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DATA EVALUATION REPORT

ZIRAM

STUDY TYPE: REPEATED DOSE DERMAL ▒ RABBIT (82-2)

8/2/2000

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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ZIRAM Repeated Dose Dermal Study (OPP 82-2; OPPTS 870.3200)

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H._____Date:_____
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Reregistration Branch I, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 21-Day Dermal ▒ Rabbit
OPPTS 870.3200 [§82-2]

DP BARCODE: D235025
P.C.CODE.: 034805

SUBMISSION CODE: S521512
TOX. CHEM, NO: 931

TEST MATERIAL (PURITY): Ziram Technical (98.5%)

SYNONYMS: Zinc dimethyldithiocarbamate

CITATION: Edwards, J., P. Baldrick, W. Gibson, et al. (1989) Twenty-one day dermal toxicity study in rabbits with Ziram. Huntingdon Research Centre, Ltd., P. O. Box 2, Huntingdon, Cambridgeshire, PE18, 6ES, ENGLAND. Study No. ZIR 4/89689, November 2, 1989. MRID 41297001. Unpublished.

SPONSOR: Ziram Task Force, Consortium No. 62405, c/o UCB Chemicals Corporation, 5365-A Robin Hood Road, Norfolk, Virginia 23513.

EXECUTIVE SUMMARY: In a 21-day repeated dose dermal toxicity study (MRID 41297001), groups of 5 male and 5 female New Zealand white rabbits were treated with Ziram Technical (98.5%) in distilled water by dermal occlusion at doses of 0, 100, 300, or 1000 mg/kg/day for 6 hours/day for 21 days.

No mortality was observed, and there were no treatment-related dermal lesions. There were also no effects on organ weights, macroscopic pathology, or histopathology. Decreased bodyweight ($p<0.05$; 9-13%) was observed in high-dose females all three weeks of the study. Decreased food consumption ($p<0.05$; 34%) was observed in high-dose females during the first week of the study. Decreased lymphocyte counts ($p<0.05$) were observed in high-dose females; however, this effect is not considered toxicologically significant. Changes in clinical pathology parameters [increased GPT (ALT), GOT (AST), bilirubin and cholesterol] in the high-dose females indicated minimal hepatotoxicity.

Under the conditions of this study, the NOAEL for systemic toxicity in females for Ziram Technical was 300 mg/kg/day. The LOAEL for systemic effects was 1000 mg/kg/day based on decreased body weight and food consumption and clinical chemistry changes suggestive of minimal hepatotoxicity (increases in GPT, GOT, bilirubin and cholesterol). The NOAEL for males is greater than 1000 mg/kg/day; the LOAEL was not identified. The NOAEL for dermal effects in both sexes was equal to or greater than 1000 mg/kg/day; the LOAEL was not identified.

This study is classified as **acceptable (guideline)** and satisfies the guideline requirements for a 21-day dermal study (82-2) in rabbits.

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Statements were present.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Technical grade Ziram

Description: creamy white powder
Batch No.: 8331 AA
Purity: 98.5 %
Stability of compound: ▒stable▒
CAS No.: 137-30-4

2. Vehicle and/or positive control

Vehicle: distilled water
Positive control: none

3. Test animals

Species: rabbit
Strain: New Zealand White
Age and weight at study initiation: males: 12 to 14 weeks, 2.55-2.83 kg; females: 12 to 14 weeks, 2.45-2.96 kg
Source: Interfauna U.K. Ltd., Huntingdon, Cambridgeshire, England
Housing: individually
Diet: SQC Rabbit Diet, *ad libitum*, occasionally supplemented with 10 g of autoclaved hay given to animals ▒showing a lack of condition▒
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 20.8-21.7°C
Humidity: 45.6-50.8%
Air changes: 19/hour
Photoperiod: 12 hour light/dark cycle
Acclimation period: 2 weeks

B. STUDY DESIGN

1. In life dates

Start: February 9, 1989; end: March 2-3, 1989

2. Animal assignment

Rabbits were randomly distributed within the experimental groups (Table 1) by a computerized method designed to ensure even weight distribution. Females were allocated to groups one day after the

males due to a delay with the computerized randomization. Groups of 5 rabbits/sex/dose were utilized.

