NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicity Studies of 2- and 4-Methylimidazole (CAS Nos. 693-98-1 and 822-36-6) Administered in Feed to F344/N Rats and B6C3F$_1$ Mice

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U.S. Department of Health and Human Services
Public Health Service
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FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Toxicity Study Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Perspectives (EHP) http://ehp.niehs.nih.gov (866-541-3841 or 919-653-2590). In addition, printed copies of these reports are available from EHP as supplies last. A listing of all the NTP Toxicity Study Reports printed since 1991 appears on the inside back cover.

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to F344/N Rats and B6C3F$_1$ Mice

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Public Health Service
National Institutes of Health

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PEER REVIEW

The draft report on the toxicity studies of 2- and 4-methylimidazole was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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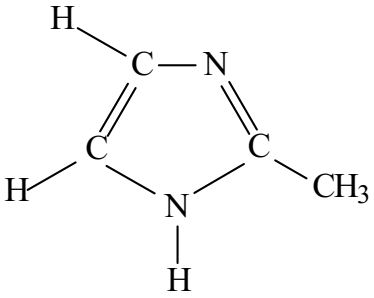
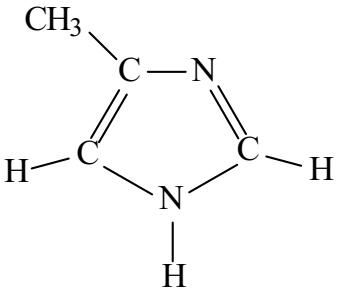
ABSTRACT

2-Methylimidazole



4-Methylimidazole



	2-Methylimidazole	4-Methylimidazole
CAS Number:	693-98-1	822-36-6
Molecular Weight:	82.11	82.11
Synonyms:	Imidazole, 2-methyl; 2-MeI; 2-methylglyoxaline; 2-MI; 2-MZ 2-methyl-1H-imidazole	Imidazole, 4-methyl; 4-MeI; 4(5)-methylglyoxaline; 5-methylimidazole; 4(5),4(5)-methylimidazole 4-methyl-1H-imidazole

2-Methylimidazole and 4-methylimidazole are intermediate/starting materials or components in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes and pigments, agricultural chemicals, and rubber; these chemicals have been identified as undesirable by-products in several foods and have been detected in mainstream and sidestream tobacco smoke. The National Cancer Institute nominated 2- and 4-methylimidazole as candidates for toxicity and carcinogenicity studies. Toxicity studies were carried out in male and female F344/N rats and B6C3F_1 mice. Animals were exposed to 2- or 4-methylimidazole in feed for 15 days or 14 weeks; clinical pathology studies were conducted in the 14-week studies on days 8, 29, and 86 and at week 14. Genetic toxicity studies were conducted in *Salmonella typhimurium*, rat and mouse bone marrow, and mouse peripheral blood.

Groups of five male and five female rats and mice were fed diets containing 0, 1,200, 3,300, or 10,000 ppm 2-methylimidazole (equivalent to average daily doses of approximately 115, 290, or 770 mg 2-methylimidazole/ kg body weight to rats; 220, 640, or 2,100 mg/kg to male mice; 300, 800, or 2,400 to female mice) for 15 days. Groups of five male and five female rats and mice were fed diets containing 0, 300, 800, or 2,500 ppm 4-methylimidazole (equivalent to average daily doses of approximately 30, 80, or 220 mg/kg for rats and 65, 170, or 500 mg/kg for mice) for 15 days. In the 15-day 2-methylimidazole studies, all animals

survived to the end of the studies. The mean body weights of 10,000 ppm male rats and female mice were significantly less than those of the controls. Feed consumption by 10,000 ppm male and female rats was reduced. Enlarged thyroid glands were observed in 3,300 and 10,000 ppm male and female rats. The incidences of diffuse hyperplasia of follicular cells of the thyroid gland in 3,300 and 10,000 ppm male and female rats and pars distalis hypertrophy of the pituitary gland in 3,300 and 10,000 ppm males and 10,000 ppm females were increased compared to the controls. In all exposed groups of male and female mice, the incidences and severities of follicular cell hypertrophy of the thyroid gland and the severities of hematopoietic cell proliferation of the spleen generally increased with increasing exposure concentration. In the 4-methylimidazole studies, all animals survived to the end of the studies, and there were no significant differences in mean body weights, clinical findings, organ weights, or gross or microscopic lesions between exposed and control groups.

