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Prechronic Inhalation Toxicity Studies of 2-Mercaptobenzimidazole (2-MBI) in F344/N Rats[1]

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*IIT Research Institute, 10 West 35th Street, Chicago, Illinois 60616; †National Institute of Environmental Health Sciences, National Toxicology Program, Research Triangle Park, North Carolina 27709; ‡University of Illinois at Chicago, Chicago, Illinois 60612; and §Pathology Associates, Inc., Chicago, Illinois 60616

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Prechronic Inhalation Toxicity Studies of 2-Mercaptobenzimidazole (2-MBI) in F344/N Rats. GAWORSKI, C. L., ARANYI, C., VANA, S., RAJENDRAN, N., ABDO, K., LEVINE, B. S., AND HALL III, A. (1991). *Fundam. Appl. Toxicol.* 16, 161–171. 2-Mercaptobenzimidazole (2-MBI), used in rubber processing, is a suspect carcinogen structurally related to ethylene thiourea. The inhalation toxicity of 2-MBI was evaluated in male and female F344/N rats exposed 6 hr/day, 5 days/week to respirable aerosols generated by spray atomization of aqueous suspensions of the 2-MBI powder and subsequent drying of the resulting aerosols. Twelve exposures at target concentrations of 0, 6.3, 12.5, 25.0, 50.0, or 100 mg/m^3 of 2-MBI produced a dose-related reduction in body weight gains, thyroid follicular cell hyperplasia, adrenal cortex fatty change, and pituitary atrophy. Subchronic exposures were conducted at target concentrations of 0, 3.1, 6.2, 12.5, 25.0, and 50.0 mg/m^3 of 2-MBI. Rats at ≥25 mg/m^3 displayed hunched posture, hypoactivity, and reduced body weight gain, with compound related mortality at the highest exposure level. Anemia; increased SGPT, SGOT, alkaline phosphatase, sorbitol dehydrogenase, BUN, and cholesterol; and reduced free fatty acid were seen in rats at ≥25 mg/m^3. Increased thyroid weight and thyroid follicular cell hyperplasia were noted in both sexes at ≥6.2 mg/m^3, with reduced triiodothyronine and thyroxine levels in both sexes at ≥12.5 mg/m^3. Thyroid follicular cell hyperplasia was also seen in rats at 3.1 mg/m^3. Thymus weights were significantly reduced in both sexes at all exposure levels with liver weight increases at ≥6.2 mg/m^3. Exposure-related histopathologic changes included pituitary cytoplasmic vacuolization, adrenal cortex necrosis, lymphoid depletion, thymic atrophy, liver cell hypertrophy, renal mineralization and tubular atrophy, and hypocellularity of the bone marrow. © 1991 Society of Toxicology.

2-Mercaptobenzimidazole ($C_7H_6N_2S$, or 1H-benzimidazole-2-thiol) is structurally related to ethylene thiourea and is suspected of being capable of producing thyroid and liver tumors. 2-Mercaptobenzimidazole (2-MBI) is used extensively as an accelerator and/or antioxidant in rubber manufacturing, with a significant potential for occupational exposure by the inhalation route.

Toxic effects of 2-MBI were recognized as early as 1950 when a single oral dose was shown to cause thyroid toxicity, as measured by a 95% decrease in iodine uptake, in rats (Searle *et al.*, 1950). Administration of 2-MBI has been used to decrease thyroid function in rats (Kellen, 1972). Thyroid enlargment, which was associated with decreased plasma concentrations of circulating thyroxine and triiodothyronine and increased thyrotropin

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levels, has been reported in rats receiving a single oral dose of 2-MBI (Janssen *et al.*, 1981). 2-MBI is readily absorbed when administered orally, has a blood half-life of approximately 83 hr when delivered intravenously, and accumulates in the thyroid (El Dareer *et al.*, 1984; Janssen *et al.*, 1981). Mice breathing 400 mg/m^3 2-MBI 2 hr/day for 15 days developed decreased RBC counts, punctate hemorrhages in the myocardium, altered morphology of lung and liver, and nervous system disorders (Mezentseva, 1968). Reduction of circulating L-thyroxine levels by benzimidazolethiol, a principle metabolite of 2-MBI (Janssen *et al.*, 1981), along with preliminary evidence of thymic involution following 2-MBI exposure (Malmfors, 1976) suggests that 2-MBI may also be immunotoxic. 2-MBI has induced embryotoxic effects in female rats inoculated intraperitoneally (Barilyak, 1974), chromosomal aberrations in rat fetal cells after administration (Barilyak and Melnik, 1979), and cytogenetic abnormalities and immunodeficiencies in the progeny of female rats (Barilyak *et al.*, 1979).

