540 ppm at weeks 13 (107% of control, $p \leq 0.01$), 26 (112% of control, $p \leq 0.05$), 52 (107% of control, $p \leq 0.05$), and 78 (107% of control, $p \leq 0.05$), as compared to controls. There was a statistically significant increase in *p*H for females treated with Ziram at 540 ppm at week 78 (106% of control, $p \leq 0.05$) as compared to controls. The volume of urine was statistically significantly decreased ($p \leq 0.05$) for females in the 540 ppm dose group at week 52. Protein levels were decreased statistically significantly ($p \leq 0.05$) for females in the 540 ppm dose group at week 104. There were no statistically significant changes identified for animals receiving Ziram at 60 ppm.

TABLE 7. SUMMARY OF STATISTICALLY SIGNIFICANT CHANGES IN URINALYSIS PARAMETERS IN RATS ADMINISTERED ZIRAM FOR 104 WEEKS.

Parameter	Week of Study	Treatment Group/Exposure Level (ppm)							
		Males (N=10)				Females (N=10)			
		0	60[a]	180	540	0	60[a]	180	540
*p*H	13	7.0	6.9	7.3	7.5**	6.4	6.4	6.5	6.5
	26	6.9	7.1	7.6**	7.7*	6.5	6.6	6.5	6.6
	52	6.8	6.9	7.3*	7.3*	6.2	6.4	6.4	6.3
	78	6.9	7.1	7.0	7.4*	6.2	6.4	6.3	6.6*
	104	6.4	6.6	6.2	6.7	6.3	6.2	6.4	6.4
Volume	13	6.2	6.1	6.0	5.6	2.6	3.1	2.7	2.0
	26	5.1	6.7	6.9	6.9	3.2	3.1	2.5	3.2
	52	6.3	7.1	5.9	5.9	6.6	5.1	5.6	3.5*
	78	10.6	10.4	9.0	7.6	6.6	8.2	8.4	6.9
	104	9.4	7.1	10.4	8.8	10.7	8.3	12.9	10.6
Protein	13	175	171	188	168	81	90	82	75
	26	201	177	135	132	73	83	70	66
	52	272	220	215	334	81	142	134	87
	78	336	116	188	114	518	107	100	100
	104	552	289	929	477	337	652	200	73*

Data taken from Table 9, pp. 107-111, MRID No. 42434001. Parameter units are: Volume (mL), Protein (mg/dL).
[a]Diet prepared to contain 66 ppm.
*$p \leq 0.05$
**$p \leq 0.01$

7. Sacrifice and pathology

Animals in the satellite and main groups were scheduled for sacrifice at weeks 52 and 104, respectively. Animals were euthanitized by carbon dioxide asphyxiation. All sacrificed animals and all animals that died during the study were subjected to gross pathological examination. Microscopic pathology was performed on all tissues for animals in the control and 540 ppm dose groups and for any animals in any dose group that died during the treatment period. Scheduled sacrifice was at 52 weeks for the satellite group and at 104 weeks for the main group. Only the liver, lungs, kidneys, and any macroscopically abnormal tissues were examined from animals in the 60 or 180 ppm dose groups at week 52. Additionally, at week 104, spleen, thyroids, skeletal muscle, sciatic nerve, spinal cord, stomach, pancreas, mesenteric lymph nodes (males), ovaries and adrenals were examined from animals in the 60 and 180 ppm dose groups. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
X	Liver*+
X	Pancreas*
Respiratory	
X	Trachea*
X	Lung*
X	Nose
X	Pharynx
X	Larynx

X	
X	Aorta*
X	Heart*
X	Bone marrow*
X	Lymph nodes*
X	Spleen
X	Thymus*
Urogenital	
X	Kidneys*+
X	Urinary bladder*
X	Testes*+
X	Epididymides
X	Prostate
X	Seminal vesicle
X	Ovaries*+
X	Uterus*
X	Vagina

X	
X	Brain*+
X	Periph. nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (optic n.)*
Glandular	
X	Adrenal gland*
X	Lacrimal gland
X	Mammary gland*
X	Parathyroids*
X	Thyroids*
Other	
X	Bone*
X	Skeletal muscle*
X	Skin*
X	All gross lesions and
X	Harderian gland

* Required for subchronic and chronic studies.
+ Organ weight required in subchronic and chronic studies.

