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WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

April 6, 2000

MEMORANDUM

SUBJECT: Ziram - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan Sanjivani Diwan P.C. code 034805
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Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: David Nixon, Toxicologist
Reregistration Branch 4
Health Effects Division (7509C)

And

Laura Parsons, Chemical Review Manager
Reregistration Branch 1
Special Review and Reregistration Division (7508C)

The Cancer Assessment Review Committee met on February 9, 2000 to evaluate the carcinogenic potential of Ziram. Attached please find the Final Cancer Assessment Document.

cc: K. Dearfield
R. Hill
Y. Woo
J. Pletcher

014085

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF *ZIRAM*

FINAL REPORT

6-APRIL-2000

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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DATA PRESENTATION: David Nixon, Toxicologist

DOCUMENT PREPARATION: Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam	
Marion Copley	
Kerry Dearfield	
Virginia Dobozy	
Yiannakis Ioannou	
Nancy McCarroll	
Linda Taylor	
Esther Rinde	
Jess Rowland	
Joycelyn Stewart	
Clark Swentzel	

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John M. Pletcher,	Pathology Consultant	
Lori Brunsman,	Statistical Analysis	for Lori

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EXECUTIVE SUMMARY

On February 9, 2000, the Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of ziram. The studies evaluated included a 2-year combined chronic toxicity/carcinogenicity study in CD (SD) BR rats, a 24-month carcinogenicity study in ICR-Crj mice as well as NTP studies in F344 rats and $B6C3F_1$ mice.

The experimental designs for Ziram studies were as follows: 1) CD (SD) BR Rats/Guideline study - 50/sex/dose at 0, 60, 180 or 540 ppm (0, 2.5, 7.7 or 23.7 mg/kg/day for males; 0. 3.4, 10.2 or 34.6 mg/kg/day for females, respectively for 104 weeks. 2) F344 Rats/ NTP study - 50/sex/group at 0, 300 or 600 ppm (0, 11 or 22 mg/kg/day for males and 0, 13 or 26 mg/kg/day, females, respectively) for 103 weeks. 3) CD-1 Mice/Guideline study - 50/sex/group received 0, 29, 75, 225 or 675 ppm (0, 3, 9, 27 or 82 mg/kg/day for males and 0, 4, 11, 33 or 95 mg/kg/day for females, respectively) for 80 weeks. 4) $B6C3F_1$ Mice/NTP study - 50/sex/dose at 0, 600 or 1200 ppm (0, 22 or 196 mg/kg/day for males and 0, 131 or 248 mg/kg/day for females, respectively) for 103 weeks.

The CARC concluded that

- **Ziram was carcinogenic to CD (SD) BR and F344/N male rats and $B6C3F_1$ female mice.**

  In CD (SD) BR male rats, there was a significant increase by pair-wise comparison with the controls for mesenteric lymph node hemangiomas and combined incidence of mesenteric lymph node and spleen hemangiomas at 540 ppm (23.7 mg/kg/day). A significant positive trend was evident for mesenteric lymph node hemangiomas and combined incidences of mesenteric lymph node and spleen hemangiomas. The incidences of these tumors were outside the historical controls range (0%-4%). The CARC therefore, concluded that these benign tumors in males were treatment-related. The dosing at 540 ppm was considered to be adequate and not excessive based on increased histopathological changes in various organs and a decrease in body weight gain at 540 ppm in both sexes.

  In F344/N male rats, there was a significant increase by pair-wise comparison with the controls for thyroid C-cell carcinomas and combined adenomas/carcinomas at 600 ppm (22 mg/kg/day). Significant dose-related increasing trends for these tumors were also evident. The incidences of these tumors were outside the historical controls range (carcinoma: 0%-8% and combined: 0%-20%). The CARC therefore, concluded that these malignant tumors were treatment-related. The Committee agrees with NTP's assessment of dosing and determined that the animals could have tolerated higher dose levels.

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In B6C3F$_1$ female mice, there was a significant increase by pair-wise comparison with the controls for alveolar/bronchiolar adenomas and combined adenomas/carcinomas of the lung at 1200 ppm (248 mg/kg/day). There were significant positive trends for alveolar/bronchiolar adenomas and combined tumors and a dose-related increase in their incidences at 600 and 1200 ppm (131 and 248 mg/kg/day, respectively). The incidence of these tumors at 1200 ppm was outside the historical controls range (adenomas: 0%-14%; combined: 0%-16%). The Committee concluded that despite the fact that Sendai virus infection can affect the rate of spontaneous tumors, the compound-related effect cannot be ruled out. Therefore, the increased incidences of alveolar/bronchiolar tumors at 600 and 1200 ppm were considered by the CARC to be treatment-related. The dosing was considered to be adequate and not excessive based on 26% decrease in body weight gain.

Ziram was not carcinogenic to CD-1 mice.

- Ziram is primarily a bacterial mutagen with the capacity to bind to macromolecules (i.e., cholinesterase). Although results of mammalian cell assays were in conflict, the preponderance of data from the cytogenetic studies favor a positive response. Based on the direct mutagenic effect on base-pair substitution strains of *S. typhimurium* and *E. coli* and the evidence of clastogenicity in mammalian cells, the Committee determined that there is a sufficient evidence for a mutagenic concern. The CARC, therefore, recommended that a dominant lethal assay be conducted by the registrant to address a possible concern for heritable effects.

According to the Agency's *Draft Guidelines for Cancer Risk Assessment* (July, 1999), the Committee classified ziram into category "Likely to be carcinogenic to humans" based on the occurrence of C-cell thyroid tumors and hemangiomas in male F344 and CD rats, respectively, and lung tumors in female B6C3F1 mice. The Committee further recommended a linear low-dose extrapolation approach for the quantification of human cancer risk based on C-cell thyroid tumors in male F344 rats. This approach is supported by the findings of benign lung tumors in female mice, lack of mode of action data, and the mutagenicity evidence for ziram.

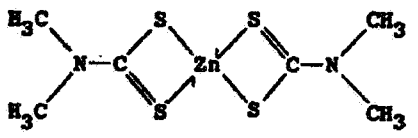
I. INTRODUCTION

On February 9, 2000, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of ziram. Dr. David Nixon of the Reregistration Branch 4 described the chronic/carcinogenicity studies in Sprague-Dawley and F344 rats as well as in CD-1 and $B6C3F_1$ mice on ziram by detailing the experimental design, reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of dose levels tested, and presenting the weight of the evidence for the carcinogenicity of ziram. No mechanistic studies were provided by the registrant.

