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MEMORANDUM

SUBJECT: ZIRAM - Report of the Hazard Identification Assessment Review Committee.

FROM: David Nixon, D.V.M., Toxicologist.
Reregistration Review Branch 4
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Susan Hummel, Branch Senior Scientist
Reregistration Review Branch 4
Health Effects Division (7509C)

PC Code: 034805

On January 11, 2000, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for **ziram** with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to **ziram** was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. The conclusions drawn at this meeting are presented in this report.

Committee Members in Attendance

Members present were: William Burnam, Vicki Dellarco, Elizabeth Doyle, Pamela Hurley, Tina Levine, Elizabeth Mendez, David Nixon, Nicole Paquette, Jess Rowland, and Brenda Tarplee.

Member(s) in absentia: None

Data evaluation prepared by: David Nixon, D.V.M., TOXICOLOGIST, RRB4

Also in attendance were: Tim Leighton (HED), Gary Otakie (HED), Ray Kent (HED), Laura Parsons (SRRD).

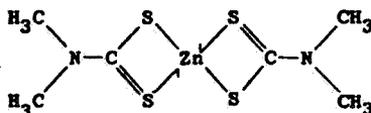
Data Evaluation / Report Presentation

David Nixon, D.V.M.
Toxicologist

Results were identified on GD 28.

1. INTRODUCTION

On January 11, 2000, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for ziram with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to ziram was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996.



Ziram

2. HAZARD IDENTIFICATION

2.1 Acute Reference Dose (RfD) - Subpopulation: Females 13-50

Study Selected: Prenatal Oral Developmental/ Rabbit OPPTS 870.3700 (§83-3b)

MRID No.: 00161916

Executive Summary: In a developmental toxicity study (MRID 00161316), ziram technical (98% a.i.) in 1% aqueous methyl cellulose was administered by gavage to pregnant New Zealand White rabbits (16/dose) at concentrations of 0, 3, 7.5, or 15 mg/kg/day on GDs 7 through 19. Does were sacrificed on GD 28.

One high-dose doe died on GD 23 and one mid-dose doe died on GD 13. Additionally, one control doe and one mid-dose doe were sacrificed *in extremis* on GDs 14 and 15, respectively; clinical signs observed prior to death in these two animals included weight loss, anorexia, and wheezing. These deaths were not considered to be the result of treatment due to the lack of a dose-response relationship. No other premature deaths occurred and no treatment-related clinical signs of toxicity were observed at any dose level.

At 15 mg/kg, decreased body weights were observed over GDs 0-28 (↓3-17%, $p \leq 0.01$ on GD 10 only). Additionally, for the overall treatment interval (GDs 7-19) and overall study interval (GDs 0-28) body weight gain, as calculated by the reviewers, were reduced as compared to the control (treatment, ↓81%; study, ↓30%, not analyzed for statistical significance). Decreases ($p \leq 0.01$) in absolute (g/animal/day) food consumption were observed beginning at the GDs 7-10 interval (↓19%) and continuing throughout the GDs 13-16 interval (↓44-49%); decreased consumption was also observed for the GDs 16-19 interval (↓24%, not statistically significant [NS]). Food

consumption was reduced for the overall treatment interval (↓34%, GDs 7-19) and for the overall study interval (↓18%, GDs 0-28).

At 7.5 mg/kg, decreased body weight gain was observed over GDs 7-19 (↓30%) and GDs 0-28 (↓19%). No other treatment-related maternal effects were noted at the mid-dose level.

No treatment-related findings were observed at gross necropsy of maternal animals.

The number of implantations/doe and percent male were similar between control and treated groups.

The maternal LOAEL is 7.5 mg/kg/day, based on decreased body weight gain. The maternal NOAEL is 3 mg/kg/day.

Reduced atrium/atria, a minor defect (variation), was observed at the mid- (fetal 2.9%; litter, 14.3%) and high-dose (fetal, 2.8%; litter, 20.0%) levels vs controls (fetal, 0.8%; litter, 6.7%); this finding was observed in a dose-dependent manner and without the %fetal and %litter incidence ranges in the historical data, this variation was considered to be equivocally treatment-related.

At the high-dose level, increases (NS) as compared to the control were observed in the total number of resorptions/doe (↑88%) and the percent postimplantation loss (↑97%). Additionally, reductions (NS) in the number of live fetuses/doe (↓15%) were noted. Upon skeletal examination, absence of the interparietal bone, a major defect (malformation), was observed at the high-dose level only (fetal, 1.9%; litter, 13.3%) vs 0 controls; since this finding was only observed at the high-dose level and without the %fetal and %litter incidence ranges in the historical data, this malformation was considered equivocally treatment-related.

The developmental LOAEL is 7.5 mg/kg/day, based on increased incidence of reduced atrium/atria. The developmental NOAEL is 3 mg/kg/day.

This developmental toxicity study is classified **acceptable/guideline (§83-3[b])** and does satisfy the guideline requirement for a developmental toxicity study in the rabbit; it would be helpful if historical control data (% fetal and % litter incidences) were provided for reduced atrium/atria and absence of the interparietal bone.

Dose and Endpoint for Establishing RfD (Females 13+): Developmental NOAEL = 3 mg/kg/day based on increased incidence of reduced atrium/atria, at 7.5 mg/kg/day (LOAEL).

Uncertainty Factor (UF): 100

Comments about Study/Endpoint/Uncertainty Factor: Fetal effects are presumed to occur from a single dose (acute exposure), and since they occur *in utero*, the selected endpoint is appropriate for this population subgroup (♀ 13-50).

Acute RfD = (Females 13+)	$\frac{3 \text{ mg/kg}}{100}$	=	0.03 mg/kg
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This risk assessment is required.

2.1.1 Acute Reference Dose (RfD) - General Population

Study Selected: Acute Oral Neurotoxicity/ Rat

OPPTS 870.6200 (§81-8)

MRID No.: 43362801

Executive Summary: In an acute neurotoxicity study (MRID 43362801), male and female Sprague-Dawley Crl:CD BR® rats received a single gavage dose of 0, 15, 300, or 600 mg/kg of Ziram (tech., 97.8% a.i.) in corn oil (7.5 mL/kg). The 0, 15, and 300 mg/kg groups consisted of 12 animals/sex, the 600 mg/kg group consisted of 16 animals/sex. Functional observational battery tests (FOB) and motor activity were recorded for all animals. FOB and motor activity evaluations were conducted pretreatment, at the time of peak effect (4 hours post-dosing), and on days 7 and 14. At necropsy, brain weights and dimensions were determined for all animals. Five animals/sex were selected for neuropathological evaluation in the control and 600 mg/kg groups.

Four males and three females in the high-dose group died on day 1; three other high-dose females died on days 2, 4, or 5 and one mid-dose female died on day 2. The cause of these deaths is unknown. Gross observations at necropsy revealed white contents of stomach/intestines (probably from corn oil), stomach distention, and in two high-dose females, emaciation. There were no findings consistent with trauma induced by gavage error. The two severely affected mid-dose males (Nos. 15355 and 15387) that survived the 2-week observation period exhibited cyanosis and hypothermia. However, neither cyanosis nor hypothermia were reported in the animals that died on study.

No effects on body weight were apparent in the low-dose group and transient effects were seen in high-dose males. In the mid-dose males, the mean body weights were significantly ($p < 0.01$) lower on days 7 (12%) and 14 (16%) for mid-dose males compared with the control group means. The decreased body weights in the mid-dose group on day 14 were attributed to two males (Nos. 15355 and 15387), with body weights of 159 g and 161 g, respectively, compared with a control group mean of 321 g. Body weights of females were not affected; however, body weight gain was transiently reduced during days 0 - 7 in both males and females at mid and high-dose.

