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DATA EVALUATION REPORT

ZIRAM

Study Type: ONCOGENICITY FEEDING ☒ MOUSE (83-2)

Prepared for

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

Prepared by

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Task Order No. 94-43G

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Disclaimer

The final Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

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[ZIRAM]

Oncogenicity Study (83-2)

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\_\_\_\_\_ Date: \_\_\_\_\_

### DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity Feeding ☒ Mouse (83-2)

TOX. CHEM. NO.: 931

P.C.CODE.: 034805

MRID NO.: 433737-01

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

SYNONYMS: Bis(dimethylcarbamodithioato-S,S')zinc; zinc dimethyldithiocarbamate; methyl cymate; Methasan; Zimate; Zirberk; Karbam White; Corozate; Fuclasin; Zerlate

STUDY NUMBER: ZIR 12/932311

SPONSOR: Ziram Task Force (Consortium No. 62405), 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: Ziram (Technical) Potential oncogenicity to mice by repeated dietary administration for 80 weeks

AUTHORS: Lindsey A.J. Powell, Sarah M. Bottomley, David Crook, S.K. Majeed, C. Gopinath, William A. Gibson, Alan Anderson

REPORT ISSUED: August 19, 1994 (Study Completion Date)

EXECUTIVE SUMMARY: In a 80-week oncogenicity feeding study (MRID No. 433737-01), Ziram (98.7%, Lot No. 8331 AA) was administered in the diet to 50 male and 50 female CrI: CD-1 (ICR) BR mice per group at 0, 29, 75, 225, or 675 ppm. The doses corresponded to overall mean doses of about 0, 3, 9, 27, and 82 mg/kg/day for males; and to 0, 4, 11, 33, and 95 mg/kg/day for females.

Significantly decreased mean weight gain was seen in males at 225 ppm (77% of control) and at 675 ppm (56% of control). In females in the 225 ppm, weight gain was decreased to about 94% of the control group. The mean weight gain in females was significantly decreased at 675 ppm compared to control values (80% of control). Dose-related decreases in mean absolute brain weights were seen in both sexes, but, although numerically greater in females, were statistically significant only in males at 225 and 675 ppm. The incidence of centrilobular hepatocyte enlargement was increased in all treated animals. The incidence reached maximums of about 50% in males and 38% in females at 75 and 225 ppm then dropped at the high dose to 39% in males and 14% in females. These effects seem to indicate an adaptive response at all doses since there was no effect on liver weight, no dose-related effect on the gradation of the pathology (minimal at all doses), and no necrosis seen even at the high dose. Significant

increases in the incidences of urinary bladder epithelial cell hyperplasia were seen in males at 225 and 675 ppm (39 and 70%, respectively, in terminal animals compared to 18% in controls), and in females at 675 ppm (20% in terminal animals compared to 0 in controls). Urinary bladder epithelial hypertrophy was significantly increased in terminal females at 675 ppm (38% compared to 8% in controls).

**The NOAEL is 75 ppm. The LOAEL is 225 ppm based on decreased absolute brain weights in both sexes and significantly increased incidence of urinary bladder epithelial hyperplasia and decreased body weight gain in males.**

There were no treatment-related increases in tumor incidences.

**This study is Acceptable/Guideline and satisfies the guideline requirements for a 83-2 oncogenicity feeding study in mice.**

Special Review Criteria (40 CFR 154.7) None

#### A. MATERIALS

##### 1. Test material: Ziram

Description: white powder

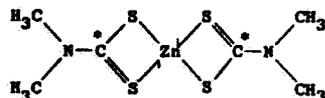
Lot/Batch No.: 8331 AA

Purity: 98.7 % a.i.

Stability of compound: Test compound was assayed at 6-month intervals; no deterioration was detected when stored in the dark at ambient temperatures.

CAS No.: 137-30-4

Structure:



\*Position of radiolabel

##### 2. Vehicle and/or positive control

Test material was mixed directly with feed; no positive control was included.

##### 3. Test animals

Species: Mouse

Strain: CrI: CD-1 (ICR) BR

Age and weight at study initiation: males and females were 42 days old; weights: males 24-34 g; females 18-27 g.

Source: Charles River Breeding Laboratories, Portage, Michigan.

Housing: Two mice of the same sex were housed in solid bottom polypropylene cages (13 cm wide, 33 cm deep, 15 cm high) with stainless steel wire tops and autoclaved sifted sawdust bedding.

Environmental conditions:

Temperature:  $21 \pm 2^\circ\text{C}$

Humidity:  $55 \pm 10\%$

Air changes: not provided

Photoperiod: 12 hours light/12 hours dark

Acclimation period: 14 days

B. STUDY DESIGN1. Animal assignment

Animals were randomly assigned to cages stratified by bodyweight to equalize the initial cage bodyweight means. The dose groups and descriptions are given in Table 1.

TABLE 1. STUDY DESIGN					
Dose Group	Doses (mg/kg/day)			No. Animals	
	Dietary Conc. Both Sexes (ppm)	Approximate Dosage Achieved mg/kg/day (Mean)		Male	Female
		Males	Females		
1	0	0	0	50	50
2	25 <sup>a</sup>	3	4	50	50
3	75 <sup>a</sup>	9	11	50	50
4	225	27	33	50	50
5	675	82	95	50	50

Data taken from MRID No. 433737-01, p. 17 and Table 5, p. 30.

