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Effects of Zinc 2-Mercaptobenzimidazolate on Pregnant Rats by Oral Treatment

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The effects of zinc 2-mercaptobenzimidadolate (ZMBI), which contains ethylene thiourea (ETU) in its structure and is used as an accelerator and/or antioxidant in rubber manufacturing, on pregnant Wistar rats, were examined. Moreover, the effects were compared with those of ETU. ZMBI was administered orally to pregnant rats during the period of organogenesis (days 7—17 of gestation) at doses of 0, 5, 15, and 45 mg/kg/day. The treatment resulted in reduced maternal thymus weight and increased thyroid gland weight at doses of ≧5 mg/kg and ≧15 mg/kg, respectively. Reduced fetal weight and visceral variations (kinked ureter and dilated renal pelvis) were seen at doses of ≧15 mg/kg, and skeletal variation (rudimentary lumber ribs) was observed at 45 mg/kg. Teratological effects were not observed at doses used in the present study. Thus, the no-observed-adverse-effect level (NOAEL) for maternal toxicity was considered to be less than 5 mg/kg/day, and that for fetal toxicity was 5 mg/kg/day. Effects of ZMBI at a dose of 73 mg/kg/day (200 μmol/kg/day) were compared with those of ETU at a dose of 41 mg/kg/day (400 μmol/kg/day). In this experiment these compounds were administered to dams during the gestational periods of days 7—10, 11—14, or 15—17. The ZMBI treatment on days 11—14 of gestation caused fetal dilated lateral ventricles and cleft plate in about 30% of the fetuses. Kinked ureter and dilated renal pelvis were also observed mainly in this group. The same visceral variations were also observed in the group treated with ETU on days 11—14 of gestation. Although cleft palate was detected in the fetuses of the dams treated with ZMBI on days 11—14 of gestation, ETU treatment on days 11—14 of gestation caused exencephaly, hydrocephaly/hydracephaly and club foot which were not observed in any group treated with ZMBI. Thus, in the present investigations, different spectra of malformations by the chemically related compounds, ZMBI and ETU, were observed.

Key words —— zinc-2-mercaptobenzimidazolate, ethylene thiourea, teratology, rat

INTRODUCTION

Zinc 2-mercaptobenzimidazolate (ZMBI) is used as an accelerator and/or antioxidant in rubber manufacturing. ZMBI is a compound with the structure of ethylene thiourea (ETU). ETU has been also used as an accelerator in rubber manufacturing. However, ETU is an antithyroid agent, which is carcinogenic in the thyroid gland[1] and is also a potent teratogen, especially in the nervous and urogenital system.[2,3]

Moreover, 2-mercaptobenzimidazole (2-MBI), a structurally related chemical to ETU, is an accelerator and/or antioxidant in rubber production and has a benzene ring attached to the 4,5-position of ETU. 2-MBI has shown embryotoxic activity in rats after intraperitoneal administration.[4] Khera and Whalen[5] classified 2-MBI together with ETU as teratogens for the nervous system using cultured neural cells. On the other hand, Ruddick *et al.*[6] designated 2-MBI as not teratogenic in rats, and carried out experiments comparing the teratogenic effects of 16 structurally related chemicals to ETU. We have also examined the effects of 2-MBI on pregnant rats and their fetuses.[7] In that report, maternal toxicity preceded fetal toxicity, and fetal malformations were seen only at a dose (60 mg/kg) which was lethal to many of the pregnant rats treated with 2-MBI.

Although ZMBI is a dimer of 2-MBI and is chemical-structurally related to ETU, there are few toxicity data available on the effects of ZMBI in adult female rats and their fetuses. Therefore, to clarify the adverse effects of ZMBI on adults and fetuses, the compound was given

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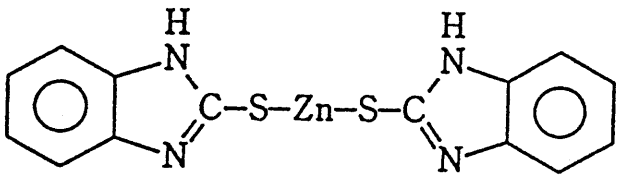
consecutively to pregnant rats by oral administration during specific periods of oranogenesis (days 7—17 of gestation). Moreover, to compare the adverse effects of ZMBI and ETU, pregnant rats were treated with ZMBI or ETU during specific periods of organogenesis (days 7—10, 11—14 or 15—17 of gestation).

