Bull. Environ. Contam. Toxicol. (1994) 53:930–936

Environmental Contamination and Toxicology

Developmental Toxicity Evaluation of Zinc Dimethyldithiocarbamate (Ziram) in Rats

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Received: 23 October 1993/Accepted: 2 May 1994

Zinc dimethyldithiocarbamate (Ziram; CAS No. 137-30-4) is a member of a class of dithiocarbamates. The dithiocarbamates, the amides of dithiocarbamic acid, are prepared by reacting carbon disulfide with primary or secondary amines in an alkaline or ammoniacal solution, and Ziram is prepared by precipitating sodium dimethyldithiocarbamate with soluble zinc salt (Fishbein 1976). The dithiocarbamates are very reactive compounds that possess strong metal-binding characteristics, interact with sulfhydryl compounds, and undergo many reactions involving oxidation and loss of sulfur (Fishbein 1976). Ziram is used extensively in agriculture as a fungicide and in the rubber industry as a vulcanization accelerator (Fishbein 1976; IARC 1976).

To date, several reports on the developmental toxicity of Ziram have been published. Van Leeuwen et al. (1986a,b) reported that Ziram possessed embryotoxic effects such as high mortality and severe spinal and vertebral abnormalities following exposure of rainbow trout during embryolarval development. Gebhardt and Van Logten (1968) noted that Ziram was extremely toxic for the early chick embryo. Weppelman et al. (1980) found that feeding Ziram stopped egg production and caused marked ovarian atrophy in hens. Serio et al. (1984) showed a correlation between antifertility action induced by Ziram in laying hens and inhibition of dopamine β-hydroxylase, and they suggested that this effect of Ziram might have resulted from its antiadrenergic action. Two reports with respect to the developmental toxicity of Ziram in mammals are available. Cilievici et al. (1983) showed that increased incidences of sterility, embryonic deaths and fetuses with skeletal malformations occurred when male mice were treated with Ziram by gavage in daily dose of 0.1 or 0.2 mg% for three weeks and were mated with normal females. Giavini et al. (1983) observed reduced fetal weight and a slight dismorphogenic effect on fetuses following oral administration of Ziram to rats during the preimplantation and organogenetic period, respectively. Thus, there is insufficient information to evaluate the developmental toxicity of Ziram. The present study was undertaken therefore to evaluate further developmental toxicity of Ziram given to pregnant rats throughout the period of organogenesis.

MATERIALS AND METHODS

Wistar rats (Std:Wistar/ST, Japan SLC, Inc., Hamamatsu) were used throughout this study. Animals were maintained in an air-conditioned room at 24 ± 1 °C, with a relative humidity of 55 ± 5%, under a controlled 12-hr light/dark cycle. The rats were reared with a basal diet (F-1, Funabashi Farm Co., Funabashi) and tap water ad

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libitum. Virgin female rats (10-15 wk old) were mated overnight for 15 hr with male rats from the same supplier. The day when sperm were detected in the vaginal smear was considered to be day 0 of pregnancy. The pregnant rats were distributed on a random basis into four groups consisting of 21 animals each and housed individually. The pregnant rats were fed zinc dimethyldithiocarbamate (Ziram; 99.9% purity), purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo), at a dietary dose of 0 (control), 0.0125, 0.025 or 0.05% from day 6 through day 15 of pregnancy. The diet containing each dose of Ziram was prepared separately every week. A predetermined amount of Ziram was weighed and added to a small aliquot of ground basal diet and hand-blended. This premix was then added to a preweighed ground basal diet and blended in a mill (V-1, Irie Shokai Co., Ltd., Tokyo) for 30 min.

The pregnant rats were observed daily for evidence of clinical signs of toxicity such as changes in the skin, fur, eyes and mucous membrane, and in the behavioral patterns. Maternal body weight and food consumption were recorded daily. Daily intake of Ziram was calculated by the method described by Tyl et al. (1988). The pregnant rats were killed on day 20 of pregnancy. The peritoneal cavity and uterus were opened, and the numbers of resorptions and live and dead fetuses were recorded. The live fetuses removed from the uterus were sexed, weighed and inspected for external malformations and malformations within the oral cavity. Approximately half of the live fetuses in each litter, randomly selected, were fixed in alcohol, stained with alizarin red S (Kawamura et al. 1990) and examined for skeletal malformations. The remaining live fetuses in each litter were fixed in Bouin's solution, sectioned with a razor blade and examined for internal malformations (Wilson 1965). Statistical analysis of the data of the offspring was carried out using the litter as a unit. Analysis of variance and Dunnett's multiple comparison test, Kruskall-Wallis test or Fisher's exact test were used as appropriate. The level of significance chosen was $p < 0.05$.