<sup>a</sup>Prepared to contain 29 ppm.

<sup>b</sup>Prepared to contain 83 ppm.

Dose selection rationale: Doses were selected based on data from a 13-week preliminary study (ZIR 11/901841) that showed histopathological changes in the stomach at dietary levels of 900 and 2700 ppm Ziram. Effects on body weight were seen at 100, 300, 900, and 2700 ppm (MRID No.433737-01, Vol. 9, p. 1695).

2. Diet preparation and analysis

Diets were prepared weekly by mixing a weighed amount of test substance with a small amount of untreated basal diet in a Turbula mixer for 2 minutes to form a relatively concentrated pre-mix. This mixture was then diluted with an appropriate amount of food to achieve the desired concentrations and then mixed for 7 minutes in a double cone blender. The mixtures were divided into aliquots for daily use and stored at 4°C until immediately before feeding. The homogeneity of the Ziram-diet mixture at 25, 50, 70, and 5000 ppm was tested prior to the beginning of treatment. Samples of each dietary mixture were taken immediately after mixing (pre-dosing) and after storage for 6 days at 4°C and an additional 24 hours at room temperature (post-dosing) for weeks 1, 13, 26, 39, 52, 65, 73, and 78. Samples at week 73 were taken from only the 25 and 75 ppm groups because of a low analyzed concentration seen at week 65 for those groups. The pure compound was reassayed by the supplier at six month intervals.

**Results** ❖

- a. Homogeneity analysis ❖ The variation in the samples tested from the top to the bottom of the container ranged from -1.9% to +5.6% (coefficient of variation 2.8%) at 70 ppm, and from +4.6% to -3.0% (coefficient of variation 2.4%) at 5000 ppm (MRID 433737-01, p. 1646). Samples that were taken from 25 ppm and 50 ppm mixtures to reflect the low concentrations utilized in this study had coefficients of variation of 7.01 and 2.21, respectively (MRID 433737-01, Table 3, p. 1592).

- b. Stability analysis ❖ Ziram losses of 23% at 25 ppm, and 13% at 50 ppm occurred under the experimental conditions of this study (storage of the dietary mixture for 6 days at 4°C plus 24 hours at room temperature) (MRID 433737-01, Table 4, p. 1593). In agreement with the sponsor, it was decided to increase the concentration of the 25 ppm mixture to 29 ppm and the 75 ppm mixture to 83 ppm to compensate for the loss and approximate the mean target concentration.
- c. Concentration analysis ❖ The pre-dose samples from all dose levels were within +16 to -12% of the target dose with only 3 samples varying greater than  $\pm 10\%$ . The post-dosing samples varied from +5 to -17% with the exception of one sample taken at the end of week 65 from the 25 ppm mixture which was 48% below the target dose. This result prompted a resampling of the 25 and 75 ppm mixtures at week 73. All but 5 of the post-dosing samples were within  $\pm 10\%$  of the target concentration (MRID 433737-01, Table 1 pp.1584-1587).

### 3. Diet

Animals received food (ground SDS Rat and Mouse No.1 modified maintenance diet) and water *ad libitum*. Animals were given a fresh aliquot of food each day.

### 4. Statistics

Statistical tests and procedures are included in Appendix 1. Statistical significance was listed as  $p \leq 0.05$ .

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

## C. METHODS AND RESULTS

### 1. Observations

Animals were inspected in early morning and afternoon on weekdays and early morning and mid-day on weekends and holidays for signs of toxicity and mortality. Comprehensive clinical examinations were carried out once daily for the first 4 weeks of the study and weekly thereafter.

**Results** ❖ The distribution of premature deaths through the 80-week treatment period is shown in Table 2. One male died at 75 ppm and one female died at 25 ppm during the post treatment terminal kill period. No statistically significant differences in mortality were seen and there was no indication of a treatment-related effect on the incidence or distribution of decedents. No statistically significant trends were seen.

Treatment Period (Weeks)	Males, Dose (ppm)					Females, Dose (ppm)				
	0 n=50	25 n=50	75 n=50	225 n=50	675 n=50	0 n=50	25 n=50	75 n=50	225 n=50	675 n=50
0-12	0	0	0	0	0	4	1	0	0	1
13-52	1	3	1	1	6	3	1	4	2	1
53-80	10	6	2	4	4	1	3	4	3	2

83-80	6	7	5	7	7	4	9	2	5	11
Total	16	16	8	12	17	12	14	10	10	15
% Survival	63	63	84	75	66	76	72	80	80	70

Data taken from NCIID No. 433737-01, Volume I, p. 27, and Table I, p. 40.

2. Body weight

Animals were weighed at the time of group assignment, on the day treatment began, once each week thereafter and on the day the animal died or was killed.