MATERUALS AND METHODS

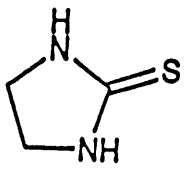
Chemicals —— ZMBI and ETU were purchased from Ouchi Shinko Chem. Co., Ltd. (Tokyo, Japan) and Wako Pure Chem. Co., Ltd. (Osaka, Japan), respectively. The chemical structures of these compounds are shown in Fig. 1.

Animals —— Wistar rats (4-week-old) of both sexes were obtained from CLEA Japan, Inc. (Tokyo, Japan) and were housed individually in stainless steel cages in a room with a constant dark and light cycle (dark period from 7 p.m. to 7 a.m.) at 23±2℃ and 60±10% relative humidity. They were given food (NMF, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum* and used for experiments when reaching 3 months of age. In this study, females were individually paired with a male of the same age, and the day on which sperm was observed in the vaginal smears was designated as day 0 of gestation.

Dose-finding study on ZMBI —— Pregnant rats were assigned to 7 groups of 5 to 6 rats each. They were treated orally with ZMBI in olive oil at 0, 3.75, 7.5, 15, 30, 60, 90 and 120 mg/kg in a volume of 5 ml/kg from days 7 through 17 of gestation. Body weight and food consumption were measured every day, and their general condition and behavior were observed. After euthanasia under diethylether anesthesia, laparotomy of the pregnant rats was performed on day 20 of gestation and the maternal thymus, thyroid gland and gravid uterus were weighed. The position and number of live and dead fetuses, including resorbed fetuses and the number of corpora lutea, were recorded. Live fetuses were weighed and examined for sex and external malformations.



ZMBI



ETU

Fig. 1. Chemical Structures of Zinc 2-Mercaptobenzimidazolate (ZMBI) and Ethylene Thiourea (ETU)

Teratology Study on ZMBI —— Dose levels of 0, 5, 15 and 45 mg/kg in a volume of 5 ml/kg in olive oil were selected based on the observations in the dose-finding study. Pregnant rats were divided into 4 groups of 22 to 23 rats each and treated by gavage with ZMBI in olive oil from day 7 through 17 of gestation. Body weight and food consumption were measured, and their general condition and behavior were observed throughout gestation. On day 20 of gestation, the rats were euthanized under diethylether anesthesia and the maternal thymus, thyroid gland and gravid uterus were weighed. Uteri and fetuses were examined grossly, the position and number of live and dead fetuses, including resorbed fetuses and the number of corpora lutea, were recorded. Live fetuses were weighed and examined for sex and external malformations. Moreover, one-half of the live fetuses were preserved in Bouin's fixative solution for subsequent visceral observations,[8] and the remaining live fetuses were fixed in alcohol for subsequent skeletal observations.[9]

Comparative Teratogenicity of ZMBI and ETU —— From the results of the dose-finding study, the dose of ≧60 mg/kg was found to be toxic to dams when given throughout the period of organogenesis (gestation days 7—17). In our previous study on 2-MBI,[4] this compound was administered for three or four consecutive days at three different periods of organogenesis (gestation days 7—10, 11—14 and 15—17) at a dose of 60 mg/kg (400 μmol/kg). One mole of ZMBI is chemically involved in two moles of ETU or 2-MBI. Therefore, pregnant rats were treated by gavage with ZMBI at a dose of 73 mg/kg (200 μmol/kg) at three different periods of organogenesis (gestation days 7—10, 11—14 and 15—17). Moreover, pregnant rats were treated orally with ETU at a dose of 41 mg/kg (400 μmol/kg) at the same three gestational periods. ETU was disolved in distilled water in a volume of 5 ml/kg. The other procedures were the same as described above. Control pregnant females were treated orally with olive oil (5 ml/kg) during days 7—17 of gestation.

