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DATA EVALUATION REPORT

8/2/2000

ZIRAM

Study Type: SUBCHRONIC FEEDING ▩ RAT (82-1a)

Prepared for

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

Prepared by

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Reregistration Branch 4 (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Feeding ▩ Rat (82-1a)

TOX. CHEM, NO: 931

P.C.CODE.: 034805

MRID NO.: 42450301

TEST MATERIAL (Purity): Ziram (98.7%)

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

CITATION: Powell, L., D. Crook, R. Gregson, C. Gopinath, W. Gibson & A. Anderson (1992) Preliminary toxicity to rats by dietary administration for 13 weeks.Huntingdon Research Centre Ltd., Cambridgeshire, England, ZIR 5/901840, August 19, 1992. MRID 42450301. Unpublished.

SPONSOR: Ziram Task Force, c/o UCB Chemicals Corporation, 5505-A Robin Hood Road, Norfolk, VA 23513

EXECUTIVE SUMMARY: In a subchronic 13-week feeding study, MRID No. 424503-01, male and female Crl:CD(SD)BR rats (10/sex/dose) were administered Ziram (Technical) in the diet at 0, 100, 300, and 1000 ppm. These doses were equivalent to 0, 7.4, 21.4, 67.8 mg/kg/day for males and 0, 8.8, 24.2, and 76.9 mg/kg/day for females.

Ziram treatment for 13 weeks did not produce clinical signs of toxicity or compound-related deaths but it resulted in dose-dependent, statistically significant decreases in body weight gain ($p < 0.01$) and food consumption ($p < 0.05$, $p < 0.01$) for males and females in the medium and high dose groups as compared to controls. Body weight gains for males treated with Ziram at 300 and 1000 ppm were 82% and 67% of controls, respectively. Body weight gains for females treated with Ziram at 300 and 1000 ppm were 82% and 68% of controls, respectively. Food consumption for males treated with Ziram at 300 and 1000 ppm was decreased to 87% and 75% of controls, respectively. Although there were no statistically significant decreases in body weight, the percentage decreases were levels generally regarded as toxicologically significant, i.e., 9-12% and 8-11% for the 300 ppm males and females, respectively; 20-21% and 13-16% for the 1000 ppm males and females, respectively. Food consumption for females treated with Ziram at 300 ppm and 1000 ppm was decreased to 83% and 75% of controls, respectively. Food efficiency for males and females was slightly decreased for the medium and high dose groups as compared to controls. There were statistically significant increases in relative organ weights of brain for males treated with Ziram at 300 ppm ($p < 0.05$) and 1000 ppm ($p < 0.01$) and of brain and spleen for females ($p < 0.01$, $p < 0.05$) at 300 ppm and 1000 ppm ($p < 0.01$ for both) that were not found in controls or in rats treated with Ziram at 100 ppm, but no concomitant histopathological findings. There were no biologically-relevant changes in hematological or chemistry parameters at any dose groups. There were microscopic pathological findings of localized areas of epithelial hyperplasia in the stomach (3 females and 1 male in the 1000 ppm

and 1 female in the 300 ppm dose group vs. 0 in control groups and centrilobular necrosis in one liver lobe in 1 female each in the 300 and 1000 ppm groups vs. 0 in the control animals). No lesions were noted for the 100 ppm dose group. Based primarily on the decreases in body weight, body weight gain, food consumption and minimal histopathological changes in the liver (females) for the mid-dose group, and the lack of findings for the low-dose group, **the LOAEL was chosen as 300 ppm (M:21.4 mg/kg/day; F: 24.2 mg/kg/day) and the NOAEL as 100 ppm (M: 7.4 mg/kg/day; F: 8.8 mg/kg/day).**

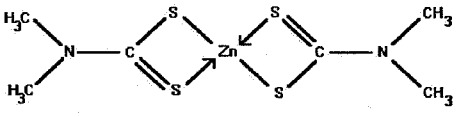
The study is classified as acceptable (guideline) and satisfies the guidelines for a subchronic feeding study (§82-1) in rats.

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% ai.
Stability of compound: at least 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. No positive control was described.