**Results** ❖ The group mean body weights at selected times through the 80-week study are given in Table 3. Graphic illustrations of body weight differences are included in Appendix 2. Males were more affected than females with significantly decreased weight gain of 23 and 44% less than the controls at 225 and 675 ppm, respectively. Males also showed a slight decrease of about 8% in overall weight gain at 75 ppm, but the decrease was not statistically significant. The body weights of all treated males were slightly lower than the control body weights from week 14 through week 80. The body weights of the high dose males ranged from about 79 to 83% of the control body weights. There was more variation in mean weight gain in females. Increases in mean weight gain of 16 and 21% were seen at 25 and 75 ppm, respectively. However, increased intragroup variation (standard deviations of 5.28 and 6.23, for 25 and 75 ppm, respectively) prevented the increases from reaching statistical significance. Decreases in mean weight gain of about 6 and 20% were seen in females at 225 and 675 ppm, respectively. A decrease in weight gain of about 22% was seen at 225 ppm compared to the mean weight at 75 ppm; however, compared to the controls, the decreased weight gain reached statistical significance only at the high dose in females. The body weights of the high dose female mice ranged from about 89 to 92% of the control body weights over the last 8 weeks of the study; whereas the body weights of the 75 ppm group were slightly but consistently higher ranging from about 105 to 109% of the control body weights.

Treatment length (Weeks)	Males, Dose (ppm)					Females, Dose (ppm)				
	0	25	75	225	675	0	25	75	225	675
0	28	28	28	28	28	21	22	22	22	22
12	30	30	30	30	34	28 (48)*	28 (49)	28	27	27 (49)
24	44	42	43	39 (49)	36 (45)	31 (46)	32 (49)	32	29	29 (49)
36	47 (49)	45	44	41 (49)	38 (45)	33 (45)	34 (49)	34	32	31 (48)
52	45 (49)	46 (47)	46 (49)	42 (49)	39 (44)	34 (49)	36 (48)	36 (46)	33 (48)	32 (48)
68	45 (53)	46 (41)	45 (47)	42 (45)	39 (40)	35 (42)	37 (45)	38 (42)	34 (45)	33 (46)
80	45 (34)	47 (34)	46 (42)	43 (32)	39 (33)	36 (33)	38 (36)	39 (40)	35 (40)	33 (35)
Mean weight gain (g) <sup>†</sup>	19.7	19.0	18.2	15.1**	11.0**	14.1	16.4	17.0	13.2	11.5*

Data taken from NCIID No. 433737-01, Volume I, Table 2, p. 41-42.

<sup>†</sup>Prepared to contain 25 ppm.

<sup>†</sup>Prepared to contain 83 ppm.

<sup>†</sup>100 animals if less than 50.

<sup>†</sup>Weight gains taken from summary table on p. 28. Weight gain statistics were performed by study authors.

\*p<0.05; \*\*p<0.01 significantly different from controls.

### 3. Food consumption and compound intake

Food consumption for each cage of animals was determined daily and the mean food consumption for each surviving mouse was calculated weekly using:  $\text{g food/mouse/week} = (\text{g total food given} - \text{g total food left} \div \text{number of animal days}) \times 7$ . The weekly food consumption values for individual experimental groups were not calculated from the individual cage means, but were calculated from the actual food consumption data according to:  $\text{group mean food consumption (g/mouse/week)} = (\text{total g food given to group} - \text{total g food left by group} \div \text{number of animal days for the group}) \times 7$ .

The achieved intake of test substance was calculated for each group weekly utilizing the group mean body weight (mid-week) and group mean food consumption and the nominal concentration of test substance in the dietary mixture (ppm).

The authors calculated food conversion ratios (food consumed  $\div$  body weight gained) using weekly food intake and body weight measurements from week 1 to 28. The reviewer calculated overall food efficiencies (weight gain  $\times$  100  $\div$  food intake) for the study utilizing the total food intake and the body weight gain from week 1-80 for each group.

#### **Results** ❖

- a. Food consumption ❖ The mean food consumption per mouse per week at various weeks through the study is given in Table 4. A trend toward decreased food intake in the treated groups can be seen throughout the experiment resulting in a dose-related decrease in total food intake, significant ( $p \leq 0.01$ ) in males at doses  $\geq 75$  ppm, and in females at all dose levels. The maximum decreases in food consumption were 23% and 30% for males and females, respectively, at 675 ppm Ziram compared to the control animals. The food consumption in females was decreased by about 26% at 225 ppm compared to the control animals.

TABLE 4. GROUP MEAN FOOD CONSUMPTION (g/ANIMAL/WEEK) IN MICE FED ZIRAM FOR 80 WEEKS										
Week of Study	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0	25	75	225	675	0	25	75	225	675
1*	41	41	42	41	38	41	39	39	40	37**
12	40	37	36	33	30	42	37	33	30	29
24	43	40	38	37	33	44	39	37	33	30
36	39	39	36	31	32	40	37	34	32	30
52	41	42	39	33	32	43	38	35	31	29
68	44	39	35	32	32	43	38	36	32	30
80	46	38	34	33	32	43	36	36	33	30
Total food consumed (week 1-80) <sup>†</sup>	3287	3117 (96)	2886** (83)	2631** (81)	2517** (77)	3556	2828** (87)	2754** (82)	2499** (74)	2341** (70)
Food conversion ratios (week 1-28) <sup>‡</sup>	64.9	71.8	63.1	63.7	102.6	118.3	92.8	83.6	101.7	102.6
Food efficiency (week 1-80) <sup>§</sup>	0.616	0.610	0.631	0.574	0.457	0.420	0.561	0.617	0.523	0.483

Data taken from NRID No. 433737-01, Table 3, pp. 44-46, and the summary table on p. 28.