Satistics —— Data from the teratology study on

ZMBI were analyzed by Dunnett's or Scheffé's multiple comparison method in a parametric or nonparametric manner. Comparison between groups in the comparative teratology study on ZMBI and ETU were made using Student's t-test or Mann-Whitney's U-test.

RESULTS

Teratology Study on ZMBI

In this dose-finding study, maternal body weight gain and food consumption were decreased at 60 mg/kg of ZMBI (data not shown). Two out of five rats treated with 60 mg/kg of ZMBI died during gestation. All dams treated with ≧90 mg/kg died during gestation. With to 30 mg/kg of ZMBI, no parameters with respect to fetuses were affected (data not shown).

While ZMBI treatment during the organogenesis (gestation days 7—17) was moderately toxic to dams, as evidenced by a substantial decrease in maternal body weight gain (Fig. 2) and food consumption (data not shown) in the group at 45 mg/kg, all dams survived throughout the experiment and had live fetuses. ZMBI was slightly more toxic to dams than fetuses: maternal thymus weight was decreased, even at the lowest dose of 5 mg/kg, and thyroid gland weight was increased at the dose of 15 mg/kg, while

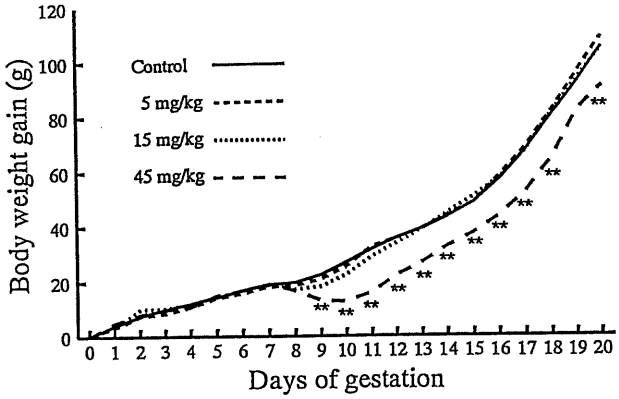


Fig. 2. Body Weight Gain of Pregnant Rats Treated Orally with Zinc 2-Mercaptobenzimidazolate (ZMBI)
**Significantly different from control at $p<0.01$.

Table 1. Effects of Oral Treatment with Zinc 2-Mercaptobenzimidazolate (ZMBI) on Pregnant Rats and Their Fetuses

	ZMBI (mg/kg)			
	0	5	15	45
Dams				
No. of dams	23	22	22	23
No. of dead dams	0	0	0	0
No. of dams with live fetuses	23	22	22	23
Maternal body weight[a] (g)	343 ±17.4	350 ±18.4	344 ±25.4	334 ±19.1
Gravid uterus weight[a] (g)	67.5±11.4	70.1±12.8	66.0±17.2	66.0±10.9
Adjusted body weight gain[b] (g)	37.6± 6.7	38.8± 9.0	38.4± 7.6	24.8± 9.3**
Thymus weight[a] (mg)	213 ±33.3	110 ±36.9**	98.0±20.6**	66.2±14.9**
Thyroid gland weight[a] (mg)	23.9± 3.2	30.1± 4.3	42.8± 6.4**	53.1± 9.2**
No. of corpora lutea[a]	15.7± 1.8	15.8± 2.3	16.0± 2.4	16.0± 1.8
No. of implants[a]	13.9± 2.9	14.0± 3.2	14.9± 2.1	14.9± 1.8
Fetuses[c]				
No. of live fetuses[a]	13.0± 2.6	13.4± 2.3	12.9± 3.7	13.1± 2.2
Incidence of dead or resorbed fetuses				
Early stage (%)	4.9	5.0	11.8	7.6
Late stage (%)	0.9	1.3	3.0	4.0
Sex ratio male/female	157/143	154/140	133/151	155/147
Fetal weight[a] (g)				
Male (g)	3.4±2.2	3.4±0.3	3.0±0.3**	2.8±0.2**
Female (g)	3.1±0.3	3.2±0.3	2.9±0.2	2.7±0.1**

a) Mean±S.D.
b) (Body weight gain from day 0 to 20 of gestation)−(gravid uterus weight).
c) The litter was used as a statistical unit for calculation of fetal values, thus these values represent means of litter means within each group.
** Significantly different from control, $p<0.01$.

