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13

DATA EVALUATION REPORT

ZIRAM

Study Type: SUBCHRONIC ORAL NEUROTOXICITY ❖ RAT (82-7SS)

8/2/2000

Prepared for

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

Prepared by

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Task Order No. 94-43D

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[ZIRAM]

Subchronic Oral Neurotoxicity (82-7SS)

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DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Neurotoxicity ☒ Rat (82-7ss)

TOX. CHEM. No.: 931

PC CODE: 034805

MRID NO.: 43413701

TEST MATERIAL: Ziram (technical, 97.8% a.i.)

SYNONYMS: Bis(dimethylcarbamodithioato-S,S')zinc; bis(dimethyldithiocarbamato)zinc; dimethyl-dithiocarbamic acid zinc salt; zinc dimethyldithiocarbamate; zinc bis(dimethylthiocarbamoyl) disulfide; methylcymate; Methasan; Zimate; Zirberk; Karbam White; Corozate; Fuclasin; Fuklasin; Zerlate

STUDY NUMBER: WIL-223004

SPONSOR: The Ziram Task Force, NPC, Inc., 22636 Glenn Drive, Suite 304, Sterling, VA

TESTING FACILITY: WIL Research Laboratories, Inc., 1407 George Road, Ashland, OH 44805-9281

TITLE OF REPORT: A Subchronic (13-Week) Neurotoxicity Study of Ziram in Rats

AUTHOR: M.D. Nemeč

REPORT ISSUED: October 7, 1994 (study completion date)

EXECUTIVE SUMMARY: In a subchronic oral neurotoxicity study (MRID 43413701), 10 Sprague-Dawley Crl:CDRBR 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.**

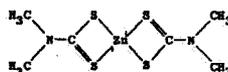
This study is classified as **Acceptable/Guideline** because it was generally well conducted and satisfies all guidelines requirements for a subchronic neurotoxicity study in rats (82-7).

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test compound: Ziram

Purity: 97.87% a.i.
Description: white powder
Lot No.: V528/8331AA
Contaminants: not specified
Structure:



2. Vehicle

Dry test material was mixed with feed; therefore, no vehicle was required.

3. Test animals

Species: rat
Strain: Sprague-Dawley CrI:CDRBR
Age and weight at study initiation: 50 days old; 207-271 g (males), 151-190 g (females)
Source: Charles River Breeding Laboratories, Inc., Portage, MI 49081
Housing: individually in stainless-steel wire cages
Environmental conditions:
Temperature: 68-75°F
Humidity: 36-78%
Air changes: not reported
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 19 days

B. STUDY DESIGN

1. Animal assignment

Animals were assigned to the test groups in Table 1 using a computer-generated randomization. Within each dose group, 5 animals/sex were allocated for cholinesterase and neurotoxic esterase determinations and the remaining 5 animals/sex were allocated for the neuropathology evaluation at study termination. Both groups were combined for the Functional Observational Battery (FOB) and locomotor activity assessments. In order to ensure that concentrations of 60 and 180 ppm for Groups 2 and 3, respectively, were maintained over the course of offering the test diet to the animals for a period of 16 hours at room temperature, the dose levels in Groups 2 and 3 were increased to 72 and 207 ppm,

respectively (see also Section B.4., stability analysis). Animals were randomized into four study replicates for conduct of neurobehavioral testing over four days.

TABLE 1. ANIMAL ASSIGNMENT			
Test Group	Dose level	Number assigned	
	ppm	male	female
1 Control	0	10	10
2 Low (LDT)	60 (72) ¹	10	10
3 Mid (MDT)	180 (207)	10	10
4 High (HDT)	540	10	10

Data taken from p. 16, MRID No. 43413701.

1 Target concentration (actual concentration prepared to adjust for instability of test material in diet)

2. Validation of test methods

Validation studies were conducted using control rats and rats administered chemicals with known neurotoxic effects to demonstrate sensitivity and reliability of laboratory neurotoxicity testing methods and instrumentation in accordance with EPA Neurotoxicity Testing Guidelines, Addendum 10. A brief summary of these studies is presented in the Appendix.

