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DATA EVALUATION RECORD – Supplemental
See TXR # 0055297 for root DER.

STUDY TYPE: Non-guideline; Developmental Neurotoxicity Study in Rats

PC CODE: 034805 **DP BARCODE**: D459226
TXR#: 0058080

TEST MATERIAL (PURITY): Ziram (96.75% a.i.)

SYNONYMS: (*T*-4)-bis(dimethylcarbamodithioato-κ*S*,κ*S*′)zinc

CITATION: Nemec, M.D. (2009) A dietary developmental neurotoxicity study of Ziram in rats. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Study No.: WIL-223006, October 22, 2009. MRID 47900401. Unpublished.

SPONSOR: Ziram Task Force
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EXECUTIVE SUMMARY: In a non-guideline developmental neurotoxicity study (MRID 47900401) Ziram (96.75% a.i.; doses adjusted for purity, Batch no. G060600605) was administered in the diet to 25 presumed pregnant female Sprague-Dawley rats/dose at nominal dose levels of 0, 72, 207, and 540/360 ppm (equivalent to 0, 8.6, 24.0, and 46.6 mg/kg/day; calculated by reviewers) from gestation day (GD) 6 through lactation day (LD) 21. Due to the magnitude of maternal toxicity observed, the 540 ppm dietary concentration was reduced to 360 ppm beginning on LD 4. Dams were allowed to deliver naturally and rear their offspring to LD 21. Any females that were found to be sperm positive and/or with a vaginal plug, but did not deliver, were sacrificed on GD 25. Females that delivered were sacrificed and necropsied on LD 21. On postnatal day (PND) 4, litters were standardized to 8 pups/litter (4/sex, when possible); the remaining offspring and dams were sacrificed and discarded without further examinations. Subsequently, 1 pup/litter/group (15 or 20 pups/sex/dose) were allocated to subsets for motor activity, brain weights, and neuropathological examination. Acceptable positive control data were included with this study.

Maternal toxicity: No treatment-related effects were observed on mortality, clinical signs of toxicity, reproductive parameters, or gross lesions in the dams.

At 540/360 ppm, mean maternal body weights were decreased ($p<0.01$) by 5-15% during GD 7 through 20. This group displayed weight loss during GD 6-9 (-14 g treated vs. 11 g controls), and decreases ($p<0.01$) in body weight gains of 33-75% compared to controls during GD 9-12, 12-15, and 18-20. Overall (GD 6-20) bodyweight gain was decreased ($p<0.01$) by 50% compared to controls at this dose. These decreases in body weight and body weight gain corresponded to decreases (14-55%; $p<0.01$) in food consumption (g/rat/day) observed throughout gestation, with overall (GD 6-20) food consumption being decreased ($p<0.01$) by 33% compared to controls. During lactation days 1 through 4, decreases ($p<0.01$) were observed in body weights (decr 14-16%) and food consumption (decr 17%). Body weight gains were also decreased during this interval; however, did not attain statistical significance. Due to the severity of maternal toxicity observed at 540 ppm, the dietary concentration of the test material was reduced to 360 ppm beginning on LD 4. Following the decrease in dietary concentration, body weights remained decreased ($p<0.05$) by 4-11% compared to controls from LD 5 through 20. However, the reductions in body weight at this dose became less severe over time, and body weight gains at this dose increased ($p<0.01$) by 225% during LD 4-7 and by 80% overall (LD 1-21) compared to controls. Food consumption at this dose remained decreased ($p<0.05$) at several intervals; however, there was no significant effect on bodyweight gains.

At 207 ppm, body weights were decreased by 2-4% from GD 7 through 20 and attained statistical significance on GD 15 and 20. Additionally at this dose, body weight gains were decreased ($p<0.01$) by 82% during GD 6-9 and by 21% during GD 18-20, and overall (GD 6-20) bodyweight gain was decreased ($p<0.01$) by 16% compared to controls. The decreased bodyweight gain noted during GD 6-9 corresponded to decreased food consumption during this interval (↓15%; $p<0.01$). During lactation, body weights were decreased ($p<0.05$) by 4-5% on LD 2-4 only.

No adverse compound-related effects were noted in the 72 or 207 ppm dams.