Results ▩

a. Organ weight ▩ For males (Table 8), absolute organ weight for adrenals were dose-dependently decreased at weeks 52 and 104. The decrease was statistically significant at week 104 for males in the high dose group for absolute (58.8% of control, $p<0.01$) and relative (66.7% of control, $p<0.05$) adrenal weights. Relative brain weights for males in the 540 ppm dose group were statistically significantly increased at week 104 (110% of control, $p<0.05$). Relative testes weights were statistically significantly increased at week 52 for males in the 540 ppm dose group (115% of control, $p<0.05$) and at week 104 for males in the 180 (112.5% of control, $p<0.05$) and 540 ppm (127% of control, $p<0.01$) dose groups. Relative organ weights were statistically significantly increased for females in the high dose group relative to controls for brain (weeks 52 and 104, 120% of control, $p<0.01$), thyroid (week 104, 133% of control, $p<0.05$), heart (week 52, 117% of control, $p<0.01$; week 104, 112% of control, $p<0.05$), kidney (week 52, 111% of control, $p<0.05$), and adrenal (week 104, 156% of control, $p<0.01$). For females, relative liver weights were dose-dependently increased at weeks 52 and 104. The increases were statistically significant at week 52 for females in the 60, 180, and 540 ppm dose groups (113%, 113%, and 120% of control, respectively, $p<0.01$) and at week 104 for females in the 540 ppm dose group (112% of control, $p<0.05$). Relative pituitary weights were statistically significantly decreased (72.5% of control, $p<0.05$) at week 52 for females in the 540 ppm dose group.

TABLE 8. GROUP MEAN ABSOLUTE (RELATIVE) ORGAN WEIGHTS FOR RATS ADMINISTERED ZIRAM FOR 52 or104 WEEKS.

Organ	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0 52 N=18 104 N=31	60 52 N=19 104 N=28	180 52 N=19 104 N=23	540 52 N=17 104 N=31	0 52 N=20 104 N=16	60 52 N=19 104 N=21	180 52 N=19 104 N=25	540 52 N=20 104 N=24
Brain --Week 52	2.16 (32)	2.15 (30)	2.19 (33)	2.10 (35)	1.94 (50)	1.94 (48)	1.97 (53)	1.90 (60**)
--Week 104	2.23 (31)	2.23 (32)	2.21 (34)	2.17 (34*)	1.99 (45)	2.00 (45)	1.99 (46)	1.94 (54**)
Pituitary--Week 52	16.6 (0.25)	14.1 (0.20)	13.5 (0.20)	13.9 (0.23)	32.4 (0.80)	21.4 (0.52)	21.8 (0.59)	18.8 (0.58*)
--Week 104	34.9 (0.48)	47.5 (0.86)	35.2 (0.53)	27.4 (0.46)	71.3 (1.78)	87.3 (2.02)	53.4 (1.40)	66.9 (1.86)
Thyroid--Week 52	29.6 (0.44)	31.3 (0.43)	29.5 (0.43)	26.5 (0.43)	23.9 (0.60)	25.7 (0.62)	24.1 (0.63)	22.6 (0.68)
--Week 104	45.6 (0.62)	49.3 (0.69)	41.9 (0.63)	50.7 (0.77)	30.9 (0.67)	32.0 (0.71)	33.2 (0.74)	33.3 (0.89*)
Heart --Week 52	1.68 (25)	1.86 (26)	1.72 (26)	1.63 (27)	1.21 (30)	1.25 (30)	1.24 (32)	1.15 (35**)
--Week 104	2.05 (28)	2.11 (30)	2.06 (31)	1.95 (30)	1.53 (34)	1.53 (34)	1.59 (36)	1.41 (38*)
Liver --Week 52	24.5 (363)	26.1 (362)	24.2 (353)	23.6 (386)	13.9 (337)	16.1 (381**)	14.6 (380**)	13.5 (405**)
--Week 104	24.7 (335)	23.0 (323)	22.8 (342)	23.0 (353)	16.6 (365)	17.3 (379)	17.5 (386)	15.5 (408*)
Kidney--Week 52	4.08 (61)	4.16 (58)	3.96 (59)	3.84 (63)	2.63 (66)	2.74 (66)	2.57 (68)	2.36 (73*)
--Week 104	5.34 (73)	4.89 (70)	4.88 (74)	4.58 (71)	3.29 (74)	3.20 (72)	3.19 (72)	2.88 (78)
Adrenal--Week 52	54.7 (0.8)	55.1 (0.8)	47.8 (0.7)	44.4 (0.7)	82.3 (2.0)	70.2 (1.7)	75.5 (2.0)	71.0 (2.2)
--Week 104	105.7 (1.5)	76.5 (1.1)	80.8 (1.2)	62.2** (1.0*)	156.4 (3.9)	107.6 (2.4)	141.8 (3.2)	210.5 (6.1**)
Testes[a] --Week 52	4.90 (73)	5.08 (72)	5.09 (77)	5.08 (84*)	---	---	---	---
--Week 104	4.64 (64)	4.81 (68)	4.74 (72*)	5.28 (81**)	---	---	---	---