3. Test animals

Species: Rat
Strain: Crl: CD(SD)BR
Age and weight at study initiation: 42 days, males 157-188 g, females 117-144g
Source: Charles River Portage, Portage, MI, USA
Housing: In suspended cages with a wire mesh floor, five animals per cage, such that mean group body weights per cage were similar.
Environmental conditions:
Temperature: 21±2°C
Humidity: 55±10%
Air changes: Not described
Photoperiod: 12 hours light/dark cycle
Acclimation period: 14 days

B. STUDY DESIGN

1. Animal assignment

Rats were weighed and assigned to cages (5 per cage/sex) such that mean body weights per cage were approximately equal. Cages of animals were assigned to treatment groups (Table 1).

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	10	10
2 Low (LDT)	100[a]	7.4	8.8	10	10
3 Mid (MDT)	300	21.4	24.2	10	10
4 High (HDT)	1000	67.8	76.9	10	10

Adapted from Table 4, p. 41, MRID No. 424503-01.
[a]Prepared at 115 ppm to compensate for losses during storage observed in pre-dosing analyses.

Dose selection rationale: Doses were selected based on a preliminary study performed by the testing facility (Huntingdon Research Centre, study designation: ZIR 2/89719). Treatment with Ziram at 500, 1000, and 2000 ppm in the diet for 4 weeks resulted in marked reductions in weight gain and food intake. Further information concerning this 4 week study (including the particular species treated or other parameters measured) was not included in the current study report, MRID No. 424503-01.

2. Diet preparation and analysis

A concentrated diet was prepared weekly by grinding Ziram into SDS Rat and Mouse No. 1 modified maintenance diet and mixing with a Turbula mixer for 2 minutes. The concentrated diet was diluted with appropriate quantities of untreated diet to provide diets with inclusion levels of 100, 300, and 1000 ppm. Diets were homogenized by mixing in a double cone blender for at least 7 minutes. The low dose diet was prepared at 115 ppm to compensate for losses determined from pre-test analytical chemistry data (information not included in the study report, MRID No. 424503-01). Diets were stored at 4°C in aliquots of 3/week. Samples of diet were analyzed at weeks 1, 6, and 13 for stability and concentration.

Results

a. Homogeneity analysis Diet was prepared with Ziram at concentrations of 70 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 Prior to initiation of the study, the stability of Ziram in diet preparations was assessed at inclusion levels of 70, 100, 300, and 5000 ppm. Ziram mixed with diet and stored at room temperature for 15 days was stable at 5000 ppm, but was not stable at 70 ppm (38.4% overall loss). Ziram stability at the 100 and 300 ppm inclusion levels was assessed during storage at 4°C for 0, 1, 4, and 7 days.

During storage of the 100 ppm diet, losses were 7.5% at day 0 (preparation losses), with an additional 10.6% loss during the 7 day storage, for an overall loss of 17.3% (relative to the nominal inclusion level). At the 300 ppm inclusion level stored at 4°C for 7 days, the storage loss was 10.1% (day 0 level compared to day 7). Exposure of the 100 or 300 ppm diet formulations to air and room temperature for 24 hours subsequent to the 7 day storage at 4°C did not result in additional losses. The losses of Ziram at the 100 or 300 ppm inclusion levels during storage of diet formulations at 4°C were not considered significant by the sponsor or the study authors. Therefore, diet formulations were prepared each week in aliquots of 3/week/dose and stored at 4°C. Diet was changed at 24 hour intervals. Despite the statement that the losses at the 100 ppm inclusion level were not considered significant, diet for the 100 ppm diet formulation was prepared at 115 ppm to account for losses during preparation. It is not clear to the reviewer why the 100 ppm diet was fortified by 15%. The preparation loss for the 100 ppm diet was 7.5% (trial 3, day 0, mean analyzed concentration corrected for procedural recovery is 92.5 ppm). Assuming a 7.5% loss, preparation of diet at an inclusion level of 115 ppm would yield an inclusion level of 106 ppm. Thus, the decision to fortify the diet to 115 ppm was apparently based upon information not presented in the study report.

c. Concentration analysis ▩ Samples of diet for the 115, 300, and 1000 ppm inclusion levels were analyzed at days 0 and 7 of study weeks 1, 6, and 13. Each sample was analyzed twice and the mean Ziram concentration determined. For the 115, 300, and 1000 ppm inclusion levels the concentrations were (overall means) 95.1% 95.7%, and 96.2% of the nominal inclusion levels.