II. BACKGROUND INFORMATION

Ziram is a dithiocarbamate compound used as a fungicide or herbicide in agricultural and residential settings, as an antimicrobial in industrial settings, or as a vertebrate repellent. The nature of its pesticidal action is unknown but may affect fungal proteins. Ziram is also being used on terrestrial food and feed crop, greenhouse food crop, and for indoor non-food uses. The PC Code of ziram is 034805 and the CAS Number is 137-30-4.

Ziram is a white powder and has a molecular weight of 305.8, a vapor pressure ~ 10^{-7}, and a water solubility of 65 ppm. The log K_{ow} is 1.086 and the melting point is 250°C.



Ziram

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III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with ziram in CD(SD)BR rats

Reference: Lindsey A. J. Powell, Sarah M. Bottomley, David Crook, Richard L. Gregson, John M. Offer, William A. Gibson, Alan Anderson (1994) Combined chronic toxicity and oncogenicity of Ziram (Technical) administered in the diet to rats. Huntingdon Research Centre Ltd. Laboratory report number: ZIR 9/942098. September 27, 1994. MRID 43404201. Unpublished.

A. Experimental Design

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 or 540 ppm for 104 weeks. These doses corresponded to achieved intakes of 0, 2.5, 7.7 or 23.7 mg/kg/day for males in the main group and 0, 3.4, 10.2 or 34.6 mg/kg/day for females in the main group.

B. Discussion of Tumor Data

The dietary administration of ziram at 540 ppm resulted in a treatment-related increased incidence of hemangiomas of mesenteric lymph nodes (5/49 or 10%) and spleen (1/49 or 2%), as well as combined hemangiomas of the mesenteric lymph node and spleen (6/49 or 12%) in male rats. These tumors rarely metastasize. There were no hemangiomas in the control group. Incidences of hemangiomas in historical controls ranged from 0 to 4% (mean = 1%) in lymph nodes and 0% in the spleen.

The statistical evaluation of hemangiomas in male rats revealed significant increasing trends at $p < 0.01$ and significant differences in the pair-wise comparisons of the 540 ppm group with the controls at $p < 0.05$ for mesenteric lymph node hemangiomas and for lymph node and spleen hemangiomas combined. No hemangiosarcomas were reported in any dose group and no treatment-related tumors were identified in males in the 60 or 180 ppm groups or in females at any dosage level. The statistical analyses of tumors in male rats are presented in Table 1.

Table 1. CD (SD) BR Male Rats: Mesenteric Lymph Node and Spleen Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (Brunsman, 2000)

ppm	0	60	180	540
mg/kg/day	0	2.5	7.7	23.7
Tumor Type				
Mesenteric Lymph Node Hemangiomas % p =	0/50 (0) 0.001**	0/49 (0) 1.000	0/50 (0) 1.000	5[a]/49 (10) 0.027*
Spleen Hemangiomas % p =	0/50 (0) 0.249	0/49 (0) 1.000	0/49 (0) 1.000	1[b]/49 (2) 0.495
Combined % p =	0/50 (0) 0.000**	0/49 (0) 1.000	0/50 (0) 1.000	6/49 (12) 0.012*

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.
[a]First lymph node hemangioma observed at week 105, dose 540 ppm.
[b]First spleen hemangioma observed at week 102, dose 540 ppm.
Note: Interim sacrifice animals are not included in this analysis. There were no hemangiomas in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-Neoplastic Lesions

There were statistically significant ($p<0.05$ or $p<0.01$) increases in the incidences of various non-neoplastic lesions. These are discussed below. In males and females from the 180 and 540 ppm dose groups, there were findings of hemosiderosis in the spleen and sinusoidal cells of the liver, bile duct hyperplasia, hyperplasia of the non-glandular epithelium of the stomach, subepithelial edema and ulcerations in the stomach, prominent ultimobranchial cysts in the thyroid, adipose infiltration/replacement of peripheral muscle fiber bundles, and narrowing of peripheral muscle fiber bundles in skeletal muscle, axonal degeneration (minimal) in the spinal cord (males), and axonal degeneration in the sciatic nerve (males, not statistically significant; females, $p<0.01$). In addition, the

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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, C-cell hyperplasia in the thyroid, hyperplasia in the parathyroids, and hypertrophy with vacuolation in the adrenal cortex. There were findings for females only in the 180 and/or 540 ppm dose groups, including an increase in lipofuscin in cortical tubular epithelial cells in the kidney, acinar hyperplasia in the mammary gland and cystic degeneration in the adrenal cortex. The findings for males in the low dose group (60 ppm) included hemosiderosis in spleen, hyperplasia of the non-glandular epithelium in the stomach, subepithelial edema in the stomach, narrowing of peripheral muscle fiber bundles in the skeletal muscle, and hypertrophy with vacuolation in the adrenal cortex. 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.

TABLE 2. MICROSCOPIC PATHOLOGY: NON-NEOPLASTIC CHANGES FOR MAIN GROUP CD (SD) BR RATS ADMINISTERED ZIRAM FOR 104 WEEKS

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0.00	60	180	540	0.00	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)[a]	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	0.00	1	0.00	3	0.00	0.00	0.00	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)	0.00	0.00	0.00	0.00

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TABLE 2 (Continued)

Pathology	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0.00	60	180	540	0.00	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.

[a]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

Organ weights were also affected by oral administration with ziram. For males, the decrease was statistically significant at week 104 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 the 540 ppm dose group were statistically significantly increased at week 104 (110% of control, $p<0.05$). 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, $p<0.05$).

D. Adequacy of the Dosing for Assessment of Carcinogenicity

The dosing was considered by the CARC to be adequate and not excessive based on histopathological changes in various tissues observed in both sexes at all dosages, effects on organ weights at 540 ppm, and decreased body weight gain in males (86% of controls) and in females (74% of controls) at 540 ppm. The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of ziram in male rats. Female rats showed a significant decreasing trend in mortality with increasing doses of ziram.

2. National Toxicology Program Two-year Carcinogenicity Study with ziram in F344/N rats

Reference: U.S. National Toxicology Program (1983) Carcinogenesis Bioassay of Ziram (CAS No. 137-30-4) in F344/N Rats and B6C3F$_1$ Mice (Feed Study; NTP Technical report series No. 238), Research Triangle Park, NC.