RESULTS AND DISCUSSION

The maternal findings in rats given dietary Ziram from day 6 through day 15 of pregnancy are presented in Table 1. Neither deaths nor clinical signs of toxicity were found in any group. The maternal body weight gains on days 6-15 and on days 0-20 of pregnancy in the 0.025 and 0.05% groups were significantly lower than those in the control group. The adjusted weight gain, which indicated the net weight gain of maternal rats during pregnancy, in the 0.025 and 0.05% groups was also significantly lower than that in the control group. The food consumption on days 6-15 and on days 0-20 of pregnancy in the 0.025 and 0.05% groups was significantly lower than that in the control group. The decreased rates of these indexes were roughly proportional to the dose. However, no statistically significant changes were detected in the body weight gain and food consumption during pregnancy in the 0.0125% group. These findings suggest that Ziram is maternal toxic at dietary doses of 0.025% and above, but not at a dietary dose of 0.0125%.

The reproductive findings in rats given dietary Ziram from day 6 through day 15 of pregnancy are shown in Table 2. No significant differences between the Ziram-treated groups and the control group were detected in the incidence of postimplantation loss per litter, the numbers of resorptions and dead fetuses per litter and live fetuses per litter, the sex ratio of live fetuses and the fetal body weight. These findings suggest that Ziram has no adverse effects on the prenatal development of rat offspring.

Table 3 shows the results of morphological examinations of fetuses of rats given dietary Ziram from day 6 through day 15 of pregnancy. External examinations revealed one fetus in the 0.0125% group with general edema. One fetus with absence of

Table 1. Maternal findings in rats given dietary Ziram on days 6-15 of pregnancy

Dose (%)	0	0.0125	0.025	0.05
No. of pregnant rats	21	21	21	21
No. of dead pregnant rats	0	0	0	0
Body weight gain during pregnancy (g)[a]				
Days 0-6	23 ± 6	20 ± 7	20 ± 6	20 ± 4
Days 6-15	45 ± 6	42 ± 8	28 ± 6*	10 ± 10*
Days 15-20	55 ± 6	55 ± 10	54 ± 8	63 ± 11*
Days 0-20	123 ± 11	118 ± 19	103 ± 11*	94 ± 12*
Adjusted weight gain[b]	43 ± 12	39 ± 13	28 ± 8*	21 ± 9*
Food consumption during pregnancy (g)[a]				
Days 0-6	98 ± 8	99 ± 11	96 ± 9	100 ± 8
Days 6-15	167 ± 11	166 ± 11	140 ± 8*	99 ± 17*
Days 15-20	99 ± 8	99 ± 9	94 ± 9	102 ± 11
Days 0-20	364 ± 22	365 ± 26	330 ± 21*	301 ± 20*
Daily intake of Ziram (mg/kg)[a,c]	0	9.5 ± 1.1	16.2 ± 1.5	23.4 ± 4.3

[a]: Values are given as mean ± SD.
[b]: Adjusted weight gain refers to maternal body weight gain excluding the gravid uterus.
[c]: [(Food consumption on days 6-15 / 9) x %Ziram] / body weight.
*: Significantly different from the control value ($p < 0.05$).

Table 2. Reproductive findings in rats given dietary Ziram on days 6-15 of pregnancy

Dose (%)	0	0.0125	0.025	0.05
No. of litters	21	21	21	21
No. of implantations per litter[a]	15.5 ± 1.8	15.0 ± 2.2	15.0 ± 1.9	14.6 ± 2.8
No. of resorptions and dead fetuses per litter[a]	0.9 ± 1.0	1.0 ± 1.3	1.2 ± 0.7	1.0 ± 1.1
Postimplantation loss per litter (%)[b]	5.3	6.4	7.9	6.1
No. of live fetuses per litter[a]	14.6 ± 1.6	14.0 ± 2.1	13.8 ± 1.8	13.6 ± 2.6
Sex ratio of live fetuses (male/female)	157 / 150	151 / 144	144 / 146	125 / 161
Body weight of live fetuses (g)[a]				
Male	3.70 ± 0.24	3.71 ± 0.36	3.75 ± 0.21	3.66 ± 0.27
Female	3.52 ± 0.23	3.51 ± 0.29	3.49 ± 0.18	3.45 ± 0.22

[a]: Values are given as mean ± SD.
[b]: (No. of resorptions and dead fetuses / no. of implantations) x 100.