3. Diet

Animals were provided with fresh food (SDS Rat and Mouse No. 1 SQC modified maintenance diet) each day. Residue from the previous day was removed and weighed for determination of food intake. Drinking water (tap) was available *ad libitum*. Diet and tap water were routinely subjected to chemical analysis (Addenda 1 and 2, pp. 160-162).

4. Statistics

Data for males and females were analyzed separately. Food and water consumption were analyzed on a per cage basis. Histopathological data was analyzed using Fisher's exact test. For food and water consumption, bodyweight, relative organ weight and clinical pathology data, the following statistical tests were used in sequence. If data consisted primarily of 1 value (relative frequency of the mode >75%) then the proportion of animals different from the mode was analyzed. Bartlett's test was used for analysis of heterogeneity of variance between treatments. If significant (1% level) heterogeneity was found, 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 in the analysis of absolute organ weight data, an analysis of covariance was used with the final body weight as the covariate.

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

C. METHODS AND RESULTS

1. Observations

Inspections were performed twice a day for signs of morbidity and mortality. For behavioral changes, reactions to treatment or illness, animals were inspected once each weekday (not on weekends) for the first four weeks. After the first 4 weeks, animals were inspected once per week.

Results One female (control group) died during scheduled blood withdrawal, likely due to ether overdose. The only other clinical findings was a slight, dose-dependent increase in incidence of hair loss in males.

2. Body weight

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

Results Although there were no statistically significant decreases in body weight, the percentage decreases were levels generally regarded as toxicologically significant, i.e., 9-12% and 8-11% for the 300 ppm males and females, respectively; 20-21% and 13-16% for the 1000 ppm males and females, respectively. There was no apparent effect on overall weight gain for rats treated with Ziram at 100 ppm. There was a statistically significant ($p<0.01$) dose-dependent reduction in weight gain over the 13-week treatment period for males and females treated with Ziram at 300 and 1000 ppm. The reduction in overall weight gain was primarily due to weight loss during the first week of treatment.

TABLE 2. GROUP MEAN BODY WEIGHTS (G) AT SELECTED WEEKS & GROUP MEAN BODY WEIGHT CHANGES (G) AT SELECTED WEEKLY INTERVALS

Week of Study	Males				Females			
	0	100	300	1000	0	100	300	1000
0	170	172	171	172	136	130	135	135
1	227	222	202 (11)'	182 (20)	159	156	142 (11)	138 (13)
12	502	515	458 (9)	398 (21)	251	251	225 (10)	212 (16)
13	505	521	445 (12)	397 (21)	243	242	223 (8)	207 (15)
Body weight change, week 0-1	57	51 (11%)	31** (46%)	10** (82%)	22	26	7** (68%)	2** (91%)
Body weight change, week 1-12	276	293	257 (7%)	216** (22%)	92	95	83 (10%)	74* (20%)
Body weight change, week 0-13	335	349	274** (18%)	225** (33%)	106	112	87** (18%)	72** (32%)

Data adapted from summary table, p.28 and Table 1, p. 38, MRID No. 424503-01.
' % decrease
*$p<0.05$, Williams' test
**$p<0.01$, Williams' test.

3. Food consumption and compound intake

Food consumption for each cage of animals was determined daily and results for each dose group presented as g/rat/week. Efficiency of food utilization (or food conversion ratio) was calculated by the study authors as cumulative food consumption/body weight gain/unit time. Compound intake (mg/kg/day) values were expressed as achieved intake of Ziram and calculated for each week as (mean food consumption (g) x ppm (nominal))/(mean bodyweight (g) x 7). Compound intakes for the treatment period were the mean of the weekly calculations and are presented in Table 1 of this report.

Results

a. Food consumption The total mean food consumption (Table 3) for males and females was similar to controls for the 100 ppm treatment group, but was less than controls for rats treated with Ziram at 300 and 1000 ppm. Thus, the decreased body weight gains were due in part to decreased food consumption.

b. Compound consumption (time-weighted average) Compound consumption (see Table 1 of this report) was proportionate for the 300 ppm dose group as compared to the 100 ppm dose group. However, compound consumption for the 1000 ppm dose group was slightly decreased when compared proportionately to the 100 ppm dose group, due primarily to decreased food consumption.

c. Food efficiency Food efficiency was calculated by the reviewer (body weight gain, g/food consumption, g per unit time X 100) for weeks 1-13 (Table 3). Food efficiency was decreased slightly for both males and females treated with Ziram at 1000 ppm as compared to controls.