<sup>\*</sup>Prepared to contain 25 ppm.

<sup>†</sup>Prepared to contain 83 ppm.

<sup>‡</sup>Taken from summary table on p. 28. Statistics on these values were performed by study authors.

<sup>§</sup>Food consumed (g) ÷ body weight gained (g). Taken from NRID No. 433737-01, Table 4, p. 47.

<sup>||</sup>Calculated by the reviewer from information in summary tables in NRID No. 433737-01, pp. 28-29.

<sup>\*\*</sup>p ≤ 0.05; <sup>\*\*\*</sup>p ≤ 0.01 significantly different from controls.

- b. Compound consumption (time-weighted average) ❖ The overall compound consumption for weeks 1-80 calculated from the food consumption and body weights is given in Table 1. The study authors stressed that these values were calculated from the nominal dosages and not from the adjusted levels in the 25 and 75 ppm groups. The achieved group mean dosages were calculated each week throughout the experiment. The factor of 3 difference between groups was well maintained during the study.
- c. Food efficiency ❖ The food conversion ratios (food consumed ÷ body weight gained) calculated by the study authors for weeks 1-28 are inversely proportional to food efficiency values. These values generally indicate a slight decrease in food utilization in treated males up to week 80, and, although variable, the results indicate a slight increase in food utilization in females through this time period. The food efficiencies for the entire experiment calculated by the reviewer showed an increase in food efficiency of about 5% compared to the control animals in males at 75 ppm Ziram. However, decreases of 5 and 28% were seen at 225 and 675 ppm, respectively. An increase in food efficiency of about 47% was seen in females at 75 ppm Ziram. The food efficiency at the high dose decreased compared with the 225 ppm dose, but was still 15% greater than the control value. The food conversion ratios and food efficiency values are summarized in Table 4.
4. Ophthalmoscopic examination
- An ophthalmoscopic examination is not required for a 83-2 oncogenicity study.
5. Venous blood was collected and blood smears were prepared from 10 males and 10 females in each group at week 52 and immediately before termination of the study. Blood smears were also prepared from all animals killed during the study whenever possible. The smears from all mice killed during the study and from 10 males and 10 females from the control

and high dose groups at both 52 weeks and study termination were stained with modified Wright's stain, and differential white cell counts were performed. The presence and description of abnormal cells was tabulated independently. The remaining smears were retained to count if any treatment-related changes were seen at the high dose. The CHECKED (X) parameters were examined.

a. Hematology

<u>X</u>	Hematocrit(HCT)	<u>X</u>	Leukocyte differential
	Hemoglobin (HGB)	X	Mean corpuscular HGB
X	Leukocyte count (WBC)		Mean corpusc. HGB
	Erythrocyte count (RBC)		Mean corpusc. volume
	Platelet count		Reticulocyte count
	Blood clotting measurements		

\* Required for oncogenicity studies in mice.

**Results** - Results of the differential counts are summarized in Table 5. Although there were some counts that were significantly different from the controls, all counts were within the range normally seen for mice of the age examined, and the differences were not biologically significant (see Charles River Technical Bulletin Summer 1986, Baseline Hematology and Clinical Chemistry Values as a Function of Sex and Age for Outbred Mice).

b. Clinical chemistry

Clinical chemistry values are not required for a 83-2 oncogenicity study.

TABLE 5. DIFFERENTIAL LEUKOCYTE COUNT										
Dose/ Treatme nt period	Percent of Total Count									
	Cell Type, Males					Cell Type, Females				
	Neut	Lym p	Eosi n	Baso	Mono	Neut	Lym p	Eosi n	Baso	Mono
Control 52 Week	30	69	1	0	0	22	75	1	0	1
675 ppm 52 Week	26	74	0	0	0	27	72	1	0	0
Control Terminal	34	64	2	0	0	44	54	2	0	1
675 ppm Terminal	40	58	2	0	1	34	64	2	0	0

Data taken from MRID No. 433737-01, Table 6, p. 51.

\* p<.05

#### 6. Urinalysis

Urinalysis is not required for an 83-2 oncogenesis study.

#### 7. Sacrifice and pathology

Following 80 weeks of treatment, all surviving animals were killed by carbon dioxide asphyxiation, weighed and subjected to gross and microscopic necropsy procedures. All animals that died or were killed prematurely during the experiment were also subjected to gross and microscopic examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed. The tissues, with the exception of the eyes, were preserved for examination in 10% formalin solution. The eyes were preserved in Davidson's fixative.

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

\* Required for oncogenicity studies in mice.

+ Organ weight required for oncogenicity studies in mice.