A. Experimental Design

In a 2-year carcinogenicity feeding study, ziram (89% pure, with 6.5% thiram) was administered in the diet to 50 male and 50 female F344/N rats per group at 0, 300, or 600 ppm for 103 weeks. The doses corresponded to overall mean doses of about 0,11, or 22 mg/kg/day for males and to 0, 13, or 26 mg/kg/day for females.

B. Discussion of Tumor Data

In male rats, the incidence of C-cell carcinomas of the thyroid in the 600 ppm group was significantly higher ($p<0.05$) than that in the controls (control, 0/50, 0%; 300 ppm, 2/49, 4%; 600 ppm, 7/49, 14%). There was also a statistically significant ($p<0.01$) positive trend for C-cell carcinomas. The incidence in the high-dose group exceeded the historical control incidences from the same laboratory (18/584, 3%; range 0% to 8%). The combined incidence of C-cell adenomas/carcinomas showed a statistically significant ($p<0.05$) positive trend (control, 4/50, 8%; 300 ppm, 9/49, 18%; 600 ppm, 12/49, 24%). There were no significant histopathologic changes noted in the thyroid follicular cells.

The statistical analyses of tumors in male rats (Table 3) were based upon the Cochran-Armitage Trend Test and the Fisher's Exact Test for pair-wise comparisons.

NTP concluded that ziram was carcinogenic for male F344/N rats, causing increased incidences of C-cell carcinomas of the thyroid gland, but was not carcinogenic for female F344/N rats.

Table 3. F344/N Male Rats: Thyroid C-cell Tumor Rates and Cochran-Armitage Trend Test and Fisher's Exact Test Results (Brunsman, 2000)

ppm	**0**	**300**	**600**
mg/kg/day	**0**	**11**	**22**
TumorType			
Thyroid C-Cell Adenoma % p =	4/50 (8) 0.422	7/49 (14) 0.251	5/49 (10) 0.487
Thyroid C-Cell Carcinoma % p =	0/50 (0) 0.003**	2/49 (4) 0.242	7/49 (14) 0.006**
Combined % p =	4/50 (8) 0.020*	9/49 (18) 0.109	12/49 (24) 0.024*

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-neoplastic lesions

C-cell hyperplasia in the thyroid gland was noted in males in the control and all dose groups, but did not appear to be dose related (control, 7/50, 14%; 300 ppm, 12/49, 24%; 600 ppm, 11/49, 22%). No treatment-related effects on C-cell histopathology were noted in females.

D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing was selected based on decreased mean body weight gain observed in a 13-week study. Since there were no treatment-related effects on mortality, clinical signs, body weight, or food consumption, and minimal non-neoplastic histopathological changes, NTP concluded that the rats of both sexes may have tolerated higher doses. Survival of rats of each sex was not adversely affected by ziram treatment. The CARC agreed with NTP's assessment of adequacy of dosing.

3. Carcinogenicity Study with ziram in CD-1 (ICR)BR mice

Reference: Lindsey A.J. Powell, Sarah M. Bottomley, David Crook, S.K. Majeed, C. Gopinath, William A. Gibson, Alan Anderson. (1994) Ziram (Technical) Potential oncogenicity to mice by repeated dietary administration for 80 weeks. Huntingdon Research Centre, Ltd., Cambridgeshire, England. Report # ZIR 12/932311, August 19, 1994. MRID # 43373701. Unpublished.

A. Experimental Design

In an 80-week carcinogenicity feeding study, ziram (98.7%, Lot No. 8331 AA) was administered in the diet to 50 male and 50 female Crl: 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 or 82 mg/kg/day for males, and to 0, 4, 11, 33 or 95 mg/kg/day for females.

B. Discussion of Tumor Data

The dietary administration of ziram up to 675 ppm did not result in an overall treatment-related increase in tumor incidences in Crl:CD-1(ICR)BR mice. No treatment-related effect on the incidence or distribution of tumors were noted.

C. Non-neoplastic lesions

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. Irregular cortical scarring of the kidneys was seen in 42.4% of males at 675 ppm compared to 14.7% in controls. The incidence of brown discoloration of the kidney was also slightly increased (9.1%) at 675 ppm compared to controls (0%) in males.

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. No dose-related microscopic pathologies were seen in the stomach or kidneys that would correspond to the changes observed on gross examination.

D. Adequacy of Dosing for Assessment of Carcinogenicity

The dosing was considered by the CARC to be adequate and not excessive based on increased incidences of urinary bladder epithelial cell hyperplasia in males at 225 and 675 ppm and in females at 675 ppm, increased incidences of urinary bladder epithelial cell hypertrophy in females at 675 ppm, decreased absolute brain weight in males at 225 and 675 ppm, and decreased body weight gain in males at 225 ppm (77% of control) and 675 ppm (56% of controls) and in females at 675 ppm (80% of controls). No statistically significant differences in mortality were noted in both sexes.

4. National Toxicology Program Two-year Carcinogenicity Study with ziram in $B6C3F_1$ mice.

Reference: U.S. National Toxicology Program (1983) Carcinogenesis Bioassay of Ziram (CAS No. 137-30-4) in F344/N Rats and $B6C3F_1$ Mice (Feed Study; NTP Technical report series No. 238), Research Triangle Park, NC.

A. Experimental Design

In a 2-year carcinogenicity feeding study, Ziram (89% pure, with 6.5% thiram) was administered in the diet to 50 male and 50 female $B6C3F_1$ mice per group at 0, 600, or 1200 ppm for 103 weeks. The doses corresponded to overall mean doses of about 22 or 196 mg/kg/day for males and to 0, 131, or 248 mg/kg/day for females.

B. Discussion of Tumor Data

The incidence of alveolar/bronchiolar adenomas of the lung in female mice in the 1200 ppm group was significantly higher ($p<0.05$) than the controls (control, 2/50, 4%; 600 ppm, 5/49, 10%; 1200 ppm, 10/50, 20%) with a statistically significant positive trend ($p<0.01$). The incidence of combined alveolar/bronchiolar adenomas/carcinomas in female mice in the 1200 ppm group was significantly higher ($p<0.05$) than the controls (control, 4/50, 8%; 600 ppm, 6/49, 12%; 1200 ppm, 11/50, 22%) with a statistically significant positive trend ($p<0.05$). The incidences of alveolar/bronchiolar adenomas and combined adenomas/carcinomas in female mice at 1200 ppm exceeded the range for the historical controls. Historical control data on alveolar/bronchiolar adenomas show an incidence of 18/501 (3.6%) from the same laboratory and 134/2788 (4.8%) with a range of 0/50 to 7/50 (0%-4%) across the Bioassay Program. The combined incidence of alveolar/bronchiolar adenomas/carcinomas in historical control females is 25/501 (5.0%) from the same laboratory and 184/2788 (6.6%) with a range of 0/50 to 8/50 (0%-16%) across the Bioassay Program.