Table 2. External, Visceral and Skeletal Observation of Fetuses from the Dams Treated with Zinc 2-Mercaptobenzimidazolate (ZMBI) from Days 7 through 17 of Gestation

	ZMBI (mg/kg)			
	0	5	15	45
External observation[a]				
No. of fetuses examined	300	294	284	302
Incidence of fetuses with external anomalies (%)	0.0	0.0	0.6[2](2)	2.0[6](6)
Anury or vestigial tail (%)	0.0	0.0	0.6[2](2)	1.2[4](4)
Omphalocele (%)	0.0	0.0	0.0	0.7[2](2)
Incidence of other fetus anomalies (%)				
Subcutaneous hemorrhage of head (%)	0.2[1](1)	0.6[2](2)	1.1[3](2)	1.2[4](3)
Visceral observation[a]				
No. of fetuses examined	138	137	133	140
Incidence of fetuses with malformations (%)	0.0	0.0	0.0	0.0
Incidence of fetuses with variations (%)	12.3[12](9)	6.1[8](6)	32.7[45](17)	49.1**[68](21)
Kinked ureter (%)	9.1[12](9)	4.3[5](4)	21.1[28](14)	30.5**[43](19)
Dilated renal pelvis (%)	3.8[6](4)	4.7[6](5)	29.2**[38](17)	37.9**[52](19)
Skeletal observation[a]				
No. of fetuses examined	162	157	151	162
Incidence of fetuses with malformations (%)	0.0	0.0	0.0	0.0
Incidence of fetuses with variations (%)	2.2[4](4)	1.1[1](1)	2.9[5](2)	25.9**[43](17)
Rudimentary lumbar ribs	2.2[4](4)	1.1[1](1)	2.9[5](2)	25.3**[42](17)
Lumbar ribs	0	0	0	0.6[1](1)
Degree of ossification[a,b]				
No. of sternebrae	4.4±0.49	4.7±0.57	4.3±0.8	3.0±0.4
No. of proximal and middle phalanges				
Fore limb	3.3±0.4	3.4±0.4	3.1±0.4	3.0±0.1
Hind limb	4.0±0.1	4.0±0.1	4.0±0.1	4.0±0.4
No. of sacral and caudal vertebrae	6.9±0.4	7.0±0.5	6.7±1.0	5.5±0.5**

a) The litter was used as a statistical unit for the calculation of fetal values, thus these values represent means of litter means with each group.
b) Mean±S.D.
** Significantly different from control, $p<0.01$.
[ ] No. of fetuses with case.
( ) No. of conceived mothers with case.

fetal body weight in both sexes was decreased in a dose-dependent manner with significance at ≧ 15 mg/kg (Table 1). As for visceral variations, kinked ureter and/or dilated renal pelvis were observed in 32.7% of the fetuses at 15 mg/kg and in 49.1% of the fetuses at 45 mg/kg. As for skeletal variations, rudimentary lumbar ribs were seen in 25.3% of the fetuses at 45 mg/kg. The degree of ossification was significantly affected at a dose of 45 mg/kg (Table 2).

Comparative Teratogenicity of ZMBI and ETU

ZMBI treatment of 73 mg/kg (200 μmol/kg) during the three different periods (gestation days 7—10, 11—14 and 15—17) was severely toxic to dams, as evidenced by a substantial decrease in maternal body weight gain and maternal death in the group treated on gestation days 11—14 and 15—17 (Table 3). Moreover, maternal thymus weight was decreased by treatment with ZMBI on gestation days 11—14 and 15—17 and maternal thyroid gland weight was increased by treatment with ZMBI during all three periods (Table 3). Fetal visceral and skeletal variations caused by ZMBI treatment at 45 mg/kg on gestation days 7—17 (Table 2) were also observed by treatment with ZMBI at 73 mg/kg (200 μmol/kg) on specific gestational days, gestation days 11—14 (Table 4). Moreover, dilated lateral ventricles and cleft palate were seen only by treatment on gestation days 11—14 (Table 4).