<sup>a</sup> The entire head was preserved which included the nasal cavity, paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, lacrimal gland, and Zymbal's gland.

### Results ❖❖

- a. Organ weight ❖❖ The absolute brain weight was decreased in both males and females with increasing dose of Ziram (4.4%, males; 9.1%, females at 675 ppm). The relative brain weight was significantly increased in males at 225 and 675 ppm (9.5 and 19.0%, respectively), but was not significantly changed in females with increasing dose. The study authors performed a covariate analysis that adjusted the brain weight for the final body weight and found the adjusted brain weight was significantly decreased at 225 and 675 ppm in males (data for males was not presented) and at 75, 225, and 675 ppm in females (96.2, 93.5, and 91.5% of the control, respectively). The relative weights of the kidneys at 675 ppm and of the testes/epididymides at 225 and 675 ppm of males were significantly increased (12.2 and 18.6%, respectively at 675 ppm compared to controls). The absolute and relative organ weights are listed in Table 6.

**TABLE 6. ABSOLUTE AND RELATIVE ORGAN WEIGHTS (mg) IN MICE TREATED WITH ZIRAM FOR 80 WEEKS**

Organ or Tissue	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0	25	75	225	675	0	25	75	225	675
Liver	2700 (589) <sup>†</sup>	2540 (560)	2500 (558)	2250 (541)	2020 (537)	1780 (525)	1750 (487)	1850 (500)	1720 (523)	1700 (553)
Kidneys	757 (165)	819 (181)	778 (174)	736 (180)	743 (199) <sup>~</sup>	511 (156)	471 (132)	485 (132)	475 (145)	460 (150)
Testes + Epidids.	336 (74.0)	336 (74.5)	326 (72.8)	341 (83.1) <sup>~</sup>	324 (87.8) <sup>~</sup>	---	---	---	---	---
Brain	479 (105)	483 (108)	473 (106)	468 <sup>~</sup> (115)	458 <sup>~</sup> (125)	505 (151)	497 (140)	488 (134)	471 (145)	459 (150)
Body Wt. (g)	46.2	45.7	45.2	41.6	37.5	34.1	36.5	37.4	32.9	31.1

Data taken from MRID No. 433737-01, Tables 7 and 8, p. 52-54.

<sup>†</sup>(Relative weight)

\* p<0.05; \*\* p<0.01 significantly different from controls.

- b. Gross pathology ❧ The incidences of selected gross lesions that changed with Ziram treatment are summarized in Table 7. Decreases in the amount of adipose tissue in decedents were seen at 675 ppm in 65% of the males and in 60% of the females compared to decedent controls (31% of males; 33% of females). However, the incidence of animals with decreased adipose tissue was not increased in animals killed at study termination. The incidences of roughened and white forestomach were increased in females at 675 ppm compared to controls (14.3% compared to 2.6% in controls for roughened stomach, 20.0% compared to 7.9% in controls for white stomach), but not in males at study termination. Irregular cortical scarring of the kidneys was seen in 42.4% of males at 675 ppm compared to 14.7% in controls at study termination. The incidence of brown discoloration of the kidneys was also slightly increased (9.1%) at 675 ppm compared to controls (0) in males. Lung petechiae were seen in 32.4% of control males and in 7.9% of females at study termination, but were not seen in any animals following 80 weeks of treatment at 675 ppm. Lung congestion was seen in all groups of animals except for high dose terminal males in a non dose-related fashion.

TABLE 7. NUMBER OF MICE FED ZIRAM IN THEIR DIET FOR 80 WEEKS WITH GROSS LESIONS										
Affected Organ or Tissue/ Gross Lesion	Treatment Group/Exposure Level (ppm)									
	Males					Females				
	0 (34/1 6) <sup>a</sup>	25 (34/ 16)	75 (41 /9)	225 (38/ 12)	675 (33/ 17)	0 (38/1 2)	25 (35/ 15)	75 (40/ 10)	225 (40/1 0)	675 (35/1 5)
Lungs/ Congested	4 <sup>b</sup> (4)	6 (7)	6 (3)	3 (5)	0 (3)	3 (3)	3 (3)	8 (2)	4 (2)	2 (4)
Lungs/ Petechiae	11 (2)	0 <sup>c</sup> (0)	2 <sup>c</sup> (0)	0 <sup>c</sup> (0)	0 <sup>c</sup> (0)	3 (0)	5 (1)	4 (0)	1 (0)	0 (1)
Adipose Tissue/ Decreased	4 (5)	3 (4)	4 (5)	3 (4)	5 (11)	13 (4)	7 (4)	7 (4)	9 (4)	8 (9)
Fore Stomach/ Roughened	2 (3)	1 (1)	3 (0)	3 (1)	2 (0)	1 (1)	2 (1)	2 (1)	3 (2)	5 (1)
Fore Stomach/ White	1 (1)	0 (3)	2 (1)	1 (4)	1 (1)	3 (2)	3 (3)	2 (2)	4 (2)	7 (3)
Kidneys/ Irregular Cortical Scarring	5 (0)	7 (0)	5 (0)	3 (3)	14 <sup>c</sup> (0)	6 (0)	1 (1)	0 <sup>c</sup> (1)	3 (0)	3 (0)
Kidneys/ Brown Coloration	0 (0)	1 (0)	1 (0)	1 (0)	3 (2)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)

Data taken from MRID No. 433737-01, Table 9, pp. 55-63.