The statistical analyses of tumors in female mice (Table 4) were based upon the Cochran-Armitage Trend Test and the Fisher's Exact Test for pair-wise comparisons.

NTP concluded that oral administration of ziram to female $B6C3F_1$ mice resulted in increased incidences of alveolar/bronchiolar adenomas and of combined alveolar/bronchiolar adenomas/carcinomas. The interpretation of this increase in lung tumors was complicated by an intercurrent Sendai virus infection.

Table 4. F344/N Female Mice: Lung Alveolar/Bronchiolar Tumor Rates and Cochran-Armitage Trend Test and Fisher's Exact Test Results.

ppm	0	600	1200
mg/kg/day	0	131	248
Tumor Type			
Lung Alveolar/ Bronchiolar Adenoma	2/50	5/49	10/50
%	(4)	(10)	(20)
p =	0.009**	0.210	0.014*
Lung Alveolar/ Bronchiolar Combined Adenoma/ Carcinoma	4/50	6/49	11/50
%	(8)	(12)	(22)
p =	0.031*	0.357	0.045*

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.
Incidences of carcinoma alone were not reported.

C. Non-neoplastic lesions

Alveolar epithelial hyperplasia of the lung was noted in females in the control as well as both dose groups and increased in a dose-related manner (control, 2/50, 4%; 600 ppm, 4/49, 8%; 1200 ppm, 10/50, 20%). Alveolar epithelial hyperplasia of the lung was not noted in males at any dosage level. Pulmonary adenomatous hyperplasia was noted in control and dosed males (control, 15/49, 31%; 600 ppm, 19/50, 38%; 1200 ppm, 16/49, 33%) and in control and dosed females (control, 18/50, 36%; 600 ppm, 27/49, 55%; 1200 ppm, 26/50, 52%). This particular histopathological finding is consistent with chronic Sendai virus infection which was confirmed by serology performed on untreated animals housed in the same room and from the same shipment. Six of the 26 females from the 1200 ppm group with the adenomatous hyperplasia had pulmonary tumors, whereas 4/24 females from the 1200 ppm group without pulmonary adenomatous hyperplasia had pulmonary tumors as well. One of 27 females from the 600 ppm group with adenomatous hyperplasia had a pulmonary tumor.

D. Adequacy of Dosing for Assessment of Carcinogenicity

The dosing was considered by the CARC to be adequate and not excessive based on decreases in mean body weight gain in males (both doses: 10-25% decrease compared to controls) and in females (1200 ppm: 13-23% decrease after day 80 compared to controls), decreased food consumption at 1200 ppm in males (78% of control) and in females (85% of control), and pulmonary histopathology findings. Dosing was selected based on decreased mean body weight gain (26% or more decrease compared to the control) in males and females receiving 2500 or 5000 ppm in a 13-week study. Survival of male and female mice was not adversely affected by ziram.

IV. TOXICOLOGY

1. Metabolism

Groups of 15 male and 15 female rats (MRID 42391001) were administered ziram or ^{14C}-ziram by gavage at doses of 15 mg/kg (Group 2, single low dose), 15 mg/kg/day for 14 days followed by a single dose of radio labeled ziram (Group 3), or 352 mg/kg (Group 4, single high dose). Controls (Group 1) received only the methyl cellulose as vehicle. Radioactivity excreted in the urine and feces was monitored for 168 hours (single low dose, multiple low dose, and single high-dose), and expired air for all three dose groups was monitored for 96 hours. Additionally, radioactivity in tissues and carcass were measured.

Overall recovery of administered radioactivity ranged from 78.9% to 92.4%. ^{14C}-Ziram derived radioactivity was excreted in the expired air, feces, and urine. There were no significant quantitative or temporal differences in excretion of radioactivity between males and females. For all three treatment groups, excretion of ^{14C}-ziram derived radioactivity (average for both sexes) was greatest in expired air (37%, 41%, and 50% for Groups 2, 3, and 4, respectively), and was associated with both CO_2 and volatile fractions. Urinary excretion accounted for 17 to 35% of the administered radioactivity and was slightly greater in the multiple low-dose group. Fecal excretion accounted for 9 to 18% of the administered radioactivity and was similar for all dose groups. Percent of radioactivity administered was low in tissues (<1%) and carcasses (≤1%) in all dose groups. Time-course data for excretion of ^{14C}-ziram indicated rapid excretion via expired air in both low-dose groups (<24 hours) and in <48 hours in the high-dose group. Urinary and fecal excretion was nearly complete within 72 hours for both low-dose groups, but appeared to be multiphasic in the high-dose group with excretion peaks at 0-8 hours, 24-72 hours, and at 96 hours.

No studies identifying the metabolites of ziram were available.

2. Mutagenicity:

Seven acceptable genetic toxicology studies on ziram have been submitted. Studies from the National Toxicology Program (NTP) and from the open literature were also available for review. The findings indicate that ziram causes base-pair substitutions in DNA-repair deficient *Salmonella typhimurium* TA1535 and TA100 and *Escherichia coli* WP2 uvrA but not in the strains (*S. typhimurium* TA102 and TA104) that show specificity for oxidative damaging agents. In general, the response in bacteria was obtained in both the presence and absence of S9 activation. Ziram produced mixed results for gene mutations in mouse lymphoma cells and was not active for forward gene mutations in Chinese hamster V79 cells. Conflicting results were also seen in the in vitro cytogenetic assays but the preponderance of assays favor a positive response at noncytotoxic doses. Ziram was also found to be negative for unscheduled DNA synthesis (UDS) both *in vitro* and *in vivo*.

The *in vivo* data from the open literature suggest that ziram is not clastogenic or aneugenic in mice. While there is evidence from an article, which provided very limited data, of dominant lethal mutations in two mouse strains and alteration of sperm morphology in mice, these findings should be viewed with caution since ziram did not cause infertility in a two-generation rat reproductive toxicity study, was not shown to be a developmental toxicant in rats but did produce equivocal evidence of malformations in rabbits. Nevertheless, based on the available database, the Committee concluded that a dominant lethal assay is required to address possible concerns for a heritable effect.