Data taken from Tables 10 and 11, pp. 112-117, MRID No. 424340-01. Values are in grams (g) for all organs, except pituitary, thyroid and adrenal which are given in milligrams (mg). Relative organ weight is calculated as ((absolute organ weight (g)/body weight (g))x100)x100.
[a]Testes weighed with epididymides
*p≤0.05
**p≤0.01

b. Gross pathology ▩ Treatment related findings (Table 9) occurring at a higher incidence in treated animals than in controls included depressions, raised areas, thickening, and white discoloration in the forestomach, cysts and cystic enlargement in adrenals (females only), and atrophy of hindlimbs. The incidence of these findings were not statistically significantly different from controls, however, there are correlates with microscopic pathological findings for stomach, adrenals, skeletal muscle, spinal cord, and sciatic nerve. There was also an increased incidence of absence of corpora lutea for females treated with Ziram at 60, 180, and 540 ppm (44%, 48%, 62%, respectively) as compared to controls (38%). This finding is not likely toxicologically significant. In the microscopic pathological examination of the ovaries, there was a similar incidence of absence of corpora lutea in treated animals as compared to controls (see Table 10 of this report).

TABLE 9. MACROSCOPIC PATHOLOGY INCIDENCE SUMMARY FOR RATS ADMINISTERED ZIRAM FOR 104 WEEKS.

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
No. animals	50	50	50	50	50	50	50	50
Ovaries								
-no corpora lutea visible	---	---	---	---	19	22	24	31
Forestomach								
-Depression	11	16	20	20	8	7	14	31
-Raised Areas	0	0	3	4	0	1	1	0
-Thickening	5	11	11	12	3	3	8	12
-White Discoloration	4	8	7	6	1	1	4	9
Overall Pathology Incidence for Forestomach	20	35	41	42	12	12	27	52
Adrenals								
-Cysts/Cystic	0	0	0	0	0	0	1	5
-Cystic enlargement	0	0	0	0	0	0	0	2
Skeletal muscle								
-Atrophied hindlimbs	4	5	3	6	0	0	0	2

Data adapted from summary table, p. 50, MRID No. 42434001.