TABLE 1. Study design

Dose Group	Dose (mg/kg/day)	No. of Animals	
		Male	Female
Control	0	5	5
Low-dose	100	5	5
Mid-dose	300	5	5
High-dose	1000	5	5

Data taken from p. 14, MRID 41297001.

3. Dose selection rationale

The dose levels were selected from available toxicity data (not presented in the report). The highest dose selected (1000 mg/kg/day) is the limit dose for a repeated dose dermal study.

4. Test substance preparation and analysis

Technical grade Ziram was used as supplied by the Sponsor. The test substance was moistened slightly with distilled water to ensure good contact with the skin.

5. Dose application

An area of fur equivalent to 10% of the body surface area was clipped from the mid-dorsal area of the trunk of each animal 24 hr before the first application of the test sample. Thereafter, the fur was clipped as necessary. Animals were treated with Ziram moistened with distilled water to ensure good contact with the skin. The treatment site was then covered with an impervious bandage consisting of gauze covered with ▒Elastoplast▒ elastic adhesive dressing backed with impervious ▒Sleek▒ plaster. After each 6-hour application, the bandages were removed and the treatment areas washed with warm tap water and blotted dry. At the end of the 21 days of treatment, animals were sacrificed by pentobarbitone sodium injection.

6. Statistics

Body weights, food consumption, terminal organ weights, hematology, and clinical chemistry values of test and control animals were initially evaluated to see if the relative frequency of the mode exceeded 75%. If so, the proportion of values different from the mode was analyzed. If not, Bartlett's test was used to determine heterogeneity of variance. If data were homogeneous, an ANOVA was performed, followed by Student's t test if significance was detected. For non-homogeneous data, a Kruskal-Wallis ANOVA was performed.

C. METHODS

1. Observations

Animals were examined for mortality and moribundity, gross signs of toxicity and for signs of irritation at the application site at least once daily.

2. Body weight

Animals were weighed at study initiation and once per week throughout the study.

3. Food consumption

Individual food consumption was calculated weekly throughout the study.

4. Ophthalmoscopic examination

Ophthalmoscopic examination was not required and was not performed.

5. Blood samples were obtained from the median artery of the ear of fasted animals prior to termination (Day 20). For specified animals (as recommended by the study director), further blood samples were obtained for reanalysis on Day 22. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)	x	Leukocyte differential count
x	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)	x	Mean corpusc. volume (MCV)
x	Platelet count		Reticulocyte count
	Blood clotting measurements		
x	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		
	(Kaolin-cephalin time)		
x	Erythrocyte morphology		

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
x	Calcium	x	Albumin
x	Chloride	x	Blood creatinine
	Magnesium	x	Blood urea nitrogen
x	Phosphorus	x	Total Cholesterol
x	Potassium	x	Globulins
x	Sodium	x	Glucose
		x	Total bilirubin
	ENZYMES	x	Total serum protein
x	Alkaline phosphatase(ALK)		(TP)
	Cholinesterase(ChE)		Triglycerides
	Creatine kinase		Serum protein electrophores
	Lactic acid dehydrogenase(LDH)		
x	Serum alanine amino-transferase (also SGPT)		
x	Serum aspartate amino-transferase(also SGOT)		
	Gamma glutamyl transferase(GGT)		
	Glutamate dehydrogenase		

6. Urinalysis

Urinalysis was not required and was not performed.

7. Sacrifice and pathology

All animals survived until scheduled termination of the study. Rabbits were sacrificed at the end of the treatment period by an intravenous overdose of pentobarbitone sodium. Microscopic examinations were carried out for control and high-dose rabbits.