Conflicting mutagenicity as well as carcinogenicity and reproductive/developmental results were also obtained for other members of the dimethyldithiocarbamate class of compounds such as thiram, ferbam, the sodium and potassium salts of dimethyldithio-carbamate and lead dimethyldithiocarbamate. The only consistent finding among the studied members of this chemical class was a direct mutagenic effect on the base-pair substitution strains of *S. typhimurium*. Therefore, until a plausible explanation is obtained for the disparate results and, in view of the mutagenic effects in bacteria, the weight-of-the-evidence indicates that a mutagenic mode of action cannot be ruled out for ziram. Studies supporting these conclusions are presented below:

GENE MUTATIONS

1) *Salmonella typhimurium*/ mammalian microsome gene mutation assay: The assay was positive with dose-related and reproducible ≈≥2-fold increases in mutant colonies of strain TA100 at 66.7-333.3 μg/plate without S9 activation or 33.3-333.3 μg/plate +S9. Greater than 2-fold increases in mutant colonies were also seen for strain TA1535 at 66.6 and 100 μg/plate +S9. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a bacterial gene mutation assay (MRID No. 00147462).

2) *S. typhimurium/* mammalian microsome gene mutation assay: Independent trials were positive with dose-related and reproducible ≥2-fold increases in mutant colonies of strain TA100 at 50, 75 and 100 μg/plate but only in the presence of 20-30% S9 in the cofactor mix. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a bacterial gene mutation assay (MRID No. 41642901).

3) *S. typhimurium/* mammalian microsome gene mutation assay: Ziram was one of 250 coded compounds evaluated in NTP's collaborative mutagenicity screening project of the S. typhimurium/ mammalian microsome gene mutation assay. Results were positive for strain TA100 at 10-333 μg/plate without and with S9 derived from Aroclor 1254-induced rat livers and at 33-333 μg/plate with hamster livers. Increases approaching or greater than 2-fold were also seen for strain TA1535 at 100 μg/plate with rat liver S9 or at 33-333 μg/plate with hamster liver S9. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a bacterial gene mutation assay (Haworth et al., 1983).

4) In vitro mammalian cell forward gene mutation assay in mouse lymphoma L5178Y cells: As part of the NTP evaluation of this mammalian cell test system, ziram was found to be positive for the induction of gene mutations at all assayed doses in Trial 1 (0.625-1.0 μg/mL) and in Trial 2 (0.1-1.8 μg/mL). Relative total growth was 18% at 1.0 μg/mL or 8% at 1.4 μg/mL; lethality was seen at levels ≥1.8 μg/mL. The test was conducted only in the absence of S9 activation and colony sizing was not performed. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a mammalian cell gene mutation assay (McGregor et al., 1988).

CHROMOSOME ABERRATIONS

5) *In vitro* mammalian cell cytogenetic assay in Chinese hamster ovary (CHO) cells: The test was negative up cytotoxic levels (doses that caused a ≥50% reduction in the mitotic index) (0.025μg/mL -S9 or 1 μg/mL + S9). The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for an *in vitro* mammalian cell cytogenetic assay (MRID 41287802).

6) *In vitro* mammalian cell cytogenetic assay in CHO cells: In contrast to the above negative results in CHO cells, the NTP-sponsored evaluation of ziram indicated significant and reproducible increases in structural chromosome aberrations at 0.025 and 0.05 μg/mL -S9 or 1.5 and 1.75 μg/mL +S9. In all trials, increases in simple chromatid or chromosome aberrations (e.g., breaks, fragments and double minutes) and complex aberrations (e.g., interchanges and rearrangements) with a preponderance of simple aberrations was reported. There was, however, no reproducible induction of sister chromatid exchanges (SCE) at 0.001-0.025 μg/mL -S9 or 0.16-1.75 μg/mL +S9. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for *in vitro* cytogenetic mutagenicity data (Gulati et al., 1989).

OTHER MUTAGENIC MECHANISMS

7) *In vitro* unscheduled DNA synthesis (UDS) in primary rat hepatocytes: Independent trials were negative up to the highest dose tested (1.0 μg/mL). The study is currently classified as Unacceptable because the highest dose tested did not cause toxicity. However, a reexamination of the data show clear evidence of cytotoxicity at higher doses ($\geq$3.16 μg/mL). Accordingly, the study should be reclassified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for an *in vitro* UDS assay (MRID No. 41287801).

INFORMATION FROM THE OPEN LITERATURE

Prokaryotic Test Systems

In agreement with the findings from the submitted assays and the NTP-sponsored study, ziram induced reverse gene mutations in *S. typhimurium* strain TA100 (Moriya et al., 1976; Franekic et al., 1994; Tinkler et al., 1998; and Crebelli et al., 1992). Mutagenesis was seen in both the presence and absence of S9 activation by all of these authors. Furthermore, Franekic et al. (1994), Tinkler et al. (1998) and Crebelli et al. (1992) reported positive results in *S. typhimurium* strain TA1535.

Crebelli et al. (1992) also reported that ziram induced a mutagenic effect in *Escherichia coli* WP2 uvrA both with and without S9 activation but was negative in *E. coli* WP2 and *S. typhimurium* strain TA102. The negative results with *S. typhimurium* strain TA102 were confirmed by Franekic et al. (1994) who also found that ziram tested negative with *S. typhimurium* strain TA104. It is of note that both of these Salmonella strains were specifically developed by Levin et al. (1982) to detect oxidative mutagens. The positive results in *S. typhimurium* TA100 and TA1535 and in *E. coli* WP2 uvrA coupled with the negative findings for *S. typhimurium* TA102 and TA104, as well as *E. coli* WP2 suggests that the mutations induced by ziram do not operate through oxidative damage since the mutagenic profile of oxidative agents shows preferential activity toward DNA repair-proficient strains such as *S. typhimurium* TA102 and TA104 and *E. coli* WP2. The lack of a positive effect in *S. typhimurium* TA102 or TA104 is in direct conflict with Rannung and Rannug's (1984) argument that the mechanism for dimethyldithiocarbamate mutagenicity in bacteria is associated with oxidative stress.