The maternal LOAEL is 540/360 ppm (equivalent to 46.6 mg/kg/day), based on decreases in body weights, body weight gains, and food consumption during gestation and lactation. The maternal NOAEL is 207 ppm (equivalent to 24 mg/kg/day).

Offspring toxicity: No compound-related effects on viability/survival, litter parameters, clinical signs, post-weaning body weights or body weight gains, sexual maturation (preputial separation or vaginal patency), brain weight or measurements, gross or microscopic lesions, or brain morphometric measurements were observed in the F_1 pups. Live birth, viability, and lactation indices were similar to controls at all doses.

At 540/360 ppm, mean pup body weights were decreased in the males (decr 10-19%, $p<0.01$) throughout pre-weaning (PND 1 through 21) and in the females (decr 11-16%, $p<0.05$) at all pre-weaning intervals except PND 17. Also at this dose, body weight gains were decreased ($p<0.05$) by 26-28% in the males during PND 1-4 and 4-7 and by 22% in the females during PND 1-4. On PND 17-21, body weight gains were decreased ($p<0.05$) by 17-19% in both sexes. Overall pre-weaning (PND 1-21) body weight gains (calculated by reviewers) were decreased by 11-12% in both sexes at this dose. Cumulative locomotor activity counts (combined sexes) were increased (not statistically significant) by 25% (total activity) and 44% (ambulatory activity) on

PND 13 and by 31% and 41%, respectively, on PND 17. On PND 21, this group showed less habituation compared to controls, resulting in increased cumulative total (40%, $p<0.05$) and ambulatory 62%, $p<0.05$) activity counts.

At 207 ppm, body weight was decreased ($p<0.05$) by 8% in the males on PND 21. On PND 17-21, body weight gains were decreased ($p<0.05$) by 15-17% in both sexes (likely due to direct consumption of the test diet). Animals in this group showed less habituation during locomotor activity evaluations on PND 21, resulting in increased cumulative total (47%, $p<0.01$) and ambulatory activity counts (66%, $p<0.05$).

At 72 ppm, male pup body weight was decreased by 7% (not statistically significant) on PND 21, and body weight gain was decreased by 13% in the males on PND 17-21. Males and females in this group showed less habituation during locomotor activity sub-session evaluations on PND 21, resulting in increased cumulative total (↑24%) and ambulatory activity counts (↑43%) relative to controls (not statistically significant).

The offspring LOAEL is 72 ppm (equivalent to 8.6 mg/kg/day), based on increased locomotor activity and decreased habituation on PND 21, as well as decreased pup body weight in males. The offspring NOAEL was not defined (<72 ppm).

This study is classified **acceptable/non-guideline** and provides useful data concerning developmental neurotoxicity in rats. The study was considered non-guideline for the following reasons: no functional observational battery was performed on the dams or pups, acoustic startle response testing was not performed, and no form of learning and memory testing (i.e. passive avoidance or water maze) was performed. Nevertheless, the study was considered acceptable.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

COMMENTS: This supplemental DER includes a revised executive summary, the conclusions of which supersede those of the previous review. The study was previously classified as unacceptable/

The previously selected maternal LOAEL was 207 ppm (equivalent to 24 mg/kg/day), based on decreases in body weights, body weight gains, and food consumption during gestation and/or lactation. The body weight changes in dams at this dose are not considered adverse according to current scientific practices because body weight decreases were <10% relative to control. At the high dose, body weights were decreased by ≥10%; therefore, the maternal LOAEL has been updated to 540/360 ppm (equivalent to 46.6 mg/kg/day). The maternal NOAEL is 207 ppm (equivalent to 24 mg/kg/day).

Previously, the offspring LOAEL was 207 ppm (equivalent to 24 mg/kg/day), based on increased locomotor activity, decreased habituation, and decreases in pup body weights and body weight gains. The offspring LOAEL has been updated to 72 ppm (8.6 mg/kg/day) based on increased locomotor activity and decreased habituation on PND 21, along with decreased pup body weight in males. Pup body weight was decreased by 7% in males on PND 21, which is considered

adverse according to current scientific practices. The increases in motor activity and ambulatory activity observed at the low dose on PND 21 were not statistically significant. The route of administration was dietary rather than gavage, which may explain the large variability in the data and lack of statistical significance. The main concern in this study is the lack of habituation. In a DNT, animals are placed in an automated measuring device designed to record movement at regular intervals. Control animals habituate relatively quickly because the environment is familiar and exploratory movement decreases with time. When examining the subsession data, it appears that pups exposed to ziram at all dose levels took longer to habituate than controls on PND 21. The corresponding increases in ambulatory and motor activity are a reflection of the lack of habituation rather than an indicator of excitability. It is also important to note that by PND 21, animals should be familiar with the testing environment and should habituate quickly; therefore, the lack of habituation at this lifestage further supports the adversity of the observations.