The most significant and biologically relevant findings of treatment were clinical signs of toxicity and effects observed during FOB and motor activity tests. Although both sexes in the mid- and high-dose groups were affected, several of the findings were limited to or occurred

most frequently in two mid-dose males (Nos. 15355 and 15387). Clinical signs were generally seen in the first week of the study (but persisted to day 15 in the two mid-dose males) and included dose-related increased incidences of gait alterations, abnormal respiration, abnormal excreta, and distended abdomen. Cyanosis and enophthalmus were limited to two mid-dose males (No. 15355 and 15387) and were seen on day 8 or later on three occasions. Rales observed on one occasion in one low-dose male cannot be clearly attributed to treatment with the test material.

In the FOB evaluations, all six of the functional domains were affected in the mid- and high-dose groups. In general, the responses occurred approximately 4 hours after dosing and were transient in nature (none persisted to day 7). Notable effects on day 0 included altered posture, palpebral closure (eye lid slightly drooping to shut), altered feces consistency, slight lacrimation, slight to severe salivation, red/crusty deposits around nose and mouth, impaired mobility and altered gait, and decreased body temperature. Impaired gait and ataxia were also noted in males at 15 mg/kg on day 0. During the FOB on day 14, several findings were noted in the two most severely affected mid-dose males (altered posture, altered palpebral closure, enophthalmus, impaired mobility, absent startle response and hindlimb extension). It should be pointed out that some findings in these two animals (gasping, mucous membrane change and color, impaired righting reflex) were not even observed on day 7 or day 0, or in animals in the high-dose group. A low incidence of effects on FOB parameters was seen in the low-dose group. These effects (affecting neuromuscular and CNS activity in 1-2 animals) were minimal and cannot be attributed unequivocally to treatment with the test material because of the subjectivity of the endpoints. Significantly ($p < 0.05$) decreased motor activity was seen in mid- and high-dose males and females. Total motor activity and ambulatory activity counts were reduced by as much as 82-87% and 76-87%, respectively, compared with controls. However, complete recovery was observed by day 7 in mid-dose males and females and in high-dose females; high-dose males recovered fully by study day 14. Even though the mean counts were not affected in the mid-dose males on day 14, the total motor activity and ambulatory activity counts for males (Nos. 15355 and 15387) were lower than the respective controls and lower than their day 7 values.

There was a dose-related decrease in absolute brain weights which was statistically significant at 300 and 600 mg/kg. No treatment-related effects on brain dimensions were noted. No treatment-related lesions were observed in central or peripheral nervous system tissues examined from the control or high-dose group.

The LOAEL is 15 mg/kg, based on ataxia and slight impairment of gait in males. No NOAEL was determined.

This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for an acute neurotoxicity study (81-8) in rats.

Dose and Endpoint for Establishing RfD (Gen. Population): 15 mg/kg/day based on ataxia and slight impairment of gait in males.

Uncertainty Factor (UF): 300

Comments about Study/Endpoint/Uncertainty Factor: An additional uncertainty factor of 3 was applied because of the use of a LOAEL (No NOAEL in this study). A 3X was chosen as compared to a 10X since two repeated dose subchronic tests (MRIDs 42450301, 43413701) did not show ataxia in rats at much higher dosages and the most pertinent route of exposure is ingestion of residues on food, not ingestion of the compound alone (the acute study is a gavage study).

$\begin{array}{l} \text{Acute RfD} = \\ \text{(Gen. Pop'n)} \end{array} = \frac{15 \text{ mg/kg}}{300} = 0.05 \text{ mg/kg}$
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This risk assessment is required.

2.2 Chronic Reference Dose (RfD)

Study Selected: Chronic Oral Toxicity/ Dog
(§83-1b)

OPPTS 870.4100

MRID No.: 42823901

Executive Summary: In a chronic feeding study (MRID No. 428239-01), 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 euthanized. In addition to the convulsive episode, the findings for the 700/500-ppm dose group include: 1) decreased body weight gain (↓181%) in females over the treatment period and 2) histologic findings for livers (aggregates of Kupffer cells and macrophages, increased foci of degenerate hepatocytes, infiltration of inflammatory cells around central veins and branches of the hepatic vein and portal areas, and increased centrilobular fibrocytes in males). The findings for the 185-ppm dose group include decreased body weight gain (↓69%) in females during the treatment period. **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.**

Classification: This study is classified as **acceptable**. The study satisfies most of the guideline requirements for a chronic feeding study in beagle dogs (§83-1).

Dose and Endpoint for Establishing RfD: 1.6 mg/kg/day based on decreased body weight gain in

females.

Uncertainty Factor(s): 100

Comments about Study/Endpoint/Uncertainty Factor: This study was chosen since there is a LOAEL and NOAEL and the endpoint, body weight gain, is also observed in other oral and dermal studies. The rat chronic/carcinogenicity study (MRID 43404201) supports the dose chosen in the chronic dog study. The LOAEL was 2.5 mg/kg/day 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. With an additional uncertainty factor of 3 applied because of the use of a LOAEL (No NOAEL in this study), the proposed RfD would result in 0.0083 mg/kg/day.

$$\text{Chronic RfD} = \frac{1.6 \text{ mg/kg/day}}{100} = 0.016 \text{ mg/kg/day}$$

This risk assessment is required.

2.3 Occupational/Residential Exposure

2.3.1 Dermal Absorption

Dermal Absorption Factor: A dermal absorption study is available, but considered unacceptable. Dermal absorption was estimated using the LOAEL of 1000 mg/kg/day in the 21-day dermal toxicity rabbit study (MRID 41297001) and the LOAEL of 7.5 mg/kg/day in the rabbit oral developmental study (MRID 00161316), both studies having the same endpoint - body weight decrement.

$$\frac{7.5}{1000} \times 100 = 0.75 \% \text{ or } \sim 1 \% \text{ dermal absorption factor}$$

2.3.2 Short-Term Dermal (1-7 days) Exposure

Study Selected: 21-day Dermal Toxicity/ Rabbit

OPPTS 870.3200 (§82-2)

MRID No.: 41297001

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.

Dose and Endpoint for Risk Assessment: 300 mg/kg/day based on decreased body weight and food consumption and clinical chemistry changes suggestive of minimal hepatotoxicity.

Comments about Study/Endpoint: The experimental conditions (dermal exposure) simulate actual exposure (dermal) scenarios and, thus, this study is appropriate for use in this risk assessment.

This risk assessment is required.

2.3.3 Intermediate-Term Dermal (7 Days to Several Months) Exposure

Study Selected: Same as Short-term Dermal

MRID No.: 41297001

Executive Summary: See Short-term Dermal

Dose/Endpoint for Risk Assessment: 300 mg/kg/day based on decreased body weight

and food consumption and clinical chemistry changes suggestive of minimal hepatotoxicity.

Comments about Study/Endpoint: See Short-term Dermal.

This risk assessment is required.

2.3.4 Long-Term Dermal (Several Months to Life-Time) Exposure

Study Selected: Chronic Oral Toxicity/ Dog
(§83-1b)

OPPTS 870.4100

MRID No.: 42823901

Executive Summary: In a chronic feeding study (MRID No. 428239-01), 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 euthanized. In addition to the convulsive episode, the findings for the 700/500-ppm dose group include: 1) decreased body weight gain (↓81%) in females over the treatment period and 2) histologic findings for livers (aggregates of Kupffer cells and macrophages, increased foci of degenerate hepatocytes, infiltration of inflammatory cells around central veins and branches of the hepatic vein and portal areas, and increased centrilobular fibrocytes in males). The findings for the 185-ppm dose group include decreased body weight gain (↓69%) in females during the treatment period. **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.**

Classification: This study is classified as **acceptable**. The study satisfies most of the guideline requirements for a chronic feeding study in beagle dogs (§83-1).

Dose and Endpoint for Risk Assessment: 1.6 mg/kg/day based on decreased body weight gain in females.