Information on the long-term toxicity of 2-MBI administered by inhalation is limited. A 13-week subchronic inhalation study was therefore conducted to investigate the toxicologic effects of 2-MBI aerosols and to identify target organs, differences in sensitivity between sexes, and dose–response relationships with repeated exposures at various concentrations. A 14-day repeated dose inhalation study was conducted prior to the subchronic study to provide a basis for dose selection.

METHODS

Chemical Analysis of 2-MBI

The 2-MBI bulk chemical was provided by the NTP through Midwest Research Institute from a commercial supplier (Mobay Chemical Corp., West Germany). For purity analysis the 2-MBI bulk chemical was dissolved in methanol and analyzed by high-pressure liquid chromatography (HPLC). Samples were eluted on a Vydac C_{18} column (AllTech Associates, Deerfield, IL) preceded by a Whatman Pellicular ODS guard column using 2 mobile phase of 75% water, 25% methanol containing 1% acetic acid and 2 variable wave length uv detector (254 nm). The 2-MBI bulk chemical was greater than 98% pure. (The 2% impurity was not identified.)

The HPLC method was also used to determine the 2-MBI content and potential degradation products of the aerosols in the exposure chamber. Samples were collected on filters and extracted using methanol. The analysis method employed the same HPLC system described above with a mobile phase of 95% water, 5% methanol containing 1% glacial acetic acid. (The mobile phase was adjusted for the filter analysis in order to obtain retention times equivalent to those obtained for purity analyses.)

2-MBI Aerosol Generation

A wet dispersion technique using a pneumatic spray nozzle was devised to generate aerosols of 2-MBI from its 10% aqueous suspension. Pneumatic dry dispersion of the 2-MBI produced inconsistant aerosol delivery rates because of the oily and sticky nature of the powder. The oiliness of the powder necessitated very high shear rates to obtain a homogeneous and uniform suspension. The suspension was prepared in two stages. In the first stage, the suspension was pumped through a 0.03-in.-diameter orifice at a pressure of 250 psig. This suspension was atomized in the second stage with a spray nozzle (Spray Setup No. 13A, Spraying Systems, Inc., Wheaton, IL) and the resulting aerosol spray was collected with a cyclone mist collector for use in the aerosol generators. To generate the 2-MBI test aerosol the pretreated suspension was spray atomized, the mist was passed through another glass cyclone and a stainless steel transport pipe heated to approximately 150°C prior to mixing with conditioned dilution air at the chamber inlet. The estimated residence time of the aerosol in the transport tube was about 1 sec. The spray nozzle operated on dry compressed air and the suspension was delivered to the nozzle by a metering pump. The cyclone was designed to remove any droplets or nonatomized liquid greater than 15 μm diameter and the heated transport tube vaporized the liquid water in the mist to facilitate drying by the dilution air. The resulting aerosol in the inhalation exposure chambers consisted of dry 2-MBI solid particles. (Detailed description of the aerosol generation system and its performance evaluation will be the subject of another paper to be published separately.)

Aerosol Monitoring

The exposure chambers were monitored for 2-MBI aerosol mass concentration and particle size. Aerosol mass concentrations were monitored continuously with RAM-S real-time aerosol monitors (MIE, Inc., Bedford, MA)

and hourly by gravimetric filter samples collected from measured volumes of the test atmospheres. The RAM-S response was used to detect potential drifts in the aerosol concentration and thereby indicate the adjustments needed to the generator's output to keep the test atmosphere concentration on target. Selected aerosol samples were analyzed chemically to ensure that the aerosol mass determined gravimetrically was all due to dry 2-MBI and not water in aerosol form. In addition, the filter samples were analyzed to establish that there were no chemical degradation products in the aerosol relative to the 2-MBI bulk chemical.