ETU treatment at 41 mg/kg (400 μmol/kg) during the different periods of organogenesis was not toxic to dams, as evidenced by the absence of effect on body weight gain in all the ETU-treated groups (Table 3). Moreover, maternal death was

Table 3. Effects of Oral Treatment of Zinc 2-Mercaptobenzimidazolate (ZMBI) and Etyhylene Thiourea (ETU) on Pregnant Rats and Their Fetuses

	Control	ZMBI (μmol/kg)			ETU (μmol/kg)		
	0	200	200	200	400	400	400
Days of treatment	7—17	7—10	11—14	15—17	7—10	11—14	15—17
Dams							
No. of dams	12	11	14	14	12	11	12
No. of dead dams	0	0	2	2	0	0	0
No. of dams with live fetuses	12	11	12	12	12	11	12
Maternal body weight[a] (g)	343.4±28.5	343.8±26.1	308.4±23.1*	309.1±27.5*	354.8±26.2	342.9±24.6	343.2±31.9
Gravid uterus weight[a] (g)	68.3±22.0	70.4±13.7	58.8±10.1	62.3±27.5	77.1± 6.2	74.5±15.2	70.1±14.7
Adjusted body weight gain[a,b] (g)	39.5± 7.8	34.6±11.4	12.2±19.9**	12.9±16.4**	40.4±11.7	37.5± 6.2	40.1± 5.6
Thymus weight[a] (mg)	244.3±55.5	116.1±29.7	71.0±14.6**	66.2±15.7**	240.0±35.0	197.6±26.7	170.6±30.6**
Thyroid gland weight[a] (mg)	21.3± 3.8	30.1± 5.3**	29.5± 3.8**	29.3± 5.7**	23.1± 3.1	23.2± 4.6	23.2± 3.7
No. of corpora lutea[a]	15.6± 2.3	15.2± 1.2	16.0± 1.7	15.7± 2.4	16.9± 2.4	16.5± 2.2	16.5± 2.0
No. of implants[a]	13.8± 3.8	14.1± 2.2	14.8± 1.2	14.6± 2.2	14.0± 3.2	14.9± 2.1	14.9± 1.8
Fetuses[c]							
No. of live fetuses[a]	13.2± 4.3	13.4± 2.4	13.1± 1.7	13.6± 2.0	14.8± 1.7	14.2± 3.2	14.3± 3.0
Incidence of dead or resorbed fetues							
Early stage (%)	9.0	2.4	3.7	1.1	3.6	3.0	2.5
Late stage (%)	0.0	2.6	7.9**	2.2	3.3	0.6	1.5
Sex ratio male/female	76/82	76/71	90/67	80/83	93/84	76/80	89/82
Fetal weight[a]							
Male (g)	3.4±0.2	3.3±0.7	2.7±0.3**	2.7±0.5**	3.3±0.2	3.0±0.2**	3.2±0.2
Female (g)	3.2±0.3	3.2±0.6	2.5±0.3**	2.6±0.5*	3.1±0.2	2.9±0.2*	3.0±0.2

a) Mean±S.D.
b) The litter was used as a statistical unit for the calculation of fetal values, thus these values represent means of litter means with each group.
*,** Significantly different from control, $p<0.05$ and $p<0.01$, respectively.

not observed in any group treated with ETU (Table 3). Maternal thymus weight was only decreased by ETU on gestation days 15—17 (Table 3). As shown in ZMBI, kinked ureter and dilated renal pelvis were seen mainly by treatment with ETU on gestation days 11—14 (Table 4). However, other major fetal anomalies caused by ETU treatment were different from those caused by ZMBI. Exencephaly, hydrocephaly/hydracephaly and club foot were observed only by treatment with ETU on gestation days 11—14 (Table 4). Moreover, dilated lateral ventricles were seen mainly by treatment with ETU on gestation days 15—17 (Table 4).