TABLE 3. MEAN INDIVIDUAL TOTAL FOOD CONSUMPTION, DAILY FOOD CONSUMPTION AND FOOD EFFICIENCY

Parameter	Exposure Level (ppm)							
	Males				Females			
	0	100	300	1000	0	100	300	1000
Total Food Consumption (g/rat)	2458	2470 (100%)[a]	2132* (87%)	1847** (75%)	1670	1661 (99%)	1393 (83%)	1256* (75%)
Daily Food Consumption[b] (g/rat/day)	27.0	27.1	23.4**	20.3**	18.4	18.3	15.3**	13.8**
Food Efficiency[c] [((bodyweight gain(g))/(total food consumed (g))]x100	13.6	14.1	12.9	12.2	6.3	6.7	6.2	5.7

Data adapted from summary tables, p. 29, and Tables 2 and 3, pp. 39-40, MRID No. 424503-01.
[a]% of control (0 ppm dose group).
[b]Calculated by reviewer, based upon 91-day treatment period. Statistical significance relative to control (0 ppm) was calculated by the reviewer with Student's *t*-test.
[c]Calculated by reviewer, using body weight gains for weeks 0-13 (see Table 2 of this report).
*$p<0.05$, Williams' test
**$p<0.01$, Williams' test

4. Water consumption

Water consumption was measured daily during Week 12. There was no evidence of a treatment-related effect.

5. Ophthalmoscopic examination

Prior to commencement of treatment, the eyes of all animals were examined. During week 13, the eyes of all the rats in the control and 1000 ppm dose groups were examined by means of a Keeler indirect ophthalmoscope. Pupils were dilated using a Tropicamide ophthalmic solution.

Results ▩ The ophthalmoscopic examination during week 13 did not reveal any lesions attributable to Ziram treatment.

6. Blood was collected for hematology and clinical chemistry from all animals after an overnight fast. During week 13, rats were anesthetized with ether and samples of blood withdrawn from the orbital sinus of all the animals in each dose group. Blood was divided into 3 tubes containing either EDTA anticoagulant (hematology), citrate anticoagulant (coagulation tests), heparin (all other biochemical tests). The CHECKED (X) parameters were examined.

a. Hematology

X		X	
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	Platelet count*		
	Blood clotting measurements		
X	(Thromboplastin time, APTT)		
	(Clotting time)		
	(Prothrombin time, PT)		

*Required for subchronic studies.

Results There were statistically significant ($p<0.05$) dose-dependent decreases in RBC levels and dose-dependent increases in MCHC for both males and females (Table 4). However, the RBC values were well within normal parameters and increases in MCHC have no clinical relevance.

TABLE 4. HEMATOLOGY: SUMMARY OF STATISTICALLY SIGNIFICANT CHANGES AT WEEK 13

Dose	Males				Females			
	PCV	RBC	MCHC	APTT	PCV	RBC	MCHC	APTT
0	55	8.0	25.7	23	52	7.4	26.3	20
100	54	7.9	25.8	22*	52	7.2	26.7*	20
300	55	7.8	26.3*	22*	52	7.2	27.2**	20
1000	54	7.7*	26.4**	22*	51*	7.1**	27.4**	20

Data adapted from Table 7 (p. 44), MRID No. 424503-01. Units are: PCV (%), RBC ($x10^6/mm^3$), MCHC (%), APTT (seconds).
*$p<0.05$, Williams' test
**$p<0.01$, Williams' test

b. Clinical chemistry

X		X	
	Electrolytes		Other

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

Enzymes

X	Alkaline phosphatase (ALK)*
	Creatinine phosphokinase (CK)*
	Lactic acid dehydrogenase (LDH)*
X	Serum alanine aminotransferase (also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*

X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
X	Globulins
X	Glucose*
X	Total serum protein (TP)*
X	Bilirubin*

* Required for subchronic studies.

Results Several clinical chemistry parameters were statistically significantly ($p < 0.05$, $p < 0.01$) different between controls and treated groups (Table 5). For both males and females, urea levels were increased and Ca^{2+} levels decreased. For females, total protein, albumin, globulin, Ca, and SGPT were significantly decreased statistically (SGPT not SS at low dose) at all doses and alkaline phosphatase levels were significantly increased at the high dose only.