Groups of 10 male and 10 female rats and mice were fed diets containing 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm 2- or 4-methylimidazole (equivalent to average daily doses of approximately 40, 80, 160, 300, or 560 mg/kg 2- or 4-methylimidazole to rats; and 100, 165, 360, 780, or 1,740 mg/kg 2-methylimidazole or 100, 240, 440, 915, or 1,840 mg/kg 4-methylimidazole to male mice; and 90, 190, 400, 800, or 1,860 mg/kg 2-methylimidazole or 110, 240, 540, 1,130, or 3,180 mg/kg 4-methylimidazole to females) for 14 weeks. All animals survived to the end of the 14-week 2-methylimidazole studies. Compared to the controls, the mean body weights were significantly decreased in groups of male rats and mice exposed to 2,500 ppm or greater and in 5,000 and 10,000 ppm female rats and mice. In rats, 2-methylimidazole induced a transient erythrocytosis in females and a minimal, exposure concentration-related, microcytic, normochromic, nonresponsive anemia. 2-Methylimidazole increased thyroid-stimulating hormone concentrations and decreased thyroxine and triiodothyronine concentrations of male and female rats in an exposure concentration-related manner. 2-Methylimidazole induced a mild to moderate, exposure concentration-related, macrocytic, hyperchromic, responsive anemia in mice. Triiodothyronine concentrations were increased in exposed male and female mice, and thyroxine concentrations were decreased in exposed females. Relative to the control groups, clinical chemistry evaluations on day 29 and at week 14 identified decreases in alanine aminotransferase concentrations and total protein and albumin concentrations of rats.

In the 2-methylimidazole studies, absolute spleen weights were significantly increased in all exposed groups of male rats. The heart and liver weights were increased in all exposed groups of male mice, as were the spleen weights of female mice exposed to 2,500 ppm or greater. Spermatid heads per testis and mean spermatid count were significantly decreased in 10,000 ppm male rats. The estrous cycle of 10,000 ppm female rats was significantly increased. Gross pathology observations included enlarged thyroid glands, small uteri, and mottled

adrenal glands (females only) in 10,000 ppm rats and enlarged and/or darkened thyroid gland and enlarged spleen in 5,000 and 10,000 ppm mice. The incidences of diffuse follicular cell hyperplasia of the thyroid gland were significantly increased in male rats exposed to 1,250 ppm or greater and female rats exposed to 2,500 ppm or greater. The incidence of testicular degeneration was significantly increased in 10,000 ppm male rats, and two males in the 10,000 ppm group had follicular cell adenoma of the thyroid gland. In mice, there were generally significant increases in the incidences of follicular cell hypertrophy of the thyroid gland, hematopoietic cell proliferation of the spleen, and hemosiderin pigmentation of the renal tubule in males exposed to 1,250 ppm or greater and females exposed to 2,500 ppm or greater.

In the 14-week 4-methylimidazole studies, one 10,000 ppm male mouse was found dead during week 4, and seven 10,000 ppm female mice were found dead during weeks 1 and 2. Mean body weights were significantly less than those of the controls for male rats exposed to 2,500 ppm or greater, 5,000 and 10,000 ppm female rats, male mice exposed to 1,250 ppm or greater, and all exposed groups of female mice. Reduced feed consumption was observed in 5,000 and 10,000 ppm male and female rats. Clinical findings included nasal/eye discharge, ruffled fur, thinness, ataxia, and abnormal breathing in rats, and ruffled fur and dull coats in female mice. On days 29 and 82, functional observations in 5,000 and 10,000 ppm rats included labored or increased respiration, mild tremors, walking on tiptoes, hunched posture, piloerection, crouching over, impaired coordination of movement, ataxia, and pupillary constriction. 4-Methylimidazole induced a transient erythrocytosis and a minimal, exposure concentration-related, microcytic, normochromic, nonresponsive anemia in male and female rats. Clinical chemistry evaluations generally showed a cholestatic effect in exposed male and female rats. At week 14, there was a significant decrease in total protein and albumin concentrations of female rats exposed to 5,000 or 10,000 ppm. In mice, 4-methylimidazole induced a macrocytic, hyperchromic, responsive anemia and, particularly in males, increases in triiodothyronine concentrations and transient decreases in thyroxine concentrations.

In the 4-methylimidazole studies, the liver weights of male rats exposed to 2,500 ppm or greater were significantly increased; spleen weights of female rats exposed to 2,500 ppm or greater were decreased. The absolute liver weight was decreased in 10,000 ppm male mice, and relative weights were significantly increased in all exposed groups of mice. In female mice, there was a significant decrease in the absolute weights and increase in the relative weights of the heart, right kidney, and liver in groups exposed to 2,500 ppm or greater. The epididymal spermatozoal concentration was significantly increased in 5,000 ppm male rats. Gross pathology observations included pale livers in male rats exposed to 2,500 ppm or greater and small testes and uteri in 10,000 ppm male and female rats. Microscopic analysis identified significantly increased incidences of cytoplasmic hepatocyte vacuolization of the liver of male rats exposed to 2,500 ppm or greater and

10,000 ppm female rats, hypospermia of the epididymis in 10,000 ppm male rats, atrophy and inflammation of the prostate gland in 10,000 ppm male rats, and degeneration of the testes in 5,000 and 10,000 ppm male rats.

