TABLE 5

SUBCHRONIC INHALATION STUDY OF 2-MERCAPTOBENZIMIDAZOLE: TRIIODOTHYRONINE (T_3) AND THYROXINE (T_4) LEVELS IN F344/N MALE RATS[a]

Hormone	2-MBI target concn (mg/m³)	Preexposure	2 Weeks	4 Weeks	8 Weeks	13 Weeks
T_3 (ng/dl)	0.0	89 ± 10	115 ± 13	78 ± 17	72 ± 22	74 ± 16
	3.1	102 ± 11	98 ± 19	72 ± 16	69 ± 19	73 ± 17
	6.2	[b]	[b]	[b]	[b]	68 ± 14
	12.5	90 ± 11	57 ± 9**	56 ± 13**	52 ± 12*	64 ± 14
	25.0	[b]	[b]	[b]	[b]	42 ± 8**
	50.0	91 ± 13	39 ± 6**	53 ± 6**	63 ± 8	60 ± 24
T_4 (μg/dl)	0.0	6.86 ± 1.28	5.97 ± 1.33	4.88 ± 0.63	5.00 ± 1.79	5.53 ± 1.35
	3.1	7.43 ± 0.94	8.33 ± 0.76	5.74 ± 0.96	5.96 ± 1.79	6.55 ± 1.95
	6.2	[b]	[b]	[b]	[b]	5.08 ± 1.14
	12.5	7.65 ± 1.19	3.30 ± 1.23	3.03 ± 1.32*	3.18 ± 1.18*	5.01 ± 1.61
	25.0	[b]	[b]	[b]	[b]	[d]
	50.0	7.65 ± 0.61	[d]	[d]	[d]	[d]

[a] Mean ± SD. N = 8 to 20 samples/group at preexposure and 4, 8, and 13 weeks, N = 3 samples/group at 2 weeks.
[b] Not examined by protocol.
[c] Not examined due to total group mortality.
[d] Below limit of detection (<1.0 μg/dl).
* Significantly different from control group ($p \leq 0.05$) by Dunnett's t test.
** Significantly different from control group ($p \leq 0.01$) by Dunnett's t test.

amined, with the most notable being organs of the endocrine system (Table 6). Thyroid hyperplasia occurred at all exposure levels, with a dose-related increase in severity. Thyroids of rats exposed at 25 or 50 mg/m³ were enlarged, the follicles were small, and the colloid was stained pale pink. There was an increase in the stroma, some of which was loose and almost myxomatous. Additionally, there were accentuated focal areas of hyperplasia where there was an increased density of follicular cells, and the epithelium was occasionally several layers thick. An increased number of normal follicles was seen in the thyroids of rats at the lower exposure concentrations. In these animals the stroma of the thyroid was decreased, but the follicles tended to be less spherical than those seen in controls. Cells of the pituitary pars distalis became enlarged with pale-stained cytoplasm. The ratio of acidophilic to basophilic cells was altered to a predominance of basophilic cells. Adrenal cortical necrosis occurred in rats at the highest exposure level. This same lesion occurred at the 25 mg/m³ level, mixed with degeneration of the zona reticularis of the adrenal cortex.

Focal accumulations of large cells, interpreted as reticuloendothelial cell hyperplasia, occurred in all four lymph nodes examined. This was most prevalent in the mesenteric lymph node. Thymic atrophy was noted with high incidence in rats exposed at 25 or 50 mg/m³. Hepatocyte hypertrophy occurred in the centrilobular areas of the liver, with increased hepatocyte size and collections of sinusoidal cells (granulomatous inflammation) noted in animals exposed at 50 mg/m³. Although mineral deposits were seen in the kidneys in all groups of females, the degree of severity was notably increased in rats at 25 or 50 mg/m³. Mineral deposits were also seen in males at the higher exposure levels, with urinary calculi

TABLE 6

SUBCHRONIC INHALATION STUDY OF 2-MERCAPTOBENZIMIDAZOLE: SUMMARY OF SIGNIFICANT HISTOPATHOLOGICAL CHANGES OBSERVED IN F344/N RATS[a]