Despite the clear evidence of mutagenicity in bacteria, ziram did not alkylate the acellular nucleophiles (4-p-nitrobenzyl)-pyridione or deoxyguanosine (Hemminiki et al., 1980). These findings (positive for mutagenicity in Salmonella but negative for electrophilicity) are consistent with Ashby's and Tennant's listing of ziram as a positive mutagen in Salmonella and as a "non-alerting" carcinogen affecting a single species, sex and site. However, the evidence of cholinesterase inhibition (ChEI) does indicate that ziram bind to macromolecules. Franekic et al. (1994) listed ziram as the most potent bacterial mutagen not requiring S9 activation among the dimethyldithiocarbamates (thiram, zineb

S-65 and ethylenethiourea) that were tested but considered ziram to be negative for mitotic chromosome malsegregation in *Saccharomyces cerevisiae D6.1M.* Ziram tested positive for DNA damage in DNA-repair deficient *Bacillus subtilis* M45 (rec-) as compared to the DNA-repair proficient strain, H17 (rec+) (Shirasu et al., 1976) but was negative for UDS in cultured hepatocytes (MRID No. 41287801) from rats pretreated with either Aroclor 1254 or 3-methylcholanthrene (Shaddock et al., 1990) or following *in vivo* exposure (Tinkler et al., 1998).

Eukaryotic Test Systems

Data from the mouse lymphoma assays with ziram produced conflicting results; it was reproducibly positive in the NTP study but yielded negative and/or inconclusive findings in the study of Tinkler et al. (1998). In the latter study, negative results were obtained without S9 activation and ziram was considered equivocal in the presence of S9 at doses that reduced cell survival to ≤15% of control. It has also been reported to be negative for gene mutations in Chinese hamster V79 cells (Donner et al.,1983). Similarly, the negative findings from the submitted *in vitro* cytogenetic assay in CHO cells neither agree with the data from the NTP study that used the same cell line nor with the dose-related and significant increases in structural chromosome aberrations in Chinese hamster epithelial liver (CHEL) and in CHO cells reported by Mosesso et al. (1994). In the study of Mosesso et al., significant and dose-related increases in the yield of cells with structural chromosome aberrations were seen at 0.22-1.00 μg/mL +S9 in CHEL cells or 1.0 or 2.15 μg/mL +S9 in CHO cells. Under both test systems, the major types of aberrations scored at noncytotoxic doses (i.e, mitotic indices were ≥85% of control for CHEL cells and ≥61% of control for CHO cells) were chromatid breaks and chromatid and chromosome exchanges. Tinkler et al. (1998) also reported a positive and reproducible dose-related clastogenic response in cultured human lymphocytes at 10-15 μg/mL+S9. Although the type of aberrations were not reported, the investigator did state that the clastogenic activity of ziram was not associated with excessive cytotoxicity as indicated by the mitotic indices, which ranged from 64 to >100% of control at 10 μg/mL to 44% of control at 15 μg/mL. In all studies reporting positive *in vitro* clastogenesis; however, most of the gross structural damage to the chromosomes (chromatid and chromosome breaks and exchanges) can be classified as unstable and would likely lead to cell death. Hence, the relevance of the positive cytogenetic assays to a direct mutagenic mode of action for ziram is not certain. Ziram was also shown to induce metaphase arrest (c-mitosis), multipolarity and anaphase disturbances as well as chromosomal aberrations such as micronuclei, bridges and polyploidy in *Allium ascalonicum* (Franekic et al., 1994). The study authors concluded that the evidence of spindle dysfunction, metaphase arrest and micronuclei induction was suggestive of aneuploidy. No other data suggesting that ziram induces aneuploidy were found.

No *in vivo* studies were submitted by the registrant. However, in the adult male feeding *Drosophila melanogaster* mutagenicity tests sponsored by NTP, Foureman et al. (1994) observed that ziram at 1000 ppm induced sex-linked recessive lethal mutations but

not reciprocal translocations. Although additional positive results have been reported in the sex-linked recessive lethal and the somatic and germinal mosaic assays in *Drosophila melanogaster* (Hemavathi et al., 1989), the studies were performed with Cuman L, a formulation containing only 27% ziram (other components were not specified). Crebelli et al. (1992) indicated that the significant induction of micronucleated polychromatic erythrocytes (MPCEs) seen in bone marrow cells harvested from male B6C3F1 mice 24 hours after the intraperitoneal administration of the mid-dose (5 mg/kg ziram, 98.5%) was inconclusive because the effect was confined to this sex, dose and sample time. No other in vivo cytogenetic assays with somatic cells were found in the open literature.

In contrast, evidence of infertility, pathology and chromosome aberrations in testicular cells and embryonic deaths, dominant lethal mutations and skeletal malformations were reported in the C3H and AK mouse strains by Cilievici et al. (1983). However, these unconfirmed findings should be viewed with caution because very limited data and study details were provided, the purity of the test substance was not specified, and the sample size was inadequate. In addition, the data indicating germinal cell effects were not supported by the two-generation reproductive study (MRID No. 43935801); there was no evidence in this study of increased infertility or embryotoxicity. Ziram did, however, produce equivocal evidence of malformations in rabbits (MRID No. 00161316) but not in rats (MRID 41908701). Nevertheless, Hemavathi et al. (1993), demonstrated sperm abnormalities in Swiss albino mice receiving intraperitoneal administrations of 50 or 100 mg/kg (single dose) or 25 mg/kg ziram (purity not specified) once daily for 5 days. While the induction of spermhead abnormalities may not be related to genetic damage in the exposed male, these findings do show that ziram or its metabolites are capable of reaching the testes. However, the Committee concluded that a dominant lethal assay should be conducted to address heritable risk concerns. The available studies submitted by the registrant satisfy the pre-1991 test guidelines for mutagenicity.