TABLE 5. CLINICAL CHEMISTRY: SUMMARY OF STATISTICALLY SIGNIFICANT CHANGES AT WEEK 13

Dose		Parameter [a]							
		Protein	Alb.	Glob.	Urea	Creat.	AP	SGPT	Ca
Males	0	6.8	3.0	3.8	14	0.5	146	27	5.2
	100	6.6	2.9	3.7	14	0.6	144	22	5.0*
	300	6.6	3.0	3.6	15	0.6	149	25	5.0*
	1000	6.6	3.0	3.6	18*	0.6**[b]	145	21*	5.1*
Females	0	7.3	3.6	3.7	18	0.6	84	34	5.2
	100	6.8**	3.3**	3.5*	22*	0.6	96	30	5.1**
	300	6.6**	3.2**	3.4**	22*	0.6	96	20**	5.0**
	1000	6.4**	3.1**	3.4**	24**	0.6	139**	20**	4.8**

Data adapted from Table 8 (p. 45), MRID No. 424503-01.
[a]Total Protein, Alb. (Albumin), and Glob. (Globulin fraction): g/dL; Urea nitrogen and Creat. (Creatinine): mg/dL; AP (Alkaline Phosphatase) and SGPT: mU/mL; Ca: mEq/L
[b]Mean value is 0.63 (calculated by reviewer from individual animal data) and is significantly different from control (reviewer calculated *t*-test, $p=0.0023$, confirms study authors' calculation of statistical significance ($p<0.01$)).
*$p<0.05$, Williams' test
**$p<0.01$, Williams' test

7. Urinalysis

Results ☒ Urinalysis was not performed.

8. Sacrifice and pathology

All animals were fasted overnight and sacrificed by carbon dioxide asphyxiation. The macroscopic appearance of tissues was noted prior to removal. Parathyroids and thyroids were weighed together as were testes and epididymides. 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.

X	
Digestive system	
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*•
	Gall Bladder*▩
X	Pancreas*
Respiratory	
X	Trachea*
X	Lung*
X	Nose
X	Pharynx
X	Larynx

X	
Cardiovasc./Hemat.	
X	Aorta*
XX	Heart*
X	Bone marrow*
X	Lymph nodes*
XX	Spleen
X	Thymus*
Urogenital	
XX	Kidneys*•
X	Urinary bladder*
XX	Testes*•
XX	Epididymides
X	Prostate
X	Seminal vesicle
XX	Ovaries*•
XX	Uterus*

X	
Neurologic	
XX	Brain*•
X	Periph. nerve*
X	Spinal cord (3 levels)*
XX	Pituitary*
X	eye (optic n.)*
Glandular	
XX	Adrenal gland*
X	Lacrimal gland
X	Mammary gland*
XX	Parathyroids*••
XX	Thyroids*••
Other	
X	Bone*
X	Skeletal muscle*
X	Skin*
X	All gross lesions and masses*

* Required for subchronic and chronic studies.
• Organ weight required in subchronic and chronic studies.
▩ Organ not present in rats.

Results ▩

a. Organ weight ▩ Absolute organ weights (Table 6) were dose-dependently decreased for pituitary (males) and heart (females). The decreases became statistically significant ($p < 0.05$) for rats in the 1000 ppm dose group. In addition, statistically significant, but not dose-dependent decreases in absolute organ weights were noted for brain (males ($p < 0.05$) and females ($p < 0.01$)) and adrenals of male ($p < 0.05$) rats in the 1000 ppm dose group. Relative organ weights (Table 7) for brain, spleen, testes and ovary of the mid and high dose groups were increased dose-dependently, relative to controls. The increases were statistically significant for brain, testes, and spleen (females only) from rats in the 300 and 1000 ppm dose groups. For spleens of males and ovaries, the increases were statistically significant for the high dose group only.

