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DATA EVALUATION REPORT

18

ZIRAM

Study Type: ACUTE ORAL NEUROTOXICITY ▩ RAT (81-8SS)

8/2/2555

Prepared for

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

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DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Neurotoxicity ▩ Rat (81-8ss)

TOX. CHEM. NO.: 931

P.C. CODE.: 034805

MRID NO.: 43362801

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

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

STUDY NUMBER: WIL-223001

SPONSOR: The Ziram Task Force, NCP, Inc., 22636 Glenn Drive, Suite 304, Sterling, VA 22107

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

TITLE OF REPORT: An Acute Neurotoxicity Study of Ziram in Rats

AUTHOR: I.C. Lamb

REPORT ISSUED: August 25, 1994 (study completion date)

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 43362801), male and female Sprague-Dawley Crl:CD BRR 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.

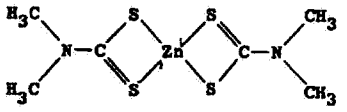
This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for an acute neurotoxicity study (81-8) in rats.

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test compound: Ziram

Purity: 97.8% a.i.; a correction factor of 1.02 was used for dose calculation.
Description: white powder
Lot No.: V528/8331AA
Contaminants: not specified
Structure:



2. Vehicle

MazolaR corn oil (Best Foods, CPC International, Inc., Englewood Cliffs, NJ)

3. Test animals

Species: rat
Strain: Sprague-Dawley Crl:CDRBR
Age and weight at study initiation: 43 days old; 189-253 g (males); 138-190 g (females)
Source: Charles River Breeding Laboratories, Inc., Portage, MI 49081
Housing: individually in stainless steel wire-mesh cages
Environmental conditions:
- Temperature: 70-74°F
- Humidity: 60-85%
- Air changes: 10/hour
- Photoperiod: 12 hours light/12 hours dark

Acclimation period: 18 days

4. Diet

Animals were fed PurinaR Certified Rodent Chow # 5002 and provided with municipal water *ad libitum*.

B. STUDY DESIGN

1. Animal assignment

Animals were assigned to treatment groups using computer-based randomization that ensured homogeneity of group means and variance for body weight (Table 1). Four study group replicates were then randomly assigned (with equal representation of each dose group and sex) for testing over four consecutive days.

TABLE 1. ANIMAL ASSIGNMENT

Test group	Dose level	No. assigned	
	mg/kg	male	female
1 Control	0	12	12
2 Low (LDT)	15	12	12
3 Mid (MDT)	300	12	12
4 High (HDT)	600	16	16

Data taken from table on p. 20, MRID No.43362801.

The test material suspension in corn oil was administered once by gavage (dose volume 7.5 mL/kg) to non-fasted animals. Doses were calculated based upon the animal body weight recorded prior to administration. Controls received an equivalent volume of the corn oil vehicle.

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 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 an acute oral range-finding study (WIL-160013) in which rats received gavage doses of 10, 20, 25, 50, 150, 300, 500, 600, 700, 800, 1250, 1500, 1750, or 2000 mg/kg of Ziram. No deaths occurred at dose levels of 10-500 mg/kg. The mortality was 2/8, 2/2, 1/2, 2/2, 2/2, 1/1, or 0/1 for males and 3/8, 2/2, 1/2, 1/2, 1/2, 1/1, or 1/1 for females administered 600, 700, 800, 1250, 1500, 1750, or 2000 mg/kg, respectively. Except for one female in the 600 mg/kg group that died on study day 3, all of these animals died or were euthanized *in extremis* within 24 hours following dosing. The predominant clinical signs included gait alterations at ≥25 mg/kg; hypoactivity at ≥50 mg/kg (except 500 mg/kg); lacrimation at 50, and 300 to 1500 mg/kg; and salivation at ≥150 mg/kg (except 500 mg/kg). The estimated time of peak effect of the treatment-related clinical signs was approximately 4 hours after dosing.