<sup>a</sup>(No. animals at termination of study/no. animals that died or were killed during the study)

<sup>b</sup>Number of animals with the lesion at termination of study (number of animals with lesion in precedents)

\* p < 0.05 significantly different from controls.

### c. Microscopic pathology ❖

- 1) Non-neoplastic ❖ A summary of findings obtained by microscopic examination of tissues and organs from treated animals and control animals in the study is given in Table 8. Significant hepatocyte enlargement occurred in all treated groups especially in the animals that completed the study. However, the incidence of hepatocyte enlargement tended to peak in the middle dose groups and decrease at the high dose. Also, most incidences of hepatocyte enlargement were graded as minimal throughout all dose groups. These effects are most likely due to an adaptive response by the liver to the test substance. Urinary bladder epithelial hyperplasia increased in a dose-related manner from total control incidences of 14.0% in males and 0 in females to incidences of 62.0% in males and 28.6% in females at the high dose. The increased incidence of urinary bladder epithelial hyperplasia was not as great in females as in males; however, the overall incidence of bladder epithelial hypertrophy increased from 12.5% in the controls to 28.6% at the high dose in females compared to no increase in males. Lung congestion was seen in both sexes in a non-dose-related pattern similar to that seen in the gross necropsy. No dose-related microscopic pathologies were seen in the stomach or kidneys that would correspond to the changes observed on gross examination.

TABLE 8. NUMBER OF MICE FED ZIRAM IN THEIR DIET FOR 80 WEEKS WITH NON-NEOPLASTIC LESIONS

Affected Organ or Tissue/ Lesion	Treatment Group Exposure Level (ppm)									
	Males					Females				
	0 (34/1 6) <sup>a</sup>	25 (34/1 6)	75 (41/9 )	225 (38/1 2)	675 (33/1 7)	0 (38/1 2)	25 (35/1 5)	75 (40/1 0)	225 (40/1 0)	675 (35/1 5)
Liver/ Centrilobular hepatocyte enlargement	1 (1) <sup>b</sup>	16 <sup>-</sup> (5)	20 <sup>-</sup> (1)	19 <sup>-</sup> (6)	13 <sup>-</sup> (6)	0 (0)	10 <sup>-</sup> (2)	15 <sup>-</sup> (4)	15 <sup>-</sup> (1)	5 <sup>-</sup> (8)
Liver/ Centrilobular hepatocyte enlargement (Total) <sup>c</sup>	2	21 <sup>-</sup>	21 <sup>-</sup>	25 <sup>-</sup>	19 <sup>-</sup>	0	12 <sup>-</sup>	19 <sup>-</sup>	16 <sup>-</sup>	13 <sup>-</sup>
Liver/ Centrilobular hepatocyte vacuolation	29 (2)	4 <sup>-</sup> (1)	1 <sup>-</sup> (0)	0 <sup>-</sup> (1)	2 <sup>-</sup> (0)	20 (1)	10 <sup>+</sup> (1)	4 <sup>-</sup> (0)	5 <sup>-</sup> (0)	0 <sup>-</sup> (0)
Liver/ Centrilobular hepatocyte enlargement and vacuolation	0 (0)	7 <sup>-</sup> (0)	9 <sup>-</sup> (0)	5 <sup>-</sup> (0)	5 <sup>-</sup> (1)	0 (0)	0 (0)	2 (0)	1 <sup>+</sup> (0)	1 (0)
Liver/ Centrilobular hepatocyte enlargement and vacuolation (Total) <sup>c</sup>	0	7 <sup>-</sup>	9 <sup>-</sup>	5 <sup>-</sup>	5 <sup>-</sup>	0	0	2	1 <sup>+</sup>	1
Liver/ Generalized enlargement	1 (0)	5 (4)	9 <sup>+</sup> (3)	13 <sup>-</sup> (2)	1 <sup>-</sup> (5)	0 (1)	4 (6)	7 (3)	5 <sup>+</sup> (2)	5 (2)
Liver/ Generalized enlargement (Total) <sup>c</sup>	1	9 <sup>-</sup>	12 <sup>-</sup>	15 <sup>-</sup>	6	1	10 <sup>-</sup>	10 <sup>-</sup>	7 <sup>+</sup>	7
Urinary bladder/ Epithelial hyperplasia	6 (1)	5 (2)	5 <sup>+</sup> (4)	15 <sup>+</sup> (5)	23 <sup>-</sup> (8)	0 <sup>+</sup> (0)	3 (2)	1 <sup>+</sup> (0)	3 <sup>+</sup> (2)	7 <sup>+</sup> (7)
Urinary bladder/ Epithelial hyperplasia (Total) <sup>c</sup>	7	7	9 <sup>+</sup>	20 <sup>-</sup>	31 <sup>-</sup>	0 <sup>+</sup>	5 <sup>-</sup>	1 <sup>+</sup>	5 <sup>+</sup>	14 <sup>+</sup>
Urinary bladder/ Epithelial hypertrophy	5 (2)	0 (2)	0 <sup>+</sup> (1)	0 (1)	2 (1)	3 <sup>+</sup> (3)	0 (0)	0 <sup>+</sup> (2)	0 <sup>+</sup> (0)	13 <sup>-</sup> (1)
Lungs/ Congestion	3 (5)	10 <sup>+</sup> (8)	7 (1)	7 (7)	3 (3)	8 (6)	9 (4)	15 (4)	9 <sup>+</sup> (2)	5 (5)

Data taken from MRID No. 433737-01, Table 10, pp. 68-108.