	2-Mercaptobenzimidazole target concn (mg/m³)											
	Males						Females					
Tissue and lesion	0	3.1	6.2	12.5	25.0	50.0	0	3.1	6.2	12.5	25.0	50.0
Thyroid folic. cell												
Hyperplasia	0/10	8/10	10/10	10/10	10/10	10/10	0/10	3/10	10/10	10/10	10/10	10/10
Pituitary												
Cytoplasm. vacuol.	0/10	[b]	[b]	0/10	10/10	10/10	0/10	[b]	0/10	2/10	10/10	10/10
Adrenal cortex												
Necrosis	0/10	0/6	0/7	0/10	1/10	8/10	0/10	0/2	0/1	0/10	2/10	9/10
Degeneration	0/10	0/6	0/7	0/10	2/10	0/10	0/10	0/2	0/1	0/10	6/10	0/10
Mesenteric LN												
Hyperplasia	0/10	[b]	[b]	0/10	2/10	8/10	0/10	[b]	[b]	0/10	8/8	10/10
Thymus												
Atrophy	0/10	[b]	0/10	4/10	9/10	9/9	1/10	[b]	[b]	0/10	8/8	9/9
Liver												
Hepat. hypertrophy	0/10	0/1	[b]	0/10	3/10	9/10	0/10	0/1	[b]	0/1	0/10	7/10
Gran. inflammation	0/10	0/1	[b]	0/10	0/10	5/10	0/10	0/1	[b]	0/1	0/10	1/10
Kidney												
Mineralization	0/10	[b]	0/10	1/10	10/10	9/10	9/10	10/10	10/10	10/10	10/10	10/10
Tubular regeneration	0/10	[b]	0/10	0/10	3/10	8/10	0/10	0/10	0/10	0/10	7/10	7/10
Bone marrow												
Hypocellularity	0/10	[b]	[b]	0/10	10/10	7/10	0/10	[b]	[b]	0/10	9/10	9/10

[a] Number with lesions/number of tissues examined.
[b] Not examined.

occasionally seen in the bladders of animals exposed at 50 mg/m³. Hematopoietic hypocellularity of the bone marrow was seen in both sexes at 25 or 50 mg/m³. No readily observable shift in the percentages of marrow hematopoietic constituents was noted, however.

DISCUSSION

2-MBI is a thiourea-derived compound with structural similarities to ethylene thiourea, a potent thyroid carcinogen (IARC, 1974). Such analogs of methimazole may also exhibit potent antithyroid goitrogenic activity. Reduced iodine uptake after 2-MBI exposure has previously been reported by Searle *et al.* (1950), with additional reports of 2-MBI-induced thyroid functional changes by Kellen (1972) and Janssen *et al.* (1981). Goitrogenic substances which alter thyroid function reportedly produce a significant increase in thyroid follicular tumors (Morris, 1955; Paynter *et al.*, 1988).

In the present studies, histopathologic changes were seen in several organs associated with the endocrine system. The 14-day repeated dose study indicated that thyroid hyperplasia was produced within a relatively short period of time (12 exposures). Thyroid weights were markedly increased following 13 weeks of exposure to 2-MBI, as was the increased incidence and severity of follicular cell hyperplasia, with a decreased presence of colloid in the lumen of the thyroid. Only the lowest 2-MBI exposure concentration demonstrated less than a 100% incidence of these thyroid tissue alterations. Decreased lumen size due to increased endocytosis of colloid and a more columnar shape of follicular cells

is generally seen during sustained TSH secretion (Capen, 1988).

One proposed mechanism for the toxic action of thiourea-related compounds on thyroid function is through blockage of T_4 synthesis by inhibition of thyroid peroxidase which catalyzes the incorporation of iodine into thyroxine (Taurog, 1976). Serial measurements of thyroid hormones during the subchronic study indicated a dose-related reduction of T_4, with levels in rats exposed to 50 mg/m^3 being quickly depleted (within 2 weeks) and remaining below detection limits for the entire study. Concomitant, although less dramatic, trends were seen in the levels of T_3 measured during the study. Following the initial reduction of circulating T_3 in rats exposed to 2-MBI, a gradual recovery was indicated. These trends are consistant with the observed effects of WY-13876, a 2-MBI-related thioureylene derivative (Janssen *et al.*, 1981). Thyroid histopathologic alterations in rats exposed to 2-MBI generally correlated with the changes in the thyroid hormone levels. Reduction in T_4 and T_3 levels would normally be expected to produce an increase in TSH, due to the feedback mechanism employed for thyroid hormone regulation. Unfortunately, the results of the TSH examinations conducted in serial blood samples obtained in this study were highly variable, precluding a definitive evaluation of TSH levels in the blood. A reduced number of acidophils in the pituitary was seen by light microscopy at the two highest 2-MBI concentrations tested, but it was not clear what functional properties of the pituitary gland were altered by 2-MBI exposure.

Clinical pathology examinations conducted following 13 weeks of exposure to 2-MBI revealed a number of effects related to thyroid toxicity, including anemia, disturbance of normal cholesterol and free fatty acid levels, and increased blood clotting time. Anemia has been associated with cases of hypothyroidism (Haynes and Murad, 1985) and altered fat metabolism is a consequence of thyroid dysfunction (Guyton, 1971). Hypocellularity of the bone marrow was considered to be related to the anemia present in exposed animals. Accumulations of reticuloendothelial cells within the lymph nodes suggested an increase in cells responsible for phagocytosis of 2-MBI particles.

Liver toxicity was suggested by increased liver weights as well as by changes seen in several liver enzymes in the animals exposed to the higher 2-MBI concentrations. Histopathologic examination confirmed liver injury in the rats at these 2-MBI exposure concentrations. Exposure to 2-MBI apparently exacerbated the severity of kidney mineralization, particularly in female rats. Increased BUN levels in rats at the higher 2-MBI exposure levels also indicated 2-MBI-related kidney effects. Possibly related to the mineralization was the presence of calculi in the bladders of rats at the 50 mg/m^3 exposure concentration.