2-Methylimidazole and 4-methylimidazole were negative in the *S. typhimurium* mutation assay when tested in strains TA97, TA98, TA100, and TA1535, with and without S9 activation enzymes. Testing of 2-methylimidazole *in vivo* for induction of chromosomal damage, as measured by micronucleated erythrocyte frequency, produced mixed results. When administered by intraperitoneal injection three times at 24-hour intervals, 2-methylimidazole produced negative results in bone marrow micronucleus tests in rats and mice. However, in the 14-week study of 2-methylimidazole, a significant exposure-related increase in the frequency of micronucleated normochromatic erythrocytes was noted in peripheral blood of male and female mice. *In vivo*, 4-methylimidazole produced uniformly negative results in three-injection bone marrow micronucleus tests in rats and mice and in 14-week peripheral blood micronucleus tests in male and female mice.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

2-Methylimidazole is a white crystal with a boiling point of 267° C and a melting point of 140° to 142° C. It is soluble in water and ethanol and sparingly soluble in cold benzene. 4-Methylimidazole is a light yellow crystal with a boiling point of 263° C and a melting point range of 46° to 48° C. It is soluble in water and alcohol (RTECS, 1994; *Chemical Economics Handbook*, 1995).

PRODUCTION, USE, AND HUMAN EXPOSURE

2-Methylimidazole is produced by cyclocondensation of aldehyde and ammonia with methylglyoxal or by platinum/alumina cyclization of ethylenediamine with acetic acid. Preparation of 4-methylimidazole involves cyclocondensation of an aldehyde and ammonia with methylglyoxal. Variations include the use of ammonium carbonate or ammonium oxalate as the ammonia source and cyclocondensation of ammonia and formamide with hydroxyacetone. Another method used to synthesize the compound is the catalytic dehydrogenation of imidazoline derivatives. 4-Methylimidazole may be formed by synthesis from propanol and formamide, by catalytic cyclization of bisformamidipropane, or by photolysis of alkenyltetrazole derived from alkenes by sequential epoxidation, ring opening, and dehydration. Production figures for 2- and 4-methylimidazole are not available (RTECS, 1994; *Chemical Economics Handbook*, 1995).

2-Methylimidazole and 4-methylimidazole are used as chemical intermediates, starting materials, or components in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes and pigments, agricultural chemicals, and rubber. 2-Methylimidazole is widely used as a polymerization crosslinking accelerator and hardener for epoxy resin systems for semiconductor potting compounds and soldering masks. It is a component of numerous polymers, including epoxy resin pastes, acrylic rubber-fluororubber laminates, films, adhesives, textile finishes, and epoxy silane coatings. It is also used as a dyeing auxiliary for acrylic fibers and plastic foams (RTECS, 1994; *Chemical Economics Handbook*, 1995).

4-Methylimidazole has been investigated for use as a starting material in the synthesis of cardiovascular stimulants, epoxy resin anticholesteremics, neurotransmitter antagonists, disinfectants, antiprotozoal antiseptic agents, and aromatase inhibitors investigated as possible antineoplastic agents. 4-Methylimidazole is also used as a component in imidazole-phenoxyalkanal oven cleaners, a crosslinking agent for epoxy resin hardeners, a

corrosion inhibitor for cooling water in heat exchangers, a component of an absorbent that removes acid gases from hydrocarbon or synthesis gas, and a starting material for inks and paper dyes (RTECS, 1994; *Chemical Economics Handbook*, 1995).

2-Methylimidazole and 4-methylimidazole have been identified as toxic by-products, formed by interaction of ammonia with reducing sugars, in ammoniated hay forage for livestock (Ray *et al.*, 1984). Ammoniation of carbohydrate-containing material, including hay, to increase the nonprotein nitrogen content is a common practice on farms.

2-Methylimidazole and 4-methylimidazole have been identified as undesirable by-products in several food products, including caramel coloring, soy sauce, Worcestershire sauce, wine, ammoniated molasses, and caramel-colored syrups (Wiggins, 1956; Yoshikawa and Fujiwara, 1981; Huang *et al.*, 1983; Matyasovszky and Jeszenszky, 1985; Wong and Bernhard, 1988). However, only caramel colors (caramel colors III and IV) manufactured with ammonia or its salts contain measurable levels of 4-methylimidazole (Chappel and Howell, 1992). 2-Methylimidazole and 4-methylimidazole have also been detected in mainstream and sidestream cigarette smoke (Moree-Testa *et al.*, 1984; Sakuma *et al.*, 1984).

Caramel color is Generally Recognized as Safe by the United States Food and Drug Administration. A joint committee of the United Nation's Food and Agriculture Organization and the World Health Organization recommended that the daily intake of caramel color III should be restricted to no more than 100 mg/kg of body weight, that the daily intake of caramel color IV made by the ammonia process should be restricted to no more than 200 mg/kg, and that ammoniated caramel colors should not contain more than 200 mg 4-methylimidazole/kg based on color intensity. The Scientific Committee for Food of the European Common Market set a maximum daily intake of 200 mg/kg for caramel colors III and IV (Chappel and Howell, 1992). A Danish law was enacted in August of 1976 to restrict the use of caramel coloring in food and beverages, citing a cancer risk.