Similar effects on offspring motor activity and habituation were observed in a previous study. Prior to the submission of this DNT, an older study treated as a DNT (MRID 43935801), which was actually a 2-generation reproduction study with neurological evaluations in F_2 pups, was classified as unacceptable for evaluating neurotoxicity because motor activity data were not analyzed statistically and brain morphometrics were not performed in offspring on PND 11 or 70. This was a dietary exposure with doses of 5, 13, and 32 mg/kg/day in the low-, mid-, and high dose groups, respectively. Although statistical significance was not determined, there were substantial increases in motor activity in all treatment groups. On PND 17, there was a dose-dependent increase in motor activity counts in males (↑55%, ↑80%, and ↑107% in the low, mid, and high dose groups, respectively) and in females (↑201%, ↑213%, and ↑396% in the low, mid, and high dose groups, respectively). On PND 21, motor activity counts were increased by 70% in low dose males and 48% in low dose females, however the data did not follow a dose response. These increases in motor activity at the low and mid dose (5 mg/kg/day and 13 mg/kg/day) on PND 17 support the increased motor activity and decreased habituation seen at 8.6 mg/kg/day in this newer DNT.

Ziram is a member of the dimethyldithiocarbamate class of fungicides. A comparison of available rat metabolism data among the dimethyldithiocarbamates suggests that ziram, thiram and ferbam either dissociate or reduce into dimethyldithiocarbamate anions and are metabolized to carbon disulfide and dimethylamine. These compounds display similar toxicity at comparable doses. Thiram has a DNT in which increased locomotor activity and decreased habituation were observed on PND 17 at 3.7 mg/kg/day. This observation further supports the lack of habituation observed in the ziram DNT at 8.6 mg/kg/day.

Based on the above considerations, the offspring LOAEL in this DNT with ziram is 8.6 mg/kg/day based on increased locomotor activity and decreased habituation on PND 21, along with decreased pup body weight in males. The offspring NOAEL was not identified.

A data table showing subsession motor activity counts (Table 5) was added to this DER, since that information was not included in the previous DER. The table of cumulative motor activity

data has also been updated to indicate statistical significance.

TABLE 1. Selected mean (±SD) body weights, body weight gains, and food consumption in dams exposed to Ziram in the diet during gestation. [a]

Observations	Dose (ppm)			
	0	72	207	540/360[b]
Body weight (g)				
GD 6	252±11.7	253±13.2	252±10.8	253±10.0
GD 7	282±13.1	282±16.4	275±12.8	267±12.0** (↓5)
GD 15	324±13.7	330±19.6	313±15.5* (↓3)	280±16.0** (↓14)
GD 20	394±16.9	397±23.0	379±19.7* (↓4)	336±20.6** (↓15)
Body weight gain (g)				
GD 6-9	11±3.8	12±5.4	2±5.3** (↓82)	-14±7.9**
GD 9-12	16±6.1	16±4.8	13±4.2	4±7.8** (↓75)
GD 18-20	33±6.7	29±4.8* (↓12)	26±5.8** (↓21)	22±5.0** (↓33)
GD 6-20	115±12.5	114±12.4	97±13.3** (↓16)	58±20.8** (↓50)
Food consumption (g/animal/day)				
GD 6-9	20±2.0	21±2.2	17±3.3** (↓15)	9±2.3** (↓55)
GD 15-18	22±3.0	24±2.3* (↑9)	23±2.8	19±2.8** (↓14)
GD 6-20	21±2.2	23±1.6* (↑10)	20±2.0	14±2.1** (↓33)

a Data were extracted from Tables 3, 4, and 7 on pages 124-132 and 146 & 147 of MRID 47900401; n=22-25. Numbers presented parenthetically are percent difference from controls.

b The 540 ppm diet concentration was offered to animals through the morning of LD 4. Thereafter, the 360 ppm diet concentration was offered.