3. Rationale for dose selection

Dose levels were selected based on the results of a previously conducted 6-week dietary range-finding study of Ziram in rats (WIL-160014) showing treatment-related effects in the clinical condition of the animals (abnormal defecation and/or gait alterations), decreased body weight gains, and decreased food consumption at dose levels of 300, 500, and 1000 ppm. No further details of this study were provided. [In a 90-day feeding study submitted to the Agency, MRID 42450301, a LOEL of 300 ppm was established based on decreased body weight gain and food consumption; no clinical signs were observed at 100, 300 or 1000 ppm).

4. Diet preparation and analysis

For dose calculations, a correction factor of 1.022 was used. The diet was prepared weekly by mixing appropriate amounts of test material with the rat chow feed. The diets were stored refrigerated due to the limited stability of the test material. For homogeneity analysis, duplicate samples were taken from the top, middle, and bottom of the test diet. The middle of the test diet was sampled for stability and/or concentration analysis. For stability analysis, several sets of diet preparations were evaluated. One set was stored at room temperature for 16 hours. In another set, Group 2 (60 ppm) was fortified to 66 ppm and stored for 24 hours at room temperature or for 10 or 15 days under refrigeration. In an additional set of formulations, Group 2 was fortified to 72 ppm and Group 3 (180

ppm) to 207 ppm and stored at room temperature for 16 hours or 11 days under refrigeration.

Samples were collected prior to study initiation for homogeneity and stability, and test material concentration analyses and approximately weekly during the study. Concentration analyses were conducted on samples collected during study weeks 0, 1, 2, 3, 7, and 11. The quantitation of Ziram in the diet was based on the decomposition of the test material and collection and analysis of the resulting carbon disulfide by gas chromatography/flame photometry.

Results ❖

- a. Homogeneity analysis ❖ The distribution of test material in the diet was found to be homogenous. The location means differed by less than 10% for the overall mean of each group.
- b. Stability analysis ❖ Selected stability data are presented in Table 2. There was a significant decrease of Ziram concentration for the Group 2 (60 ppm) and Group 3 (180 ppm) test diets when they were stored for 16 hours at room temperature. When the test diets for Groups 2 and 3 were fortified to 72 and 207 ppm, respectively, they were stable when kept at room temperature for 16 hours or refrigerated for 11 days. In order to ensure that a concentration of 60 and 180 ppm was maintained in the course of offering of the test diet, the test material concentrations for Group 2 and Group 3, therefore, were increased to 72 and 207 ppm, respectively.

TABLE 2. STABILITY OF ZIRAM IN RODENT FEED		
Dose Group	Percent of Target Concentration	
	16 Hours at Room Temperature	Refrigerated for 11 days
Group 2 (60 ppm) ^a	73.8	ND
Group 3 (180 ppm) ^a	75.8	ND
Group 4 (540 ppm) ^a	86.1	ND
Group 2 (72 ppm) ^b	92.0	90.9 ^e
Group 3 (207 ppm) ^b	98.9	107 ^d
Group 4 (540 ppm) ^b	95.2	96.7

Data taken from pp. 23, 945, and 946, MRID No. 43413701.

^aTarget dose; ^bAdjusted dose

^cCompared to desired dose of 60 ppm; ^dcompared to desired dose of 180 ppm

ND = no data

- c. Concentration analysis ❖ The mean concentrations of the test material in the diet were 96.3% (RSD = 4.5), 98.5% (RSD = 3.6) and 101% (RSD = 2.2) for nominal concentrations of 72, 207 and 540 ppm. Of the analyses performed, only one batch

exceeded 10% of target concentration (112%, 10/22/93 preparation of 72 ppm). This variation does not adversely affect the outcome of the study.

4. Diet

Animals were fed PurinaR Certified Rodent Chow #5002 and water ad libitum. Based on the results of the stability analyses, fresh treated diets were provided daily from the refrigerated weekly preparations.

5. Statistical analysis

Statistical analyses were performed by a DigitalRMicroVAX 3400 computer with appropriate software except for the FOB and motor activity. All statistical analyses were two-tailed (except as noted) for significance levels of 5% and 1%. Body weights, body weight changes, food consumption, cholinesterase and neurotoxic esterase values, absolute and relative brain weights, and brain dimensions were analyzed by a one-way analysis of variance (ANOVA). If significant differences were found, Dunnett's test was used to compare the control and treated groups. Histopathological findings in treated groups were compared to control group data by the one-tailed Kolmogorov-Smirnov test.