4. Preparation and analysis of dosing solutions

Dosing suspensions of Ziram in MazolaR corn oil were prepared by direct dilution at the appropriate doses on the day of dosing. Dosing suspensions were analyzed for stability and homogeneity prior to administration and concentration of dosing suspensions administered were determined for Replicate #1 only. The concentrations of test material in corn oil were determined by gas chromatography/flame photometry.

Results The dosing suspensions were stable for 8 hours at room temperature. Homogeneity of three samples taken from the low-, mid-, and high- dose suspensions was acceptable (within 10% of target concentration). The actual concentrations of doses administered were 2.15 mg/mL, 42.6 mg/mL, or 87.5 mg/mL for the low-, mid-, or high-dose groups, respectively (104-107% of target concentrations). Spiked sample mean recoveries ranged from 44.8% - 99.8%; recoveries were used to correct analysis values for the appropriate group.

5. Statistical analysis

Statistical analyses of body weight, histopathologic and brain data were performed by a Digital MicroVAX 3400R computer with appropriate software. 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 control and treated groups. Histopathological findings in treated groups were compared to control 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 (8/25/94) Quality Assurance and Good Laboratory Practice Statements were present.

C. METHODS AND RESULTS

1. Clinical observations and mortality

Animals were examined twice daily for mortality and/or moribundity. Detailed clinical signs of toxicity were recorded daily for all animals. No clinical signs were recorded when the FOB was conducted.

Results One female in the 300 mg/kg group died on study day 2. Four males and seven females died following dosing with 600 mg/kg (the males and three of the females died on study day 1; the other three females died on study day 2, 4, or 5). All other animals in the 300 and 600 mg/kg groups, all animals in the 15 mg/kg group, and all controls survived the 14-day observation period.

Representative clinical observations are shown in Table 2. Clinical signs observed for those animals that died on study in the 600 mg/kg group were generally limited to 2/4 males and 5/7 females and included gait alterations, abnormal respiration, abnormal excretion, ptosis, hypothermia and hypoactivity. In the animals that survived to study termination effects were generally seen during the first week of the study. Notable clinical signs in the surviving 300 and 600 mg/kg groups included decrease body temperatures (Day 0 after dosing), gait alterations (rocking, lurching, or swaying and/or high carriage), abnormal respiration (rales, labored respiration, and/or gasping),

TABLE 2. REPRESENTATIVE CLINICAL OBSERVATIONS DURING 14-DAY OBSERVATION PERIOD

Observations [1]	0 mg/kg		15 mg/kg		300 mg/kg		600 mg/kg	
	male	female	male	female	male	female	male	female
Gait alterations	0	0	0	0	10/3	2/2	8/5	17/8
Labored respiration	0	0	0	0	10/2	0	4/3	6/3
Gasping	0	0	0	0	13/2	0	1/1	2/1
Rales	0	0	2/2	0	20/3	1/1	6/3	6/3
Abnormal excreta								
soft stool	1/1	0	0	0	2/2	4/3	7/5	6/5
diarrhea	0	0	0	0	2/1	0	7/7	5/4
mucoid feces	0	0	0	0	1/1	1/1	9/8	10/7
decr. defecation	0	0	0	0	14/3	2/2	6/3	9/6
decr. urination	0	0	0	0	14/3	0	6/3	9/5
Distended abdomen	0	0	0	0	10/2	0	7/3	6/4
Body surface staining[1]	0	0	0	0	2	4	7	5
Cyanosis	0	0	0	0	6/2	0	0	0
Cool body temp.	0	0	0	0	3/2	0	2/2	0
Enophthalmus	0	0	0	0	3/2	0	0	0
Unkempt appearance	0	0	0	0	3/2	0	0	0
Hypoactivity	0	0	0	0	6/2	0	4/1	2/1
Ptosis								
right eye	0	0	0	0	1/1	1/1	1/1	1/1
left eye	0	0	0	0	2/2	1/1	1/1	1/1

Data taken from Table 1, pp. 50-55, MRID No. 43362801.
[1]Data expressed as total occurrence/number of animals affected.
[1]Data taken from p. 27; expressed as number of animals affected.