<sup>a</sup>(No. animals at termination of study/no. animals that died or were killed during the study)

<sup>b</sup>Incidence in animals at termination of study (Incidence in precedents)

<sup>c</sup>Taken from MRID No. 433737-01, summary tables on p. 33; statistics performed by study authors.

<sup>d</sup>Only 37 animals were examined for this lesion at the termination of the study.

<sup>e</sup>Only 39 animals were examined for this lesion at the termination of the study.

<sup>f</sup>Only 36 animals were examined for this lesion at the termination of the study.

<sup>g</sup>Only 38 animals were examined for this lesion at the termination of the study.

<sup>h</sup>Only 34 animals were examined for this lesion at the termination of the study.

<sup>i</sup>Only 33 animals were examined for this lesion at the termination of the study

\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 significantly different from controls. Fisher exact test performed by reviewer unless stated otherwise.

- 2) Neoplastic  There were no treatment-related increases in tumors, benign or malignant seen in the study. All tumor incidences were within the expected tumor incidences of laboratory-maintained mice of this strain. The most common neoplastic

lesions were lymphoid and pulmonary tumors in both sexes and liver tumors in male mice. The number of mice with tumors most commonly seen in this study are given in Table 9.

TABLE 9. NUMBER OF MICE FED ZIRAM IN THEIR DIET FOR 80 WEEKS WITH NEOPLASTIC LESIONS										
Affected Organ or Tissue/ Lesion	Treatment Group Exposure Level (ppm)									
	Males <sup>a</sup>					Females <sup>a</sup>				
	0 (34/1 6) <sup>b</sup>	25 (34/ 16)	75 (41 /9)	225 (38/ 12)	675 (33/ 17)	0 (38/1 2)	25 (35/1 5)	75 (40/ 10)	225 (40/1 0)	675 (35/1 5)
Liver/ Adenoma	8 (1) <sup>c</sup>	5 (1)	6 (1)	2 (0)	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Liver/ Carcinoma	3 (4)	1 (0)	3 (0)	2 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lymphatics/ Multicentric tumors, total	0 (0)	0 (1)	1 (1)	2 (1)	0 (2)	3 (3)	1 (2)	2 (1)	2 (5)	0 (2)
Lungs/ Adenoma	8 (1)	6 (1)	11 (0)	7 (1)	6 (3)	6 (0)	5 (1)	6 (3)	6 (1)	6 (2)
Lungs/ Adenocarcinoma + sarcoma	3 (4)	0 (1)	2 (0)	0 (0)	5 (0)	2 (0)	0 (0)	1 (1)	1 (1)	2 (1)

<sup>a</sup>Data taken from MRID No. 433737-01, Appendix 8, Vol. 3-5, pp. 324-936.

<sup>b</sup>Data taken from MRID No. 433737-01, Table 10, pp. 65-67.

<sup>c</sup>(No. animals at termination of study/no. animals that died or were killed during the study)

<sup>d</sup>Incidence in animals at termination of study (Incidence in precedents)

#### D. DISCUSSION

Groups of 50 male and 50 female Crl: CD-1 (ICR) BR mice were fed diets containing 0, 25, 75, 225, or 675 ppm Ziram for 80 weeks. These doses correspond to mean achieved intakes of 0, 3, 9, 27, and 82 mg/kg/day for males, and 0, 4, 11, 33, and 95 mg/kg/day for females. Ziram was found to be unstable when mixed with food especially at the lower concentrations. The initial weekly preparations of 25 and 75 ppm Ziram in food were prepared to contain 29 and 83 ppm Ziram to adjust for the unavoidable loss of the test chemical during the feeding period. The average dose was shown to be within  $\pm 10\%$  of the target dose for all but 5 samples at 25 and 75 ppm and all but 3 samples at the higher doses.

No significant differences were seen in the distribution of premature deaths among the controls and various dose groups. The highest survival rates (males, 84 and 76% at 75 and 225 ppm, respectively; females, 80% at 75 and 225 ppm) were seen in the middle dose groups in both sexes. Decreased weight gain in males was dose dependent and reached statistical significance at 225 and 675 ppm (77 and 56% of control weight gain, respectively). The body weight gain in females was much less affected and significant only at the high dose (80% of control weight gain). The mean body weight of females in the treated groups increased at 25 and 75 ppm and decreased at the higher doses, but never varied more than  $\pm 11\%$  from the control body weights. However, the mean body weight of the 675 ppm dose group was 15.4% lower than the 75 ppm group. The overall food efficiency increased to about 105% and 147% of control levels in males and females, respectively at 75 ppm then decreased at 225 and 675 ppm. The food efficiency

in females remained slightly above control levels, however in males it decreased to about 73% of control levels at the high dose. There seemed to be no correlation between food consumption and body weight in females.