Aerosol particle size distribution in the chamber atmosphere was measured in each exposure chamber with a Quartz Crystal Microbalance (QCM)-based cascade impactor (California Measurements, Inc., Sierra Madre, CA).

Spatial homogeneity in the exposure chambers was determined by measuring the aerosol mass concentrations with a RAM monitor at six locations within the chamber in the approximate animal breathing zones.

Animals and Animal Care

Male and female F344/N rats, approximately 6 to 7 weeks of age, were obtained from Simonson Laboratories (Gilroy, CA), for the 14-day study and from Taconic Farms, Inc. (Germantown, NY), for the subchronic study. Animals were maintained in stainless steel wire-mesh cages in 2-m^3 inhalation chambers (Lab Products, Inc., Maywood, NJ), with 2- to 3-week quarantine periods prior to exposure initiation. Cage units were rotated on a weekly basis within the chambers. Exposure chambers were maintained at 75 ± 3°F and 55 ± 15% relative humidity, with air flows of 15 ± 2 changes per hour. NIH-07 open formula diet (Zeigler Brothers, Inc., Gardners, PA) was available *ad libitum*, except during exposures. Filtered City of Chicago drinking water was supplied *ad libitum* via an automatic watering system. A 12-hr light/dark cycle (6 AM to 6 PM light) was provided. At terminal necropsy, serum samples were obtained from rats housed in the control chamber during the subchronic study for a standard NTP virus antibody screen. All samples were negative for the diseases screened.

Experimental Design and Toxicology Procedures

Fourteen-day repeated dose study. Groups of five rats/sex were exposed at target concentrations of 6.3, 12.5, 25.0, 50.0, or 100.0 mg/m^3 2-MBI 6 hr/day, 5 day/week, for a total of 12 exposures. Three consecutive exposures were given immediately prior to scheduled termination. A control group of five rats/sex was exposed to filtered air.

Subchronic study. Groups of 10 rats/sex (core study) were exposed at target concentrations of 3.1, 6.2, 12.5, 25.0, or 50.0 mg/m^3 2-MBI 6 hr/day, 5 day/week, for 13 weeks. A control group of 10 rats/sex was exposed to filtered air. At least two consecutive exposures were given immediately prior to the scheduled termination. Nineteen additional male rats were included in each of the control and the 3.1, 12.5, and 50 mg/m^3 groups (special study) for scheduled collection of serum samples for thyroid hormone radioimmunoassay (RIA).

Observations and body weights. Animals were observed twice each day for mortality/moribundity, with formal clinical observations performed daily during the 14-day study and weekly during the subchronic study. Body weights were measured at exposure initiation, weekly thereafter, and at necropsy.

Clinical pathology and hormone analysis. All blood samples were collected from nonfasted rats anesthetized with 70% CO_2. Blood was collected via the abdominal vena cava from all surviving core study rats at the terminal necropsy of the subchronic study. Hematology parameters examined included erythrocyte count and indices; leucocyte count and differentials; hemoglobin, hematocrit, reticulocyte, and platelet counts; and prothrombin and activated partial thromboblastin times. Clinical chemistry tests included albumin, total protein, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatatase, glucose, total cholesterol, serum cholinesterase, sorbitol dehydrogenase, lactic dehydrogenase, total bilirubin, and free fatty acids. Hematologic determinations were performed with a Baker 9000 hematology analyzer, and the clinical chemistry tests were performed with a Baker Centrifichem 500 automated analyzer (Serono-Baker, Allentown, PA).

Blood samples for radioimmunoassay of the thyroid hormones triiodothyronine (T_3), thyroxine (T_4), and thyroid-stimulating hormone (TSH) were collected via the retroorbital sinus from each of three special study rats necropsied at 2, 4, or 8 weeks of exposure. Special study rats scheduled to complete the entire 13-week exposure period were sampled preexposure, at 4 and 8 weeks of exposure, and at termination. Following collection, the serum was stored at −70°C until analyzed. T_3 and T_4 were measured using commercially available RIA kits (ICN Biomedical, Inc., Carson, CA). TSH was measured in serum by a double antibody method using the RIA reagents and procedure provided by Dr. S. Raiti through the National Hormone and Pituitary Program (Baltimore, MD).