abnormal excreta (soft stool, diarrhea, mucoid feces, decreased defecation and urination, and small feces), distended abdomen, and tan, red, orange and/or brown staining of body surfaces. Some of these findings were limited to or occurred most frequently in two males in the 300 mg/kg group (Nos. 15355 and 15387); clinical signs observed only in these two males included cyanosis and enophthalmus. [The authors indicated that unkempt appearance also occurred only in males Nos. 15355 and 15387. Data in Tables 50-55 of the study report, however, show a low incidence of these findings also in high-dose males.] Cyanosis, cool body temperature, enophthalmus, and unkempt appearance were generally seen on study day 8 or later in these two males. Abnormal respiration, gait alterations, abnormal excreta, and distended abdomen in the two 300 mg/kg males generally persisted through to scheduled termination on day 15. Rales were observed in two males in the 15 mg/kg group on study day 1. [Individual data (pp. 908 and 912 of Appendix A) show that rales occurred on study days 3 and 5.] The author indicated and the reviewer concurs that this finding cannot be unequivocally attributed to treatment due to lack of an apparent dose response in males at 300 and 600 mg/kg and the absence of a similar effect in females at 15 mg/kg for this study day.

2. Body weights

Body weights were recorded pre-study (day -10), on study days 0, 7, and 14, during the FOB evaluations, and prior to necropsy (day 15). Animals found dead were weighed prior to necropsy. The final body weights of the 600 mg/kg group male and female found dead were inadvertently not recorded.

Results Mean body weights are presented in Table 3. Mean body weights were significantly ($p<0.01$) lower for study days 7, 14 and 15 in the 300 mg/kg males (12-16% lower than controls) and on day 7 in the 600 mg/kg males (9% lower than controls). Gains were reduced by 63% and 67% at 300 and 600 mg/kg, respectively ($p<0.01$) and cumulative gain was lower than controls due to these decreases. The reduced body weight gain in mid-dose males was attributed mainly to two animals (Nos. 15355 and 15387). Body weights in the 15 mg/kg males were comparable to controls.

Mean body weight at day 14 was comparable among all groups of females. However, transient statistically significant decreases in body weight gain were observed during the first week (-55% and -45% of controls at 300 and 600 mg/kg, respectively). Although the mean body weights were comparable in all groups at day -10, the mean weight of high dose females was 10 g heavier on day 0. After the initial decrease, gain was similar or greater than controls. The transient decreases are considered to be an effect of treatment. No effects were seen at 15 mg/kg.

TABLE 3. MEAN BODY WEIGHTS (g) OF RATS GIVEN A SINGLE GAVAGE DOSE OF ZIRAM IN CORN OIL

	0 mg/kg		15 mg/kg		300 mg/kg		600 mg/kg	
	male	female	male	female	male	female	male	female
Day -10	122±6.2	106±4.7	122±6.3	106±4.9	122±5.8	106±4.7	124±7.0	109±6.5
Day 0	221±15.1	154±10.1	222±9.9	156±10.2	220±11.6	158±9.0	227±15.5	164±9.8*
Day 7	273±23.3	176±10.1	272±10.5	174±15.8	239±15.6**	168±9.5	248±23.1**	177±9.4
Day 14	321±28.8	195±12.0	322±14.4	191±18.0	271±56.6**	190±13.5	314±25.8	199±14.4
Day 15	327±28.8	196±11.7	328±15.3	192±17.4	275±60.7**	192±12.7	321±24.6	200±14.1

Data taken from Table 2, pp. 56-57, MRID No. 43362801.
*Significantly different from controls, $p<0.05$ (Dunnett's test)
**Significantly different from controls, $p<0.01$ (Dunnett's test)

3. Food consumption

Food consumption data were not provided. Test material intake was by gavage and, therefore, unaffected by food intake.

4. Functional observational battery (FOB)

An FOB was conducted pretest, at the approximate time of peak effect (4 hours after dosing) and at days 7 and 14 of the study. FOB tests were performed by the same technicians without knowledge of the animal group assigned. The following parameters were observed according to the method of Moser (1991):

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 Representative FOB parameters from the session on day of dosing (day 0) are shown below in Table 4. In general, responses occurred 4 hours after treatment.