The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
x	Tongue	x	Aorta	x	Brain
x	Salivary glands	x	Heart		Periph. nerve
x	Esophagus	x	Bone marrow		Spinal cord (3 levels)
x	Stomach	x	Lymph nodes	x	Pituitary
x	Duodenum	x	Spleen	x	Eyes (optic n.)
x	Jejunum	x	Thymus		
x	Ileum				GLANDULAR
x	Cecum		UROGENITAL	xx	Adrenal gland
x	Colon	xx	Kidneys*		Lacrimal gland
	Rectum	x	Urinary bladder	x	Mammary gland
xx	Liver*	xx	Testes	x	Parathyroids
x	Gall bladder	x	Epididymides	x	Thyroids
x	Pancreas	x	Prostate		
			Seminal vesicle		OTHER
	RESPIRATORY	xx	Ovaries	x	Bone
x	Trachea	x	Uterus	x	Skeletal muscle
x	Lung			x	Skin (treated and untreated)
	Nose				
x	Pharynx			x	All gross lesions and masses
x	Larynx				

*Required for subchronic studies based on Subdivision F Guidelines

II. RESULTS

A. OBSERVATIONS

No treatment-related mortality or clinical signs were observed. Infrequent and transient diarrhea, decreased quantity of normal feces, and a thin appearance were observed in some treated rabbits. However, the effects are not considered treatment-related. No treatment-related dermal effects were observed. Slight irritation from the bandage was observed in one mid-dose male and one high-dose female. Additionally, infrequent and transient staining of the dose site were observed in high-dose males and females.

B. BODYWEIGHT AND WEIGHT GAIN

Decreased ($p<0.05$ or $p<0.01$) body weight was observed in high-dose females during the entire study when compared to controls. In addition, the body weights of the high dose females was less than the pretreatment period during the first three weeks of dosing. No other effects on body weight were noted. Body weight data are summarized in Table 2.

TABLE 2. Group mean body weights (g)

	MALES				FEMALES			
Dose (mg/kg/day)	0	100	300	1000	0	100	300	1000
WEEK 0	2649	2678	2628	2689	2742	2601	2728	2704
WEEK 1	2752	2830	2687	2772	2844	2705	2850	2587** (9%)[a]
WEEK 2	2897	2991	2819	2867	2958	2807	2996	2683* (9%)
WEEK 3	3003	3134	2938	2999	3043	2874	3157	2660** (13%)

Data taken from p. 29 MRID, 41297001.
*p<0.05; **p<0.01
[a] Percent decrease from control value

C. FOOD CONSUMPTION

1. Food consumption

Significant (p<0.05) decreases in food consumption were observed in high-dose females when compared to controls during the first week of the study only. Food consumption was also decreased in high-dose females during weeks 2 and 3; however, statistical significance was not achieved. No significant differences were observed in any other animals. Summary food consumption data are presented in Table 3.

TABLE 3. Group mean food consumption (g)								
	MALES				FEMALES			
Dose (mg/kg/day)	0	100	300	1000	0	100	300	1000
WEEK 1	878	934	875	851	1021	1044	1013	677* (34%)[a]
WEEK 2	1825	1935	1841	1761	2006	2030	2072	1509
WEEK 3	2840	2980	2829	2747	3009	2924	3147	2335

Data taken from p. 30, MRID 41297001.
*p<0.05
a Percent decrease from control value

2. Food efficiency

Feed efficiency ({body weight gain [kg]/food consumption [kg per unit time]} x 100) values (calculated by reviewers) are presented in Table 4. Negative food efficiencies occurred in high-dose females for week 1 and week 3.

TABLE 4. Group mean food efficiency[a]								
	MALES				FEMALES			
Dose (mg/kg/day)	0	100	300	1000	0	100	300	1000
WEEK 1	+12	+16	+6.7	+9.7	+10	+10	+12	-17
WEEK 2	+7.9	+8.3	+7.2	+5.4	+5.7	+5.0	+7.0	+6.4
WEEK 3	+3.7	+4.8	+4.2	+4.8	+2.8	+2.3	+5.1	-0.99

calculated by the reviewer.

D. OPHTHALMOSCOPIC EXAMINATION

An ophthalmoscopic examination was not performed and was not required.