Comments about Study/Endpoint: See comments in Chronic RfD discussion. The 1% dermal absorption factor will need to be applied since this is an oral chronic study.

This risk assessment is required.

2.3.5 Inhalation Exposure (Short-term)

Study Selected: Prenatal Oral Developmental/ Rabbit OPPTS 870.3700 (§83-3b)

MRID No.: 00161916

Executive Summary: See Acute RfD (♀ 13-50).

Dose/Endpoint for Risk Assessment: Developmental: 3 mg/kg/day based on increased incidence of reduced atrium/atria.

Comments about Study/Endpoint: An inhalation absorption factor will need to be applied since this is an oral study. Assume a 100% inhalation absorption factor.

This risk assessment is required.

2.3.6 Inhalation Exposure (Intermediate/ Long-term)

Study Selected: Chronic Oral Toxicity/ Dog OPPTS 870.4100
(§83-1b)

MRID No.: 42823901

Executive Summary: See Chronic RfD.

Dose/Endpoint for Risk Assessment: 1.6 mg/kg/day based on decreased body weight gain in females.

Comments about Study/Endpoint: See comments in Chronic RfD discussion. The chosen dose is protective of the developmental endpoints of concern, and is taken from a study of appropriate duration of exposure (intermediate/long-term). An inhalation absorption factor will need to be applied since this is an oral study. Assume a 100% inhalation absorption factor.

This risk assessment is required.

2.3.7 Margins of Exposure for Occupational/Residential Risk Assessments

A MOE of 100 is required for occupational dermal and inhalation exposure risk assessment. The MOEs for residential exposure risk assessment will be determined by the FQPA Safety Factor Committee.

2.4 Recommendation for Aggregate Exposure Risk Assessments

For **acute** aggregate exposure risk assessment, combine the **high end** exposure values from food + water and compare it to the acute RfD.

For **chronic** aggregate exposure risk assessment, combine the **average** exposure values from food plus water and compare it to the chronic RfD.

Short-term dermal and inhalation risk assessments must be done separately due to different endpoints; dermal = decrease in body weight and inhalation = developmental effects.

For **intermediate- and long-term** aggregate exposure risk assessment, the aggregate systemic (oral), dermal and inhalation exposure risk assessments are appropriate due to the common toxicological endpoint (body weight decrements) seen via the three routes.

3 CLASSIFICATION OF CARCINOGENIC POTENTIAL

3.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats

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

There was no excess mortality in any of the treated groups relative to controls. Group mean body weight gains were decreased for males (86% of control, $p < 0.01$) and females (74% of control, $p < 0.01$) in the high dose group (540 ppm). Food consumption was decreased compared to controls for males (540 ppm: 91%, $p < 0.01$) and females (180 ppm: 92%, $p < 0.05$; 540 ppm: 94%, $p < 0.05$). Hematology parameters (RBC, HGB, and PCV) were decreased relative to controls for females in the 540 ppm (weeks 26-104, $p < 0.05$, $p < 0.01$) and 180 ppm (weeks 26-52, $p < 0.05$, $p < 0.01$) dose groups. There were statistically significant decreases ($p < 0.05$, $p < 0.01$) in clinical chemistry parameters (calcium, total protein, albumin, calcium and SGPT) during weeks 13-52 for females. For males (540 ppm, week 104) organ weight for the adrenals was decreased (absolute, 59% of control, $p < 0.01$; relative, 67% of control, $p < 0.05$). There were macroscopic pathological findings (not statistically significant) for animals in the 180 and 540 ppm-dose groups for the stomach and skeletal muscle (males and females), and the adrenals (females only). There were microscopic pathological findings for males and females in the 180 and 540 ppm dose groups for spleen ($p < 0.01$), liver ($p < 0.01$, $p < 0.05$), stomach ($p < 0.05$, $p < 0.01$), thyroid ($p < 0.01$, $p < 0.05$), skeletal muscle ($p < 0.01$), spinal cord (males only, $p < 0.05$), sciatic nerve (females only, $p < 0.01$), and adrenal cortex ($p < 0.05$, $p < 0.01$). As there were histopathological findings for males in the 60 ppm dose group for spleen ($p < 0.01$), stomach ($p < 0.01$, $p < 0.05$), skeletal muscle ($p < 0.05$), and adrenal cortex ($p < 0.05$), a NOAEL for males could not be identified. For females, there was an increase in prominent ultimobranchial cysts in the thyroid

in all dose groups (Controls: 3/50; 60 ppm: 12/50, $p < 0.05$; 180 ppm: 22/50, $p < 0.01$; 540 ppm: 27/50, $p < 0.01$), precluding the identification of a NOAEL for females. **The NOAEL could not be identified for either males or females, due to histopathological findings for animals in the low dose group (60 ppm).**

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

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

MRID No. 43404201

Discussion of Tumor Data: Carcinogenic potential was evidenced by the finding of treatment-related tumors (benign hemangioma) in mesenteric lymph nodes (5/50, $p < 0.05$) and in spleen (1/50) in males in the 540-ppm dose group. There were no treatment-related tumors identified in males in the 180- or 60-ppm dose groups, or in females in any dose group. There were no treatment-related malignant tumors in either sex.

Adequacy of the Dose Levels Tested: Doses were adequate to assess carcinogenicity based on histopathological findings at the low dose and decreased body weight gains at the high dose with no increasing trends in mortality with increasing doses of ziram.

3.2 Carcinogenicity Study in Mice

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 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, 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 (9 mg/kg/day for males, 11 mg/kg/day for females). The LOAEL is 225 ppm (27 mg/kg/day for males, 33 mg/kg/day for 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.

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.

MRID No.: 43373701

Discussion of Tumor Data: No increased incidence of tumors.

Adequacy of the Dose Levels Tested: Dosing was considered adequate to assess carcinogenic potential based on decreased absolute brain weights in both sexes and increased incidence of urinary bladder epithelial hyperplasia and decreased body weight gain in males as 225 ppm, decreased body weight gain in females at 675 ppm, and no increasing trends in mortality with increasing doses of ziram.

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

Executive Summary: 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. 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.

There were no treatment-related effects on mortality, clinical signs, body weight, or food consumption, and minimal non-neoplastic histopathology. 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.

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

Citation: U.S. National Toxicology Program (1983) Carcinogenesis Bioassay of Ziram (Cas No. 137-30-4) in F344/N Rats and B6C3F₁ Mice (Feed Study)(NTP Technical report series No. 238), Research Triangle Park, NC.

Discussion of Tumor Data: C-cell carcinomas of the thyroid in male rats occurred with a statistically significant positive trend ($p < 0.01$) and the incidence 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%). The incidence in the high-dose group also exceeded historical control incidences from the same laboratory (18/584, 3%; range 0% to 8%). The combined incidence of males with either C-cell adenoma or carcinoma also 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 follicular cells.

Adequacy of the Dose Levels Tested: Since there were no treatment-related effects on mortality, clinical signs, body weight, or food consumption, and minimal non-neoplastic histopathology, NTP concluded that the amount of dosing may not have been adequate and rats of both sexes may have tolerated higher doses. Dosing was selected based on decreased mean body weight gain in a 13-week study.

3.4 National Toxicology Program Two-year Carcinogenicity Study with ziram in B6C3F₁ mice

Executive Summary: 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 B6C3F₁ mice per group at 0, 600, or 1200 ppm. 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.

No treatment-related effects on mortality or clinical signs were noted. There were treatment-related 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), decreases in food consumption at 1200 ppm in males (78% of control) and in females (85% of control), and histopathology findings.