TABLE 6. GROUP MEAN ABSOLUTE ORGAN WEIGHTS (G)

Dose (ppm)	Males				Females			
	BR	Pit	H	Ad	BR	Pit	H	Ad
0	2.12	0.0152	1.46	0.0537	1.88	0.0159	0.87	0.0605
100	2.12	0.0143	1.51	0.0502	1.85	0.0143	0.87	0.0567
300	2.08	0.0137	1.40	0.0530	1.87	0.0156	0.83	0.0567
1000	1.93*	0.0132*	1.23	0.0455*	1.77**	0.0142	0.73**	0.0517

Data are adapted from Table 9 (p. 46), MRID No. 424503-01. Abbreviations are: BR (brain), Pit (pituitary),
H (heart), Ad (Adrenal)
*p<0.05, Williams' test
**p<0.01, Williams' test

TABLE 7. GROUP MEAN RELATIVE ORGAN WEIGHTS (G)

Dose (ppm)	Males				Females			
	Body Weight [a]	Brain	Spleen	Testes	Body Weight [a]	Brain	Spleen	Ovaries
0	497	43	16	95	241	78	20	2.9
100	514	42	16	95	243	76	22	2.8
300	443*	48*	18	107*	219**	86**	23*	3.1
1000	394**	49**	19**	116**	208**	86**	24**	3.3*

Data are adapted from Table 9 (p. 47), MRID No. 424503-01. Relative organ weights are presented as % x 100.
[a]Terminal body weight, these weights are slightly different than the values for week 13 presented in Table 2 of this report. The differences may be the result of blood sampling (see Discussion).
*p<0.05, Williams' test
**p<0.01, Williams' test

b. Gross pathology The hair loss (alopecia) noted during the study was, upon pathologic examination, confined to 1 control male and 5 females (100 ppm: 1; 300 ppm: 4). There were findings for stomach (white nodules near limiting ridge) and for liver (pale subcapsular areas near the median cleft, small or mottled lobes) and these are detailed in Table 8.

TABLE 8. MACROSCOPIC PATHOLOGY INCIDENCE SUMMARY

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	100	300	1000	0[a]	100	300	1000
LIVER Pale subcapsular area(s)-median cleft	0/10	1/10	0/10	0/10	1/9	1/10	2/10	1/10
Small lobe	0/10	0/10	0/10	0/10	0/9	0/10	1/10	1/10
Mottled lobe	0/10	0/10	0/10	0/10	0/9	0/10	1/10	1/10
STOMACH White nodule(s) near to limiting ridge	2/10	1/10	3/10	0/10	0/9	3/10	1/10	1/10

Data adapted from Table 10 (pp. 48-50), MRID No. 424503-01.
[a]Female rat No. 46 in the control group died during blood collection in week 13. The pathologic findings for this animal are not included
in this table.

c. Microscopic pathology

1) Non-neoplastic Histopathological examinations were performed on all tissues from animals in the control and high dose groups. For rats in the low and intermediate dose groups, the following tissues were examined: lungs, liver, kidneys and stomach. Macroscopically abnormal tissues from any animal were also examined. Microscopic pathology findings (Table 9) included centrilobular hepatocyte necrosis in the liver of 2 females (1 each in the 300 and 1000 ppm dose groups), hyperplasia of the non-glandular epithelium of the stomach (dose-dependent increase in treated females and in 1/10 males in the 1000 ppm group), and ectopic foci of non-glandular epithelium within the glandular mucosa of the stomach (dose-dependent increase for females).

2) Neoplastic None recorded.

TABLE 9. MICROSCOPIC PATHOLOGY INCIDENCE

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	100	300	1000	0[a]	100	300	1000
LIVER								
(1) Subcapsular area of finely vacuolated hepatocytes at the median cleft	0/10	0/10	0/10	0/10	1/10	1/10	2/10	1/10
(2) Centrilobular hepatocyte necrosis in one lobe	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10
STOMACH								
(1) Focus of ectopic non-glandular epithelium within the glandular mucosa	1/10	1/10	2/10	0/10	0/10	1/10	1/10	2/10
(2) Hyperplasia of the non-glandular epithelium near the limiting ridge	0/10	0/10	0/10	1/10	0/10	0/10	1/10	3/10

Data adapted from Table 11 (pp. 51-56), MRID No. 424503-01.
[a]Data for control group female No. 46 (died during blood collection in week 13) is included in table.