TABLE 4. FUNCTIONAL OBSERVATIONAL BATTERY (DAY OF DOSING)

Observation [1]	0 mg/kg		15 mg/kg		300 mg/kg		600 mg/kg	
	male	female	male	female	male	female	male	female
No. of animals	12	12	12	12	12	12[1]	12	12[1]
Home cage observations								
Posture								
sitting, head held low	0	0	1	0	4	3	5*	2
rearing	0	0	0	0	2	0	1	2
flattened, limbs may be extended	0	0	0	0	0	1	0	0
Palpebral closure								
eye lids slightly drooping	0	0	1	0	2	0	0	1
half-closed eye lids	0	0	0	0	1	2	3	1
eye lids shut	7	8	7	7	9	7	8	7
Feces consistency								
pellets partially formed	0	0	0	0	1	0	2	0
pellets unformed, diarrhea	0	0	0	0	0	1	4	3
Handling observations								
Lacrimation, slight	0	0	0	0	3	5*	0	1
Salivation								
slight	0	0	1	0	7*	8*	5*	2
severe	0	0	0	0	0	0	1	1

Fur appearance								
slightly soiled	0	0	1	0	4	4*	4	2
very soiled, crusty	0	0	0	0	1	1	2	2
Palpebral closure								
slight	0	0	0	0	6*	5*	8*	5*
half-closed eye lids	0	0	0	0	0	0	0	1
Respiration, decreased	0	0	0	0	3	2	5*	2
Decr. muscle tone	0	0	0	0	0	1	2	0
Red deposits								
eyes	0	0	1	0	3	0	1	0
nose	0	0	2	0	5*	2	2	1
mouth	0	0	0	0	6*	1	2	2
Crusty deposits								
nose	0	0	0	0	2	3	5*	3
mouth	0	0	0	0	4	6*	8*	7*

TABLE 4. Continued

Observation [1]	0 mg/kg		15 mg/kg		300 mg/kg		600 mg/kg	
	male	female	male	female	male	female	male	female
Open field observations								
Impaired mobility								
slight	0	0	0	0	3	4*	4	2
moderate	0	0	0	0	1	1	1	1
Time to first step (sec)	0.6	0.4	0.5	0.4	0.8	0.5	0.6	0.6
Altered gait								
walks on tiptoes	0	0	0	1	4	7*	7*	5*
ataxia, excessive sway, rocks or lurches	0	0	2	0	4	1	2	2
Gait score								
slight impairment	0	0	2	1	7*	4*	3	3
considerable impairment	0	0	0	0	1	4*	6*	4*
Sensory and physiological observations								
Tail pinch response, absent	0	0	0	0	1	0	0	1
Olfactory response, absent	0	1	0	0	1	2	2	2
Startle response, absent	0	0	0	0	0	0	1	1
Forelimb extension, absent	0	0	0	0	0	0	0	1
Hindlimb extension, absent	0	1	0	0	0	2	0	1
Body temperature (°C)[2]	38.8	38.9	38.6	38.8	35.1**	35.2**	35.2**	35.1**

Data taken from Table 6, pp. 66-67; Table 14, pp. 91-97; Table 22, pp. 140-143; Table 30, pp. 173-176; and Table 45, pp. 217-218, MRID No. 43362801.
*Significantly different from controls, $p<0.05$ (Fisher exact test)
**Significantly different from controls, $p<0.01$ (Dunnett's test)
[1]Data expressed as number of animals showing each observation
[1]During handling and open field observations, only 11 and 9 females were tested in the 300 and 600 mg/kg groups, respectively.
[2]Group means

Home cage observations on study day 0 showed treatment-related effects at 300 and 600 mg/kg consisting of alterations in posture, palpebral closure, and feces consistency in both sexes. No treatment-related effects were observed on study day 7 at any dose level. On study day 14, two 300 mg/kg group males (Nos. 15355 and 15387) had altered posture and one of the two had altered palpebral closure (slightly drooping). There was a decreased ($p<0.05$) number of alert 600 mg/kg males on study day 14 when posture was evaluated; however, this finding was not attributed to treatment, because the distribution of posture findings in this group were identical to that in the pretest group.