Alveolar epithelium hyperplasia in the lungs was noted in females in the control group and both dose groups and was dose-related (control, 2/50, 4%; 600 ppm, 4/49, 8%; 1200 ppm, 10/50, 20%). Alveolar epithelium hyperplasia in the lungs 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 1200-ppm group females with the adenomatous hyperplasia had pulmonary tumors, whereas four of the 24 1200-ppm group females without pulmonary adenomatous hyperplasia had pulmonary tumors also. One of 27 600-ppm group females with adenomatous hyperplasia had a pulmonary tumor. Cystic follicles in the thyroid occurred at increased incidences in females at 1200 ppm (controls, 0/47; 1200 ppm, 21/48, 44%). Lymphoid hyperplasia was seen at increased incidences at 600 and 1200 ppm in females (controls, 0/50; 600 ppm, 2/50, 2%; 1200 ppm, 7/50, 14%).

NTP concluded that oral administration of ziram to female B6C3F₁ mice resulted in increased incidences of alveolar/bronchiolar adenomas and of combined alveolar/bronchiolar adenomas or carcinomas. The interpretation of this increase in lung tumors, however, was complicated by an intercurrent Sendai virus infection.

Citation: U.S. National Toxicology Program (1983) Carcinogenesis Bioassay of Ziram (Cas No. 137-30-4) in F344/N Rats and B6C3F₁ Mice (Feed Study)(NTP Technical report series No. 238), Research Triangle Park, NC.

Discussion of Tumor Data: Alveolar/bronchiolar adenomas of the lung in female mice occurred with a statistically significant positive trend ($p < 0.01$) and the incidence in the 1200-ppm group was significantly higher ($p < 0.05$) than that in the controls (control, 2/50, 4%; 600 ppm, 5/49, 10%; 1200 ppm, 10/50, 20%). Combined alveolar/bronchiolar adenomas or carcinomas in female mice occurred with a statistically significant positive trend ($p < 0.05$) and the incidence in the 1200-ppm group was significantly higher ($p < 0.05$) than that in the controls (control, 4/50, 8%; 600 ppm, 19/50, 38%; 1200 ppm, 16/49, 33%). The incidences of alveolar/bronchiolar adenomas and combined adenomas or carcinomas in female mice at 1200 ppm exceeded the range for the historical controls also. Historical control data on alveolar/bronchiolar adenomas show an incidence of 18/501 (3.6%) from the same laboratory and 134/2788 (4.8%) in female mouse controls across the Bioassay Program with a range of 0/50 to 7/50 (14%). The combined incidence of alveolar/bronchiolar adenomas or carcinomas in 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 (16%) across the Bioassay Program.

Adequacy of the Dose Levels Tested: Dosing was considered adequate 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.

3.5 Classification of Carcinogenic Potential

The Cancer Assessment Review Committee met on February 9, 2000, to evaluate the carcinogenicity of ziram. The Committee classified ziram as "**likely to be carcinogenic to humans**" based on increased incidences of thyroid C-cell tumors in male F344/N rats and mesenteric lymph node and splenic hemangiomas in male CD(SD)BR rats and supported by increased incidences of pulmonary alveolar/bronchiolar tumors in female B6C3F₁ mice.

4 MUTAGENICITY

Six genetic toxicology studies on Ziram have been submitted. Studies from the National Toxicology Program (NTP) and from the open literature were also available. 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. Nevertheless, the induction of unstable chromosome aberrations by Ziram cast doubts on the relevance of this finding as an influence on the initiation of carcinogenesis. 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.

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 dimethyldithiocarbamate 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 can not 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 $\approx \geq 2$ -fold increases in mutant colonies of strain TA100 at 66.7-333.3 $\mu\text{g}/\text{plate}$ without S9 activation or 33.3-333.3 $\mu\text{g}/\text{plate} + \text{S9}$. Greater than 2-fold increases in mutant colonies were also seen for strain TA1535 at 66.6 and 100 $\mu\text{g}/\text{plate} + \text{S9}$.

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 microsomes 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 $\mu\text{g}/\text{plate}$ 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 microsomes gene mutation assay: Ziram was one of 250 coded compounds evaluated in NTP's collaborative mutagenicity screening project of the *S. typhimurium*/ mammalian microsomes gene mutation assay. Results were positive for strain TA100 at 10-333 $\mu\text{g}/\text{plate}$ without and with S9 derived from Aroclor 1254-induced rat livers and at 33-333 $\mu\text{g}/\text{plate}$ with hamster livers. Increases approaching or greater than 2-fold were also seen for strain TA1535 at 100 $\mu\text{g}/\text{plate}$ with rat liver S9 or at 33-333 $\mu\text{g}/\text{plate}$ 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 $\mu\text{g}/\text{mL}$) and in Trial 2 (0.1-1.8 $\mu\text{g}/\text{mL}$). Relative total growth was 18% at 1.0 $\mu\text{g}/\text{mL}$ or 8% at 1.4 $\mu\text{g}/\text{mL}$; lethality was seen at levels $\geq 1.8 \mu\text{g}/\text{mL}$. 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 to cytotoxic levels (doses that caused a $\geq 50\%$ reduction in the mitotic index) (0.025 $\mu\text{g}/\text{mL}$ -S9 or 1 $\mu\text{g}/\text{mL}$ +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 $\mu\text{g}/\text{mL}$ -S9 or 1.5 and 1.75 $\mu\text{g}/\text{mL}$ +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 $\mu\text{g}/\text{mL}$ -S9 or

0.16-1.75 $\mu\text{g}/\text{mL}$ +S9. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for *in vitro* cytogenetic mutagenicity data (Gulati 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 $\mu\text{g}/\text{mL}$). 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 (≥ 3.16 $\mu\text{g}/\text{mL}$). 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. 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.

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 $\leq 15\%$ 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 $\mu\text{g}/\text{mL}$ +S9 in CHEL cells or 1.0 or 2.15 $\mu\text{g}/\text{mL}$ +S9 in CHO cells. Under both test systems, the major types of aberrations scored at noncytotoxic doses (i.e, mitotic indices were $\geq 85\%$ of control for CHEL cells and $\geq 61\%$ 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 $\mu\text{g}/\text{mL}$ +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 $\mu\text{g}/\text{mL}$ to 44% of control at 15 $\mu\text{g}/\text{mL}$. 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 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 *D. 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 (MPCs) 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.

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 rat hepatocytes (MRID No. 41287801), in hepatocytes harvested from rats pretreated with either Aroclor 1254 or 3-methylcholanthrene (Shaddock *et al.*, 1990) or following *in vivo* exposure (Tinkler *et al.*, 1998).

5 FOPA CONSIDERATIONS

5.1 Adequacy of the Data Base

The following studies are available:

- Acute and subchronic neurotoxicity studies
- Developmental toxicity studies in Rat & Rabbit
- Two-Generation Reproduction Study
- Developmental Neurotoxicity Study

The database is adequate for FQPA evaluation.

5.2 Neurotoxicity

Acute Delayed Neurotoxicity - Hen N/A

Acute Neurotoxicity -§81-7 : In an acute neurotoxicity study (MRID 43362801), male and female Sprague-Dawley Crl:CD BR® rats received a single gavage dose of 0, 15, 300, or 600 mg/kg of Ziram (tech., 97.8% a.i.) in corn oil (7.5 mL/kg). The 0, 15, and 300 mg/kg groups consisted of 12 animals/sex, the 600 mg/kg group consisted of 16 animals/sex. Functional observational battery tests (FOB) and motor activity were recorded for all animals. FOB and motor activity evaluations were conducted pretreatment, at the time of peak effect (4 hours post-dosing), and on days 7 and 14. At necropsy, brain weights and dimensions were determined for all animals. Five animals/sex were selected for neuropathological evaluation in the control and 600 mg/kg groups.

Four males and three females in the high-dose group died on day 1; three other high-dose females died on days 2, 4, or 5 and one mid-dose female died on day 2. The cause of these deaths is unknown. Gross observations at necropsy revealed white contents of stomach/intestines (probably from corn oil), stomach distention, and in two high-dose females, emaciation. There were no findings consistent with trauma induced by gavage error. The two severely affected mid-dose males (Nos. 15355 and 15387) that survived the 2-week observation period exhibited cyanosis and hypothermia. However, neither cyanosis nor hypothermia were reported in the animals that died on study.