FOB and locomotor activity data were analyzed using a personal computer installed with SAS/STAT software. The animal that died on study was not included in the calculation for any test period. Continuous FOB and locomotor activity data were analyzed using a two-way repeated measures ANOVA. If significant treatment or treatment-time interactions occurred, a one-way ANOVA was conducted at each time point. If significant differences were observed at a time point, Dunnett's multiple t-test was performed. FOB parameters yielding scalar (ordinal) or descriptive data were analyzed using the repeated measures SAS CATMOD procedure. If significant treatment or treatment-time interactions occurred, Fisher's exact test or Dunnett's test was employed.

6. Signed and dated (10/7/94) GLP and quality assurance statements were present.

C. METHODS AND RESULTS

1. Clinical observations and mortality

The animals were observed twice daily for mortality and/or moribundity. Detailed clinical observations were recorded daily for all animals. On study days when the FOB was conducted, no additional signs were recorded, except for an inadvertent additional evaluation of the control and low-dose groups during study week 3.

Results ❖ One male treated with 72 ppm was found dead on study day 14 (not treatment-related; cause of death undetermined). The animal exhibited hypoactivity and labored respiration prior to death. No test material-related clinical signs of toxicity were noted in the animals that survived to study termination.

2. Body weights

Body weights were recorded weekly, beginning approximately two weeks prior to treatment and on treatment days when the FOB and locomotor activity evaluations were conducted. The body weights were recorded on all animals prior to scheduled necropsy (week 13) and on the male rat that was found dead.

Results ❖ Weekly mean body weights are presented in Table 3. No effects on body weights were observed in the groups treated with 72 or 207 ppm. The mean body weights in males and females treated with 540 ppm were lower than the control groups from week 1 through week 13. The decreases at weekly intervals ranged from 8% to 10% of control values for males (statistically significant from week 1 through 8) and from 7% to 11% of control values in females (statistically significant from week 1 through 13, except for week 4). The lower mean body weights in males and females treated with 540 ppm were attributed mainly to the lower mean body weight gains from week 0 to 1. The weekly mean body weight gains in the high dose group were generally comparable to controls for the remainder of the study. The cumulative body weight gain (weeks 0 to 13) was significantly lower in the high dose group (18% and 32% lower than control values for males and females, respectively). Although the body weights and weekly body weight gains in females receiving 207 ppm were similar to controls, the cumulative weight gain was 10% lower (not statistically significant) than the control value.

TABLE 3. WEEKLY MEAN BODY WEIGHTS AND BODY WEIGHT GAINS (g) IN MALE AND FEMALE RATS FED ZIRAM FOR 13 WEEKS								
Week of Study	Exposure Concentration (ppm)							
	Males				Females			
	0	72	207	540	0	72	207	540
0	242	239	244	245	163	163	170	167
1	292	290	291	264**	181	183	184	169*
2	332	330	333	300*	196	197	196	183*
3	365	362	368	334*	210	211	211	192**
4	386	385	392	349*	211	215	218	197
5	411	410	421	371*	227	228	227	208**
6	431	434	436	389*	234	236	232	211**
7	448	455	456	407*	239	242	239	217**
8	455	455	466	413*	242	246	241	220**
9	463	466	474	422	241	246	242	222*
10	483	489	490	439	253	254	250	227**
11	497	503	508	453	261	260	254	232**
12	506	511	508	464	264	265	261	234**
13	507	508	510	463	261	262	258	234**
Body Weight Gain 0-13	266	270 (102) ^a	266 (100)	218* (82)	98	99 (101)	88 (90)	67** (68)

Data taken from Table 2, pp. 40-47 and Table 3, pp. 56 and 59, MRID No. 43413701.

^aCalculated by reviewer; numbers in parenthesis are percent of control weight gain.

*Significantly different from control, $p < 0.05$ (Dunnett's test)

**Significantly different from control, $p < 0.01$ (Dunnett's test)

3. Food consumption

Beginning two weeks prior to treatment, individual food consumption was measured daily, and the weekly averages were reported. Food intake was calculated as g/animal/day and g/kg/day. Test material consumption (mg/kg/day) for each sex/group was calculated from the mean amount of food consumed (g/kg/day) and the appropriate nominal concentration of test material in the food (ppm). Food efficiency [(body weight gain in g/food consumption in g per unit time) x 100] was not calculated.