E. CLINICAL PATHOLOGY

Significantly ($p<0.05$) decreased lymphocyte counts were observed in high-dose females compared to controls. Decreased lymphoctye counts were also observed in high-dose males; however, statistical significance was not reached. A general trend of increasing GPT and GOT activities were observed in mid- and high-dose males and females. The increase was statistically significant for GPT ($p<0.01$) in high-dose females and for GOT in mid-($p<0.05$), and high-dose ($p<0.01$) females. Significant ($p<0.05$) increases in bilirubin were observed in high-dose females and increased cholesterol was observed in high-dose males ($p<0.01$) and females ($p<0.05$) compared to controls. The data are presented in Table 5.

TABLE 5. Selected Clinical Pathology Results[a]

	MALES				FEMALES			
Dose (mg/kg/day)	0.00	100	300	1000	0.00	100	300	1000
Lymphocytes ($x10^3/mm^3$)	5.72	5.04	5.27	4.17	5.27	4.96	5.97	3.65+
SGPT (mU/ml)[b]	31	35	46	78	22	33	53	101++
SGOT (mU/ml)[b]	20	13	20	60	12	16	42+	99++
Bilirubin (mg/dl)[c]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2+
Cholesterol (mg/dl)	35	49	40	71++	84[d]	73	87	171+

a Extracted from pages 31-32 of the study report.
+ $P<0.05$ in comparison with the controls using Williams' test
++ $P<0.01$ in comparison with the controls using Williams' test
b Kruskal-Wallis analysis
c Frequency analysis applied; more than 75% of the data has the same value.
d Data log transformed prior to analysis.

F. URINALYSIS

Urinalysis was not required and was not performed.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

There were no compound-related effects on organ weight.

2. Gross pathology

No treatment-related lesions were observed. Cortical scarring of the kidneys was reported in all groups, including controls.

3. Microscopic pathology

a) Non-neoplastic ▒ No treatment-related lesions were observed.

b) Neoplastic ▒ No neoplastic lesions were observed.

III. DISCUSSION

A. New Zealand White rabbits were treated with technical grade Ziram by dermal occlusion at doses of 0, 100, 300, or 1000 mg/kg/day, 6 hours/day for 21 days.

No mortality was observed and there were no treatment-related clinical signs or dermal effects. Decreased bodyweight ($p<0.05$ and 0.01; 9-13%) was observed in high-dose females during all three weeks of the study. Decreased food consumption was observed in high-dose females throughout the study, reaching statistical significance only during the first week of the study. Negative food efficiencies were calculated for high-dose females during weeks 1 and 3 only. Decreased lymphocyte counts ($p<0.05$) were observed in high-dose females; however, this effect is not considered toxicologically significant due to the small magnitude of the effect. A trend of increasing GPT (ALT) and GOT (AST) were observed in mid- and high-dose males and females. Significant ($P<0.01$) increases in GPT and GOT was observed in the high-dose females. There was also a statistical increase ($P<0.05$) in GOT in the mid-dose females. Significantly increased levels of bilirubin ($p<0.05$) were observed in high-dose females, while increased cholesterol levels ($p<0.01$) were observed in high-dose males and females. Examination of the individual animal data showed that the increase in GPT and GOT in the high-dose males was due to the effect in one animal. Therefore, these changes are not considered toxicologically significant. The increases in GOT, GPT, cholesterol and bilirubin together could indicate some hepatoxicity, however there was no histopathological evidence of liver changes. Therefore, they should be considered evidence of minimal hepatotoxicity in the high-dose females only.

Under the conditions of this study, the NOAEL for systemic toxicity in females for Ziram Technical was 300 mg/kg/day. The LOAEL for systemic effects was 1000 mg/kg/day based on decreased body weight and food consumption and clinical chemistry changes suggestive of minimal hepatotoxicity (increases in GPT, GOT, bilirubin and cholesterol). The NOAEL for males is greater than 1000 mg/kg/day; the LOAEL was not identified. The NOAEL for dermal effects was equal to or greater than 1000 mg/kg/day; the LOAEL was not identified.

B. STUDY DEFICIENCIES

Minor- Serum lactic dehydrogenase was not measured. This does not compromise the validity of this study.

SignOff Date:	8/2/00
DP Barcode:	D172447
HED DOC Number:	014277
Toxicology Branch:	RAB2