Necropsy, organ weights, and histopathology. All animals (excluding special study groups in the subchronic study) received a complete necropsy and were examined for gross lesions. Liver, thymus, thyroid, right kidney, right testis, heart, brain, and lung weights were measured. The following tissues were collected for histopathologic examination: gross lesions and tissue masses, lymph nodes (bronchial, mediastinal, mandibular, and mesenteric), mammary gland with adjacent skin, thigh muscle, salivary gland, femur including marrow, rib (costochondral junc-

TABLE 1

SUBCHRONIC INHALATION STUDY OF 2-MERCAPTOBENZIMIDAZOLE: AEROSOL EXPOSURE CONDITIONS[a]

Aerosol target concn (mg/m^3)	Experimentally determined aerosol mass concentrations[b]			Aerosol particle size distribution[c]	
	Mean (mg/m^3)	%RSD[d]	N	MMAD[e]	σ_g[e]
3.1	3.1	12.1	66	2.0	2.9
6.2	6.2	8.0	66	2.3	2.9
12.5	12.5	11.5	66	2.2	2.8
25.0	25.1	11.2	66	2.0	2.7
50.0	51.1	7.5	66	2.3	2.4

[a] 6 hr/day, 5 days/week.
[b] Determined from six daily gravimetric filter-collected aerosol samples over 66 exposure days.
[c] The values are means of four determinations.
[d] Relative standard deviation.
[e] Mass median aerodynamic diameter and geometric standard deviation.

tion), nasal cavity and turbinates, tongue, larynx, pharnyx and trachea, lung and mainstem bronchi, heart and aorta, thymus, thyroids, parathyroids, esophagus, stomach, large and small intestines, liver, pancreas, spleen, kidneys, adrenals, urinary bladder, preputial or clitoral glands, prostate, testes, epididymides, seminal vesicles, scrotal sac, vagina, ovaries, uterus, brain and pituitary, spinal cord, sciatic nerve, eyes, and Zymbal's glands. Tissues were fixed in 10% neutral buffered formalin, trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. A complete histopathologic evaluation inclusive of gross lesions was conducted on all animals in the control and high concentration exposure groups. Additionally, all of the required tissues in the 25 mg/m^3 exposed female rats in the subchronic study were examined due to the mortality in the 50 mg/m^3 group. Tissues demonstrating chemically related lesions (target organs) were identified, and these organs plus gross lesions were examined in lower doses until a no-observed effect level was determined.

Statistics

Organ weights and organ weight/body weight ratios were analyzed by one-way (by sex) analysis of variance (ANOVA) followed by a Dunnett's test when a significant F ratio was obtained. Thyroid hormone data were analyzed by ANOVA followed by either a Dunnett's test, a one-sample t test, or a Mann–Whitney test (RS/Explore software, version 1.1, Serial No. V-658, BBN RS/Expert Limited Partnership, BBN Software Products Corp., Cambridge, MA). TSH results were analyzed following exclusion of outliers, as determined by the method of Dixon (1953). Clinical chemistry results were analyzed by ANOVA and a Dunnett's test using LABCAT software (Innovative Programming Associates, Inc., Princeton, NJ). The level of significance was $p \leq 0.05$.

RESULTS

The 2-MBI Aerosol

For the 13-week subchronic study, aerosol mass concentrations determined gravimetrically were maintained within 15% relative standard deviation (RSD) of the means for the entire range of dose levels throughout the 66 exposure days (Table 1). For the 14-day repeated dose study (12 exposure days), the mean aerosol concentrations were within 20% RSD of target concentrations in all chambers (data not shown).

The aerosol mass concentrations determined by gravimetric method and through chemical analysis were in good agreement for the entire range of concentrations. The ratio of aerosol concentrations calculated from chemically analyzed and gravimetrically determined amounts of 2-MBI ranged from 0.98 to 1.12, proving that the aerosol mass determined gravimetrically was all due to dry 2-

MBI. Results of the chemical analysis of the aerosol for potential 2-MBI degradation products showed the test article to be unaltered by the generation process.

Particle size measurements over the duration of the studies demonstrated that the size distribution of the 2-MBI aerosol was in the inhalable range and that the distribution did not vary with the aerosol concentration. In general, the mass median aerodynamic diameter (MMAD) was less than 3.0 μm and the geometric standard deviation (σ_g) was in the range of 1.9 to 3.0 for both studies. The values shown in Table 1 are means of four determinations made during the course of the study. The 2-MBI aerosol concentrations monitored by RAM-S sensors at six locations within the exposure chambers revealed a spatially homogeneous distribution. Typically, the spatial variations between these six sampling locations were within 10% for all the exposure chambers for both the 14-day repeated dose and the subchronic studies.

