RESULTS

Acute oral toxicity study

First stage treatment (n=3): Treatment with 10 mg/kg of 2-MBI did not induce any acute toxic signs. At 100 mg/kg, decreases in spontaneous movement, ataxic gait, eyelid closure, and lacrimation were observed within 1 hr of treatment. The rats then recovered within 24 hr and all survived. Treatment with 1000 mg/kg of 2-MBI immediately caused paralytic gait, prone position with coma, lacrimation and hypothermia, and 2 male rats and all 3 female rats died within 32 hr. The remaining one male rat died within 72 hr.

Second stage treatment (140-600 mg/kg, n=2): Depending on the dose administered, loss of spontaneous activity, ataxic gait, eyelid closure, lacrimation, paralytic gait, prone position and coma were observed. Rats treated with doses less than 225 mg/kg recovered within 24 hr but all rats treated with 370 and 600 mg/kg died within 2 days.

LD_{50} (Oral): According to the calculation method proposed by Lorke (1983) using the numbers of rats that died during the 14 days, the LD_{50} values for 2-MBI in this experiment were determined to be 300 mg/kg for both male and female rats.

Autopsy findings in the acute toxicity study: Slight congestion in lung, congestion in glandular stomach mucosa, and retention of administered oil in the stomach through to the colon were found in animals which died within 3 days of dosing. Rats that survived for 14 days exhibited no abnormalities.

Twenty eight-day repeated dose oral toxicity study

Clinical signs

No mortality due to 2-MBI treatment occurred for either male or female rats. No clinical signs related to 2-MBI administration were observed except for decrease in body weight gain and food consumption. The rats of both sexes receiving 50 mg/kg showed emaciation and severe suppression in body weight gain along with decreased food consumption one week after treatment started, as shown in Figs 2 & 3. No significant differences in both body weight gain or food consumption were observed in the groups that received 10 mg/kg 2-MBI or less.

Hematology

At the termination of treatment, significant decreases in WBC in the 10 and 50 mg/kg female rats and in PLT in 50 mg/kg male rats were observed. Significant WBC decrease in male rats was still noted as observed 2-weeks after termination of 2-MBI treatment. At the end of the recovery period, RBC, Hb and HCT were significantly decreased in both the male and female 50 mg/kg groups showing delayed onset of anemia (Table 1).

Increased active partial thromboplastin time (APTT, blood clotting time) was observed for both the males and females receiving 50 mg/kg.

Clinical biochemistry

At the termination of the 2-MBI administration, serum levels of TP, BUN, PL, T-CHO, F-CHO, CHE and γ -GTP were significantly increased in both males and females given 50 mg/kg. ALP, K^+ and Pi were decreased significantly in male rats receiving more than 10 mg /kg, and Na^+ was increased significantly in the 50 mg/kg treated animals. CHO and PL levels remained significantly high 2 weeks after termination of 2-MBI administration (Table 2 & 3). Serum levels of T_3, T_4 and TSH measured 2 weeks after repeated dose treatment with 2-MBI or the antithyroid agents, TU and ETU, are shown in Fig.4.

Organ Weight

At the termination of 2-MBI administration, dose related increases in absolute and relative weights of thyroid, liver and kidney were observed. At 10 mg/kg, the mean absolute and relative thyroid weights in both sexes were approximately 3 times those of the respective control rats, and in the 50 mg/kg dose group the increase in relative thyroid weight was more than 10-fold. Even after the 2-week recovery period, relative thyroid weights were approximately 6 times the control values. Dose-related decreases in absolute and relative thymus weights in all treatment groups of male and female rats and a decrease in relative spleen weight in both males and females receiving 50 mg/kg were also observed. Significant increases in brain, lung, adrenal and pituitary gland weights and decreases in heart and sabmaxillary glands of relative organ weights were also observed with 50 mg/kg. Some of these organ weight changes may have been due to the severe reduction in body weight gain as shown in Fig 2 & 3 and Tables 4 & 5.

Histopathology

As gross findings, marked enlargement of thyroid glands and thymus involution were evident in treated rats. Diffuse hyperplasia of tall and columnar epithelial cells of follicles, and decrease in colloid and thickening of fibrous capsule appeared dose-dependently(Photo. 1). In association with the thyroid changes, hypertrophic cells in the anterior pituitary glands were found, the so-called thyroidectomy cells. Calcification of the collecting tubules in kidney and fatty changes in adren-

al cortex were also observed. These histopathological changes mostly disappeared after the 2-week recovery period, as shown in Table 6. Despite the thymus weight being decreased dose-dependently, there were no obvious changes in its architecture.

DISCUSSION

The present subacute toxicity study of 2-MBI by gavage administration for 28 consecutive days followed by 2-week recovery period in Wistar rats demonstrated marked thyroid enlargement associated with characteristic diffuse hyperplasia in both male and female rats receiving a dose of 10 mg/kg 2-MBI or above. This thyroid toxicity was accompanied by significant decreases in circulating thyroid hormone (T_3&T_4) levels and a marked increase in the serum TSH level in the ancillary 14-day gavage administration study, the changes being more pronouced than with TU and ETU (Paynter *et al.*, 1988).The antithyroid effects in the present gavage study are consistent with the results observed in the inhalation study of Gaworski *et al.* (1991).The possibility of tumor promotion potential therefore arises (Onodera *et al.*, 1994). Hypertrophic cells increased in the anterior pituitary glands of male rats treated with more than 10 mg/kg 2-MBI, suggesting stimulated TSH generation due to the decrease in thyroid hormone levels by negative feedback regulation (Haynes, 1990). The pituitary and thyroid lesions induced by 2-MBI treatment were identical to those reported after thyroidectomy (Norford *et al.*, 1993).