2-MBI immunotoxic properties were suggested by thymic atrophy, significant reductions in thymus weight, and decreased WBC counts, with lower numbers of circulating lymphocytes in males. Malmfors (1976) has previously reported thymic involution in rats, but not in mice, guinea pigs, or rabbits, following a single dose of 2-MBI. Additional immunotoxicological testing would be necessary to establish specific 2-MBI effects.

In summary, this prechronic inhalation toxicity study with 2-MBI demonstrated dose-related endocrine system toxicity. Additionally, several other target tissues were identified, including liver, kidney, thymus, and bone marrow. On the basis of the presence of hyperplasia of the thyroid gland and thymus weight reductions at the lowest 2-MBI concentration tested, the no-observable-effect level was less than 3.1 mg/m^3.

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The Adverse Effects of Oral 2-Mercaptobenzimidazole on Pregnant Rats and Their Fetuses

TETSUO YAMANO,[1] TSUTOMU NODA, MITSURU SHIMIZU, AND SHIGERU MORITA

Osaka City Institute of Public Health and Environmental Sciences, 8-34 Tojo-cho, Tennoji-ku, Osaka 543, Japan

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The effects of oral 2-mercaptobenzimidazole (2-MBI) on pregnant Wistar rats were examined. In a preliminary dose-finding study, pregnant rats treated with 2-MBI over Days 7–17 of gestation showed reduction in maternal thymus weights with compound-related mortality at doses ≥40 mg/kg. No adverse effects on fetuses were found at doses ≤40 mg/kg. However, anasarca, cleft palate, and dilated lateral ventricles were present in all fetuses from the only survivor among the dams treated with 60 mg/kg of 2-MBI. In the teratology study, pregnant rats were treated with 2-MBI at doses of 0, 3.3, 10, and 30 mg/kg during the period of organogenesis (Gestation Days 7–17). In addition, pregnant rats of three groups were also treated with 60 mg/kg of 2-MBI for 3 or 4 days during specific periods of organogenesis (Days 7–10, 11–14, or 15–17 of gestation). Treatment on Gestation Days 7–17 resulted in reduced maternal thymus weights at doses of ≥3.3 mg/kg. In addition to reduced fetal weights, visceral variations (kinked ureter and dilated renal pelvis) and delayed ossification were seen in the fetuses at doses ≥10 mg/kg, and skeletal variations (rudimentary lumbar ribs) were seen at 30 mg/kg. In the fetuses from the dams treated with 60 mg/kg of 2-MBI, rudimentary lumbar ribs were seen mainly in the group treated on Days 7–10 of gestation, whereas kinked ureter and dilated renal pelvis were evident mainly in the group treated on Gestation Days 15–17. Dilated lateral ventricles and cleft palate were present only in the group treated with 60 mg/kg on Days 11–14 of gestation, though 5 out of 16 dams died during the study. In conclusion, maternal toxicity preceded fetal toxicity and major fetal malformations were seen only at a dose (60 mg/kg) which was lethal to many of the treated dams. © 1995 Society of Toxicology.

The compound 2-mercaptobenzimidazole (2-MBI) is used as an accelerator and/or an antioxidant in rubber manufacturing. The risk of 2-MBI to workers is a matter of concern because it is structurally related to ethylene thiourea, an antithyroid agent, which is carcinogenic in the thyroid gland (IARC, 1974), as well as a potent teratogen especially in the nervous and urogenital system (Khera, 1973; Ruddick and Khera, 1975). Thyroid toxicity induced by 2-MBI, such as a decrease in iodine uptake and circulating thyroid hormones, and thyroid enlargement, has been recognized for a long time (Searle *et al.*, 1950; Kellen, 1972; Janssen *et al.*, 1981). Recently, in addition to its thyroid toxicity, numerous other adverse effects of 2-MBI have been found in rats after long-term administration by the inhalation route, including reduced serum fatty acid levels, increased GOT, GPT, and ALP activities, and histopathological changes in several organs other than the thyroid (Gaworski *et al.*, 1991).

2-MBI is embryotoxic in rats after intraperitoneal administration (Barilyak, 1974, 1976). Khera and Whalen (1988) classified 2-MBI together with ethylene thiourea as teratogenic in the nervous system by means of an *in vitro* assay using cultured neural cells. On the other hand, Ruddick *et al.* (1976) designated 2-MBI as nonteratogenic in rats from a comparison of the teratogenicity of 16 chemicals that were structurally related to ethylene thiourea. However, this conclusion was based on the results derived from a single dose administered on a single day to a small number (four) of animals. In order to fully explore the teratogenic potential of 2-MBI, we undertook this study: a much larger number of animals were used, four doses were set, and the treatment period covered the entire period of organogenesis.