The National Occupational Exposure Survey conducted from 1981 to 1983 estimated that 7,023 workers at 318 facilities were potentially exposed to 2-methylimidazole (NIOSH, 1990). No standard or guideline has been set for occupational exposure or environmental concentrations of 2- or 4-methylimidazole in the United States.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

2-Methylimidazole is metabolized by monooxygenase. Following an intravenous administration of ^{14}C-2-methylimidazole at 3 μg/kg to male Wistar rats, radioactivity associated with 2-methylimidazole and its metabolites was excreted in the urine. About 78% of the injected dose was excreted in 24 hours of which 60% was unchanged 2-methylimidazole, and 4.1% was identified as the metabolite 2-methyl-4(5H)- or 5(4H)-imidazolone. Pretreatment with SKF-525A or cimetidine had little effect on the urinary metabolite level of rats (Ohta *et al.*, 1998).

In rats, the uptake at 5 minutes after a single intraperitoneal dose (unspecified) of 4-methylimidazole was greatest in the intestines, followed by the blood, liver, stomach, and kidney. The compound was excreted unchanged in urine beginning about 30 minutes after injection; the amount of unchanged compound excreted reached about 90% in 8 hours (Hidaka, 1976).

In ewes, half of an oral dose (20 mg/kg) of 4-methylimidazole was absorbed in about 27 minutes; the bioavailability calculated from plasma data was 0.69, and the biological half-life was 9.37 hours. Only 0.07 mg/kg of the dose was recovered in urine as unchanged 4-methylimidazole. Metabolites of 4-methylimidazole were not detected by high-performance liquid chromatography (Karangwa *et al.*, 1990).

In goats and heifers, the mean residence time of 20 mg/kg 4-methylimidazole administered orally or intravenously was about 5 hours, and the volume of distribution was 0.9 L/kg body weight. 4-Methylimidazole was distributed mainly to the liver, kidney, and lung (Nielsen *et al.*, 1993). 4-Methylimidazole and its metabolites were excreted mainly in urine, but also in milk and feces. Metabolites identified included 5-methyl hydantoin and 2-methylhydantoic acid, an unidentified metabolite, and urea. In pregnant and postpartum cows and mice, 4-methylimidazole was excreted in milk following oral administration (Morgan and Edwards, 1986).

Following gavage administration of [^{14}C]-4-methylimidazole at 50 mg/kg to male F344 rats, about 85% of the administered radioactivity was recovered in urine within 48 hours. The majority of the radioactivity in urine or plasma was associated with the parent compound, and only one minor hydrophilic metabolite was present in urine and in plasma. Elimination of radioactivity via fecal, biliary, or respiration was negligible (Yuan and Burka, 1995).

TOXICITY

Experimental Animals

The LD_{50} values of 2-methylimidazole for mice were 1,400 mg/kg orally and 480 mg/kg intraperitoneally (Nishie *et al.*, 1969). The LD_{50} values of 4-methylimidazole were 370 mg/kg orally and 165 mg/kg intraperitoneally for mice, 120 mg/kg intraperitoneally for rabbits, and 590 mg/kg orally and 210 mg/kg intraperitoneally for chickens. LD_{50} values of 2- and 4-methylimidazole for rats were not found.

2-Methylimidazole and 4-methylimidazole have been associated with acute toxicity to foraging animals fed commercially ammoniated grasses or grains. Animals exposed to 2- or 4-methylimidazole exhibited convulsant activity including restlessness, bellowing, frothing at the mouth, and paralysis (Wiggins, 1956). Ewes fed ammoniated hay showed facial twitching and general body tremors initially, followed by opisthotonus and convulsion. Death may ensue (Weiss *et al.*, 1986). Neurological signs and convulsant activity have been observed in cattle fed ammoniated molasses (Nishie *et al.*, 1969; Morgan and Edwards, 1986). Calves nursing from cows fed ammoniated hay ran in circles and into walls and were easily excited by noise and touch (Weiss *et al.*, 1986; Perdok and Leng, 1987); 4-methylimidazole was implicated, but not identified, for the toxicosis (Weiss *et al.*, 1986). In goats and heifers, intravenous administration of 20 mg/kg 4-methylimidazole induced coughing, salivation, urination, or defecation within 30 minutes; 40 to 60 mg/kg doses induced convulsions or clonic seizure (Nielsen *et al.*, 1993). Oral administration of 4-methylimidazole at doses of 200 mg/kg or greater resulted in death of calves (Fairbrother *et al.*, 1987).

In mice, 2- and 4-methylimidazole induced similar acute toxic neurologic effects. The convulsant doses (CD_{50}) were 1,300 mg/kg orally and 500 mg/kg intraperitoneally for 2-methylimidazole and 360 mg/kg orally and 155 mg/kg intraperitoneally for 4-methylimidazole. At subconvulsant doses (50 to 100 mg/kg intraperitoneally), 4-methylimidazole decreased spontaneous motor activity measured with a Woodard animal activity cage with six photocells and a circular raceway. Convulsions were also induced by 4-methylimidazole in rabbits and day-old chicks (Nishie *et al.*, 1969). The results from studies in mice, rabbits, and chicks suggest that 4-methylimidazole is responsible for the findings of toxicity in cattle fed ammoniated feeds.