Fourteen-Day Repeated Dose Study

No mortalities resulted from exposure to 2-MBI. Clinical signs of toxicity were limited to the higher exposure levels and included mild transient lethargy and hunched posture. Reduced body weights occurred in rats exposed at 50 or 100 mg/m^3. Organ weight changes attributed to 2-MBI exposure included increased liver weight and decreased heart, lung, and thymus weights. Enlarged thyroids were noted in exposed rats at necropsy which corresponded with microscopically observed thyroid follicular cell hyperplasia in rats at the four highest 2-MBI concentrations. Additional changes included adrenal cortex fatty accumulation in males at ≥12.5 mg/m^3 and in females at ≥50 mg/m^3 and pituitary atrophy in both sexes at ≥25 mg/m^3.

Subchronic Study

Ten of the 19 male rats and all 10 females exposed to 50 mg/m^3 2-MBI died, or were euthanized in a moribund condition, during the study. Moribund animals were often noted to be hypothermic, ataxic, or comatose. These mortalities were considered to be test article related. One female control rat also died. The principle clinical signs of toxicity seen in rats exposed at 25 or 50 mg/m^3 included hunched posture, emaciation, and hypoactivity. Females generally had a greater overall incidence as well as an earlier time of onset of these clinical signs compared to males.

A dose-related decrease in body weight gain occurred in both sexes exposed to 25 mg/m^3 2-MBI, or greater (Table 2), with adverse changes generally apparent after 3–4 weeks of exposure. Comparison of initial and final body weight group means indicated essentially no weight gain during the exposure period in ei-

TABLE 2

SUBCHRONIC INHALATION STUDY OF 2-MERCAPTOBENZIMIDAZOLE: MEAN BODY WEIGHTS OF F344/N RATS

2-MBI target concn (mg/m^3)	Mean body wt		Body wt change	
	Initial (g)	Final (g)	Absolute (g)	Relative[a] (%)
		Male rats		
0	172	371	+199	—
3.1	173	372	+200	+0.5
6.2	173	374	+201	+1.0
12.5	170	383	+213	+7.0
25.0	172	273	+101	−49.2
50.0	172	177	+5	−97.5
		Female rats		
0	132	221	+89	—
3.1	134	226	+92	+3.4
6.2	133	235	+102	+14.6
12.5	135	224	+89	0
25.0	134	136	+2	−97.8
50.0	133	[b]	—	—

[a] Relative = (exposed body wt change − control body wt change)/(control body wt change) × 100.

[b] Total group mortality.

ther sex exposed at the 50 mg/m^3 level or in females exposed at 25 mg/m^3. No significant adverse body weight effects were seen in the rats exposed at 2-MBI concentrations of 12.5 mg/m^3, or less.

Necropsy observations included dark/red adrenal glands, discolored skin of the toes, and enlarged thyroid glands in exposed rats. Selected organ weights are shown in Table 3. Dose-related increases in absolute and relative thyroid weights occurred in both sexes of rats. At 6.2 mg/m^3, the mean absolute and relative thyroid weights of either sex were approximately twice the values of the respective controls, with exposure at 25 or 50 mg/m^3 resulting in increased relative weights of these organs of approximately 5 to 10 times the control weights. Increased relative liver weights were seen in males exposed at 6.2 mg/m^3, or greater, and in females exposed at 3.1 mg/m^3, or greater. Absolute and relative thymus weights were significantly reduced in both sexes exposed to 2-MBI. This effect was dose-related and was evident even at the lowest concentration tested. Other organ weight changes noted were considered incidental or related to body weight changes.