METHODS

Chemicals. 2-MBI was purchased from Ouchi Shinko Chem. Co., Ltd. (Tokyo, Japan) (>97.0% purity by HPLC).

Animals. Four-week-old SPF Wistar rats of both sexes obtained from CLEA Japan Inc. (Tokyo, Japan) were housed individually in stainless-steel cages in a room with a constant photoperiod (dark period from 7:00 PM to 7:00 AM) at 23 ± 2°C and 60 ± 10% relative humidity. They were given feed (NMF, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum* and studied at 3 months of age. For the teratology study, females were individually paired overnight with a male of similar age, and the day upon which sperm was found in vaginal smears was designated as Day 0 of gestation.

Dose-finding study. Mated (pregnant) rats were assigned to seven groups of five or six animals each. They were treated by gavage with 2-MBI

[1] To whom correspondence should be addressed.

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TABLE 2

Visceral and Skeletal Observations of Fetuses from the Dams Treated Orally with 2-Mercaptobenzimidazole

	2-MBI (mg/kg)						
	0	3.3	10	30	60	60	60
Days of treatment	7–17	7–17	7–17	7–17	7–10	11–14	15–17
	Visceral observations[a]						
No. of fetuses examined	148	135	135	140	122	66	107
Incidence of fetuses with malformations (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Incidence of fetuses with variations (%)	3.3[5](5)[b]	3.3[5](5)	20.5**[27](15)	47.6**[65](18)	8.9[11](6)	15.8[11](4)	44.6[49](14)
Dilated lateral ventricles (%)	0.0	0.0	0.0	0.0	0.0	12.9[9](2)	0.0
Kinked ureter							
Unilateral (%)	2.6[4](4)	2.1[3](3)	8.6[12](11)	23.4**[32](16)	5.7[7](5)	6.0[4](3)	24.7[25](12)
Bilateral (%)	0.0	0.6[1](1)	3.3[4](3)	8.8**[12](7)	0.0	0.0	6.2[7](6)
Total (%)	2.6[4](4)	2.6[4](4)	12.0*[16](13)	32.1**[44](17)	5.7[7](5)	6.0[4](3)	31.0[32](12)
Dilated renal pelvis							
Unilateral (%)	0.7[1](1)	0.7[1](1)	9.8[13](7)	28.9**[38](17)	5.7[7](4)	2.9[2](2)	20.8[23](12)
Bilateral (%)	0.0	0.6[1](1)	1.7[2](2)	6.5**[9](7)	1.7[2](2)	1.3[1](1)	16.8[20](10)
Total (%)	0.7[1](1)	1.3[2](2)	11.4[15](7)	35.4**[47](17)	7.4[9](5)	4.1[3](3)	37.6[43](13)
	Skeletal observations[a]						
No. of fetuses examined	137	137	142	137	107	62	96
Incidence of fetuses with malformations (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Incidence of fetuses with variations (%)	0.7[1](1)	5.8[8](4)	7.8[11](7)	23.8**[33](12)	19.3[21](11)	11.8[6](3)	8.1[7](6)
Cervical ribs							
Unilateral (%)	0.0	0.0	1.3[2](2)	0.8[1](1)	1.0[1](1)	0.0	0.8[1](1)
Rudimentary lumbar ribs							
Unilateral (%)	0.7[1](1)	4.6[6](4)	4.4[6](4)	13.3**[20](9)	8.8[9](8)	2.0[1](1)	5.0[5](4)
Bilateral (%)	0.0	1.3[2](1)	2.2[3](3)	8.9**[12](7)	4.1[5](4)	4.0[2](1)	2.2[1](1)
Total (%)	0.7[1](1)	5.8[8](4)	6.6[9](5)	22.2**[32](11)	12.9[14](9)	6.0[3](1)	7.3[6](5)
Lumbar ribs							
Unilateral (%)	0.0	0.0	0.0	0.0	1.7[2](2)	0.0	0.0
Splitting of vertebral bodies							
Lumbar (%)	0.0	0.0	0.0	0.7[1](1)	1.8[2](2)	4.2[2](2)	0.0
Thoracic (%)	0.0	0.0	0.0	0.0	1.8[2](2)	1.7[1](1)	0.0
Degree of ossification[c]							
No. of sternebrae	3.7 ± 0.24	3.7 ± 0.31	3.4 ± 0.28*	3.0 ± 0.45**	3.2 ± 0.67	2.9 ± 0.93	3.2 ± 0.66
No. of proximal and middle phalanges							
Fore limb	3.1 ± 0.14	3.1 ± 0.20	3.0 ± 0.06	3.1 ± 0.44	3.2 ± 0.71	3.2 ± 0.61	3.1 ± 0.46
Hind limb	4.0	4.0 ± 0.07	4.0 ± 0.04	4.0 ± 0.03	4.0 ± 0.15	3.9 ± 0.23	3.9 ± 0.29
No. of ossification centers of vertebrae							
Thoracic	12.7 ± 0.32	12.7 ± 0.29	12.4 ± 0.29*	11.9 ± 0.31**	12.2 ± 0.33	12.0 ± 0.38	12.3 ± 0.32
Sacral and caudal	6.5 ± 0.23	6.7 ± 0.39	6.4 ± 0.42	5.6 ± 0.70**	5.8 ± 0.93	5.5 ± 1.33	5.5 ± 1.30

[a] The litter was used as a statistical unit for calculation of fetal values, thus these values represent means of litter means within each group.