Liver hypertrophy in rats following intraperitoneal injection of 4-methylimidazole has been reported (Hidaka, 1976). Intraperitoneal injections of 2- or 4-methylimidazole induced aggressive behavior in male Wistar rats treated with lisuride; 4-methylimidazole was more potent than 2-methylimidazole (Ferrari *et al.*, 1987). Hargreaves *et al.* (1994) reported that 2- and 4-methylimidazole inhibited rat liver $P_{450}2E1$ activities; 4-methylimidazole was a stronger inhibitor than 2-methylimidazole. Dierickx (1989) determined 2-methylimidazole cytotoxicity by measuring the PI_{50} (the concentration required to induce a 50% reduction in

cell protein content) in cultured Hep G2 cells and reported that 2-methylimidazole (PI_{50}: 18 mM) was 2.5 times more cytotoxic than the parent compound, imidazole (PI_{50}: 45 mM).

Humans

No data on human toxicity of 2- or 4-methylimidazole were found in the literature.

CARCINOGENICITY

Information on the carcinogenicity of 2- and 4-methylimidazole in experimental animals or humans was not found in the literature.

GENETIC TOXICITY

Yamaguchi and Nakagawa (1983) reported that 2-methylimidazole exhibited no suppressing effects on the mutagenicity of 3-amino-1-methyl-5H-pyrido[2,3-b]indol, 2-acetylaminofluorene, or benzo[a]pyrene in *Salmonella typhimurium* strains TA98 and TA100; 1-methylimidazole showed a marked suppressing effect. No mutagenicity data for 4-methylimidazole were found in the literature.

OTHER BIOLOGICAL EFFECTS

4-Methylimidazole selectively inhibits thromboxane synthetase but does not inhibit platelet-fibrin clot retraction *in vitro* (DiMinno *et al.*, 1982). Neither 2- nor 4-methylimidazole significantly affected human platelet aggregation *in vitro*, whereas imidazole and 1-methylimidazole did (Horton *et al.*, 1983).

In a study of antioxidant activity in a 2,2′-azobis 2-amidinopropane dihydrochloride-induced lipid oxidation system, 2- and 4-methylimidazole reduced the rate of phosphatidylcholine oxidation by 28% and 50%, respectively; imidazole produced a 39% reduction, and 1-methylimidazole had little antioxidant activity (Kohen *et al.*, 1988).

4-Methylimidazole is a strong inhibitor of cytochrome P450-mediated drug oxidation. The effect was demonstrated by hepatic metabolism of tolbutamide (measuring plasma hydroxytolbutamide concentration by high-performance liquid chromatography) *in vivo* in adult male Wistar rats. In contrast, 2-methylimidazole does not inhibit microsomal oxidation (Back and Tjia, 1985). 4-Methylimidazole also acted as a noncompetitive

inhibitor of tolbutamide hydroxylase activity in human liver microsomes, but 2-methylimidazole did not (Back *et al.*, 1988). 4-Methylimidazole stimulated the phosphorylation of rabbit kidney (Na^+ and K^+)-ATPase, while 2-methylimidazole did not (Schuurmans *et al.*, 1988).

STUDY RATIONALE

2-Methylimidazole and 4-methylimidazole were nominated for toxicity testing by the National Cancer Institute. The nominations were based on the widespread use, potential for widespread human exposure in food products, and lack of chronic toxicity data. Dosed feed was chosen as the route of exposure because this is the most common route by which humans are exposed.

2-Methylimidazole was among a broad selection of chemical substances evaluated in a class study of chemicals used in the electronics industry. In the preparation of a more detailed report of information on this compound, attention turned to its positional isomer, 4-methylimidazole, a toxicologically more active chemical that is also widely used, particularly as a pharmaceutical intermediate.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 2- AND 4-METHYLIMIDAZOLE

2-Methylimidazole (lot 323734/1 193) was supplied by Fluka Chemie AG (Buchs, Switzerland) and 4-methylimidazole (lot 08302BF*) was supplied by Aldrich Chemical Company (Milwaukee, WI). Identity and purity analyses were performed by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO); chemical identities were confirmed by the study laboratory. Reports on analyses performed in support of the 2- and 4-methylimidazole studies are on file at the National Institute of Environmental Health Sciences.

2-Methylimidazole, a white crystalline solid, and 4-methylimidazole, a light yellow powder, were identified by proton nuclear magnetic resonance and infrared spectroscopy. Purity of lots 323734/1 193 and 08302BF* was determined by Karl Fischer water analysis and high-performance liquid chromatography (HPLC). For lot 323734/1 193, Karl Fischer water analysis indicated 0.03% ± 0.02% water. HPLC indicated a purity of 100.3% ± 0.2% and no impurity peak with an area greater than or equal to 0.1% relative to the major peak. For lot 08302BF*, Karl Fischer water analysis indicated 0.13% ± 0.03% water. HPLC indicated a purity of 99.0% ± 0.1% and one impurity peak with area equal to 0.1% relative to the major peak.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

For the 15-day studies, a single set of dose formulations for each chemical was prepared 6 days before the studies began. For the 14-week studies, dose formulations were prepared at the beginning of the studies, weekly for the first 4 weeks of the studies, and every 2 weeks thereafter.