Concentration-dependent anemia with decreased RBC counts and hemoglobin and hematocrit values was seen at levels of 12.5 mg/

TABLE 3

SUBCHRONIC INHALATION STUDY OF 2-MERCAPTOBENZIMIDAZOLE: SELECTED ORGAN WEIGHT DATA OF F344/N RATS[a]

Organ	2-MBI target concn (mg/m^3)	Male		Female	
		Absolute wt (g)	Relative wt[b]	Absolute wt (g)	Relative wt[b]
Liver	0	13.004 ± 0.863	34.315 ± 1.605	7.076 ± 0.387	32.082 ± 1.558
	3.1	13.351 ± 1.116	35.969 ± 2.103	7.915 ± 0.527	35.103 ± 2.409*
	6.2	14.154 ± 0.604	37.938 ± 2.029**	8.418 ± 0.826**	35.867 ± 2.663
	12.5	15.191 ± 1.601*	38.989 ± 1.539**	8.142 ± 1.040*	36.305 ± 2.839**
	25.0	10.360 ± 2.297*	40.270 ± 2.197**	6.206 ± 0.899	45.647 ± 4.065**
	50.0	7.349 ± 0.784[c]**	40.270 ± 2.197[c]**	[d]	[d]
Thymus	0	0.231 ± 0.029	0.610 ± 0.065	0.202 ± 0.010	0.917 ± 0.041
	3.1	0.125 ± 0.040**	0.338 ± 0.108**	0.149 ± 0.011**	0.660 ± 0.062**
	6.2	0.130 ± 0.017**	0.348 ± 0.046**	0.150 ± 0.019**	0.639 ± 0.078**
	12.5	0.122 ± 0.028**	0.312 ± 0.061**	0.134 ± 0.013**	0.599 ± 0.033**
	25.0	0.068 ± 0.029**	0.239 ± 0.078**	0.042 ± 0.017**	0.302 ± 0.083**
	50.0	0.025 ± 0.019**	0.136 ± 0.097**	[d]	[d]
Thyroid	0	0.019 ± 0.004	0.049 ± 0.012	0.021 ± 0.003	0.095 ± 0.015
	3.1	0.025 ± 0.004	0.068 ± 0.010	0.029 ± 0.002	0.129 ± 0.008
	6.2	0.038 ± 0.006**	0.102 ± 0.016*	0.042 ± 0.003**	0.179 ± 0.015**
	12.5	0.057 ± 0.008**	0.146 ± 0.016**	0.053 ± 0.010**	0.238 ± 0.045**
	25.0	0.103 ± 0.023**	0.385 ± 0.073**	0.090 ± 0.013**	0.664 ± 0.091**
	50.0	0.094 ± 0.025**	0.511 ± 0.118**	[d]	[d]

[a] Values represent means ± SD; N = 10/group/sex at 3.1, 6.3, 12.5, and 25 mg/m^3, 10 males and 9 females at 0 mg/m^3, and 5 males at 50 mg/m^3.

[b] Relative wt = organ weight/terminal body weight × 1000.

[c] N = 4.

[d] Total group mortality.

* 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.

m^3 and above (Table 4). A macrocytosis was noted in these groups and was considered to be a physiologic compensatory response to the anemic state. Although the effect was not strictly dose related, total WBC counts were depressed for males, but not females, at all concentration levels (Table 4). Differential analysis indicated this response was due to a reduction in lymphocytes. Kidney damage was suggested from increased BUN levels for both sexes, while elevated cholesterol and/or reduced free fatty acid levels suggested altered lipoprotein metabolism. Although the data are not shown, SGPT, SGOT, alkaline phosphatase, and sorbitol dehydrogenase were increased in males exposed at 25 mg/m^3. Prothrombin times and activated partial thromboblastin times were also increased at the highest exposure level. Other clinical pathology changes noted were considered incidental.

A dose-related decrease in T_3 was observed in male rats at the 2-, 4-, and 8-week sampling periods (Table 5). After initial depression at 2 weeks, T_3 concentration showed a progressive recovery in rats exposed to 50 mg/m^3 2-MBI. 2-MBI also produced a marked dose-related reduction in T_4 levels. At the highest 2-MBI exposure level no serum thyroxine was detected after 2 weeks of exposure, with levels remaining below the limit of detection for the remainder of the exposure. Rats exposed to 12.5 mg/m^3 2-MBI had decreased T_4 at 2, 4, or 8 weeks, with recovery by termination of the study, while exposure at 3.1 mg/m^3 did not significantly reduce T_4 levels. TSH levels were highly variable and revealed no consistent trends in relation to the 2-MBI dosage or length of exposure (data not shown).