Handling observations on study day 0 revealed treatment-related signs of toxicity in males and females receiving 300 or 600 mg/kg including lacrimation, salivation, changes in fur appearance, altered palpebral closure, changes in respiratory rate and/or character, red deposits around the mouth, and crusty deposits around the nose and mouth. Also seen was decreased muscle tone in 600 mg/kg males only. Except for rales observed in two 300 mg/kg group males (Nos. 15355 and 15387), there were no treatment-related effects while handling the animals on study day 7. The only remarkable findings on study day 14 were limited to the same two males in the 300 mg/kg group and included altered palpebral closure, gasping, and changes in mucous membrane and skin color. One of the two males

had changed fur appearance and crusty deposits around the nose and mouth; the other male exhibited enophthalmus (change in eye prominence).

Open field observations on study day 0 revealed treatment-related signs of toxicity consisting of impaired mobility and altered gait in males and females that received 300 or 600 mg/kg and males in the 15 mg/kg group. No treatment-related effects were noted on study day 7 at any dose level. On study day 14, considerably impaired gait (without falling) was observed in two 300 mg/kg males (Nos. 15355 and 15387). In addition, animal No. 15355 had moderately impaired mobility on study day 14. In the 15 and 600 mg/kg groups on study day 14, the time to first step means were significantly ($p<0.05$) lower than the control group means. The lower times to first step were not considered a treatment-related effect because the differences were slight and higher times to first step, rather than lower, are usually considered to be an adverse effect. Mean urination counts in the 600 mg/kg males were significantly ($p<0.05$) increased compare to controls on study day 14. The values were similar to historical control data and were not considered treatment-related.

Sensory observations that were attributed to treatment on study day 0 included absent tail pinch and olfactory responses in 300 and 600 mg/kg group males, absent startle response in 600 mg/kg group males and females, and reduced startle response in 300 and 600 mg/kg group males and females. In addition, absent forelimb and/or hindlimb extension was seen in the 300 and 600 mg/kg group females. No treatment-related effects were noted on study day 7 at any dose level. On study day 14, impaired air righting reflex (lands on side) and absence of hindlimb extension was observed in 300mg/kg group male No. 15355. On study day 14, the numbers of females in the 600 mg/kg group that had slow approach and touch responses were significantly increased ($p<0.05$) compared to controls; however, the values were similar to pretest values and were not considered treatment-related.

Neuromuscular observations did not reveal treatment-related effects in any dose group on study days 0, 7, and 14. On study day 7, mean rotarod performance was decreased in 600 mg/kg group males (-48%, not significant), but was similar to the pretest values. On study day 14, mean forelimb grip strength of the 600 mg/kg males was significantly increased (+28%; $p<0.01$). This effect was considered the result of biological variation, because decreases rather than increases are usually considered adverse effects.

Physiologic observations on study day 0 revealed treatment-related reductions ($p<0.01$) in body temperature in the 300 and 600 mg/kg group males and females. On study day 7, slightly decreased mean body temperatures in the 300 and 600 mg/kg group males were not considered treatment-related because they were similar to pretreatment values. On study day 14, two 300 mg/kg group males (Nos. 15355 and 15387) had markedly decreased body temperatures (34.9°C and 33.2°C, respectively) when compared to control group means (38.8°C). No treatment-related effects on catalepsy were observed in any dose group at study day 0, 7, or 14.

5. Motor and locomotor activity

Observations were made on all animals during the pretest period, 4 hours after dosing, and on study days 7 and 14. Locomotor activity was recorded after the completion of the FOB in an automated motor activity unit (Digiscan Micro Animal Activity System, Omnitech Electronics, Inc.) which utilizes a series of infrared photobeams surrounding a clear plastic cage to quantify an animal's motor activity. The movements were recorded for each animal during 41-minute sessions at 1-minute epochs in four 10-minute subsessions. Locomotor activity was divided into two categories, total motor and ambulatory activity.