Table 3. External, skeletal and internal examinations of fetuses of rats given dietary Ziram on days 6-15 of pregnancy

Dose (%)	0	0.0125	0.025	0.05
External examination				
No. of fetuses (litters) examined	307(21)	295(21)	290(21)	286(21)
No. of fetuses (litters) with malformations	0	1(1)	0	0
No. of fetuses (litters) with:				
General edema	0	1(1)	0	0
Skeletal examination				
No. of fetuses (litters) examined	158(21)	153(21)	152(21)	150(21)
No. of fetuses (litters) with malformations	0	0	2(2)	0
No. of fetuses (litters) with:				
Absence of cervical vertebral arches	0	0	1(1)	0
Fusion of thoracic vertebral arches	0	0	1(1)	0
No. of fetuses (litters) with variations	10(8)	7(5)	4(4)	7(6)
No. of fetuses (litters) with:				
Lumbar ribs	0	3(3)	0	1(1)
Shortening of 13th ribs	0	0	1(1)	0
Shift of lumbosacral vertebral border	5(4)	7(4)	2(2)	4(3)
Asymmetry of sternebrae	4(4)	1(1)	2(2)	2(2)
Splitting of sternebrae	1(1)	0	0	1(1)
Degree of ossification[a]				
No. of sternebrae	5.9 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.9 ± 0.2
No. of ossification centers of caudal vertebrae	4.8 ± 0.6	4.7 ± 0.7	5.0 ± 0.6	5.0 ± 0.7
Internal examination				
No. of fetuses (litters) examined	149(21)	142(21)	138(21)	136(21)
No. of fetuses (litters) with malformations	0	0	0	0

[a]: Values are given as mean ± SD.

the cervical vertebral arches and one fetus with fusion of the thoracic vertebral arches were observed in the 0.025% group. No fetuses with internal malformations were found in any group. The malformations observed in the present study are not thought to be attributable to the administration of Ziram, because the incidences of these malformations were very low and these malformations are of types seen in full-term fetuses in rats (Kameyama et al. 1980; Morita et al. 1987). Several types of skeletal variations in the vertebrae, ribs and sternebrae were observed in all groups, but no consistent trend was detected in the incidences of these variations. Skeletal variations are frequently observed in fetuses of rats at term (Fritz and Hess 1970; Kimmel and Wilson 1973; Kameyama et al. 1980; Khera 1981; Morita et al. 1987). The appearances of these alterations are not uncommon in the developmental toxicity studies. In addition, the incidences of fetuses and litters with skeletal malformations and variations were not significantly different between the Ziram-treated groups and the control group. The degree of ossification indicated by the numbers of the sternebrae and ossification centers of the caudal vertebrae was not significantly different between the Ziram-treated groups and the control group. The morphological alterations observed in the present study were not compound-related and thought to be spontaneous. Therefore, these findings do not indicate a teratogenic response and suggest that Ziram has no teratogenic potential in rats.

The present study extends the previous findings (Giavini et al. 1983) and presents an effort to better understand the characterization of the developmental toxicity of Ziram. Giavini et al. (1983) administered relatively high doses of Ziram to CD rats by tube from day 6 through day 15 of pregnancy (sperm positive = day 1 of pregnancy), and found a low teratogenic property of Ziram that only became effective at the highest dose (100 mg/kg) that was lethal to 50% of pregnant rats and a significant decrease in the maternal body weight gain at all tested doses (above 12.5 mg/kg). However, we administered relatively low doses of Ziram to Wistar rats during the organogenetic period, and found a significant decrease in the maternal body weight gain at doses of 0.025% and above, but not at a dose of 0.0125%, in the present study. The results presented here indicate that the minimally maternal toxic dose of Ziram is between 9.5 (average daily intake of Ziram at 0.0125%) and 16.2 mg/kg (average daily intake of Ziram at 0.025%) and the maximal maternal no-observable-effect level (NOEL) of Ziram is considered to be 9.5 mg/kg.

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