3. Structure-Activity Relationship

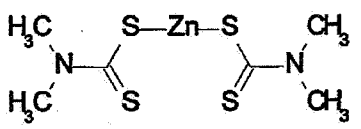
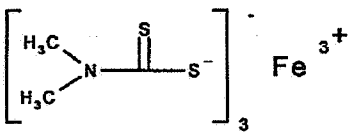
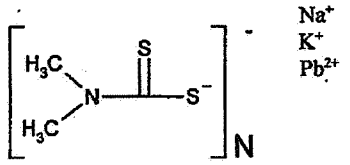
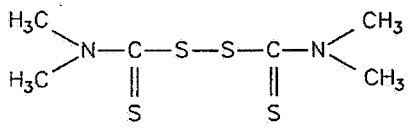
Ziram is a member of the dimethyldithiocarbamate class of compounds, which includes thiram, the environmental degradation product of ziram as well as the ferric dimethyldithiocarbamate, ferbam. Table 5 summarizes the carcinogenicity and mutagenicity of compounds structurally related to ziram and ferbam. Literature studies on ferbam showed no evidence of carcinogenicity. The genotoxicity profile for thiram is similar to ziram (i.e., positive for gene mutations in *S. typhimurium* TA 1535 and TA100, clastogenic in CHEL and CHO cells at noncytotoxic S9-activated doses but with a preponderance of unstable structural chromosomes and no alkylating activity toward select nucleophiles) and causes increased thyroid gland C-cell hyperplasia in male rats (MRID No. 4215601). In contrast to ziram however, thiram was shown to induce reproducible increases in micronuclei in the bone marrow cells of male mice. There is also evidence from the open literature and the submitted studies that thiram causes decreased fertility in rats, embryo toxicity in rats and hamsters and teratogenicity in mice and hamsters. Other structural analogues (e.g., ferbam and the sodium and potassium salts of dimethyldithiocarbamate) were also positive in *S. typhimurium* TA 1535 and

TA100; negative for SCE induction in mammalian cells; and either negative or yielded equivocal results for mammalian cell gene mutations in CHO cells. Both the sodium and potassium salts produced conflicting results in the developmental toxicology studies that were performed (i.e, Na was negative in both rabbits and rats while K was negative in the rat but induced malformations and other adverse fetal effects in the rabbit). No data were available on the carcinogenic potential of either salt. Ferbam was also mutagenic in *S. typhimurium* TA1535 and TA 100. Short et al. (1976) found that ferbam has little or no adverse effect on reproduction and was judged to be not teratogenic in mice or rats. The International Agency for Research on Cancer (IARC, 1976) indicated that ferbam did not induce a carcinogenic effect in mice or rats but listed ferbam as Group 3 (i.e., unclassifiable as to carcinogenicity in humans).

Despite the wealth of data on ziram and other dimethyldithiocarbamates, the only consistent finding among the studied members of this chemical class was a direct mutagenic effect on the base-pair substitution strains of *S. typhimurium*. In agreement with the latter statement, Moriya et al. (1983) in their evaluation of pesticides in microbial systems found that 7 of the 13 dimethyldithiocarbamates studied did not require exogenous metabolic activation to induce mutations in *S. typhimurium* TA1535 or TA100 and that all of the positive dimethyldithiocarbamates share a common moiety, (CH3)2NCSS-, which appears to be essential for mutagenicity. While the dimethyl moiety may confer genotoxic activity, its role in the process of carcinogenicity and the potential electrophilicity of ziram is not clear since lead dimethyldithiocarbamate and possibly ferbam are not carcinogenic but are positive in *S. typhimurium* TA100 (Zeiger, 1987). Additionally, Ashby and Tennant (1991) list ziram as a "non-alerting" carcinogen affecting a single species, sex and site. A similar conclusion can be reached for thiram.

Since the structure of ziram does not suggest electrophilicity and there was no evidence of acellular alkylation of nucleotides, attempts by several investigators to uncover the mechanism of mutagenic action toward bacteria have failed. Nevertheless, ziram does induce ChEI indicating that the test substance has the capacity to bind to macromolecules. Hence, the underlying basis for mutagenicity is unclear. However, until a plausible explanation is obtained for the disparate results, the weight-of-the-evidence indicates that a mutagenic mode of action cannot be ruled out for ziram. Also, the mutagenicity evidence for close analogues supports the mutagenicity concern for ziram. There are no carcinogenicity studies available for the sodium, potassium, and lead dimethyldithiocarbamate salts. Ferbam and all three salts are positive in the Ames TA 100 mutagenic assay. Ferbam is also positive in the Ames TA 1535 mutagenic assay. Thiram is classified by the CARC as "not likely to be carcinogenic in humans." Thiram is positive in the Ames TA 1535 and TA 100 mutagenic assays and the mouse micronucleus assay and is clastogenic in CHEL and CHO cells.

Table 5. Carcinogenicity of ziram / ferbam and structurally related compounds.

Compound	Structure	Carcinogenic Effect	Carcinogen Class/ Mutagen
Ziram		Hemangiomas in lymph nodes and spleen in male rats. NTP studies showed thyroid gland C-cell adenomas in male rats and lung tumors in female mice.	Not classified IARC - Group 3 Positive: Ames TA 1535, TA 100 Mouse lymphoma Clastogenic in CHO, CHEL, Human lympho.
Ferbam		No studies submitted to the Agency. Literature studies did not show any evidence of carcinogenicity.	Not classified IARC - Group 3 Positive: Ames TA 1535, TA 100
Na, K, Pb salts		No studies available.	Not classified Positive: Ames TA 100 All 3 salts
Thiram		No significant increase in tumor incidence in mice or rats.	Classified as "Not likely to be carcinogenic in humans" Positive: Ames TA 1535, TA 100 Micronucleus Clastogenic in CHEL and CHO cells

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4. Subchronic and Chronic Toxicity

A) Subchronic Toxicity

Rat

In a subchronic oral toxicity study (MRID 42450301), treatment-related increases in brain and spleen weights relative to body weight were seen in both sexes at 300 ppm and 1000 ppm ziram, but with no concomitant histopathology. There was a slight increase in the incidence of centrilobular hepatocyte necrosis in one lobe in females at 300 ppm (1/10) and 1000 ppm (1/10) as compared to the controls (0/10). Also, increases in the incidence of localized epithelial hyperplasia in the stomach was noted in both sexes at 1000 ppm (1/10 males, 3/10 females) and in females at 300 ppm (1/10). Body weight gain decreased in both sexes at 300 ppm (82% of controls for both) and at 1000 ppm (67% for males, 68% for females). The LOAEL was 300 ppm (21.4/24.2 mg/kg/day in male/female) based on decreased body weight, body weight gain, and food consumption, and minimal histopathological changes in the liver (females). The NOAEL was 100 ppm (7.4/8.8 mg/kg/day in male/female). This study was classified as acceptable/guideline.

B) Chronic Toxicity

Rat

Refer to Rat Combined Chronic Toxicity/Carcinogenicity Study on page 1 of this report.