Results Mean total motor activity and mean ambulatory activity data are presented in Table 5 and 6, respectively. During the pretest evaluation, locomotor activity in the 15, 300, and 600 mg/kg groups was comparable to control values for both sexes.

Treatment with the test material produced significantly ($p < 0.05$) decreased total motor activity and mean ambulatory activity counts in both sexes at 300 and 600 mg/kg on study day 0. The values for all four subsessions, particularly the first three, were remarkably reduced in these two dose groups. Full recovery occurred by study day 7 in males and females treated with 300 mg/kg and in females treated with 600 mg/kg; males treated with 600 mg/kg recovered fully by study day 14.

The total motor activity on day 0 was only 14% and 18% of controls in the 300 and 600 mg/kg groups males, respectively, and 21% and 13% of controls in females, respectively. In the 15 mg/kg group females on study day 0, the subsession 2 mean motor activity count was significantly lower than the control mean (-40%; $p < 0.05$). However, all other subsession values were comparable to control group values. In addition, the subsession 2 mean ambulatory count was not significantly decreased. Therefore, the lower subsession mean was not considered test material related. The total motor activity was 80% of controls for the 600 mg/kg males on study day 7 and was comparable to controls for the 300 and 600 mg/kg groups (both sexes) on study day 14.

On day 0, the ambulatory activity was 13% and 20% of controls in the 300 and 600 mg/kg groups males, respectively, and 24% and 13% of controls in females, respectively. At 15 mg/kg, the ambulatory activity was comparable or slightly lower than control values, respectively. On study day 7, the ambulatory activity in males exposed to 600 mg/kg was reduced to 76% of control values. All other ambulatory activity counts were comparable to control on study days 7 and 14.

TABLE 5. MEAN TOTAL MOTOR ACTIVITY: TOTAL MOVEMENT COUNTS/40 MIN TEST SESSION

	0 mg/kg		15 mg/kg		300 mg/kg		600 mg/kg	
	male	female	male	female	male	female	male	female
Pre-test	1687	1812	1811 (107)[a]	1855 (102)	1593 (94)	2139 (118)	1797 (107)	1861 (103)
Day 0	1504	1703	1375 (91)	1449 (85)	205* (14)	360* (21)	267* (18)	228* (13)
Day 7	1775	1998	2043 (115)	2012 (101)	1642 (93)	1919 (96)	1415 (80)	1977 (99)
Day 14[b]	1659	2255	1888 (114)	1929 (86)	1727 (104)	2170 (96)	1589 (96)	2165 (96)

Data taken from Tables 48-51, pp. 224-239, MRID No. 433628-01.
a Numbers in parenthesis represent percent of control activity (calculated by reviewer).
b Excludes counts from male #15378 due to monitor malfunction
*Significantly different from controls, $p<0.05$ (Dunnett's test)

TABLE 6. MEAN AMBULATORY ACTIVITY/40 MIN TEST SESSION

	0 mg/kg		15 mg/kg		300 mg/kg		600 mg/kg	
	male	female	male	female	male	female	male	female
Pre-test	848	839	934 (110)[a]	896 (107)	772 (91)	1036 (123)	961 (113)	950 (113)
Day 0	800	873	768 (96)	724 (83)	106* (13)	212* (24)	161* (20)	117* (13)
Day 7	1037	1147	1243 (120)	1184 (103)	908 (88)	1056 (92)	784 (76)	1054 (92)
Day 14[b]	1004	1346	1193 (119)	1140 (85)	1017 (101)	1268 (94)	875 (87)	1277 (95)

Data taken from Tables 48-51, pp. 225-239, MRID No. 433628-01.
a Numbers in parenthesis represent percent of control activity (calculated by reviewer).
b Excludes counts from male #15378 due to monitor malfunction
*Significantly different from controls, $p<0.05$ (Dunnett's test)

6. Clinical chemistry

Clinical chemistry analyses were not performed.