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

TABLE 2. Selected mean (±SD) body weights, body weight gains, and food consumption in dams exposed to Ziram in the diet during lactation. [a]

Observations	Dose (ppm)			
	0	72	207	540/360[b]
Body weight (g)				
LD 1	300±19.0	301±20.5	291±17.1	258±16.0** (↓14)
LD 2	304±17.6	305±17.9	292±15.4* (↓4)	260±16.6** (↓15)
LD 3	310±17.5	307±19.5	296±16.6* (↓5)	262±16.3** (↓16)
LD 4	314±19.1	313±20.2	299±14.5* (↓5)	266±16.0** (↓15)
LD 5	317±21.8	318±21.0	309±16.8	282±16.6** (↓11)
LD 15	344±19.2	349±22.2	337±16.6	323±16.5** (↓6)
LD 20	341±16.7	345±22.9	339±18.9	328±14.4* (↓4)
LD 21	339±16.7	346±20.3	341±20.0	332±12.5
Body weight gain (g)				
LD 1-4	14±10.2	13±10.0	8±9.4	9±10.0 (↓36)
LD 4-7	8±7.8	9±8.1	12±11.2	26±8.1** (↑225)
LD 17-21	-8±10.6	-6±13.8	-4±8.2	3±9.6**
LD 1-21	41±14.8	45±11.6	50±13.4	74±15.0** (↑80)
Food consumption (g/animal/day)				
LD 1-4	35±5.6	36±6.0	32±4.1	29±4.2** (↓17)
LD 4-7	39±5.4	40±5.0	39±4.1	38±3.9
LD 11-14	60±5.5	58±6.4	57±5.5	54±4.4** (↓10)
LD 1-21	53±5.2	52±5.5	50±3.5	49±2.5* (↓8)

a Data were extracted from Tables 5, 6, and 9 on pages 133-142 and 157 & 158 of MRID 47900401; n=22-25. Numbers presented parenthetically are percent difference from controls.

b The 540 ppm diet concentration was offered to animals through the morning of LD 4. Thereafter, the 360 ppm diet concentration was offered.

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

TABLE 3. Mean (±SD) pre-weaning F_1 pup body weights and overall body weight gains (g). [a]

Postnatal Days	Dose (ppm)			
	0	72	207	540/360[b]
Males				
1	7.0±0.75	6.9±0.44	6.8±0.61	6.2±0.55** (↓11)
4[c]	9.5±1.43	9.3±1.07	9.2±0.88	8.0±0.78** (↓16)
7	14.9±2.45	14.2±2.40	13.8±1.98	12.1±1.69** (↓19)
17	39.0±3.73	37.1±5.09	36.7±3.63	35.0±3.87** (↓10)
21	50.9±6.34	47.6±6.87 (↓7)	46.6±4.34* (↓8)	44.7±4.81** (↓12)
1-4	2.5±0.97	2.4±0.77	2.3±0.49	1.8±0.43** (↓28)
4-7	5.4±1.39	4.8±1.66	4.6±1.61	4.0±1.03** (↓26)
17-21	11.9±3.11	10.3±2.16* (↓13)	9.9±1.40** (↓17)	9.6±1.25** (↓19)
Overall (1-21) gain [d]	43.9	40.7	39.8	38.5 (↓12)
Females				
1	6.6±0.73	6.6±0.43	6.4±0.52	5.9±0.53** (↓11)
4[c]	8.9±1.42	8.8±1.12	8.6±0.79	7.7±0.81** (↓14)
7	13.7±2.68	13.4±2.53	13.1±2.04	11.5±1.58** (↓16)
17	36.6±4.27	36.0±5.40	35.0±3.42	33.5±3.63
21	47.4±6.85	45.9±6.72	44.2±4.38	42.4±4.32* (↓11)
1-4	2.3±0.89	2.3±0.84	2.2±0.53	1.8±0.45* (↓22)
17-21	10.8±3.23	9.9±1.93	9.2±1.59* (↓15)	9.0±1.14* (↓17)
Overall (1-21) gain [d]	40.8	39.3	37.8	36.5 (↓11)

a Data were extracted from Tables 21 & 22 on pages 184-190 of MRID 47900401; n=22-25 litters. Numbers presented parenthetically are percent difference from controls.

b The 540 ppm diet concentration was offered to animals through the morning of LD 4. Thereafter, the 360 ppm diet concentration was offered.

c Pre-culling

d Calculated by reviewers using body weight data in this table.