[b] Nos. in brackets represent No. of fetuses with variations. Nos. in parentheses represent No. of mothers that conceived young with variations.

[c] Mean ± SD.

*,** Significantly different from control group at $p < 0.05$ and $p < 0.01$, respectively, by Dunnett's multiple comparison test.

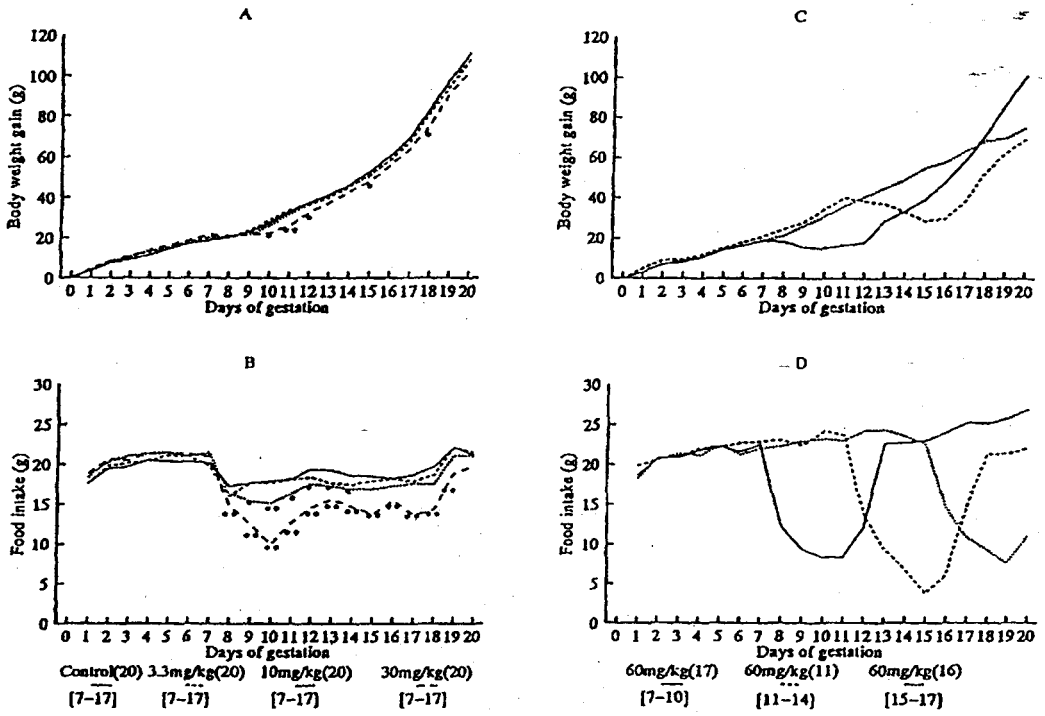

FIG. 1. Body weight gain and food consumption of pregnant rats treated orally with 2-mercaptobenzimidazole. Parentheses represent no. of rats; brackets represent days of treatment. *,**Significantly different from control group at $p < 0.05$ and $p < 0.01$, respectively, by Dunnett's multiple comparison test.

survived and had eight live fetuses, all of which had anasarca and cleft palate. These fetuses also had dilated lateral ventricles.

Teratology study. All dams treated with 0, 3.3, 10, and 30 mg/kg of 2-MBI survived and had live fetuses. It was obvious that 2-MBI was more toxic to dams than to fetuses: maternal thymus weight was decreased even at the lowest dose of 3.3 mg/kg, whereas fetal body weights were significantly decreased only in the litters of dams dosed with 10 mg/kg or higher of 2-MBI (Table 1). Visceral variations consisting of unilateral or bilateral kinked ureter and/or dilated renal pelvis were noted in 20.5% of fetuses at 10 mg/kg and in 47.6% of the fetuses at 30 mg/kg. Skeletal variations, unilateral or bilateral rudimentary lumbar ribs, were observed in 22.2% of the fetuses at 30 mg/kg. The degree of ossification was significantly reduced in the litters of dams treated with ≥10 mg/kg of 2-MBI (Table 2).

Because of the severe resulting toxicity, 60 mg/kg dose could not be administered throughout the period of organogenesis (Gestation Days 7–17). Instead, the test animals were dosed for shorter periods of time, viz., Gestation Days 7–10, 11–14, or 15–17. Even under these dosing conditions, 2-MBI was severely toxic to dams, as evidenced by a substantial decrease in maternal body weight gain and food consumption in all three groups (Figs. 1C and 1D) and maternal death (5 out of 16 dams) in the group treated on Days 11–14 of gestation (Table 1). Vaginal bleeding was observed in 10 and 2 dams in the group treated on Days 11–14 and 15–17 of gestation, respectively. All fetuses were resorbed in the litters of each dam of these two groups.