No effects on body weight were apparent in the low-dose group and transient effects were seen in high-dose males. In the mid-dose males, the mean body weights were significantly ($p < 0.01$) lower on days 7 (12%) and 14 (16%) for mid-dose males compared with the control group means. The decreased body weights in the mid-dose group on day 14 were attributed to two males (Nos. 15355 and 15387), with body weights of 159 g and 161 g, respectively, compared with a control group mean of 321 g. Body weights of females were not affected; however, body weight gain was transiently reduced during days 0 - 7 in both males and females at mid and high-dose.

The most significant and biologically relevant findings of treatment were clinical signs of toxicity and effects observed during FOB and motor activity tests. Although both sexes in the mid- and high-dose groups were affected, several of the findings were limited to or occurred most frequently in two mid-dose males (Nos. 15355 and 15387). Clinical signs were generally seen in the first week of the study (but persisted to day 15 in the two mid-dose males) and included dose-related increased incidences of gait alterations, abnormal respiration, abnormal excreta, and distended abdomen. Cyanosis and enophthalmus were limited to two mid-dose males (No. 15355 and 15387) and were seen on day 8 or later on

three occasions. Rales observed on one occasion in one low-dose male cannot be clearly attributed to treatment with the test material.

In the FOB evaluations, all six of the functional domains were affected in the mid- and high-dose groups. In general, the responses occurred approximately 4 hours after dosing and were transient in nature (none persisted to day 7). Notable effects on day 0 included altered posture, palpebral closure (eye lid slightly drooping to shut), altered feces consistency, slight lacrimation, slight to severe salivation, red/crusty deposits around nose and mouth, impaired mobility and altered gait, and decreased body temperature. Impaired gait and ataxia were also noted in males at 15 mg/kg on day 0. During the FOB on day 14, several findings were noted in the two most severely affected mid-dose males (altered posture, altered palpebral closure, enophthalmus, impaired mobility, absent startle response and hindlimb extension). It should be pointed out that some findings in these two animals (gasping, mucous membrane change and color, impaired righting reflex) were not even observed on day 7 or day 0, or in animals in the high-dose group. A low incidence of effects on FOB parameters was seen in the low-dose group. These effects (affecting neuromuscular and CNS activity in 1-2 animals) were minimal and cannot be attributed unequivocally to treatment with the test material because of the subjectivity of the endpoints.

Significantly ($p < 0.05$) decreased motor activity was seen in mid- and high-dose males and females. Total motor activity and ambulatory activity counts were reduced by as much as 82-87% and 76-87%, respectively, compared with controls. However, complete recovery was observed by day 7 in mid-dose males and females and in high-dose females; high-dose males recovered fully by study day 14. Even though the mean counts were not affected in the mid-dose males on day 14, the total motor activity and ambulatory activity counts for males (Nos. 15355 and 15387) were lower than the respective controls and lower than their day 7 values.

There was a dose-related decrease in absolute brain weights which was statistically significant at 300 and 600 mg/kg. No treatment-related effects on brain dimensions were noted. No treatment-related lesions were observed in central or peripheral nervous system tissues examined from the control or high-dose group.

The LOAEL is 15 mg/kg, based on ataxia and slight impairment of gait in males. No NOAEL was determined.

This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for an acute neurotoxicity study (81-8) in rats.

Subchronic Neurotoxicity- §82-5 : In a subchronic oral neurotoxicity study (MRID 43413701), 10 Sprague-Dawley Crl:CD@BR rats/sex/dose group received Ziram (tech., 97.87% a.i.) in the diet at concentrations of 0, 72, 207, or 540 ppm for 13 weeks. The

average consumption of test material was 5, 14, or 34 mg/kg/day (males) and 6, 16, or 40 mg/kg/day (females). Functional observational battery (FOB) and motor activity tests were conducted on all animals during weeks 3, 7, and 12. In each group, 5 animals/sex were allocated to cholinesterase/neurotoxic esterase evaluations and 5 animals/sex to neurohistopathologic evaluations.

At 540 ppm, the mean weekly body weights in males and females were 7% to 11% lower compared with controls beginning at week 1 and throughout the study period. The cumulative body weight gains (weeks 0 to 13) in males and females, respectively, were 18% and 32% lower than control values due in part to reduced food consumption, particularly during the initial study week (31% and 24% of controls for males and females, respectively). The LOAEL for systemic toxicity is 540 ppm (34 mg/kg/day in males, 40 mg/kg/day in females) based on decreased body weights and body weight gains; the corresponding NOAEL is 207 ppm (14 mg/kg/day in males, 16 mg/kg/day in females).

At 13 weeks, statistically significant brain inhibition of brain neurotoxic esterase activity was observed at 540 ppm compared with controls (-47%, males and -38%, females). Decreased brain cholinesterase activity was seen in males at 540 ppm (16%) and in females at 207 ppm (15%) and at 540 ppm (23%). No treatment-related effects were observed in the FOB, motor activity tests or microscopic examinations. The LOAEL for brain cholinesterase inhibition was 540 and 207 ppm in males and females respectively. In addition, inhibition of brain neurotoxic esterase activity was noted in both sexes at 540 ppm. The NOAEL was 207 and 72 ppm in males and females, respectively.

This study is classified as **Acceptable/Guideline** because it was generally well conducted and satisfies all guidelines requirements for a subchronic neurotoxicity study in rats (82-7).

Developmental Neurotoxicity- §83-6: Ziram (97.8% a.i.) was evaluated for developmental neurotoxicity during the conduct of a two-generation reproduction study. Test article was administered to male and female Sprague-Dawley CD rats in the diet at concentrations of 0, 72, 207, or 540 ppm for two generations (MRID 43935801). These concentrations resulted in F₁ maternal doses of 5, 13, and 32 mg/kg/day, respectively, during gestation and 11, 30, and 79 mg/kg/day, respectively, during lactation. The developmental neurotoxicity of ziram was evaluated in the F₂ offspring. Behavioral alterations, motor activity measures, auditory startle response, learning and memory, and the age of sexual maturation (vaginal perforation and balanopreputial separation) were examined. Brain weights and dimensions were recorded, and gross and histopathological evaluation of the nervous system tissue was conducted.

No treatment-related maternal or offspring toxicity was observed in the 72 or 207 ppm groups as compared with controls.

All F₁ dams survived until scheduled sacrifice and there were no treatment-related clinical signs of toxicity or neurobehavioral alterations. The high-dose F₁ females had significantly lower body weights throughout gestation ($p \leq 0.05$ or 0.01) and lactation ($p \leq 0.01$) as compared to controls. Body weight gains were significantly lower in the high-dose ($p \leq 0.01$) group as compared to controls during days 14-20 of gestation. No significant differences occurred for body weight gains during lactation for any treated group as compared to controls. On gestation day 20 and lactation day 21 body weights of the high-dose F₁ animals were 89% and 93%, respectively of the control level. Food consumption was significantly ($p \leq 0.05$ or $p \leq 0.01$) lower than controls in the high-dose group throughout gestation and lactation. At necropsy, there were no treatment-related gross- or histopathological abnormalities observed in the dams, and differences in absolute and relative organ weights of the high-dose group as compared to controls were consistent with reduced body weights of these animals.

High-dose F₂ pups also had lower body weights than the controls throughout lactation, with significance reached on postnatal days 1, 4 precull (92-93%; $p \leq 0.05$), 14, and 21 (88-91%; $p \leq 0.01$). Mean body weights of the high-dose F₂ males and females were also statistically significantly ($p \leq 0.05$ or 0.01) less than the controls throughout the post-weaning period. However, final (postnatal day 70) body weights of F₂ males and females were 93 and 96%, respectively, of the control values. Overall body weight gain of the high-dose males was 94% of the controls while overall weight gain of the high-dose females was 99% of the control value. The age of sexual maturation for F₂ pups was not affected by treatment.