Results ❖

- a. Food consumption ❖ Data at weekly intervals (g/animal/day) are presented in Table 4. Food consumption in males and females administered 72 or 207 ppm was unaffected by treatment with the test material throughout the study period. Food consumption in males administered 540 ppm was significantly decreased for weeks 0-1 ($p < 0.01$) and

weeks 3-6 and 12-13 ($p < 0.05$). In females administered 540 ppm, food consumption was significantly decreased weeks 0-2, 9-13 ($p < 0.01$) and weeks 2-3 and 5-6 ($p < 0.05$). The g/kg/day food consumption values (not shown) were comparable to control values except during week 0 - 1, which was significantly less than controls for both males (-29%) and females (-25%) at 540 ppm (and -10% at 207 ppm for females). Reduced food consumption after week 1 was therefore attributed to the lower body weights in the high dose animals.

TABLE 4. MEAN FOOD CONSUMPTION (G/ANIMAL/DAY) AT WEEKLY INTERVALS IN MALE AND FEMALE RATS FED ZIRAM FOR 13 WEEKS

Week of Study	Exposure Concentration (ppm)							
	Males				Females			
	0	72	207	540	0	72	207	540
0-1	26	26	25	18**	17	17	16	13**
1-2	27	27	27	24	18	19	18	16**
2-3	27	28	27	25	18	19	18	16*
3-4	26	26	27	23*	17	17	18	15
4-5	27	28	27	24*	18	18	18	17
5-6	26	27	27	23*	17	18	17	16*
6-7	26	28	26	24	17	19	18	16
7-8	26	25	26	23	17	18	17	16
8-9	27	27	27	24	18	18	18	16
9-10	26	27	26	24	18	18	17	15**
10-11	26	27	27	24	19	18	17	16**
11-12	26	26	26	24	18	18	17	15**
12-13	25	25	27	23*	17	16	16	15**
Week 0-13 ^a	341 (2387) ^b	347 (2429)	345 (2415)	303 (2121)	229 (1603)	233 (1631)	225 (1575)	202 (1414)

Data taken from Table 4, pp. 60-65, MRID No. 434137-01.

^aCalculated by reviewer.

^bTotal food consumption; calculated by multiplying daily food consumption (week 0-13) by 7 days.

*Significantly different from control, $p < 0.05$ (Dunnett's test); **Significantly different from control, $p < 0.01$ (Dunnett's test)

- b. Compound consumption (time-weighted average) ❖ Males received doses of 5, 14, or 34 mg/kg/day and females 6, 16, or 40 mg/kg/day for dietary concentrations of 72, 207, or 540 ppm.
- c. Food efficiency ❖ Based on total weight gain and on the total amount of food consumed, the overall efficiency calculated by the reviewer was 11.14, 11.12, 11.01, or 10.28 for males and 6.11, 6.07, 5.59, or 4.74 for females at dietary concentrations of 0, 72, 207, or 540 ppm. Thus, male rats exhibited a slight decrease in food efficiency that appeared to be dose-related. In female rats, food efficiency was also decreased in a dose-related manner; the decrease was slight at 72 and 207 ppm and more pronounced at 540 ppm. Food efficiency was sharply decreased during the first

week at 540 ppm in both males (15% vs 28%, controls) and females (2% vs. 15%, controls).

4. Functional observational battery (FOB)

An FOB was conducted on 10 animals/sex/group pretest and at weeks 3, 7, and 12 of the study. The following parameters were observed:

- a. Home cage observations including postures, convulsions/tremors, feces consistency, biting, and palpebral (eye lid) closure.
- b. Handling observations including ease of removal from cage, lacrimation/ chromodacryorrhea, piloerection, palpebral closure, red/crusty deposits, eye prominence, ease of handling animals in hand, salivation, fur appearance, respiratory rate/character, mucous membranes/eyes/skin color, and muscle tone.
- c. Open field observations (evaluated over a 2-minute observation period) including mobility, rearing, convulsions/tremors, grooming, bizarre/stereotypical behavior, time to first step (seconds), gait, arousal, urination/defecation, gait score, and backing.
- d. Sensory observations including approach response, startle response, pupil response, forelimb extension, air righting reflex, touch response, tail pinch response, eye blink response, hindlimb extension, and olfactory orientation.
- e. Neuromuscular observations including hindlimb extensor strength, hindlimb foot splay, grip strength (hind- and forelimb), and rotarod performance.
- f. Physiological observations including catalepsy, body temperature, and body weight.