Exposure to 2-MBI produced histopathologic alterations in several of the organs ex-

TABLE 4

SUBCHRONIC INHALATION STUDY OF 2-MERCAPTOBENZIMIDAZOLE: SELECTED CLINICAL PATHOLOGY DATA IN F344/N RATS[a]

2-MBI target concn (mg/m³)	RBC (×10⁶/mm³)	HGB (g/dl)	HCT (%)	WBC (×10³/mm³)	BUN (mg/dl)	Cholesterol (mg/dl)	Free fatty acid (mmol/liter)
				Male			
0	9.23 ± 0.30	16.8 ± 0.6	46.4 ± 1.8	9.3 ± 0.8	17.3 ± 2.3	82.3 ± 8.8	0.837 ± 0.135
3.1	9.03 ± 0.39	16.4 ± 0.7	45.5 ± 1.9	7.0 ± 0.5**	18.8 ± 2.5	82.4 ± 6.2	0.936 ± 0.228
6.2	8.81 ± 0.30	15.8 ± 0.4	44.4 ± 1.5	7.2 ± 1.2**	18.1 ± 2.5	92.1 ± 10.6	1.000 ± 0.247
12.5	8.55 ± 0.33	15.7 ± 0.6	44.1 ± 1.8	8.0 ± 1.2*	15.2 ± 1.6	96.3 ± 11.1	0.958 ± 0.211
25.0	7.51 ± 0.55**	14.1 ± 1.1**	40.2 ± 3.2**	5.9 ± 0.9**	25.7 ± 9.1**	223.3 ± 52.2**	0.552 ± 0.095**
50.0[c]	6.36 ± 0.11**	11.6 ± 3.8**	34.3 ± 10.7**	6.3 ± [b]	31.1 ± [b]	188.3 ± [b]	0.667 ± 0.152
				Female			
0	8.56 ± 0.27	16.6 ± 0.6	45.1 ± 1.7	5.9 ± 1.7	16.6 ± 2.1	114.2 ± 10.4	0.557 ± 0.132
3.1	8.20 ± 0.38	16.1 ± 0.6	43.6 ± 2.1	6.8 ± 1.1	16.2 ± 2.5	116.8 ± 6.7	0.621 ± 0.152
6.2	8.40 ± 0.35	16.3 ± 0.6	44.8 ± 1.7	5.5 ± 1.1	16.1 ± 2.0	112.1 ± 10.2	0.609 ± 0.156
12.5	7.96 ± 0.25**	15.7 ± 0.5*	43.4 ± 1.7	6.0 ± 1.1	13.0 ± 1.7	105.6 ± 11.7	0.560 ± 0.151
25.0	6.17 ± 0.40**	11.6 ± 0.8**	32.6 ± 2.3**	6.3 ± 1.1	32.5 ± 5.9**	241.7 ± 31.9**	0.411 ± 0.075*
50.0[c]	—	—	—	—	—	—	—

[a] Mean ± SD, n = 7 to 10 samples/group/sex.
[b] N = 1 to 5 samples (SD not calculated where $N \leq 2$).
[c] No samples available—total group mortality.
* 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.

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 follic. 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

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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.

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

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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.