No clinical signs of neurotoxicity were observed in the F₂ offspring during daily cageside observations or at detailed physical examinations. Motor activity (total and/or ambulatory counts) was increased at all treatment levels, often 2- to 3-fold greater than control and in a dose-related manner, in pups of both sexes. At the low dose, these increases are apparent beginning at postnatal day 17 and continuing through postnatal day 21, while at the mid and high doses, they initiate at postnatal day 13 and continue through both days 17 and 21. Motor activity counts for postnatal day 60 were similar for control and treated rats of both sexes. Mean peak startle response was decreased (approximately 30% from control) in an apparently dose- and treatment-related manner in high-dose pups of both sexes at postnatal day 22; this finding was not observed on postnatal day 60. Mean latency to peak response, response duration, and average response values appeared to be unaffected in treated animals as compared with controls on postnatal days 22 and 60. Learning and memory evaluations (in a water T-maze) at postnatal days 11 and 70 were similar for control and treated offspring. Brain weights (whole and regional) and dimensions (length and width) were not affected by treatment at postnatal days 11 or 70. Qualitative histopathological evaluation of the nervous system tissues did not reveal any treatment-related findings.

The maternal LOAEL is 540 ppm (32 mg/kg/day) based on reduced body weights and/or body weight gains, and decreased food consumption during gestation and lactation. The maternal NOAEL is 207 ppm (13 mg/kg/day).

The offspring LOAEL is 72 ppm (5 mg/kg/day) based on increased motor activity on postnatal days 17 and 21 for both sexes. The offspring NOAEL is <72 ppm (5 mg/kg/day).

Although this study contains useful information regarding the developmental neurotoxic potential of ziram, it is classified as **Guideline Unacceptable** (§83-6; OPPTS 870.6300) due to the following major deficiencies: 1) Neurobehavioral data (motor activity, startle response, and cognitive function) were not presented as percent change from control or analyzed statistically. 2) Simple morphometric analysis of representative locations within the neocortex, hippocampus, and cerebellum was not performed for F₂ offspring during histopathological examination of the brain at postnatal days 11 and 70. This study can be upgraded upon the submission and review of acceptable statistical analysis and morphometric data.

Evidence of neurotoxicity from other oral toxicity studies:

Chronic toxicity rat study (MRID 43404201) - A statistically significant increase in axonal degeneration of the sciatic nerve was noted in females at the highest dose tested (540 ppm). There was also a non-statistically significant increase of the same finding in males at the HDT and a statistically significant increase in minimal axonal degeneration of the spinal cord in males at the MDT and HDT.

Carcinogenicity mouse study (MRID 43373701) - Dose-related decreases in mean absolute brain weight in both sexes, but only statistically significant in males at 225 and 675 ppm.

Chronic toxicity dog study (MRID 42823901) - Treatment-related convulsive episode in one female at the highest dose tested (700/500 ppm), resulting in euthanasia.

Developmental toxicity rat study (MRID 41908701) - Dose-related post-dosing salivation in maternal rats which is most likely related to the irritant properties of the compound.

5.3 Developmental Toxicity

Rat - Presumed pregnant CrI:CD® (SD) BR VAF/Plus rats, randomly assigned to one control and four treatment groups of 25 animals each, were administered Ziram by gavage at doses of 0, 1, 4, 16, or 64 mg/kg on gestation days (GD) 6-15 inclusive. Cesarean section examinations were performed on all surviving dams on GD 20, followed by external examination of all fetuses. Approximately one-half of each litter was examined

for visceral anomalies and the remainder was fixed and stained for skeletal examinations. There were at least 22 pregnant animals per group.

All animals survived to terminal sacrifice on GD 20. Post-dosing salivation, generally during the last few days of dosing, was associated with treatment at 16 mg/kg in 2 of 25 animals and at 64 mg/kg in 9 of 25 animals. Generalized hair loss occurred in 2/25 control and 6/25 high-dose animals. No clinical signs were associated with treatment with 1 or 4 mg/kg/day. Significantly ($p \leq 0.05$) reduced body weights (approximately 92% of control) occurred in the 64 mg/kg/day-group as compared to controls during the treatment interval and continuing until sacrifice. The 16 mg/kg-group also had significantly ($p \leq 0.05$) reduced mean body weight (94%) as compared to the control group during the treatment period; however, recovery occurred after treatment ended. Mean food consumption was significantly ($p \leq 0.01$) decreased in the 16 and 64 mg/kg-groups as compared to controls beginning with GD 6-7 during the treatment interval and continuing to GD 16-17 for the high-dose group. In contrast, water consumption was significantly ($p \leq 0.01$) increased in the 16 and 64 mg/kg groups during the treatment period as compared to the control group. Water intake continued to be greater ($p \leq 0.05$) than controls for these two groups after the treatment interval but returned to the control level on the last day of the study. **Therefore, the maternal toxicity LOAEL is 16 mg/kg/day based on decreased body weights, reduced food consumption, and salivation during the treatment interval and the maternal toxicity NOAEL is 4 mg/kg/day.**

Mean fetal body weights of the high-dose litters were significantly ($p \leq 0.01$) lower than controls (89%). There were no differences between treated and control groups for number of fetuses per litter, implantations per dam, number of resorptions per dam, or fetal sex ratios, and there were no dams with whole litter resorption. Overall, there was no significant difference or dose-related trend in the number of treated litters affected as compared to control when the incidences of external, visceral, and skeletal malformations/ variations were combined. The number of litters affected in the control, 1, 4, 16, and 64 mg/kg-groups was 13 of 23, 9 of 24, 8 of 22, 19 of 23, and 11 of 24, respectively. No treatment-related external or skeletal malformations/variations were seen in any fetuses from any group. However, there was a dose-related increase in the incidence of diaphragmatic lesions. The incidence of thinning of the diaphragm with protrusion of the liver occurred in 0/23, 0/24, 1/22, 4/23, and 6/24 ($p \leq 0.05$) litters in the 0, 1, 4, 16, and 64 mg/kg-groups, respectively. **Therefore, the developmental toxicity LOAEL is 16 mg/kg/day based on diaphragmatic thinning, and the developmental toxicity NOAEL is 4 mg/kg/day.**

Classification: Acceptable/Guideline

This study satisfies the guideline requirement for a developmental toxicity study (83-3) in rats.

Rabbit - In a developmental toxicity study (MRID 00161316), ziram technical (98% a.i.) in 1% aqueous methyl cellulose was administered by gavage to pregnant New Zealand White rabbits (16/dose) at concentrations of 0, 3, 7.5, or 15 mg/kg/day on GDs 7 through 19. Does were sacrificed on GD 28.

One high-dose doe died on GD 23 and one mid-dose doe died on GD 13. Additionally, one control doe and one mid-dose doe were sacrificed *in extremis* on GDs 14 and 15, respectively; clinical signs observed prior to death in these two animals included weight loss, anorexia, and wheezing. These deaths were not considered to be the result of treatment due to the lack of a dose-response relationship. No other premature deaths occurred and no treatment-related clinical signs of toxicity were observed at any dose level.

At 15 mg/kg, decreased body weights were observed on GDs 0-28 (↓13-17%, $p \leq 0.01$ on GD 10 only). Additionally, overall treatment interval (GDs 7-19) and overall study interval (GDs 0-28) body weight gain, as calculated by the reviewers, were reduced (treatment, ↓81%; study, ↓30%, not analyzed for statistical significance). Decreases ($p \leq 0.01$) in absolute (g/animal/day) food consumption were observed beginning at the GDs 7-10 interval (↓19%) and continuing throughout the GDs 13-16 interval (↓44-49%); decreased consumption was also observed for the GDs 16-19 interval (↓24%, not statistically significant [NS]). Food consumption was reduced for the overall treatment interval (↓34%, GDs 7-19) and for the overall study interval (↓18%, GDs 0-28).