DISCUSSION

Although we did not estimate exactly the no-observed-adverse-effect level (NOAEL) of ZMBI for maternal toxicity in the this study, it was considered to be less than 5 mg/kg, because of a significant decrease in maternal thymus weight at this dose. In particular, the maternal thymus was the most sensitive organ to this compound, suggesting that immunotoxicity of ZMBI should be noticed. Treatment with 15 and 45 mg/kg of ZMBI also resulted in increased maternal thyroid gland weight. We[7] and another laboratory[10] have both reported that 2-MBI, which is chemically similar to ZMBI, results in decreased thymus weight and increased thyroid gland weight in non-pregnant and pregnant rats. In our previous report,[7] the maternal thymus weight was decreased at 3.3 mg/kg of 2-MBI and maternal thyroid gland weight was increased at 10 mg/kg of 2-MBI. Since ZMBI is a dimer of 2-MBI, the potency of the maternal toxicity by ZMBI might be similar to that by 2-MBI.

Treatment with 45 mg/kg of ZMBI caused apparent maternal toxicity such as decreases in body weight gain and thymus weight and an increase in thyroid gland weight. This dose reduced fetal body weights and increased the incidence of certain anomalies of the urogenital system and rudimentary lumbar ribs. These find-

Table 4. External, Visceral and Skeletal Observations of Fetuses from the Dams Treated with Zinc 2-Mercaptobenzimidazolate (ZMBI) or Ethylene Thiourea (ETU)

	Control	ZMBI (μmol/kg)			ETU (μmol/kg)		
	0	200	200	200	400	400	400
Days of treatment	7–17	7–10	11–14	15–17	7–10	11–14	15–17
Dams							
External observation[a]							
No. of fetuses examined	158	147	157	163	177	156	171
Incidence of fetuses with external anomalies (%)	0.0	0.0	0.6[1] (1)	0.0	0.0	95.7**[149](11)	0.6[1] (1)
Exencephaly (%)	0.0	0.0	0.0	0.0	0.0	22.1**[34] (9)	0.0
Hydrocephaly (%)	0.0	0.0	0.0	0.0	0.0	73.6**[115](11)	0.0
Anury (%)	0.0	0.0	0.6[1] (1)	0.0	0.0	0.0	0.6[1] (1)
Club foot (%)	0.0	0.0	0.0	0.0	0.0	28.1**[42] (9)	0.0
Kinky tail (%)	0.0	0.0	0.0	0.0	0.0	1.8[2](1)	0.0
Incidence of other fetus anomalies (%)	0.0	0.0	0.0	0.8[1] (1)	0.5[1] (1)	0.0	0.0
Subcutaneous hemorrhage of head (%)	0.0	0.0	0.0	0.8[1] (1)	0.5[1] (1)	0.0	0.0
Visceral observation[a]							
No. of fetuses examined	82	76	84	85	94	84	86
Incidence of fetuses with malformations (%)	0.0	0.0	29.4**[24](6)	0.0	0.0	54.3**[46] (10)	0.0
Hydrocephaly/hydracephaly (%)	0.0	0.0	0.0	0.0	0.0	54.3**[46] (10)	0.0
Cleft palate (%)	0.0	0.0	29.4**[24](6)	0.0	0.0	0.0	0.0
Incidence of fetuses with variations (%)	11.1[9](7)	16.6[14](7)	70.7**[59](12)	19.0[15](8)	28.8[25](10)	71.7**[62] (11)	34.4[33](9)
Dilated lateral ventricles (%)	0.0	0.0	28.3* [22](5)	0.0	0.0	3.4[6](3)	30.1**[29](7)
Kinked ureter (%)	4.6[4](4)	8.4[7] (3)	21.8[20](7)	12.1[10](6)	11.6[10](6)	65.0**[56] (11)	0.0
Dilated renal pelvis (%)	7.6[6](4)	13.1[10](6)	43.3* [36](11)	16.8[13](7)	22.8[20](9)	44.1**[37] (11)	6.3[6] (5)
Skeletal observation[a]							
No. of fetuses examined	76	71	73	78	83	72	85
Incidence of fetuses with malformations (%)	0.0	0.0	0.0	0.0	6.2[5] (4)	3.3[2](2)	0.0
Fusion of ribs (%)	0.0	0.0	0.0	0.0	6.2[5] (4)	3.3[2](2)	0.0
Incidence of fetuses with variations (%)	3.3[2](2)	10.7[8] (5)	13.8[9] (4)	3.4[3] (3)	45.7* [36](12)	0.0	6.1[4] (4)
Cervical ribs (%)	0.0	2.6[1] (1)	0.0	0.0	0.0	0.0	0.0
Rudimentary lumbar ribs (%)	3.3[2](2)	10.7[8] (5)	13.8[9] (4)	3.4[3] (3)	45.7* [36](12)	0.0	6.1[4] (4)
Degree of ossification[a,b]							
No. of sternebrae	4.5±0.47	4.2±1.0	3.0±0.1**	3.2±1.1**	4.6±0.6	4.8±0.2	3.4±0.3**
No. of proximal and middle phalanges							
Fore limb	3.2±0.3	3.2±0.3	2.7±0.9*	2.8±0.5	3.4±0.4	2.7±0.3*	3.4±0.3
Hind limb	4.0±0.1	4.0±0.3	3.6±1.1	3.7±0.4	4.0±0.1	3.8±0.5	3.9±0.2
No. of sacral and caudal vertebrae	7.0±0.6	6.6±1.1	5.5±1.4*	5.4±1.2*	6.6±0.4	4.5±0.7**	6.9±0.5