Premixes were prepared by mixing 2- or 4-methylimidazole with feed (Table G1). Final dose formulations were obtained by blending additional feed with the premixes in a twin-shell blender for 15 minutes, using an intensifier bar for the first 5 minutes. Dose formulations were stored in double plastic bags at a temperature of approximately 4° C and used within 4 weeks of preparation.

Homogeneity and stability studies of 2-methylimidazole (625, 666, 6,000, and 10,000 ppm) and 4-methylimidazole (167, 300, 625, 1,500, 2,500, and 10,000 ppm) formulations were performed by the study

*__ERRATUM:__ There is an error in the lot number: “lot 08302BF” should be “lot 08202PF” (November 5, 2019)

laboratory using high-performance liquid chromatography. Homogeneity was verified. Stability was confirmed for up to 28 days.

The study laboratory analyzed the dose formulations for the 15-day studies (Tables G2 and G3) and the initial, midpoint, and final dose formulations for the 14-week studies (Tables G4 and G5) prior to their use with high-performance liquid chromatography. Animal room samples of the same dose formulations were also analyzed. All dose formulations and animal room samples in the 15-day 2-methylimidazole studies were within 10% of the target concentrations. For the 15-day 4-methylimidazole studies, all dose formulations were within 10% of the target concentrations; one of three animal room samples for rats and two of three for mice were more than 10% below the target concentrations. In the 14-week 2-methylimidazole studies, 14 of 15 dose formulations were within 10% of the target concentrations. All animal room samples for rats and 13 of 15 for mice were within 10% of the target concentrations. In the 14-week 4-methylimidazole studies, all dose formulations were within 10% of the target concentrations; 14 of 15 animal room samples for rats and 12 of 15 for mice were also within 10% of the target concentrations.

15-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the 5-week-old animals were quarantined for 11 to 12 days for the 2-methylimidazole studies and for the 4-methylimidazole studies. Before the studies began, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease.

Groups of five male and five female rats and mice were fed diets containing 0, 1,200, 3,300, or 10,000 ppm 2-methylimidazole or 0, 300, 800, or 2,500 ppm 4-methylimidazole for 15 days. Exposure concentrations were selected based on published LD_{50} values. Rats and female mice were housed five per cage and male mice were housed individually. Feed and water were available *ad libitum*. Clinical findings were recorded and animals were weighed initially, on day 8, and at the end of the studies. Feed consumption was measured weekly. Details of the study designs and animal maintenance are summarized in Table 1.

A necropsy was performed on all animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed and examined for gross lesions. Histopathologic examinations of selected tissues were performed on animals that died early, control and 10,000 ppm rats and mice in the 2-methylimidazole studies, and control and 2,500 ppm rats and mice in the 4-methylimidazole studies. Tissues were examined in the lower exposure

groups at each study until a no-observed-adverse-effect level was determined. Organs and tissues examined microscopically are listed in Table 1.

14-WEEK STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the 5-week-old animals were quarantined for 14 to 17 days for the 2-methylimidazole studies or for 12 to 15 days for the 4-methylimidazole studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Blood samples were collected from five male and five female control rats and five male and five female sentinel mice at the end of the 14-week studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results for the 2-methylimidazole studies were negative. One female rat from the 4-methylimidazole study had positive titers for parvovirus and Toolan's H-1 virus. Further testing with an immunofluorescence assay indicated that one male and three additional females had positive titers for parvovirus. Parvoviruses, including Toolan's H-1 virus, are pathogenic for fetal and suckling rats (Jacoby *et al.*, 1996). However, because weaned rats were used in the current study and because this study did not involve reproduction, possible infection with parvovirus was not considered significant for the interpretation of the study.

In the core studies, groups of 10 male and 10 female rats and mice were fed diets containing to 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm 2- or 4-methylimidazole. Rats and female mice were housed five per cage and male mice were housed individually. Feed and water were available *ad libitum*. Clinical findings were recorded and animals were weighed initially, weekly, and at the end of the studies. Functional observation batteries were performed at weeks 5 and 12 on rats exposed to 0, 2,500, 5,000, or 10,000 ppm 2- or 4-methylimidazole. Feed consumption was measured weekly. Details of the study design and animal maintenance are summarized in Table 1.

Additional groups of 10 male and 10 female rats and mice per exposure concentration were included in the 14-week 2- and 4-methylimidazole studies and were designated for interim hematology (rats only) and clinical chemistry evaluations and immunohistochemistry analyses. Animals were anesthetized with a CO_2:O_2 mixture, and blood was collected from the retroorbital sinus of 10 male and 10 female clinical pathology study rats on days 8 and 29 and from core study rats at the end of the study for hematology and clinical chemistry analysis. Blood was collected from the retroorbital sinus of 10 male and 10 female clinical pathology study mice on days 8, 29, and 86 for clinical chemistry and from core study mice at the end of the study for hematology.

Blood for hematology analysis was collected into tubes containing potassium EDTA as the anticoagulant. Blood for clinical chemistry analysis was collected in untreated tubes and allowed to clot, and the serum was separated by centrifugation.