c. Microscopic pathology ▩

1) Non-neoplastic ▩ There were changes seen for spleen, liver, stomach and pancreas in satellite group animals treated with Ziram at 540 ppm for 52 weeks that were not found in control animals. In main group animals sacrificed at week 104 (Table 10), these changes were generally more severe, were extended to additional dose groups, and the incidence of these changes was statistically significant ($p<0.05$, $p<0.01$). For main group males and females in the 180 and 540 ppm dose groups, there were findings of hemosiderosis in the spleen ($p<0.01$) and sinusoidal cells of the liver ($p<0.01$), bile duct hyperplasia ($p<0.05$, $p<0.01$), hyperplasia of the non-glandular epithelium of the stomach ($p<0.05$, $p<0.01$), subepithelial edema ($p<0.05$) and ulcerations ($p<0.05$) in the stomach, prominent ultimobranchial cysts in the thyroid ($p<0.01$), adipose infiltration/replacement of peripheral muscle fiber bundles ($p<0.01$), and narrowing of peripheral muscle fiber bundles ($p<0.01$) in skeletal muscle, axonal degeneration (minimal) in the spinal cord (males, $p<0.05$) [not shown in Table 10 p. 24 of DER; see p. 264 of the study], and axonal degeneration in the sciatic nerve (males, not statistically significant; females, $p<0.01$). In addition, the degeneration was of greater severity in treated animals than in controls and generally increased in severity with increasing dose of Ziram. There were findings for males only in the 180 and/or 540 ppm dose groups, including adipose replacement of pancreatic tissue ($p<0.05$, $p<0.01$), C-cell hyperplasia in the thyroid ($p<0.05$), hyperplasia in the parathyroids ($p<0.05$), and hypertrophy with vacuolation in the adrenal cortex ($p<0.05$). There were findings for females only in the 180 and/or 540 ppm dose group, including an increase in lipofuscin in cortical tubular epithelial cells in the kidney ($p<0.01$), acinar hyperplasia in the mammary gland ($p<0.05$) and cystic degeneration in the adrenal cortex ($p<0.01$). The findings for males in the low dose group (60 ppm) included hemosiderosis in spleen ($p<0.01$), hyperplasia of the non-glandular epithelium in the stomach ($p<0.01$), subepithelial edema in the stomach ($p<0.05$), narrowing of peripheral muscle fiber bundles in the skeletal muscle ($p<0.05$), and hypertrophy with vacuolation in the adrenal cortex ($p<0.05$). The only microscopic pathological finding occurring at a statistically significant increased incidence for females in the low dose group (60 ppm) was prominent ultimobranchial cysts in the thyroid ($p<0.05$).

TABLE 10. MICROSCOPIC PATHOLOGY: NON-NEOPLASTIC CHANGES FOR MAIN GROUP RATS ADMINISTERED ZIRAM FOR 104 WEEKS

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
No. animals (main group)	50	50	50	50	50	50	50	50
Spleen								
No abnormalities detected	22	13*	13*	14	14	9	2**	3**
Hemosiderosis	10 (2.3)	22** (1.6)	29** (2.0)	23** (2.0)	24 (2.8)	29 (2.2)	38** (2.3)	39** (2.9)
Liver								
Pigment (hemosiderin) in sinusoidal cells	2 (1.5)	5 (1.2)	22** (1.3)	26** (1.4)	0 (0)	3 (1.3)	13** (1.8)	15** (1.9)
Bile duct hyperplasia	7 (1.9)	7 (2.0)	13 (2.1)	15* (2.1)	5 (1.8)	6 (1.8)	9 (2.1)	17** (2.1)
Stomach-Non-Glandular Region								
No abnormalities detected	31	28	18**	20*	32	35	27	11**
Epithelial hyperplasia	6 (2.5)	18** (2.7)	25** (2.6)	25** (2.7)	7 (2.3)	6 (2.5)	15* (2.5)	37** (2.5)
Subepithelial edema	2 (3.0)	8* (2.75)	8* (2.75)	10* (2.7)	2 (2.5)	3 (3.3)	3 (2.3)	11** (2.7)
Ulceration	5 (2.4)	8 (2.4)	14* (2.4)	14* (2.4)	3 (2.3)	5 (2.8)	6 (2.5)	12* (2.2)
Perforating ulceration, marked	0	1	0	3	0	0	0	1
Hyperplasia at the limiting ridge	1 (2.0)	2 (2.0)	3 (2.0)	3 (2.3)	1 (2.0)	2 (2.5)	4 (2.8)	1 (2.0)
Pancreas								
Replacement by adipose tissue	7 (2.4)	14 (2.4)	15* (2.3)	21** (2.7)	0/50 (0)	2/50 (1.5)	4/49 (2.0)	3/50 (2.7)
Thyroid								
No abnormalities detected	29	31	25	24	37	31	20**	17**
C-cell hyperplasia	1 (2.0)	3 (2.3)	5 (2.2)	8* (2.5)	5 (2.8)	5 (2.2)	5 (2.2)	3 (2.3)
Prominent ultimobranchial cysts	4	5	14**	15**	3	12*	22**	27**
Parathyroids								
No abnormalities detected	48/49	40*/47	44/48	39*/46	47/48	46/46	46/47	47/47
Hyperplasia	1/49 (2.0)	4/47 (2.5)	4/48 (2.3)	7*/46 (2.3)	0	0	0	0