Significant changes in organ weights were seen in males including increases in the relative weights of kidneys (121% of control at 675 ppm), testes + epididymides (112 and 119% at 225 and 675 ppm, respectively), and brain (110 and 119% at 225 and 675 ppm, respectively). The absolute brain weights were slightly, but significantly decreased at 225 and 675 ppm compared to controls (98 and 96% of controls, respectively). The mean absolute brain weights of females were also decreased, but did not reach statistical significance (96.6, 93.3, and 90.9% of controls at 75, 225, and 675 ppm respectively). Covariant analysis of female brain weights correcting for terminal body weights also indicated slight, but significant, decreases in brain weight (96.2, 93.5, and 91.5% of control brain weight at 75, 225 and 675 ppm, respectively). Although the adjusted brain weights of terminal females receiving 75 ppm Ziram were slightly decreased, there were no increases in microscopic lesions found associated with the decreased brain weight. The significance of the adjusted brain weight at 75 ppm would seem more likely to reflect the varying body weight changes than the decreased brain weight. At higher doses the mean female body weights were less than the control level, and the mean absolute brain weight was decreased compared to controls. There were no significant additional organ weight changes in females. It would seem that the relative organ weight changes can largely be explained on the basis of decreased body weight seen especially in males at 225 and 675 ppm Ziram.

Gross pathological examination of tissues and organs found random lung congestion with a decrease in lung petechiae, especially in treated males; a slight decrease in adipose tissue in females; increases in roughened and white appearance of the stomach in females at the high dose; and increases in irregular cortical scarring ( $p < 0.05$ ) and brown coloration of the kidneys in males. Only the cortical scarring of the kidneys in high dose males reached statistical significance, and no microscopic lesions were found in the stomachs or kidneys of the test animals. Microscopic examination revealed dose-dependent urinary bladder epithelial cell hyperplasia in both sexes. This lesion was found in 62.0% of high dose males in the study compared with 14.0% in the controls, and in 28.0% of high dose females compared to 0 in the controls. An increase in the incidence of urinary bladder epithelial hypertrophy was seen in terminal females at the high dose (38% compared to 8% in controls), but was not seen in males or in decedents. Significantly increased incidences of centrilobular hepatocyte enlargement were seen in livers of all treated groups. The incidences in terminal animals increased in a dose-related manner from about 3% in controls to about 50% at 75 and 225 ppm in males, and from 0 in female controls to about 38% at 75 and 225 ppm. The incidences decreased to about 39 and 14% at 675 ppm in terminal males and females, respectively, compared to the 75 and 225 ppm groups. Incidences of centrilobular hepatocyte vacuolation significantly decreased from control levels of 85 and 53% in terminal males and females, respectively, to 2.4 and 10% for terminal males and females at 75 ppm, 0 and 12.5% for males and females at 225 ppm, and 6 and 0% at 675 ppm. The incidences of generalized liver enlargement increased from about 2% in the total male and female controls to 30% at 225 ppm in males and 20% at 25 and 75 ppm in females. The incidence dropped to 12% in high dose males and to 14% at 225 and 675 ppm in females. The incidences of hepatocyte and general liver enlargement tended to increase in a dose-related manner from 0 to 75 ppm, were constant or decreased at 225 ppm, and decreased at 675 ppm especially in animals completing the study. These effects seem to indicate an adaptive response at all doses since there was no effect on liver weight, no dose-related effect on the gradation of the pathology (minimal at all doses), and no necrosis seen even at the high dose.

A No-Observed-Adverse-Effect-Level (NOAEL) of 75 ppm (9 mg/kg/day for males, 11 mg/kg/day for females) was identified, and a Lowest-Observed-Adverse-Effect-Level (LOAEL) of 225 ppm (27 mg/kg/day for males, 33 mg/kg/day for females) was determined

based on decreased weight gain, urinary bladder epithelial hyperplasia, and decreased brain weight in females and decreased brain weight and body weight gain in males.

The most common neoplastic lesions were pulmonary and lymphatic lesions in both sexes and liver tumors in male mice. All tumor incidences were within the expected tumor incidences of laboratory-maintained CD-1 mice. No treatment-related increases in benign or malignant tumors were seen in the study.

#### E. STUDY DEFICIENCIES

In general, this study was well organized with clear and concise summary tables. However, the neoplastic summary for male mice was missing. The data for this report were obtained from the pathology reports on individual mice. Covariate analysis of brain weights adjusting for terminal body weights for male mice was discussed in the results section of the report, but the data were not included in Table 7. This is confusing since the authors based the NOAEL for females on the adjusted brain weight. All the relevant data were not analyzed statistically.

These deficiencies did not significantly affected the overall conclusions or validity of the study.

[ZIRAM]

Oncogenicity Study (83-2)

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DP Barcode:  
HED DOC Number:  
Toxicology Branch:

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