At 7.5 mg/kg, decreased body weight gain was observed on GDs 7-19 (↓30%) and GDs 0-28 (↓19%). No other treatment-related maternal effects were noted at the mid-dose level.

No treatment-related findings were observed at gross necropsy of maternal animals.

The number of implantations/doe and percent male were similar between control and treated groups.

**The maternal LOAEL is 7.5 mg/kg/day, based on decreased body weight gain.
The maternal NOAEL is 3 mg/kg/day.**

Reduced atrium/atria, a minor defect (variation), was observed at the mid- (fetal 2.9%; litter, 14.3%) and high-dose (fetal, 2.8%; litter, 20.0%) levels vs controls (fetal, 0.8%; litter, 6.7%); this finding was observed in a dose-dependent manner and without the %fetal and %litter incidence ranges in the historical data, this variation was considered to be equivocally treatment-related.

At the high-dose level, increases (NS) were observed in the total number of resorptions/doe (↑88%) and the percent postimplantation loss (↑97%). Additionally,

reductions (NS) in the number of live fetuses/doe (\downarrow 15%) were noted. Upon skeletal examination, absence of the interparietal bone, a major defect (malformation), was observed at the high-dose level only (fetal, 1.9%; litter, 13.3%) vs 0 controls; since this finding was only observed at the high-dose level and without the %fetal and %litter incidence ranges in the historical data, this malformation was considered equivocally treatment-related.

The developmental LOAEL is 7.5 mg/kg/day, based on increased incidence of reduced atrium/atria.

The developmental NOAEL is 3 mg/kg/day.

This developmental toxicity study is classified **acceptable/guideline** (§83-3[b]) and does satisfy the guideline requirement for a developmental toxicity study in the rabbit.

5.4 **Reproductive Toxicity**

Ziram (97.8% a.i.) was administered to male and female Sprague-Dawley CD rats in the diet at concentrations of 0, 72, 207, or 540 ppm for two generations (MRID 43935801). Premating doses for the F0 males were 5.3, 14.8, and 37.5 mg/kg, respectively and for the F0 females were 6.1, 16.8, and 42.8 mg/kg, respectively. Premating doses for the F1 males were 5.6, 16.7, and 42.7 mg/kg, respectively, and for the F1 females were 6.3, 18.4, and 47.5 mg/kg, respectively. Each generation contained 30 animals/sex/dose which were given test or control diet for at least 10 weeks then mated within the same dose group. F1 animals were weaned on the same diet as their parents. Sibling matings were avoided and at least 23 litters were produced in each generation. All animals were exposed to test material either in the diet or during lactation until sacrifice. The time course for the study was as follows: weeks 1-10, F0 premating; weeks 11-18, F0 breeding, gestation, and lactation; weeks 19-30, F1 premating; week 39, end of study.

All F0 and F1 parental animals survived to scheduled necropsy. Generalized, clinical signs in the adult animals, such as hair loss and sores, were observed in the control and treated animals equally and there was no correlation with dose.

No treatment-related effects were seen in the 72 or 207 ppm groups of either generation as compared with controls. High-dose F0 males initially had lower body weights (90-93%) than controls at weeks 1, 2 ($p \leq 0.01$), and 3 ($p \leq 0.05$) due to a significantly ($p \leq 0.01$) lower body weight gain (71%) during week 0-1. Throughout the remainder of the study, there were no significant differences in absolute body weights of the treated F0 male groups as compared to controls. Food consumption by the high-dose F0 males was significantly ($p \leq 0.01$) less than controls for the first 4 weeks of the study and at weeks 8-9, 9-10 ($p \leq 0.05$), and 10-11. Body weights of the high-dose F0 females were significantly ($p \leq 0.01$) less than the controls for the entire premating period (92-94%). However, body weight gains were significantly less than controls only during week 0-1.

(44%; $p \leq 0.01$), week 1-2 (76%; $p \leq 0.05$), and week 6-7 (67%; $p \leq 0.01$). High-dose F0 females ate significantly ($p \leq 0.01$) less than the controls throughout the entire prematuring period.

High-dose F1 males had significantly ($p \leq 0.01$) lower body weights (97-90%) as compared to controls throughout the entire prematuring period and continuing until study termination. Body weight gains in the high-dose males were significantly less than the controls during study weeks 18-19, 20-21 (83%; $p \leq 0.01$), and 21-22 (90%; $p \leq 0.05$) of the prematuring period. Food consumption was significantly less than the controls for the high-dose F1 males ($p \leq 0.01$) throughout the entire prematuring period. Absolute body weights of the high-dose F1 females were significantly lower than the controls for the entire prematuring period (89-92%; study weeks 19-23, $p \leq 0.05$; weeks 24-30, $p \leq 0.01$); significantly lower body weight gains (67-87%) occurred only during study weeks 18-19 ($p \leq 0.05$), 23-24, and 24-25 ($p \leq 0.01$). Food consumption by the high-dose F1 females was also significantly less than the controls throughout prematuring ($p \leq 0.01$; weeks 21-22 and 28-29, $p \leq 0.05$).

There were no treatment-related gross- or histological abnormalities observed in either generation. Differences in absolute and relative organ weights of the high-dose male and female F0 and F1 groups as compared to controls are consistent with reduced body weights of these animals.

Therefore, the systemic toxicity LOAEL is 540 ppm (37.5 mg/kg/day) based on reduced body weights, body weight gains, and decreased food consumption by F0 and F1 males and females. The systemic toxicity NOAEL is 207 ppm (14.8 mg/kg/day).

High-dose F0 animals had significantly ($p \leq 0.01$) lower body weights as compared to controls throughout gestation and until day 14 of lactation; body weight gains were significantly ($p \leq 0.05$) less than controls during the day 10-14 interval of gestation. Some recovery was apparent in the high-dose F0 females with body weight gains significantly ($p \leq 0.01$) greater than the controls during lactation days 14-21; this resulted in overall body weight gains during lactation significantly greater than the controls. On gestation day 20 and lactation day 21 body weights of the high-dose F0 animals were 90% and 98% of the control level. High-dose F0 females also had significantly ($p \leq 0.01$) lower food consumption as compared to controls throughout gestation and during days 4-7 ($p \leq 0.05$) and 7-14 of lactation. The high-dose F1 females had significantly lower body weights throughout gestation (days 0 and 7, $p \leq 0.05$; day 10, 14, and 20, $p \leq 0.01$) and lactation ($p \leq 0.01$) as compared to controls. Body weight gains were significantly lower in the high-dose ($p \leq 0.01$) group as compared to controls during days 14-20 of gestation. No significant differences occurred for body weight gains during lactation for any treated group as compared to controls. On gestation day 20 and lactation day 21 body weights of the high-dose F1 animals were 89% and 93% of the control level. Food

consumption was significantly ($p \leq 0.05$ or $p \leq 0.01$) lower than controls by the high-dose group throughout gestation and lactation.

No dose- or treatment-related effects were noted on the reproductive performance of adults from either generation. F1 pups from high-dose group dams had consistently lower body weights than controls beginning at day 4 precull with significance (92%; $p \leq 0.01$) reached on day 14. High-dose F2 pups also had lower body weights than the controls throughout lactation with significance reached on days 1, 4 precull (92-93%; $p \leq 0.05$), 14, and 21 (88-91%; $p \leq 0.01$).

Therefore, the LOAEL for offspring toxicity is 540 ppm (42.8 mg/kg/day) based on reduced pup body weights at birth in F2 pups and during lactation in both F1 and F2 pups. The corresponding NOAEL for offspring toxicity is 207 ppm (16.8 mg/kg/day).