7. Sacrifice/necropsy/neurohistopathology

Animal sacrifice and processing of tissues ▩ A complete necropsy was conducted on all animals found dead during the study. The necropsy included an examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities including the viscera. All gross lesions were collected and preserved in 10% buffered formalin. After at least 14 days of observation, all surviving animals in each group were euthanized by carbon dioxide inhalation and then perfused *in situ*. 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. Remarkable gross lesions observed internally/externally during perfusion/ dissection processes were also 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 specify how many brain sections were prepared but based on the regions examined, it appears that 5 may have been prepared. The following nerve tissues and brain regions from 5 animals/sex in the control and 600 mg/kg groups were examined microscopically:

Brain		Spinal cord		Peripheral nerves	
X	Forebrain	X	Cervical (C_1-C_t)	X	Sciatic nerve
X	Cerebrum, center	X	Lumbar (T_{tt}-L_t)	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 ▩

a. Brain weight ▩ Dose-related decreases in absolute brain weights were seen in males treated with Ziram (relative brain weights were not provided). At doses of 15, 300, or 600 mg/kg, the brain weights were 4%, 6%, or 7%, respectively, lower than control values and statistically significant ($p<0.01$) at 300 and 600 mg/kg.

b. Gross observations ▩ Findings in the animals that died on study included the following: white contents in the stomach and/or intestine in the female in the 300 mg/kg group and in three of the males and five of the females in the 600 mg/kg group; distention of the stomach/intestine in female in the 300 mg/kg group and in one male and three females in the 600 mg/kg group; and emaciation (no abdominal adipose tissue present) in two females in the 600 mg/kg group. No treatment-related gross lesions were noted at necropsy in surviving animals.

c. Neurohistopathology ▩ No treatment-related lesions were observed in central or peripheral nervous system tissues examined from the control or high dose group.

In the central nervous system tissues, digestion chambers, a spontaneous nerve fiber degeneration, were observed in the lumbar root of one control male, in the lumbar ventral root fibers of another control male, and in the lumbar dorsal root fibers of one

male in the 600 mg/kg group. In the peripheral nervous system, digestion chambers were observed in the sciatic nerve of one control male. The study author noted that digestion chambers have been observed in the central and peripheral nervous systems of control and treated rats (Eisenbrandt et al., Toxicol. Pathol. 18:154-164, 1990) and should not be considered uncommon.

D. DISCUSSION

In an acute neurotoxicity study, male and female Sprague-Dawley Crl:CD BRR rats received a single gavage dose of 0, 15, 300, or 600 mg/kg of Ziram 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.

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 study author did not indicate why the doses used were selected. The high dose of 600 mg/kg caused mortality in the range-finding study (and also one death at 300 mg/kg), which should indicate that for a neurotoxicity screening study, a lower dose should be selected. Furthermore, a rationale for spacing of the low and mid doses at 15 and 300 mg/kg, respectively, was not provided. If the range-finding study indicated some clinical signs at 25 mg/kg, lower mid and high doses may have been more appropriate for testing this compound. Testing at lethal dose levels may complicate interpretation of neurobehavioral data.

The effects observed in this study during clinical, FOB, and motor activity evaluations are indicative of test-material related neurotoxicity. The LOAEL is 15 mg/kg based on ataxia and impaired gait in males. No NOAEL was determined.

E. STUDY DEFICIENCIES

The study appears to have been properly designed and conducted. Minor deficiencies include lack of food consumption data and a few discrepancies between clinical signs reported in Tables and summary data provided by the study author. Food consumption data would have been helpful, especially since two mid-dose males had significantly decreased body weights. However, these deficiencies do not detract from the validity of the study.

APPENDIX

VALIDATION STUDIES (Appendix J, pp. 1014-1044, MRID No. 433628-01)

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 of one day, 0, 2.5, 5.0, or 10 mg/kg chlorpromazine hydrochloride. Pronounced, transient increases in total and ambulatory motor activity in animals treated with D-amphetamine sulfate and pronounced decreased activity was seen in animals treated with chlorpromazine hydrochloride were observed compared with controls. The study demonstrated sensitivity of the system employed as well as reliability of operation of the system. 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, 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; and 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 reported). 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, digestion chambers, 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)

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To demonstrate inter-observer reliability for FOB studies, 3'-3'iminodipropionitrile (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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