Results ❖ No test material-related effects indicative of neurotoxicity were observed between treated and control group animals when home cage observations, handling observations, or sensory observations were evaluated during the pretest period and during study weeks 3, 7, and 12.

When the open field observations were evaluated, one male and one female in the 540 ppm group displayed a slight, but definite gait alteration (walking on tiptoes) at study weeks 3 and 7, respectively. These isolated occurrences of gait alteration were not attributed to the test material because this finding has been previously observed in female WIL historical control animals and no gait alterations were observed during week 12 open field observations or during the daily clinical examinations at any dose level (however, it is noted that gait abnormalities were observed at 300 mg/kg in the acute neurotoxicity study on ziram, MRID 43362801). No other remarkable differences were observed between treated and control groups in the open field observations.

No remarkable differences in neuromuscular observations were apparent between control and treated groups that were evaluated during the pretest period and study weeks 3, 7, and 12. At the study week 7, the mean forelimb grip strength in females exposed to 540 ppm was significantly lower compared with the control group ($p < 0.05$; 860.9 g vs. 1100 g for controls). The forelimb grip strength in these females was similar to controls at the week 3 and 12 evaluations. A reduction in forelimb grip strength was not observed in males exposed to 540 ppm or to the lower two doses in either sex at any evaluation period. Therefore, the reduced forelimb grip strength in females at 540 ppm was not considered treatment-related and was possibly related to decreased weight gain.

Physiologic evaluations revealed no cataleptic effects or effects on body temperature at any dose level. Body weights during the pretest period and weeks 3, 7, and 12 were consistent with those noted for the weekly body weight data (see also Section C.2.). Body weights were decreased in males and females exposed to 540 ppm during study weeks 3, 7, and 12. With the exception of the week 12 value for males, all of the decreases were statistically significant ($p < 0.05$ or 0.01) but of marginal biological significance, ranging from 8% to 9% of control values for males and from 9% to 11% for females. No effects on body weights were seen at 72 or 207 ppm at any evaluation interval.

5. Motor activity

Observations were made on 10 animals/sex/group pre-study and during the study weeks 3, 7, and 12 prior to dosing. Locomotor activity was measured using the Digiscan "Micro" Animal Activity System (Omnitech Electronics, Inc., Columbus, OH) which utilizes a series of infrared photobeams in a rectangular cage to quantify an animal's motor activity. The test session duration was 41 minutes, divided into 10-minute subsessions; the data were collected in 1-minute epochs (the first minute of data was deleted to account for placement of the animal in the activity cage). Data for ambulatory and total motor activity were tabulated. Total motor activity was defined as a combination of fine motor skills (i.e., grooming, interruption of one or two adjacent photobeams) and ambulatory motor activity (interruption of three or more consecutive photobeams).

Results ❖ Mean ambulatory and total motor activity values in the three dose groups for both males and females were similar to controls during the pre-test period and at weeks 3, 7, and 12. Animals in all groups during each evaluation period showed normal habituation profiles to the test chambers during the locomotor activity sessions.

6. Clinical chemistry

Plasma and red blood cell (RBC) cholinesterase (ChE) evaluations were conducted on 5 animals/sex pre-study, at study weeks 3 and 7, and at study termination (week 13). Blood samples were collected from the caudal (tail) vein of the same animals for the intervals prior to study termination and from the inferior vena cava at the time of necropsy. For study weeks 3 and 7, blood collections followed the completion of the FOB and locomotor activity evaluations. ChE activity was analyzed based on a photometric method (Ellman *et al. Biochem. Pharmacol.*, 1961, 7:88) which utilizes an acetylthiocholine substrate.

Following euthanization and exsanguination, whole brain ChE and neurotoxic esterase (NTE) evaluations were conducted for each of the 5 animals/sex/group in this study component. Whole brain weights were also recorded for each of the animals in this group. Plasma and RBC ChE evaluations were performed by WIL Research Laboratories and whole brain ChE and NTE evaluations by AniLytics, Inc., Gaithersburg, MD.

Results ❖ Cholinesterase and NTE values are presented in Table 5. Compared with controls, slight decreases in plasma ChE activity were seen in males at week 13 and in females at week 3, 7, and 13. No effects on RBC ChE activity were evident in females. In males, decreased RBC ChE activity was seen only at week 3 (29% decrease at 207 ppm; 8% decrease at 540 ppm). However since no dose-response was observed and the 29% decrease was not significant due to variability in individual measurements, it was not considered treatment-related. Brain ChE activity was decreased in males at 540 ppm (16%) and in females at 207 (15%) and 540 ppm (23%) compared with controls.