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a multigeneration reproduction feeding study (83-4) in rats.

5.5 Developmental Neurotoxicity

See Executive Summary in Neurotoxicity Section (5.2).

5.6 Additional Information from Literature Sources

Ema, M., T. Itami, Y. Ogawa, and H. Kawasaki. 1994. Developmental toxicity evaluation of zinc dimethyldithiocarbamate (ziram) in rats. Bull. Environ. Contam. Toxicol. 53: 930-936.

In a developmental toxicity study, zinc dimethyldithiocarbamate (ziram, 99.9% purity) was administered to 21 pregnant rats (10-15 weeks old) /dose in the diet at dosage levels of 0, 0.0125, 0.025, or 0.05% (0, 9.5, 16.2, or 23.4 mg/kg/day, respectively) from days 6 through day 15 of gestation.

No treatment-related effects on mortality or clinical signs were observed. Treatment-related decreases in body weight gain, adjusted body weight gain (for gravid uterine weight), and food consumption occurred at 0.025 and 0.05% on days 6-15 and on day 0-20 of gestation. Body weight gain and food consumption were not affected at 0.0125%. **The maternal LOAEL is 0.025% (16.2 mg/kg/day) based on decreased body weight gain and food consumption. The NOAEL is 0.0125% (9.5 mg/kg/day).**

No treatment-related effects on the incidence of postimplantation loss per litter, the numbers of resorptions and dead fetuses per litter and live fetuses per litter, the sex ratio of live fetuses, and the fetal body weight were noted.

The incidences of fetuses and litters with skeletal malformations and variations were not significantly different between the compound-treated groups and the control group. No internal malformations (other than skeletal malformations) were observed in any group. **The developmental NOAEL is 0.05% (23.4 mg/kg/day). No LOAEL was determined since no developmental effects were noted as a result of oral administration of ziram.**

5.7 Determination of Susceptibility

Data from the oral developmental studies suggest that there is no increased susceptibility to the developing fetus when the dams are fed ziram in the diet. There is also no evidence of offspring or reproductive toxicity at doses below maternal systemic toxicity levels.

There is quantitative evidence of increased susceptibility in the rat developmental neurotoxicity study, but the observations are questionable since the actual dosage of the compound to the pups is unknown. The effects of concern occurred late during lactation and may be a result of increased compound uptake due to exposure from milk and feed.

6 HAZARD CHARACTERIZATION

The primary target organs of ziram appear to be the liver and the nervous system, and possibly the thyroid. Liver histopathology, sometimes accompanied by increases in hepatic serum enzyme levels, was seen at various doses in the subchronic and chronic rat studies (MRIDs 42450301 and 43404201, respectively) and the carcinogenicity mouse study (MRID 43373701). Changes in hepatic serum enzyme levels alone were noted in the 21-day rabbit dermal study (MRID 41297001). Evidence of neurological impairment include: neurological signs including ataxia, salivation, lacrimation, impaired gait, abnormal posture, and decreased absolute brain weights in the acute neurotoxicity rat study (MRID 43362801); inhibition of brain cholinesterase and brain neurotoxic esterase in the subchronic neurotoxicity rat study (MRID 43413701); convulsions in a test animal of the high dose treatment group in the chronic toxicity dog study (MRID 42823901); decreased brain weights in the carcinogenicity mouse study. Thyroid C-cell hyperplasia in males and prominent thyroid ultimobranchial cysts in both sexes were noted in the chronic rat study (MRID 43404201). Thyroid C-cell hyperplasia was also observed in male rats in the NTP carcinogenicity study.

Long-term dietary administration of ziram resulted in an increased incidence of benign hemangiomas in male rats (MRID 43404201). No effect was observed in female rats. The levels of the doses tested appeared adequate. No tumors were noted in male or female mice after long-term dietary administration of ziram (MRID 43373701). In the NTP study, long-term dietary administration of ziram resulted in an increased incidence of thyroid C-cell tumors in male rats and pulmonary alveolar/bronchiolar tumors in female mice. No effect was observed in female rats or male mice. The Cancer Assessment Review Committee classified ziram as "likely to be carcinogenic in humans."

Ziram does show positive evidence of mutagenicity in the Ames test and conflicting evidence of mutagenicity in CHO gene mutation tests and is negative in unscheduled DNA synthesis assays. *In vivo* mutagenicity tests are negative.

The oral rat developmental study (MRID 41908701) did not show an increased susceptibility of the fetus to ziram *in utero*. Diaphragmatic thinning was seen at a dose of 16 mg/kg/day in fetuses while maternal toxicity resulted in reduced food consumption and salivation at the same dose.

The oral rabbit developmental study (MRID 00161316) revealed an increased susceptibility of the fetus to ziram *in utero*. Increased incidence of reduced atrium/atria was noted at 7.5 mg/kg/day in fetuses while maternal toxicity resulted in decreased body weight, body weight gain, and food consumption at 15 mg/kg/day.

A two-generation rat reproduction study (MRID 43935801) did not show an increased susceptibility to offspring. Reduced pup body weights at birth in F₂ pups and during lactation in both F₁ and F₂ pup were noted at a dose of 42.8 mg/kg/day while systemic parental toxicity resulted in reduced body weights, body weight gains, and decreased food consumption in F₀ and F₁ males and females at 42.8 mg/kg/day.

Both the acute and subchronic neurotoxicity studies show that ziram does have an adverse effect on the nervous system as noted above.

One rat metabolism study showed that Ziram was excreted in the expired air, feces, and urine. Excretion of ziram was greatest in the expired air (37-50%) and was associated with both CO₂ and volatile fractions. Urinary excretion: 17-35%. Fecal excretion: 9-18%. Residual radioactivity was low in tissues (<1%) and carcasses (≤ 1%) in all dose groups. Excretion via air was rapid (24 - 48 hours) and via urine and feces was complete within 72 hours for low-dose groups, but bi-phasic in the high-dose group with excretion peaks at 0-8 hours, 24-72 hours, and 96 hours. No metabolites were identified.

7 DATA GAPS

Data gaps include a metabolite identification study and a dominant lethal study requested by the Cancer Review Assessment Committee in order to evaluate any heritable effects via the germ cells as a result of exposure to ziram.

9 SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (Females 13 +)	NOAEL = 3 UF = 100	Increased incidence of reduced atrium/atria.	Prenatal Oral Developmental / Rabbit
		Acute RfD (Females 13 +) = 0.03 mg/kg/day	
Acute Dietary (Gen. Population)	LOAEL = 15 UF = 300	Ataxia and slight impairment of gait.	Acute Oral Neurotoxicity / Rat
		Acute RfD (Gen. Population) = 0.05 mg/kg/day	
Chronic Dietary	NOAEL = 1.6 UF = 100	Decreased body weight gain.	Chronic Oral Toxicity / Dog
		Chronic RfD = 0.016 mg/kg/day	
Dermal, Short-Term	NOAEL = 300	Decreased body weight and food consumption. Clinical changes suggestive of minimal hepatotoxicity.	21-Day Dermal Toxicity / Rabbit
Dermal, Intermediate-Term	NOAEL = 300	Decreased body weight and food consumption. Clinical changes suggestive of minimal hepatotoxicity.	21-Day Dermal Toxicity / Rabbit
Dermal, Long-Term ¹	NOAEL = 1.6	Decreased body weight gain.	Chronic Oral Toxicity / Dog
Inhalation, Short-Term ²	NOAEL = 3	Increased incidence of reduced atrium/atria.	Prenatal Oral Developmental / Rabbit
Inhalation, Intermediate/Long-Term ²	NOAEL = 1.6	Decreased body weight gain.	Chronic Oral Toxicity / Dog

1. Use the appropriate dermal absorption factor (1%) since the NOAEL is from an oral study.
2. Use the appropriate inhalation absorption factor (100%) since the NOAEL is from an oral study.