"Split dosing" with 60 mg/kg of 2-MBI helped us determine the critical periods for the major anomalies observed in this study. Following a treatment schedule that covered the entire period of organogenesis (Gestation Days 7–17), rudimentary lumbar ribs, kinked ureter, and dilated renal pelvis were the major anomalies observed in the litters of dams treated with 10 or 30 mg/kg of 2-MBI (Fig. 2). Only rudimentary lumbar ribs were observed when the treatment period (with 60 mg/kg) was shortened to Gestation Days 7–10, while kinked ureter and dilated renal pelvis were observed only following treatment with 60 mg/kg on Days 15–17. Finally, dilated lateral ventricles and cleft palate were observed only in the litters of dams treated with 60 mg/kg on Days 11–14; these anomalies were not observed at lower dosages of the chemical.

DISCUSSION

Under the conditions of the present study, the no-observed-adverse-effect level (NOAEL) of 2-MBI for maternal

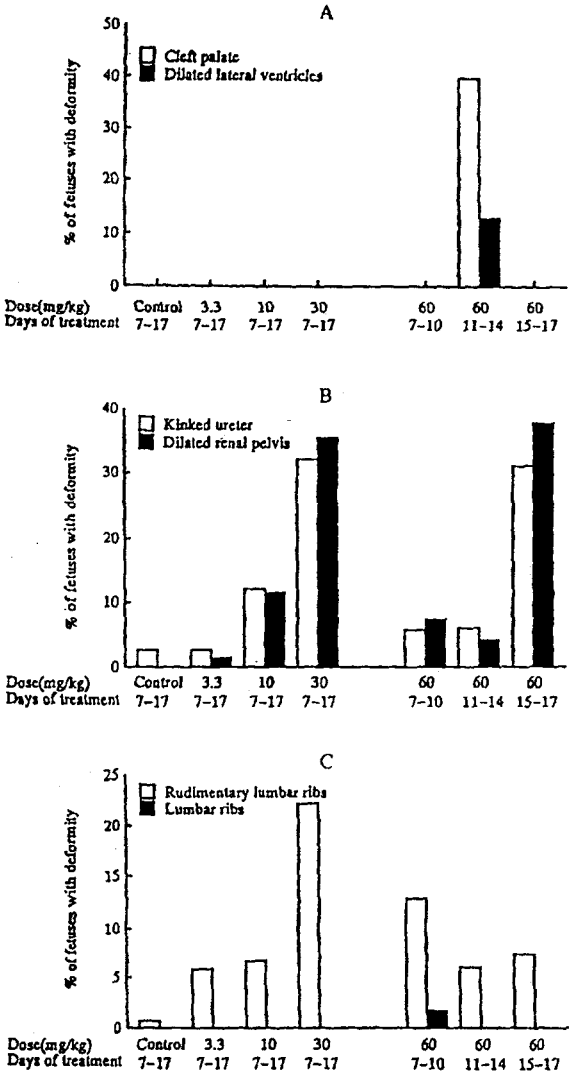

FIG. 2. The incidence of fetuses with major anomalies in relation to dose and treatment day. (A) Open bar, cleft palate; solid bar, dilated lateral ventricles. (B) Open bar, kinked ureter; solid bar, dilated renal pelvis. (C) Open bar, rudimentary lumbar ribs; solid bar, lumbar ribs.

toxicity was considered to be less than 3.3 mg/kg, because of significant decrease in maternal thymus weights at this dose, and that for fetal toxicity was determined to be 3.3 mg/kg.

Adverse fetal effects were observed only at doses which were clearly maternotoxic. Treatment with 10 and 30 mg/kg of 2-MBI resulted in decreased thymus weights, increased thyroid weights, decreased body weight gain, and decreased food consumption in the treated dams. These dosages also reduced fetal body weights and increased the incidence of certain anomalies of the urogenital system and of rudimentary lumbar ribs. It has been shown that supernumerary lumbar ribs are secondary to maternal stress (Beyer and Chernoff, 1986) and tend to disappear during the postnatal period (Marr *et al.*, 1992). Similarly, dilated renal pelvis has been claimed to be a reversible finding (Woo and Hoar, 1972). Palmer (1978) has classified convoluted (kinked?) ureter as a normal developmental variability and felt that these "may represent compensatory mechanisms of advantage to the individual in maintaining the spatial relationships of tissues during growth." None of the fetal anomalies in the litters of dams treated with 10 or 30 mg/kg of 2-MBI should be considered to be a major malformation. In our study, the more serious fetal malformations, cleft palate and dilatation of the lateral ventricles, were observed only at 60 mg/kg, a dose which was lethal to many of the treated dams.