It is well documented that thyroid hormones stimulate metabolism of cholesterol to bile acid and that hypercholesterolemia is characteristic of hypothyroid states (Haynes, 1990). In the present study, a 2-3 fold increase in serum cholesterol was observed in the 50 mg/kg treated rats and full return to the control level was not evident after the 2-week recovery period. Serum levels of phospholipid were also increased significantly upon 2-MBI administration, although the free fatty acids which were significantly decreased in the inhalation study (Gaworski *et al.*, 1991) did not appear to be affected by gavage administration.

Thyroid hormones are also known to participate regulation of the balance of intracellular and intercellular Na^+ and K^+ levels by active transport (Asano, 1982). The serum Na^+/K^+ ratios in this study were markedly altered from 26.4(control) to 40.6(50 mg/kg) for males and from 30.7 to 46.1 for females, respectively. This may be due to the severe hypothyroid state induced by 2-MBI administration. It is also well known clinically that serum levels of Ca^{2+}, Pi and ALP are altered by a parathyroid hormone imbalance which affects bone metabolism (Fujita,1987). In the present rat study, serum levels of ALP and Pi were significantly decreased , but Ca^{2+} was not changed by 2-MBI treatment. In contrast, Gaworski *et al.*(1991) observed an increased serum level of ALP, so this alteration may not be directly related to a hypothyroid state.

Anemia has been associated with hypothyroidism (Haynes and Murad, 1985) and was observed at termination in the 13-week inhalation study conducted by Gaworski *et al.* (1991). Clinical symptoms of anemia (decreases in RBC, Hb and HCT) were also detected 2 weeks after the termination of high dose 2-MBI administration in the present study. This delayed change may be partially due to the long retention of 2-MBI by hemoglobin (El Dareer *et al.*, 1984) and the long half-life of RBC.

2-MBI is known to be a metabolite of the immunomodulator, 3-(p-chlorophenyl)-2,3-dihydro-3-hydroxythiazolo[3,2a]benzimidazole-2-acetic acid (Janssen *et al.*, 1981). In the present 28-day gavage study in rats, a clear dose-related involution of the thymus was observed in all treatment groups. Severe decrease of body weight gain affects the thymus weight (Levin *et al.*, 1993) but the thymus change was observed even at doses which had no effects on body weight. Although manifest morphological alterations were not found in either the thymus or the spleen, 2-MBI might thus have potential immunotoxicity. A significant decrease in WBCs occurred in female rats at doses higher than 10 mg/kg and a decrease in the relative spleen weight in 50 mg/kg dose rats was also demonstrated. In the 13-week inhalation study (Gaworski *et al.*, 1991), a similar significant decrease in thymus weight associated with atrophy and decreased WBC counts due to lower numbers of circulating lymphocytes in male rats were also observed. Malmfors (1976) reported thymic involution even after a single dose of 2-MBI in rats but not in mice, guinea pigs or rabbits. Such thymic involution in rats was not observed after thyroidectomy (Malmfors, 1976). In our 14-day ancillary study (data not shown), TU and ETU also induced significant thymus involution without notable decrease in body weight gain. These facts suggest antithyroid function-linked thymus involution in rats. Further immunotoxicological investigation is required to clarify this prominent effect of 2-MBI and other antithyroid agents on the thymus.

A significant increase in relative liver weight was observed in the 10 and 50 mg/kg dose groups of both sexes, which is in agreement with the results of the

inhalation study (Gaworski,1991) in which liver injury associated with hepatic hypertrophy and granulomatous inflammation was found. Increased blood clotting time (APTT) also suggests an adverse effect on liver functions. However, in the present gavage study, no overt hepatocyte injury was apparent, which is consistent with no increases in serum AST and ALT levels. In addition, the relative liver weight increase in 50 mg/kg dose rats returned to the control level within the 2-week recovery period. Therefore, the observed liver changes appeared to be an adaptive response.

Slight, but statistically significant increases of BUN level in males, and of CRN level in females associated with a slight increase in relative kidney weight were observed in the higher 2-MBI dose groups. In consideration of the previous report demonstrating renal toxicity (Gaworski,1991), the present finding of calcification in the collecting tubules in one animal of each sex may be significant.

The non-observed-effect level (NOEL) of 2-MBI in rats in the present gavage study was evaluated to be less than 2 mg/kg based on the significant decreases in absolute and relative thymus weights of the lowest treatment dose group. Obviously, prudence must be taken in extrapolating animal data to the human situation. In safety evaluation of chemicals, an uncertainty factor of "100x" is usually adopted. If the available NOEL is based on a subacute study like that conducted here, an additional uncertainty factor of "5x " should be considered (to give 500x). Based on this safety evaluation, 4, μg/kg/day might be acceptable for the human exposure level of 2-MBI. However, the contamination level of 2-MBI in drugs must be reduced as far as possible or should be replaced by a less toxic rubber antioxidant. This might be particularly important in the case of drugs administered over long periods such as insulin. Furthermore, 2-MBI is a fatty soluble compound, and thus its accumulation in the body may occur with repeated administration. Toxicokinetic studies of 2-MBI and related compounds such as methylated derivatives are now under way.

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

CHARLES L. GAWORSKI,*,[2] CATHERINE ARANYI,*,[3] STANLEY VANA,* NARAYANAN RAJENDRAN,* KAMAL ABDO,† BARRY S. LEVINE,‡ AND ALAN HALL III§

*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 acrodynamic 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 $\geq$12.5 mg/m^3 and in females at $\geq$50 mg/m^3 and pituitary atrophy in both sexes at $\geq$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/m^3)	RBC ($\times 10^6$/mm^3)	HGB (g/dl)	HCT (%)	WBC ($\times 10^3$/mm^3)	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[b]	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.