Hematology determinations were provided by Professional Corporations Laboratory (Chevy Chase, MD) using a Serono-Baker System 9000 hematology analyzer (Serono-Baker Diagnostics, Allentown, PA). Hematocrit values were determined manually with an Adams CT2900 microhematocrit centrifuge (Clay Adams, Sparks, MD). Differential leukocyte counts and erythrocyte, leukocyte, and platelet morphologic evaluations were determined by light microscopy from blood smears stained on a Ames Hema-Tek Slide Stainer (Miles Laboratory, Ames Division, Elkhart, IN) using a modified Wright's stain. Smears made from preparations of equal volumes of new methylene blue (Sigma Chemical Company, St. Louis, MO) and whole blood were examined microscopically for the quantitative determination of reticulocytes.

All clinical chemistry variables except triiodothyronine and thyroid-stimulating hormone were measured with a Hitachi 717® chemistry analyzer (Boehringer Mannheim, Indianapolis, IN) by Professional Corporations Laboratory; reagents were obtained from the manufacturer with the exception of the reagents for sorbitol dehydrogenase and total bile acids, which were obtained from Sigma Chemical Company. Triiodothyronine concentrations were measured with an ES-300 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). Thyroid-stimulating hormone concentrations were measured by Anilytics Inc. (Gaithersburg, MD). The hematology and clinical chemistry parameters evaluated are listed in Table 1.

At the end of the 14-week studies, samples were collected from core study animals for sperm motility and vaginal cytology evaluations on up to eight male and 10 female rats and 10 male and 10 female mice exposed to 0, 2,500, 5,000, or 10,000 ppm 2-methylimidazole, eight male and 10 female rats and 10 female mice exposed to 0, 1,250, 2,500, or 5,000 ppm 4-methylimidazole, and 10 male mice exposed to 0, 2,500, 5,000, or 10,000 ppm 4-methylimidazole. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and

nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethylsulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on up to 10 male and 10 female rats and mice per group in the 14-week core studies. The heart, right kidney, liver, lung, right testis, spleen (except male mice exposed to 2-methylimidazole), and thymus were weighed and examined for gross lesions. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on control animals, 5,000 ppm female mice exposed to 4-methylimidazole, 10,000 ppm animals, and animals that died before the end of the studies. Selected organs were examined in the lower exposure groups until a no-observed-adverse-effect level was determined. Organs and tissues examined microscopically are listed in Table 1.

Upon completion of the laboratory pathologist's histopathologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results for 2-methylimidazole were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). The results for 4-methylimidazole were similarly reviewed and evaluated by the NTP.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 2- and 4-Methylimidazole

15-Day Studies	14-Week Studies
Study Laboratory	
Microbiological Associates, Inc. (Bethesda, MD)	Microbiological Associates, Inc. (Bethesda, MD)
Strain and Species	
Rats: F344/N	Rats: F344/N
Mice: $B6C3F_1$	Mice: $B6C3F_1$
Animal Source	
Taconic Farms (Germantown, NY)	Taconic Farms (Germantown, NY)
Time Held Before Studies	
2-Methylimidazole	2-Methylimidazole
Rats: 11 days	Rats: 16 to 17 days
Mice: 12 days	Mice: 14 to 15 days
4-Methylimidazole	4-Methylimidazole
Rats: 11 days	Rats: 14 to 15 days
Mice: 12 days	Mice: 12 to 13 days
Average Age When Studies Began	
6 weeks	7 weeks
Date of First Exposure	
2-Methylimidazole	2-Methylimidazole
Rats: September 13, 1993	Rats: January 20, 1994 (males) or January 21, 1994 (females)
Mice: September 14, 1993	Mice: January 18, 1994 (males) or January 19, 1994 (females)
4-Methylimidazole	4-Methylimidazole
Rats: September 20, 1993	Rats: February 3, 1994 (males) or February 4, 1994 (females)
Mice: September 21, 1993	Mice: February 1, 1994 (males) or February 2, 1994 (females)
Duration of Exposure	
15 days	14 weeks (7 days/week)
Date of Last Exposure	
2-Methylimidazole	2-Methylimidazole
Rats: September 27, 1993	Rats: April 21, 1994 (males) or April 22, 1994 (females)
Mice: September 28, 1993	Mice: April 19, 1994 (males) or April 20, 1994 (females)
4-Methylimidazole	4-Methylimidazole
Rats: October 4, 1993	Rats: May 5, 1994 (males) or May 6, 1994 (females)
Mice: October 5, 1993	Mice: May 3, 1994 (males) or May 4, 1994 (females)
Necropsy Dates	
2-Methylimidazole	2-Methylimidazole
Rats: September 27, 1993	Rats: April 21 and 22, 1994
Mice: September 28, 1993	Mice: April 19 and 20, 1994
4-Methylimidazole	4-Methylimidazole
Rats: October 4, 1993	Rats: May 5 and 6, 1994
Mice: October 5, 1993	Mice: May 3 and 4, 1994
Average Age at Necropsy	
8 or 9 weeks	20 weeks
Size of Study Groups	
5 males and 5 females	10 males and 10 females