TABLE 10 (Continued)

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
Skeletal muscle								
No abnormalities detected	38	31	13**	4**	50	43**	22**	16**
Adipose infiltration/replacement of peripheral muscle fiber bundles	5 (2.4)	10 (1.9)	27** (2.2)	43** (2.5)	0 (0)	4 (1.8)	21** (2.1)	26** (2.2)
Narrowed peripheral muscle fiber bundles	2 (2.5)	10* (1.8)	30** (2.1)	37** (2.2)	0 (0)	4 (1.8)	15** (1.7)	22** (2.0)
Spinal cord								
Axonal degeneration	15 (1.3)	23 (1.4)	20 (1.9)	22 (1.9)	13 (1.1)	10 (1.5)	15 (1.6)	13 (1.5)
Sciatic nerve								
Axonal degeneration	14 (1.6)	12 (1.5)	15 (1.7)	22 (2.0)	3 (1.0)	5 (1.2)	4 (1.3)	16** (1.4)
Kidney								
Brown pigment (lipofuscin) in cortical tubular epithelial cells	2 (1.0)	6 (1.2)	6 (1.3)	4 (1.8)	5 (1.8)	8 (1.0)	9 (1.7)	19** (1.4)
Adrenals								
Cortical hypertrophy with vacuolation	4 (2.3)	11* (2.1)	11* (2.2)	12* (2.0)	1 (1.0)	1 (2.0)	0 (0)	2 (3.0)
Cortical cystic degeneration	2 (3.0)	3 (2.0)	1 (2.0)	2 (3.5)	7 (3.6)	9 (3.2)	12 (2.9)	29** (3.4)
Lymph nodes-Cervical								
No abnormalities detected	49/50	19**/26	22**/29	42*/50	48/50	26/29	23/26	46/50
Plasmacytosis	1	5*	4	6	2	3	3	3
Ovaries								
Absence of corpora lutea	--	--	--	--	26/50	27/50	29/50	32/49
Mammary Gland								
Acinar hyperplasia	4/50 (2.3)	1/23 (2.0)	1/29 (3.0)	3/50 (2.7)	20/50 (3.0)	20/47 (2.7)	18/48 (2.6)	30*/50 (2.6)

Data adapted from Table 13, p. 142-296, MRID No. 42434001. Unless otherwise noted in the Table, the incidence of a lesion is given per 50 animals examined.
[1]Numbers in parentheses are the average severity rating of the lesion per number of affected animals, as calculated by the reviewer using the following numerical equivalents to the grade of the pathology: 1=trace, 2=minimal, 3=moderate, 4=marked, 5=severe.
*$p<0.05$
**$p<0.01$

2) Neoplastic ▒ The control animals developed a variety of tumors in different tissues including the liver, pancreas, testes, thyroid, adrenal, pituitary and mammary gland (See Appendix 2). The tumors with increased or decreased incidence relative to control are presented in Table 11. The incidence of males with tumors was decreased dose-dependently compared to controls (540 ppm, $p<0.05$). This decrease was primarily due to a dose-dependent decrease in malignant tumors for males (540 ppm, $p<0.01$). The only tumors considered by the study authors to be treatment-related were hemangiomas in mesenteric lymph nodes and spleens of males. These tumors were benign, showing no evidence of malignancy. The incidence of animals with this tumor type was statistically significantly increased for males treated with Ziram at 540 ppm ($p=0.024$), as compared to controls. In addition, the dose-related trend in the number of animals with this tumor type was statistically significant ($p=0.0001$). Historical control data obtained by the testing facility indicated that the incidence for this tumor type in males ranged from 0/50 to 2/50. The overall incidence for this tumor type in the current study was 6/50. For females, there were no tumors found with a higher incidence in treated animals than in controls that were considered by the study authors to be treatment-related. Appendix 2 summarizes tumor types and incidences in male and female rats of the main study groups.