D. DISCUSSION

For animals treated with Ziram at 300 and 1000 ppm, there were changes in food consumption, body weights, body weight gains, clinical chemistry, histopathology and relative organ weights that were not generally found in controls or in animals treated with Ziram at 100 ppm. The NOAEL was chosen as 100 ppm due to the lack of significant toxicological findings in any category.

The LOAEL was chosen as 300 ppm. There were statistically significant decreases in body weight gain, food consumption and increases in relative organ weights for males and females treated with Ziram at 300 ppm that were generally not found in the control or low dose groups and were found in greater severity in the high dose group. For males and females, body weight gains ($p < 0.05$) and food consumption ($p < 0.01$) were statistically significantly decreased relative to controls. Although there were no statistically significant decreases in body weight, the percentage decreases were levels generally regarded as toxicologically significant, i.e., 9-12% and 8-11% for the 300 ppm males and females, respectively; 20-21% and 13-16% for the 1000 ppm males and females, respectively. For males treated with Ziram at 300 ppm, there were statistically significant ($p < 0.05$) increases in relative organ weights for brain and testes, relative to controls. For females treated with Ziram at 300 ppm, there were statistically significant increases in relative organ weights for brain ($p < 0.01$) and spleen ($p < 0.05$), relative to controls. The effect on brain weight was not considered to be a toxic effect on the brain

itself, but an effect related to decreased body weight resulting in decreases in absolute brain weight and increases in relative brain weight.

The statistically significant decreases in total protein and albumin levels and increases in AP levels for treated females may point to liver pathology since the values were at or below low normal values. The macroscopic and microscopic histopathology findings for liver included pale subcapsular areas, finely vacuolated hepatocytes, and centrilobular necrosis, primarily associated with 2 females (67 and 79). However, the individual clinical chemistry values for these animals were not substantially altered when compared to control values. The changes in clinical chemistry values are of uncertain toxicological significance as there were no associated histopathological changes.

It should be noted that the calculations of relative organ weights were based upon the terminal body weights (not the body weights at week 13, see Table 2 vs. Table 7 of this report). The terminal body weights were different from the weights at week 13 possibly due to variations caused by blood sampling. However, the body weights at termination were similar enough to the weights at week 13 that the relative organ weight changes noted in Table 7 of this report would retain statistical significance.

E. STUDY DEFICIENCIES

Assays for creatinine phosphokinase (CK) and serum lactate dehydrogenase (LDH) activity were not performed in the clinical chemistry evaluations. In the pathological exams, the lungs were not weighed and the oviduct was not examined. The inclusion of this data would add little information to the study report.

The diet for the 100 ppm dose group was prepared to contain 115 ppm to account for losses during preparation. The data supporting the decision to fortify the diet was not included in the study report.

The death of the control female during the periorbital bleeding procedure in week 13 was apparently accidental and likely due to ether toxicity.

The study authors used the Williams' test for statistical analysis of body weights, food consumption and organ weight data. One of the limitations of this statistical test is the requirement that the data over the dose groups be part of a trend, such as a dose-dependent decrease or increase. This requirement is not satisfied in the analysis of body weights, food consumption, clinical chemistry (male SGPT levels) or organ weights. In each of these instances, there is a value for either males or females (or both) in the 100 ppm dose group that is higher (in the case of a dose-dependent decrease) or lower (in the case of a dose-dependent increase) than the value for the respective control (0 ppm) group. In the case of the SGPT levels, the value for the 300 ppm dose group is higher than the values for the 100 and 1000 ppm dose groups. In addition, due to bias of this test to attempt to identify a dose-dependent trend, there can be cases where a statistically significant difference is not identified. One striking example of a discrepancy in statistical significance occurs in the comparison of Food Consumption data (statistical significance calculated by the study authors using

Williams' test) and data for Daily Food Consumption (statistical significance calculated by the reviewer). Daily Food Consumption is essentially Total Food Consumption divided by the number of days in the study period. The data for both calculations are drawn from Appendix 2, p. 61-61, MRID No. 424503-01. For females treated with Ziram at 300 ppm, food consumption is 87% of control (0 ppm), but this difference is not considered significant according to Williams' test. Daily food consumption, again comparing females treated with 300 ppm vs. controls, is statistically significantly different ($p < 0.01$), according to the *t*-test. It would be of value for the study authors to analyze their data with a statistical test other than Williams' test. to verify the level (*p* value) of statistical significance for all of the parameters measured.

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