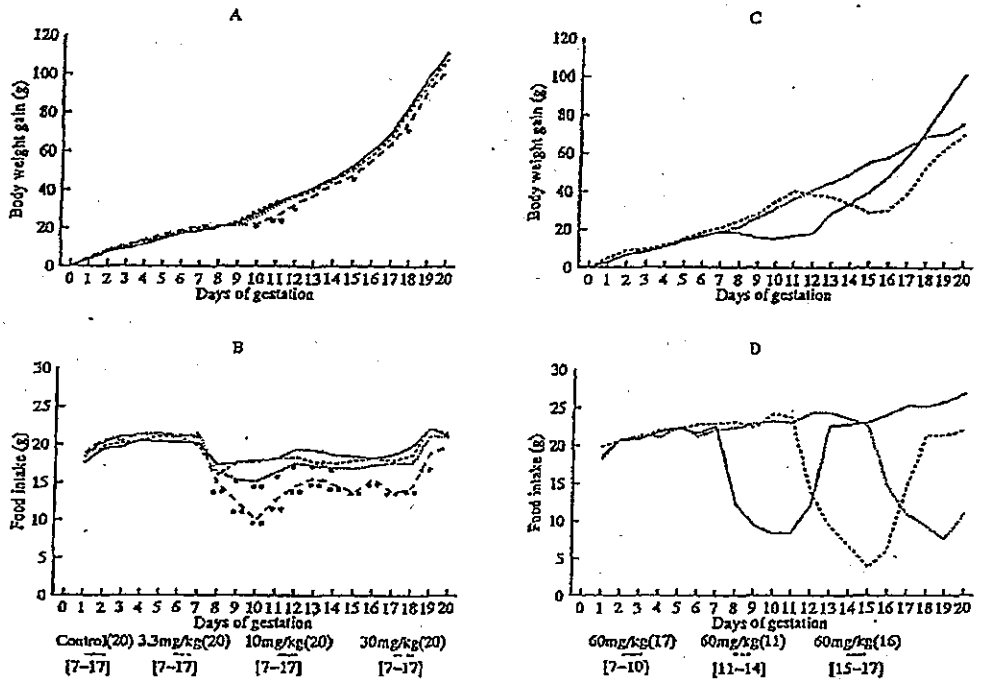

FIG. 1. Body weight gain and food consumption of pregnant rats treated orally with 2-mercaptobenzimidazole. Parentheses represent no. of rats; ackets represent days of treatment. *,**Significantly different from control group at $p < 0.05$ and $p < 0.01$, respectively, by Dunnett's multiple mparison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

rvived and had eight live fetuses, all of which had ana-rca and cleft palate. These fetuses also had dilated lateral :ntricles.

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

Because of the severe resulting toxicity, 60 mg/kg dose uld not be administered throughout the period of organo-esis (Gestation Days 7–17). Instead, the test animals re dosed for shorter periods of time, viz., Gestation Days 10, 11–14, or 15–17. Even under these dosing condi-ns, 2-MBI was severely toxic to dams, as evidenced by a stantial decrease in maternal body weight gain and food sumption in all three groups (Figs. 1C and 1D) and ma-ternal 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

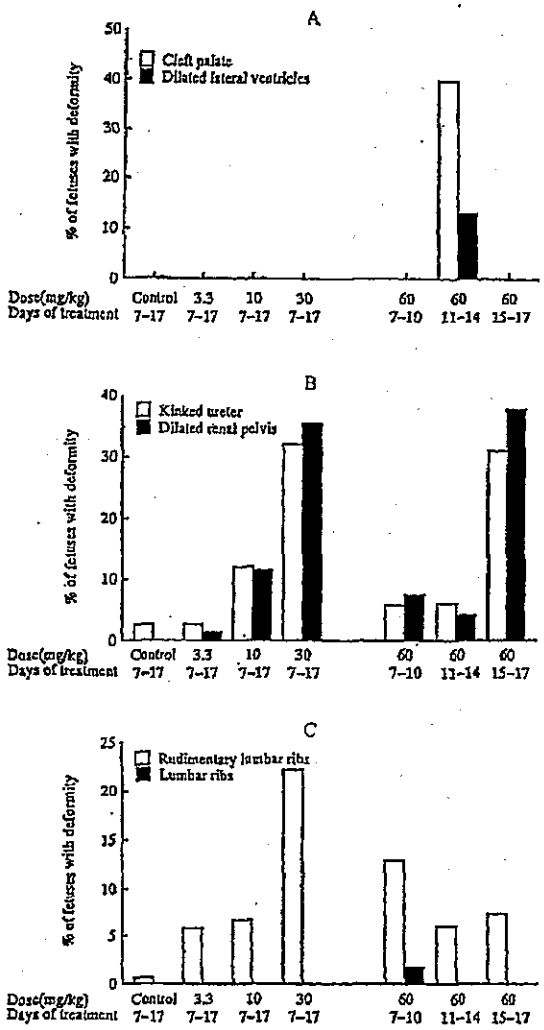

FIG. 2. The incidence of fetuses with major anomalies in relation to dose and treatment day. (A) Open bar, cleft palate; solid bar, dilated lateral ventricles. (B) Open bar, kinked ureter; solid bar, dilated renal pelvis. (C) Open bar, rudimentary lumbar ribs; solid bar, lumbar ribs.

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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