TABLE 11. MICROSCOPIC PATHOLOGY: NEOPLASTIC CHANGES FOR MAIN GROUP RATS ADMINISTERED ZIRAM FOR 104 WEEKS

Neoplasia	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	60	180	540	0	60	180	540
No. animals	50	50	50	50	50	50	50	50
No. of animals with tumors[a]	45	44	41	37*	48	46	49	47
No. of animals with malignant tumors[a]	23	20	17	8**	12	15	21*	13
No. of animals with benign tumors[a]	35	34	32	35	46	41	42	43
No. of animals with metastatic tumors[a]	3	1	1	2	0	0	2	1
Lymph Nodes-Mesenteric								
Hemangioma (Benign)[b]	0	0	0	5*	0	0	0	0
Spleen								
Hemangioma (Benign)[b]	0	0	0	1	0	0	0	0

Data adapted from Table 13, p. 142-296, MRID No. 42434001.
[a]Statistical significance calculated by the reviewer, *t*-test.
[b]Statistical significance calculated by the study authors.
*$p<0.05$
**$p<0.01$

D. DISCUSSION

From the results presented in the current study report, MRID No. 434042-01, oral administration of Ziram at 60, 180, and 540 ppm for 104 weeks to male and female rats

resulted in toxicologically significant effects. There was no treatment-related mortality. The dose selection rationale was based upon a subchronic (13-week) feeding study (MRID No. 424503-01, summarized in the Appendix to this report). Although the toxicological findings for the low dose group (100 ppm) were minimal and virtually indistinguishable from controls during the 13-week treatment period, the study authors felt that treatment with Ziram at 100 ppm for 104 weeks would result in significant toxicological findings. The low dose of Ziram for the 104-week chronic feeding/oncogenicity study was chosen as 60 ppm in an attempt to determine a NOAEL. The medium and high doses of Ziram were chosen as 180 and 540 ppm (3 and 9 times the low dose, respectively).

The MTD of Ziram for males and females was chosen as 540 ppm, based upon the decreased group mean body weight gains from weeks 0-104 for males (86% of controls, $p<0.01$) and females (74% of controls, $p<0.01$) in the high dose group (540 ppm), decreased food consumption for males (91% of control, $p<0.01$) and females (94%, $p<0.05$), statistically significant decreases ($p<0.05$, $p<0.01$) in hematology parameters (RBC, HGB, PCV) during weeks 26-104 for females, and statistically significant decreases ($p<0.05$, $p<0.01$) in clinical chemistry parameters (total protein, albumin, calcium and SGPT) during weeks 13-52 for females. In addition, there were statistically significant decreases in absolute ($p<0.01$) and relative ($p<0.05$) organ weight for the adrenals for males. There was an increased incidence (not statistically significant) of macroscopic pathological findings for males and females for stomach (raised areas, thickening and white discoloration), skeletal muscle (atrophy of hindlimbs), and for females for adrenals (cysts). There were microscopic pathological findings for males and females for spleen (hemosiderosis, $p<0.01$), liver (hemosiderosis, $p<0.01$, bile duct hyperplasia, $p<0.05$, $p<0.01$), stomach (epithelial hyperplasia, $p<0.01$, subepithelial edema, $p<0.05$, and ulcerations, $p<0.05$), pancreas (males only: adipose tissue replacement, $p<0.01$), thyroid (males and females: prominent ultimobranchial cysts, $p<0.01$, males only: C-cell hyperplasia, $p<0.05$), parathyroids (males only: hyperplasia, $p<0.05$), skeletal muscle (adipose infiltration/replacement of peripheral muscle fiber bundles, $p<0.01$, narrowing of peripheral muscle fiber bundles, $p<0.01$), spinal cord (males only, axonal degeneration-minimal, $p<0.05$), sciatic nerve (axonal degeneration, males, not statistically significant, females, $p<0.01$), kidney (females only: lipofuscin in cortical tubular epithelial cells, $p<0.01$), adrenal cortex (males: hypertrophy with vacuolation, $p<0.05$, females: cystic degeneration, $p<0.01$), and mammary gland (females only: acinar hyperplasia, $p<0.05$). The lesions and the hyperplasia noted for stomach epithelium were likely a result of irritation from the compound administration. The microscopic pathologic findings for other organs (liver, spleen, kidneys, adrenals, spinal cord and sciatic nerve, and thyroid) are associated with Ziram treatment, although the mechanism of toxicity is not immediately evident. Splenic pigmentation (hemosiderosis) is common in older animals and is found in controls and the Ziram treatment exacerbated the incidence of this finding.