a) The litter was used as a statistical unit for the calculation of fetal values, thus these values represent means of litter means with each group.
b) Mean±S.D.
** Significantly different from control at $p<0.05$ and $p<0.01$, respectively.
[ ] No. of fetuses with case
( ) No. of conceived mothers with case

ings are similar to our previous teratology study on 2-MBI.[7] It has been shown that rudimentary lumbar ribs are secondary to maternal stress[11] and disappear during the postal period.[12] Moreover, a kinked ureter has been classified as a normal development variability. Thus, these fetal anomalies in litters of dams treated with 45 mg/kg should not be considered to be major malformations. On the other hand, the NOAEL of ZMBI for fetal toxicity was considered to be 5 mg/kg, because no effect on fetuses were observed at this dose.

In the comparative study of ZMBI and ETU, treatment with 200 μmol/kg of ZMBI during each different period of organogenesis caused apparent maternal toxicity because of a significant decrease in maternal body weight gain, thymus weight and an increase in thyroid gland weight, while treatment with ETU of 400 μmol/kg did not cause any apparent maternal toxicity.

Although dilation of the lateral ventricles and cleft palate in fetuses were observed at 200 μmol/kg of ZMBI during gestation days 11—14, these malformations were not observed in fetuses of dams treated with ETU. However, major anomalies such as exencephaly and hydrocephaly/hydracephaly and club foot caused by ETU[2,3] were observed by treatment with ETU during gestation days 11—14 in the present study. Therefore, while ZMBI is a compound related to ETU, these compounds produce a significantly different spectrum of malformations.

The different effects of ZMBI and ETU on the fetuses may be related to the difference in incorporation, distribution and excretion of these compounds. It is well known that ETU is a principal degradation product of ethylenebisthiocarbamate fungicides.[13] Moreover, ETU is distributed among maternal tissues and embryos following administration of a single oral dose of ETU to pregnant rats.[3] Ruddick et al.[14] have reported that transplacentally distributed ethylene thiourea may have a direct role in the occurrence of malformation. Although the metabolism and distribution of ZMBI is unknown, benzene rings attached the 4,5-position of ETU may result in a loss of the teratologenic activity of ETU.[6] Further study on the metabolism and transplacental distribution of ZMBI is necessary to elucidate the fetal effect of ZMBI.

Other factors, such as reduced thyroid hormones affecting the differentiation of neuronal tissues, also play a role in facilitating the anomalies caused by ETU.[15] However, in the present study, the effect of ZMBI on maternal thyroid gland weight was stronger than that of ETU. Thus, the different fetal malformations caused by ZMBI and ETU may not be due to the effect of these compounds on the maternal thyroid gland.

In summary, ZMBI had no teratological effect in the rat at the doses used, NOAEL for maternal toxicity was considered to be less than 5 mg/kg/day, and the NOAEL for fetal toxicity was 5 mg/kg/day in the teratology study. Moreover, while ZMBI is a related compound of ETU, these two chemicals produce a significantly different spectrum of malformations.

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