The LOAEL was 60 ppm (2.5/3.4 mg/kg/day in male/female) based on increased hemosiderosis in the spleen, increased epithelial hyperplasia and subepithelial edema in the non-glandular region of the stomach, increased incidence of narrowed peripheral muscle fiber bundles in skeletal muscle, and increased cortical hypertrophy with vacuolation in the adrenals in males and an increased incidence of prominent ultimobranchial cysts in the thyroid in females. No NOAEL was determined in this study.

Mouse

Refer to Mouse Carcinogenicity Study on page 7 of this report.

The LOAEL was 225 ppm (27/33 mg/kg/day for males/females) 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. The NOAEL was 75 ppm (9 and 11 mg/kg/day in males and females, respectively).

Dog

In a chronic feeding study (MRID No. 42823901), ziram (98.5%; Lot No. 8331 AA) was administered for 52 weeks in the diet to four male and four female beagle dogs per dose at concentrations of 0, 50, 185, and 700 ppm (700 ppm dose reduced to 500 ppm at day 3 of week 12), equivalent to doses of 0, 1.6, 6.6, 17.4 mg/kg/day for males and 1.9, 6.7, and 20.6 mg/kg/day for females, respectively.

There was a treatment-related convulsive episode at week 11 for a female in the 700/500 ppm dose group that required the animal to be euthanitized. In addition to the convulsive episode, the findings for the 700/500 ppm and 185 ppm dose groups include: 1) decreased body weight gain for females over the treatment period, 2) changes in clinical chemistry parameters-decreases in albumin (males and females) and total protein levels (females) and increases in SGPT (males) and alkaline phosphatase (males), and 3) histologic findings for livers (aggregates of Kupffer cells and macrophages and increased infiltration of inflammatory cells in males and females) and spleens (pigmented macrophages; males). The NOAEL is 50 ppm based on the lack of significant toxicological effects. The LOAEL is 185 ppm based on decreased body weight gain in females, and increased liver pathology accompanied by corresponding clinical chemistry changes and the occurrence of pigmented macrophages in the spleen in males.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity:

- **The CARC concluded that ziram was carcinogenic in male CD (SD) BR and F344/N rats.**

  In CD (SD) BR male rats, there were significant ($p<0.05$) differences in the pair-wise comparisons of the 540 ppm group with the controls for mesenteric lymph node hemangiomas 5/49, 10% vs 0/50, 0% in controls) and mesenteric lymph node and spleen hemangiomas combined (6/49, 12% vs 0/50, 0% in controls). Significant ($p<0.01$) increasing trends were also evident for these tumors. Hemangiomas are benign tumors and rarely progress to malignancy. The incidence of hemangiomas exceeded the historical controls range (0%-4%). The CARC considered these tumors to be treatment-related. The dosing at the highest dose was considered to be adequate and not excessive in both sexes based on decrease in body weight gain ($\geq$14%), increased incidence of C-cell hyperplasia and histopathological changes in various organs (kidney, bile duct, mammary gland, stomach, spinal cord etc.) in one or both sexes. The selection of dose levels was supported by similar findings in a 90-day study.

In F344/N male rats, there was a significant increase by pair-wise comparison with the controls for C-cell carcinomas (7/49, 14%, $p<0.01$ vs 0/50, 0% in controls) and combined C-cell adenomas/carcinomas (12/49, 24%, $p<0.05$ vs 4/50, 8% in controls) of thyroid at 600 ppm (22 mg/kg/day). A significant dose-related significant ($p<0.01$ or $p<0.05$) increasing trend for these tumors was also evident. The incidences of these tumors at 600 ppm were outside the historical controls range (carcinoma: 0%-8% and combined: 0%-20%). The CARC, therefore, concluded that these malignant tumors in males were treatment-related. The Committee agrees with NTP's assessment of dosing and determined that the animals could have tolerated higher dose levels.

There was no treatment-related increase in tumors in female Sprague-Dawley and F344/N rats.

- **Ziram was carcinogenic to female B6C3F1 mice because** there was a significant ($p<0.05$) increase by pair-wise comparison with the controls for pulmonary alveolar/bronchiolar adenomas (10/50, 20% vs 2/50, 4% in controls) and combined adenomas/carcinomas (11/50, 22% vs 4/50, 8% in controls) at 1200 ppm (248 mg/kg/day, respectively) in females. There was a significant positive trend for alveolar/bronchiolar adenomas ($p<0.01$) and combined tumors ($p<0.05$) and a dose-related increase in the incidences at 600 and 1200 ppm (adenomas: 10% and 20%, respectively; combined: 12% and 22%, respectively). The incidences of these tumors at 1200 ppm were outside the historical control range (adenomas: 0%-14%; combined: 0%-16%). Lung tumors were seen in treated females but not in males. The Committee concluded that despite the fact that Sendai virus infection can affect the rate of spontaneous tumors (Cera, 1992), the compound-related effect in female can not be ruled out. Therefore, the increased incidences of alveolar/bronchiolar tumors at 600 and 1200 ppm in females were considered by the CARC to be treatment-related. The dosing was considered to be adequate and not excessive based on ≥26% decrease in body weight gain in both sexes in a 13-week study. There was no adverse effect on the survival of animals.

  Ziram was not carcinogenic to male B6C3F1 mice and CD-1 male and female mice.

2. Mutagenicity

- Ziram is primarily a bacterial mutagen with the capacity to bind to macromolecules (i.e., ChE). Although results for mammalian cell assays were in conflict, the preponderance of data from the cytogenetic studies favor a positive response. Based on the direct mutagenic effect on base-pair substitution strains of *S. typhimurium* and *E. coli* and the evidence of clastogenicity in mammalian

cells, the Committee determined that there is a sufficient evidence for a mutagenic concern. The CARC, therefore, recommended that a dominant lethal assay be conducted by the registrant to address a possible concern for heritable effects.

3. Structure Activity Relationship

- Structurally related dithiocarbamate compounds including thiram, ferbam as well as sodium, potassium and lead dithiocarbamate salts were also mutagenic in the base-pair substitution strains of *Salmonella*. Thiram and ferbam (published literature only) were also not carcinogenic and no data were available regarding the carcinogenicity of dithiocarbamate salts.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the Agency's *Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the Committee classified ziram into category "Likely to be carcinogenic to humans" based on the occurrence of C-cell thyroid tumors and hemangiomas in male rats and lung tumors in female mice.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended a linear low-dose extrapolation approach for the quantification of human cancer risk based on C-cell thyroid tumors in male rats. This approach is supported by the findings of benign lung tumors in female mice, lack of mode of action data, and the mutagenicity evidence for ziram.

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