The LOAELfor males and females was 60 ppm (2.5 mg/kg/day in males, 3.4 mg/kg/day in females). No NOAEL could be determined. There was an increased incidence of microscopic pathological findings for males treated with Ziram at 60 ppm for spleen (hemosiderosis, $p<0.01$), stomach (epithelial hyperplasia, $p<0.01$, subepithelial edema, $p<0.05$), pancreas (adipose tissue replacement, not statistically significant), skeletal muscle (narrowing of peripheral muscle fiber bundles, $p<0.05$), and adrenal cortex (hypertrophy with vacuolation, $p<0.05$). These histopathologic changes were present in generally greater severity in the higher dose groups (180 and 540 ppm), were not present in controls, and thus are toxicologically significant. For females treated with Ziram at the low dose (60 ppm), the only treatment-related effect was a statistically significant increase ($p<0.05$) in the incidence of prominent ultimobranchial cysts in the thyroid. The toxicological significance of this finding is not clear. There were no statistically significant differences between controls and females treated with Ziram at 60 ppm in absolute or relative organ weights for the thyroid, T4 levels, or incidence of thyroid tumors. However, the presence of the histopathological finding for the thyroid in the low dose group

and the increased incidence above the controls in the higher dose groups precludes the identification of a NOAEL for females.

There were microscopic pathology findings that may be of interest for other studies of Ziram toxicity. There was an increased incidence of foci of axonal degeneration in the spinal cord and sciatic nerve. It will be of interest to note if similar changes are seen in neurotoxicological studies for Ziram. Also of interest were the presence of prominent ultimobranchial cysts in the thyroid. These structures are considered to be of embryonic origin, thus it is possible that Ziram treatment may affect developing tissues. It may be of interest to note if similar changes are found in a developmental toxicity study for Ziram.

There was an increased incidence of benign tumors (hemangioma) in the spleen (1 male) and in the mesenteric lymph nodes (5 males) for males treated with Ziram at the MTD. The incidence of the neoplastic change for the mesenteric lymph nodes was increased statistically significantly ($p<0.05$) as compared to controls, and the incidence in this study was higher than in previous studies at this testing facility. The hemangioma showed no evidence of malignancy. These tumors were not found in females, in males in the medium or low dose groups, or in controls. There were no malignant tumors attributable to Ziram treatment. Dosing for males and females was adequate. The MTD for oral administration of Ziram to males and females for 104 weeks is 540 ppm, based upon the changes in body weights, food consumption, hematology, organ weights, and macroscopic and microscopic pathology.

E. STUDY DEFICIENCIES

A NOAEL could not be identified for males or females (see Discussion section above). The lack of a definitive NOAEL will require supplementary studies to be performed, even though a valid attempt was made to define the limit in the current study, MRID No. 434042-01. Creatinine phosphokinase and lactate dehydrogenase levels were not determined in the clinical chemistry evaluations. The appearance of urine or how urine was collected was not mentioned in the urinalysis section. The addition of data concerning these parameters would not change the conclusions, or the identification of the MTD.

APPENDIX 1

Dose Selection Study in Rats

MRID No.: 424503-01
Study Type: Subchronic Feeding-Rat (§82-1a), use for range-finding
Test Material: Ziram
Study No.: ZIR 5/901840
Sponsor: Ziram Task Force, c/o UCB Chemicals Corporation, 5505-A Robin Hood Road, Norfolk, VA 23513 USA
Testing Facility: Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England
Study Title: Preliminary toxicity to rats by dietary administration for 13 weeks.
Authors: Lindsey A. J. Powell, David Crook, Richard Gregson, Chirukandath Gopinath, William A. Gibson, Alan Anderson
Study Completed: August 19, 1992

Methods:
Test Animals: Rats, Crl:CD(SD)BR, 42 days, male 157-188 g, female 117-144 g
Group Size: 10 males, 10 females per dose group
Test material concentrations: Daily diet of Ziram at 0, 100, 300, or 1000 ppm.