Dilatation of the lateral ventricles and cleft palate, noted by us at 60 mg/kg of 2-MBI, were also reported by Khera (1973) in his teratology study of ethylene thiourea. However, a number of other fetal malformations (exencephaly, hydrocephaly, micrognathia, limb and digital defects, etc.) noted by Khera were not observed in the present study. Thus, while 2-MBI and ethylene thiourea may be chemically similar, the two chemicals produce significantly different spectrum of malformations.

The mechanisms of teratogenesis for 2-MBI remains to be elucidated. However, it is clear that, at least in the rat, maternal toxicity precedes fetal toxicity and major fetal malformations are seen only at a dose (60 mg/kg) which is lethal to many of the treated dams.

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1,4-ジシアノベンゼンのラットを用いる単回経口投与毒性試験

Single Dose Oral Toxicity Test of 1,4-dicyanobenzene in Rats

要約

1,4-ジシアノベンゼンの354，500，707，1000，1414および2000 mg/kgを5週齢のCrj:CD(SD)系雌雄ラットに経口単回投与し，その毒性を試験した．

死亡は，雄の500，1000，1414および2000 mg/kg群ならびに雌の1414および2000 mg/kg群で5例中1～2例に投与後2～4日に認められた．LD_{50}値は雌雄ともに約2000 mg/kgと推定された．1,4-ジシアノベンゼン投与による症状として，死亡例で間代性痙攣，強直性痙攣，自発運動の減少，横臥および呼吸緩徐が，生存例で間代性痙攣，流涎，自発運動の減少および被毛汚染が認められた．また，1,4-ジシアノベンゼン投与群で低体重あるいは体重減少が認められた．病理学的検査では，1,4-ジシアノベンゼンの刺激による反応性変化と考えられる所見として，前胃粘膜層の肥厚，潰瘍あるいは過角化などが認められ，死亡例ではさらに，肝臓に中心性の肝細胞の空胞化，脾臓および肺にうっ血などが認められた．

材料および方法

1. 被験物質

1,4-ジシアノベンゼンは燻蒸剤として使用される殺菌・殺虫用化学物質であり，油溶性(水溶解度：0.03 g/100 g)の白色結晶である．本試験では昭和電工(株)より提供されたロット番号930825(純度99%以上)のものを使用した．被験物質は試験期間中安定であることを確認した．投与には，被験物質を1%メチルセルロース溶液(MC：品名メトロース，SM-15，信越化学工業(株)；日本薬局方精製水：ヤクハン製薬(株))で用時に懸濁調製したものを使用した．

2. 試験動物および飼育条件

生後4週齢のCrj:CD(SD)系のSPFラット雌雄を日本チャールス・リバー(株)より受け入れ，9日間の馴化飼育を行い，順調な発育を示した動物を試験に用いた．動物は，温度23±3℃，湿度55±10%，換気回数10～15回／時および照明12時間／日に設定されたバリアシステムの飼育室において，ブラケット式金属製金網床ケージに群分け前は5匹以内，群分け後は3匹以内収容した．飼料は固型飼料(CRF-1，オリエンタル酵母工業(株))，飲料水は水道水(札幌市水道水)を自由に摂取させた．飼料の混入物質および飲料水の水質を検査し，異常がないことを確認した．

3. 試験群の設定

本試験の投与量設定のために実施した試験において，2000および1000 mg/kg群で死亡が認められ，500 mg/kg群では死亡はみられなかったが，投与後の体重に減少あるいは増加抑制が認められ，死亡する可能性も考えられた．以上のことから，本試験では雌雄ともに投与(調製)可能な最大量である2000 mg/kgを最高用量とし，以下，公比√2で1414，1000，707，500および354 mg/kg，さらに1%メチルセルロース溶液を投与する対照を設け，計7群とした．動物数は1群当たり雌雄各5匹とし，群分けは投与前日に体重別層化無作為抽出法により行った．

4. 投与方法

投与は，動物を約17～18時間絶食させた後，胃ゾンデを用いて強制経口投与した．投与容量は，体重1 kg当り10 mlとして投与日に測定した体重に基づいて算出した．投与時の週齢は雌雄ともに5週齢で，その平均体重(体重範囲)は雄で129.4 g(118～141 g)，雌で109.9 g(105～114 g)であった．

5. 観察，測定および検査項目

全例について，一般状態を投与日は投与後6時間までは頻繁に，投与後1日以降は1日1回以上の頻度で投与後14日まで観察した．また，体重を投与日，投与後1，3，5，7，10および14日に測定した．死亡例は発見後直ちに，生存例は投与後14日に体外表を観察した後，エーテル麻酔下で放血致死させ，剖検した．このうち，死亡例は全例，生存例は対照群および1,4-ジシアノベンゼン投与各群の雌雄各2例の肝臓，腎臓，脾臓，心臓，肺，脳(大脳・小脳)，胃(前胃・腺胃)，十二指腸，空腸，回腸，盲腸，結腸，直腸および異常所見部位を10%中性緩衝ホルマリン液で固定し，常法に従いパラフィン切片を作製し，ヘマトキシリン・エオジン染色あるいは特殊染色[PAS染色，α-アミラーゼ消化試験，PTAH染色，GFAP免疫染色，エラスチカ・ワンギーソン染色および鍍銀(渡辺変法)染色]標本を作製し，病理組織学的検査を行った．また，500および1000 mg/kg群の雌の生存例各1例の前胃，1414 mg/kg群の雄の死亡例1例の皮膚および骨格筋につい