Brain NTE activity at week 13 was lower than that observed in controls at all three dose levels of Ziram, with significant decreases occurring at 540 ppm in males ($p < 0.01$) and

females ($p < 0.05$). At dietary concentrations of 72, 207, or 540 ppm, the respective brain NTE activities were 16%, 15%, or 47% (males) and 27%, 24% or 38% (females) lower than control values. The decreases at 540 ppm were attributed to the test material.

TABLE 5. CHOLINESTERASE (ChE) AND NEUROTOXIC ESTERASE (NTE) ACTIVITY IN MALE AND FEMALE RATS FED ZIRAM FOR 13 WEEKS

Parameter	Exposure Concentration (ppm)							
	0	SD	72	SD	207	SD	540	SD
Males								
Plasma ChE (U/L)								
week 3	424	65.4	498	86.7	461	144.3	426	43.9
week 7	410	78.9	533	73.7	438	147.5	443	49.8
week 13	388	79.7	408	53.9	391	154.4	362 (7%) [†]	40.3
RBC ChE (U/L)								
week 3	2087	592.9	2265	307.8	1492 (29%)	1118	1929 (8%)	457.5
week 7	2022	212.4	1982	246.7	2031	199.6	1973	248.9
week 13	1950	278.3	2016	331.9	2104	195.7	2138	128.7
Brain ChE (U/G)	1.55	0.334	1.66	0.588	1.58	0.314	1.30 (16%)	0.556
Brain NTE (NM/MN/MG)	16.5	2.27	13.9 (16%)	3.34	14.0 (15%)	2.56	8.7**(47%)	1.85
Females								
Plasma ChE (U/L)								
week 3	1564	501.3	1689	698.4	1768	536.0	1459 (7%)	383.2
week 7	2090	739.9	2271	981.7	2467	774.0	1890 (10%)	711.0
week 13	2235	646.4	2531	1172	2642	828.6	2075 (7%)	608.3
RBC ChE (U/L)								
week 3	1634	594.8	1830	671.5	1650	693.2	1900	635.8
week 7	1751	372.9	1963	328.9	1821	322.1	1870	322.1
week 13	1802	290.6	1543	520.2	1859	178.2	1849	178.2
Brain ChE (U/G)	1.75	0.630	1.75	0.829	1.48 (15%)	0.490	1.35 (23%)	0.270
Brain NTE (NM/MN/MG)	15.5	2.05	11.3 (27%)	4.13	11.8 (24%)	3.56	9.6* (38%)	1.39

Data taken from Tables 54-56, pp. 246-253, MRID No. 43413701.

[†]N=5, except Males: plasma and RBC ChE 72ppm at Week 7, N=4; Females: RBC ChE control at Week 3, N=4.

[‡]Numbers in parenthesis are percent inhibition relative to controls (calculated by reviewer).

^{*}Significantly different from control, $p=0.05$ (Dunnett's test)

^{**}Significantly different from control, $p=0.01$ (Dunnett's test)

7. Sacrifice/necropsy/neurohistopathology

Animal sacrifice and processing of tissues ❖ A complete necropsy was performed on the animal that died on study day 14. At study termination, 5 animals/sex/group (allocated for neuropathologic examination) were euthanized by carbon dioxide inhalation and then perfused *in situ* with a buffered sodium nitrite solution followed by a solution of 1.5% glutaraldehyde-4.0% formaldehyde. The central and peripheral nervous system tissues were dissected and preserved. Brain weight (excluding olfactory bulbs) and brain dimensions (length and width) were recorded. Any observable gross changes, abnormal coloration, or lesions of the brain and spinal cord were recorded. The nerve tissues were embedded in plastic (central nervous system tissues) or paraffin (peripheral nervous system

tissues), sectioned, and then stained with hematoxylin and eosin. The study report did not state how many sections of the brain were examined, but it would appear that at 5 may have been taken based on the regions observed. The following nerve tissues and brain regions in the control and 540 ppm groups were examined microscopically:

Brain		Spinal cord		Peripheral nerves	
X	Forebrain	X	Cervical (C ₁ -C ₇)	X	Sciatic nerve
X	Cerebrum, center	X	Lumbar (T ₁₁ -L ₄)	X	Sural nerve
X	Midbrain	X	Gasserian gang./trigeminal nerve	X	Tibial nerve
X	Cerebellum	X	Lumb. dors. root gang.	X	Peroneal nerve
X	Pons	X	Lumb. dors. root fib.	X	Forelimbs
X	Medulla obl.	X	Lumb. ventr. root fib.	X	Tail
		X	Cerv. dors. root gang.	X	Optic nerve
		X	Cerv. dors. root fib.		Other
		X	Cerv. ventr. root fiber	X	Eyes

Results ❖

- Brain weights ❖ No test material-related changes in absolute brain weights or brain weights relative to body weight were observed in any of the treated or control groups. No effects were apparent on brain dimensions (length and width) in any of the perfused animals.
- Gross observations ❖ Macroscopic examination of the male rat that died on study day 14 revealed a large mass on the thymus gland and enlarged mediastinal and bronchial lymph nodes. No other gross lesions were observed at necropsy in treated or control animals.
- Neurohistopathology ❖ Histopathologic examination of the 540 ppm group did not reveal any microscopic lesions in central or peripheral nervous system tissues. The only lesions observed occurred in two control animals. Digestion chambers were seen in the sciatic nerve of one male and one female control and in the peroneal nerve of the female.

D. DISCUSSION

Groups of 10 male and 10 female rats were used to assess the neurotoxic potential of Ziram following dietary administration at concentrations of 72, 207, or 540 ppm for 13 weeks. Survival was not affected by treatment with Ziram. The death of one low dose male on study day 14 (showing a large mass on the thymus gland) was probably not a test material related effect, because all animals receiving the higher doses survived to termination of the study.

Effects on growth, manifested by decreased mean body weights and body weight gains in both sexes at 540 ppm, were of marginal biological significance. Beginning at week 1 and throughout the study period, the body weights were 7% to 11% lower than control values. The lower body weights were mainly due to the lower body weight gains seen during the first study week. The overall weight gain was significantly lower in males ($p < 0.05$) and females ($p < 0.01$). The effect on body weight was due in part to reduced food consumption which was significantly decreased in both sexes, particularly during the initial week of the study and was

generally slightly lower during the remainder of the study. Palatability of the test diet may have been a factor affecting food consumption at the high dose. Effects on food efficiency at 540 ppm (slight decrease in males and more pronounced decrease in females) suggest that the depressed growth was due to a toxic effect of the test material.

No test-material related effects indicative of neurotoxicity were observed at any dose level when clinical signs of toxicity, FOB and motor activity parameters, brain weights and dimensions and histopathologic examinations of neural tissues were evaluated in animals that survived to scheduled sacrifice. Transient occurrences of slight gait effect in one male and one female at high dose were insufficient to indicate a treatment-related effect. It should be noted, however, that abnormal defecation and/or gait abnormalities were reported in the range-finding study at 300 ppm. Gait abnormalities were reported at 300 mg/kg in the acute neurotoxicity study on ziram (MRID 43362801).

Treatment with Ziram produced a slight, not statistically significant, inhibition of brain ChE activity in males at 540 ppm (16%) and in females at 207 ppm (15%) and 540 ppm (23%). Brain NTE activity was significantly reduced at 540 ppm in males (47% lower than controls) and females (38% lower than controls). Brain NTE activity was also decreased at 72 and 207 ppm in both sexes but was not statistically significant. In females, inhibition of brain NTE activity at 72 and 207 ppm was equivocal and may have been treatment-related, but since it was not dose-related or statistically significant, it was not considered biologically relevant. RBC ChE activity appeared to be unaffected by treatment with Ziram in females at any dose level and the 29% inhibition of RBC ChE activity in males at 207 ppm (week 3 only) is of uncertain toxicological significance. The slightly decreased ($\leq 10\%$) plasma ChE activity observed in males at week 13 and in females at week 3, 7, and 13 is considered within normal variation. The study author concluded that the NOAEL is 540 ppm for plasma and RBC ChE inhibition and 207 ppm for brain NTE inhibition. The reviewer considers the threshold LOAEL for brain ChE inhibition to be 540 in males and 207 ppm in females based on the marginal effect seen in both sexes.