Results:
Clinical signs: Increased incidence of hair loss in rats treated with Ziram at 300 and 1000 ppm. This was not noted for controls or rats treated with Ziram at 100 ppm.

Mortality: One female in the control group died during scheduled blood withdrawal. The cause of death is unknown.

Bodyweight: There were statistically significant ($p<0.01$), dose-dependent reductions in body weight gain for males and females treated with Ziram at 300 and 1000 ppm. Body weight gain for rats treated with Ziram at 100 ppm was similar to controls.

Food Consumption:
Food consumption was dose-dependently decreased for males and females. The decreases were statistically significant ($p<0.01$) for males and females treated with Ziram at 300 and 1000 ppm.

Clinical Pathology:
Hematology:
RBC and MCHC levels were dose-dependently decreased for both males and females. The decrease in RBC was statistically significant for males ($p<0.05$) and females ($p<0.01$) treated with Ziram at 1000 ppm. The decrease in MCHC was statistically significant for females at 100 ppm ($p<0.05$), and for males and females at 300 ($p<0.05$) and at 1000 ppm ($p<0.01$).

Biochemistry:
For males treated with Ziram at 1000 ppm, urea ($p<0.05$) and creatinine ($p<0.01$) levels were increased and SGPT levels were decreased ($p<0.05$), relative to controls. For males and females, Ca^{2+} levels were dose-dependently decreased. The decreases were statistically significant for all dose groups for males ($p<0.05$) and females ($p<0.01$). For females, total protein, albumin, globulin, and SGPT levels were dose-dependently decreased. The changes were statistically significant ($p<0.05$, $p<0.01$) for the 100, 300 and 1000 ppm dose groups. Alkaline phosphatase and urea levels for females were dose-dependently increased. The increases were statistically

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significant ($p<0.01$) for the 1000 ppm dose group for AP and for the 100 ($p<0.05$), 300 ($p<0.05$), and 1000 ($p<0.01$) ppm dose groups for urea levels.

Urinalysis:
Not performed.

Organ Weight Gain:
Relative organ weights for brain, spleen, testes and ovary were increased dose-dependently, relative to controls. The increases were statistically significant ($p<0.05$, $p<0.01$) for brain, testes, and spleen for the 300 and 1000 ppm dose groups, and for ovary in the high dose group.

Macroscopic and Microscopic Pathology:
Macroscopically, there were findings for stomach (white nodules near limiting ridge) and liver (pale subcapsular area, small or mottled lobes). Upon microscopic examination, the findings of note were for females: subcapsular area of finely vacuolated hepatocytes at the median cleft of the liver (1 control female, 1 in the 100, 2 in the 300, and 1 in the 1000 ppm dose groups), centrilobular hepatocyte necrosis in the liver (1 female in each of the 300 and 1000 ppm dose groups), focus of ectopic non-glandular epithelium within the glandular mucosa of the stomach (0 females in the control, 1 in the 100 ppm, 1 in the 300 ppm, and 2 in the 1000 ppm dose groups), and hyperplasia of the non-glandular epithelium near the limiting ridge of the stomach (0 in control, 0 in 100 ppm, 1 in the 300 ppm and 3 in the 1000 ppm dose groups).

Conclusions: The toxicity data for the 13-week feeding study included findings of statistically significant decreases in body weight gain, food consumption, changes in hematology and clinical chemistry parameters, and increases in relative organ weights. The microscopic pathology data did not provide strong evidence for toxicity of Ziram. As the findings that included the 100 ppm dose group were often also found in the control group, the NOAEL was chosen as 100 ppm. The LOEL was chosen as 300 ppm based upon the decreases in body weight, food consumption and increases in relative organ weights. On the basis of this study, levels of Ziram at 60, 180, and 540 ppm were chosen for use in the chronic feeding/oncogenicity study (MRID No. 434042-01).

Core Classification: Core-minimum (§82-1a), use for dose range-finding in chronic feeding/ oncogenicity study, MRID No. 434042-01.

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There are no electronic copies of Appendix 2

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Toxicology Branch:	RAB2

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