TABLE 4. Mean (±SD) cumulative locomotor activity (counts, combined sexes) in F_1 pups [a]

Interval (PND)	Dose (ppm)			
	0	72	207	540/360
Total activity				
13	1063.8±779.7	979.2±273.9	1105.8±412.5	1330±724.0 (↑25)
17	3557.7±1710.5	3057.7±1996.2	3658.6±1874.1	4650.4±2521.5 (↑31)
21	2739.3±1137.7	3396.1±1590.5 (↑24)	4017.5±1875.6** (↑47)	3830.5±1274.4* (↑40)
61	4600.3±1324.1	4941.1±1479.5	5445.3±1227.6** (↑18)	5065.8±1246.1* (↑10)
Ambulatory activity				
13	258.1±347.6	157.7±74.4	204.4±176.2	371.4±455.6 (↑44)
17	1498.8±837.5	1233.4±1044.8	1602.7±1024.7	2112.1±1340.1* (↑41)
21	908.5±419.8	1303.8±875.6 (↑43)	1510.2±837.1* (↑66)	1471.3±628.2* (↑62)
61	1134.4±445.2	1227.9±549.6	1437.5±530.5* (↑26)	1383.1±502.9* (↑22)

a Data were obtained from Table 33 on pages 207-222 of MRID 47900401; n=20 for PND 13, 17, and 21 and n=38-40 on PND 61.

* $p<0.05$, ** $p<0.01$

TABLE 5. Motor activity sub-session counts in F_1 pups - [males and females] (mean count ±S.D.) [a]

Sub-session (minutes)		Dose (ppm)			
		0	72	207	540/360
PND 13	0-10	223.3±128.01	241.3±117.83	244.6±99.44	224.5±124.92
	11-20	122.6±92.80	136.6±115.96	151.1±70.29	206.1±153.47
	21-30	177.4±133.19	120.9±57.93	157.9±96.96	209.0±191.06
	31-40	189.9±208.28	141.9±70.22	179.1±121.01	208.4±120.36
	41-50	171.3±206.92	151.8±92.87	185.5±133.97	246.7±207.65
	51-60	179.5±206.92	186.6±92.87	187.7±133.97	235.5±207.65
PND 17	0-10	1064.7±414.10	977.2±415.47	1174.7±296.83	1161.3±472.06
	11-20	587.5±387.83	466.9±366.80	641.6±290.2	777.7±453.72
	21-30	515.6±379.78	485.8±437.09	459.1±372.69	705.4±469.37
	31-40	479.5±386.88	421.6±440.05	507.1±436.92	708.9±515.74
	41-50	405.2±435.63	382.0±367.13	428.1±412.56	633.0±497.57
	51-60	505.4±362.86	324.2±413.29	448.1±455.50	664.2±513.27
PND 21	0-10	1148.2±245.99	1355.7±246.88	1368.5±320.26	1377.4±275.73
	11-20	426.8±267.69	476.4±323.64	658.6±305.72	561.3±255.47
	21-30	348.9±260.98	476.0±295.64	535.7±390.57	478.0±300.01
	31-40	249.3±239.90	368.2±315.30	487.8±358.22	458.6±282.09
	41-50	303.7±288.85	350.8±386.54	494.4±415.10	501.2±322.18
	51-60	262.5±203.13	369.1±340.18	472.6±376.45	454.0±341.01
PND 61	0-10	1554.3±321.07	1620.4±324.92	1592.7±278.05	1599.0±228.45
	11-20	1117.2±343.96	1097.4±399.29	1186.8±282.00	1176.1±244.10
	21-30	717.1±294.35	819.0±327.57	905.6±269.82	834.6±305.24
	31-40	490.8±344.63	630.7±323.86	737.1±329.21	621.8±377.95
	41-50	426.8±387.99	424.7±351.71	553.9±340.05	435.8±354.36
	51-60	294.2±304.43	349.0±316.15	469.2±341.76	398.6±386.48

[a] Data obtained from Table 33 on pages 207-212 and 291 of MRID 47900401; n=20 for PND 13, 17, and 21 and n=38-40 on PND 61.