The doses selected for the 13-week neurotoxicity study were based on the results of a 6-week dietary study with rats showing effects on body weight, food consumption and clinical signs of toxicity at doses of ≥ 300 ppm. Based on these findings, the doses selected for the 13-week study appear to be justified even though marginal indications of toxicity was observed in this study.

Classification: Core-minimum.

E. STUDY DEFICIENCIES

The relative humidity (36% to 78%) was slightly higher than the range specified by protocol (30% to 70%); food efficiency was not calculated.

These deficiencies were considered minor and did not affect the quality or outcome of the study.

APPENDIX

VALIDATION STUDIES (Appendix G, pp. 1248-1280, MRID No. 434137-01)

D-Amphetamine Sulfate and Chlorpromazine Hydrochloride (Study No. WIL-99026)

To show that the Motor Activity System employed in the conduct of the motor activity test is sensitive (i.e., capable of detecting both increases and decreases in activity), rats received single intraperitoneal injections (i.p.) of 0, 0.5, 1.0, or 2.0 mg/kg D-amphetamine sulfate and following a rest period, 0, 2.5, 5.0, or 10 mg/kg chlorpromazine hydrochloride. Pronounced, transient increases in total and ambulatory motor activity were seen in animals treated with D-amphetamine sulfate and pronounced decreased activity was seen in animals treated with chlorpromazine hydrochloride compared with controls. The study demonstrated sensitivity of the system employed as well as reliability of operation across devices and across days of operation for both sexes. In addition, the system was capable of detecting changes in activity associated with the photoperiod, characteristic for this species.

Carbaryl (Study No. WIL-99032)

FOB and motor activity examinations were performed on rats after i.p. injection of 0, 2, 10, or 50 mg/kg carbaryl in 0.5% methyl cellulose. FOB and motor activity responses occurred primarily 30 minutes after dosing, were dose-related, and transient in nature. Specific FOB responses included altered posture, palpebral closure, convulsions during home cage observations; altered ease of removal (from home cage) and handling, salivation during handling, salivation, fur appearance and eye prominence during handling; increased time to first step, alterations in mobility, gait, gait score, arousal, convulsions, and number of rearing episodes during open-field observations; alterations in the approach, touch, startle, tail pinch and pupil responses, olfactory orientation and air righting reflex during the sensory observations; alterations in hindlimb extensor strength, grip strength, and rotarod performance during neuromuscular observations; alterations in catalepsy and body temperature. During the motor activity test, reductions in total and ambulatory activity were observed.

Acrylamide and Trimethyltin Chloride (Study No. WIL-99034)

To validate procedures used in the neurotoxicity screening battery, male and female rats received doses of 0, 5, 10, or 20 mg/kg/day acrylamide, 5 days/week for four consecutive weeks (route not indicated). Functional effects observed were dose-related and were more apparent with cumulative exposure (time). They included alterations in muscle tone during handling; alterations in the startle and tail pinch responses and air righting reflex during sensory observations; alterations in hindlimb extensor strength, fore- and hindlimb grip strength, and rotarod performance (decreases) and hindlimb footsplay (increases); and decreases in body temperature and body weight. Motor activity appeared to be unaffected by acrylamide. Both sexes treated with acrylamide developed lesions indicative of neurotoxicity (axonal degeneration, swollen axon cylinders or demyelination) in the trigeminal nerve, lumbar dorsal and ventral root fibers, cervical dorsal root fibers, sciatic nerve, sural nerve, tibial nerve, peroneal nerve, lumbar root (females only), and cervical ventral root fibers (females only).

To provide a positive control group exhibiting central nervous system pathology in which to validate neuropathology procedures, male and female rats were administered i.p. injections of 0 or 7.5 mg trimethyltin chloride and neural tissues were processed for histopathologic examination. Neuronal loss in the dentate gyrus was seen in 2/5 and chromatolysis in the gasserian ganglion neurons in 1/5 male rats.

3'-3'Iminodipropionitrile (Study No. WIL-99035)

To demonstrate inter-observer reliability for FOB studies, 3'-3'imino dipropionitrile (IDPN) was administered to rats by gavage as a single dose of 2000 mg/kg (controls received water). FOB tests were performed by eight trained technicians following the onset of the clinical signs characteristic

of IDPN exposure (continuous circling and head rolling behavior). The observations/alterations recorded for controls and IDPN-treated rats were considered consistent between observers.

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