て同様に検査した．

死亡例数より死亡率を算出し，体重についてBartlettの等分散検定の後，一元配置分散分析法あるいはKruskal-Wallis法により解析し，有意な場合，Dunnettの検定法あるいはMann-WhitneyのU-検定法により対照群と1,4-ジシアノベンゼン投与各群との比較を行った．なお，対照群との検定については，危険率5%以下を統計学的に有意とした．また，プロビット法によりLD_{50}値が算出できなかったため，死亡状況からおおよそのLD_{50}値を推定した．

成績

1. 死亡状況およびLD_{50}値(Table 1)

雄では死亡が500，1000，1414および2000 mg/kg群で投与後2～4日に認められ，LD_{50}値は約2000 mg/kgと推定された．雌では死亡が1414および2000 mg/kg群で投与後2あるいは3日に認められ，LD_{50}値は約2000 mg/kgと推定された．

2. 一般状態観察

死亡例では，1414 mg/kg群の雄1例で投与後2日に間代性痙攣，呼吸緩徐，自発運動の減少がみられ，その日に死亡が認められた．2000 mg/kg 群の雌1例では，投与後1日に強直性痙攣，横臥および呼吸緩徐が認められ，投与後2日に死亡が認められた．その他の例では症状はみられず，投与後3あるいは4日に死亡が認められたのみであった．

生存例では，1000 mg/kg群の雄1例で投与後4～6日に間代性痙攣，流涎，自発運動の減少，口周囲の被毛汚染が，同群の雌1例で投与後2～5日に外尿道口周囲および腹部の被毛汚染，腹部に外傷および痂皮が認められた．1414 mg/kg群の雌1例で投与後4日に外尿道口周囲の被毛汚染が認められた．その他の例では症状は認められなかった．

3. 体重推移

雌雄ともに，1,4-ジシアノベンゼン投与群の投与後約1週間の体重は対照群よりも低く推移し，雄の500 mg/kg以上の群で投与後1～7日，雌の354 mg/kg以上の群で投与後1～5日に有意な低値が認められた．また，雌雄ともに500mg/kg以上の群で投与後1ないし3日に投与日体重を下回る例も認められ，その程度は生存例よりも死亡例で重度であった．一方，1,4-ジシアノベンゼン投与群の生存した例では，ほぼ全例で投与後14日には対照群と同程度にまで回復した．

4. 病理学的検査

死亡例の剖検では，前胃に粘膜の白色顆粒状がほぼ全例に，腺胃粘膜に暗赤色斑あるいは赤色化ならびに消化管内容物の暗赤色化が数例に認められた．病理組織学的には前胃粘膜層の肥厚や潰瘍などが認められたのみで，腺胃や腸管に異常は認められなかった．また，肝臓に小葉中心性の肝細胞の空胞化，脾臓及び肺にうっ血，頸部あるいは背部の皮膚に筋層における筋線維の巣状壊死，皮下織における出血，肩甲部骨格筋に筋線維の壊死などが認められたが，脳には異常は認められなかった．このうち，皮膚や骨格筋における所見は死亡当日に間代性痙攣のみられた例に認められ，痙攣の際の衝撃によることが予想された．

生存例では，剖検で前胃粘膜の一部肥厚が500 mg/kg以上の群の雌の一部の例に認められ，病理組織学的には粘膜層の肥厚，過角化などが認められた．

Table 1　Mortality and LD_{50} values of rats treated orally with 1,4-dicyanobenzene in the single dose toxicity test

Sex	1,4-dicyano-benzene (mg/kg)	Distribution of dead animals 0	1	2	3	4	5-14 a)	Mortality b)	Approximate LD_{50} value (mg/kg)
Male	0	0	0	0	0	0	0	0/5	
	354	0	0	0	0	0	0	0/5	
	500	0	0	0	1	0	0	1/5	
	707	0	0	0	0	0	0	0/5	2000
	1000	0	0	0	1	0	0	1/5	
	1414	0	0	1	0	0	0	1/5	
	2000	0	0	0	0	2	0	2/5	
Female	0	0	0	0	0	0	0	0/5	
	354	0	0	0	0	0	0	0/5	
	500	0	0	0	0	0	0	0/5	
	707	0	0	0	0	0	0	0/5	2000
	1000	0	0	0	0	0	0	0/5	
	1414	0	0	0	1	0	0	1/5	
	2000	0	0	1	1	0	0	2/5	

a : Day after administration.

b : No. of dead animals / No. of animals dosed.