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 2- and 4-Methylimidazole

15-Day Studies	14-Week Studies
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 15-day studies
Animals per Cage Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 5 Mice: 1 (males) or 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo
Diet NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 15-day studies
Water Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system, available *ad libitum*	Same as 15-day studies
Cages Polycarbonate	Same as 15-day studies
Bedding Sani-Chip® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed twice weekly (rats and female mice) or weekly (male mice)	Same as 15-day studies
Cage Filters Reemay spunbound 100% polyester (Snow Filtration Co., Westchester, OH)	Same as 15-day studies
Racks Stainless steel, changed every 2 weeks	Same as 15-day studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Exposure Concentrations 2-Methylimidazole: 0, 1,200, 3,300, or 10,000 ppm in feed 4-Methylimidazole: 0, 300, 800, or 2,500 ppm in feed	0, 625, 1,250, 2,500, 5,000, or 10,000 ppm in feed
Type and Frequency of Observation Observed twice daily; animals were weighed initially, on day 8, and at the end of the studies. Clinical findings and feed consumption were recorded weekly.	Observed twice daily; animals were weighed and clinical findings were recorded initially and weekly until the end of the studies. Feed consumption was recorded weekly.
Method of Sacrifice CO_2 asphyxiation	Same as 15-day studies
Necropsy Complete necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Complete necropsies were preformed on core study rats and mice. Organs weighed were the heart, right kidney, liver, lung, spleen (except 2-methylimidazole male mice), right testis, and thymus.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 2- and 4-Methylimidazole

15-Day Studies	14-Week Studies
Clinical Pathology	
None	Blood was collected from the retroorbital sinus of clinical pathology study rats on days 8 and 29 for hematology and clinical chemistry analyses. Blood was collected from retroorbital sinus of clinical pathology study mice on days 8, 29, and 86 for clinical chemistry analyses. Blood was collected from the retroorbital sinus of core study rats and mice at the end of the studies for hematology analyses and core study rats for clinical chemistry analyses. The liver of clinical pathology study rats on day 29 and a portion of the liver of core study rats at the end of the studies were removed for uridine diphosphate glucuronosyltransferase enzyme assays. ***Hematology:*** automated and manual hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and total leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, total bile acids, thyroid-stimulating hormone, triiodothyronine, thyroxine, and uridine diphosphate glucuronosyltransferase
Histopathology	
Histopathology was performed on control and 10,000 ppm rats and mice exposed to 2-methylimidazole, on control and 2,500 ppm rats and mice exposed to 4-methylimidazole, and all animals that died early. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain, heart, kidney, liver, lung, pituitary gland, ovary, spleen, stomach (forestomach and glandular), testes, thymus, and thyroid gland.	A complete histopathology evaluation was performed on all core study control, 5,000 ppm female mice exposed to 4-methylimidazole, 10,000 ppm rats and mice, and all animals that died before the end of the studies. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (with adjacent skin), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. Additionally, in the 2-methylimidazole studies, the mesenteric lymph nodes, testis, thyroid gland, and uterus of rats, heart and thymus of male rats, adrenal gland of female rats 5,000 ppm), and the kidney, spleen, and thyroid gland of mice in the lower exposure groups were examined. In the 4-methylimidazole studies, the liver, prostate gland, and testes of 1,250, 2,500, and 5,000 ppm male rats, liver and uterus of 2,500 and 5,000 ppm female rats, the liver of all lower exposure groups of mice, and the lung, mandibular lymph node, salivary gland, and urinary bladder of 5,000 ppm female mice were examined.
Functional Observations	
None	Functional observation batteries were carried out on 0, 2,500, 5,000, and 10,000 ppm rats at weeks 5 and 12. The following parameters were observed: body position, convulsions, activity level, coordination, gait, general behavior, head-flick, head-searching, compulsive biting or licking, backward walking, self-mutilation, circling, lacrimation or chromodacryorrhea, piloerection, pupillary dilation or constriction, salivation, diarrhea, tremors, unusual respiration, excessive or diminished urination, and vocalization.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 2- and 4-Methylimidazole

15-Day Studies	14-Week Studies
Immunohistochemistry Evaluations	
None	The brains of male and female special study mice on day 86 were rapidly collected, bisected, frozen on dry ice, stored at -70° C, and shipped to the NTP for glial-fibrillary acidic protein and proliferating cell nuclear antigen analyses. At the end of the studies, one hemisphere of the brain was collected from two male and two female core study rats anesthetized and cardiac perfused with 4% paraformaldeyde and from two male and two female core study mice at routine histopathologic examination.
Sperm Motility and Vaginal Cytology Evaluations	
None	At the end of the core studies, rats and mice administered 0, 2,500, 5,000, or 10,000 ppm 2-methylimidazole and rats and female mice administered 0, 1,250, 2,500, or 5,000 ppm and male mice administered 0, 2,500, 5,000, or 10,000 ppm 4-methylimidazole were selected for evaluation. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid count, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies. Estrous cycle length and the percentage of time spent in the various estrous stages were measured.

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

The incidences of lesions presented in Appendixes A and B are given as the numbers of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. The Fisher exact test (Gart *et al.*, 1979), a procedure based on the overall proportion of affected animals, was used to determine significance.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and vehicle control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified

by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations.

QUALITY ASSURANCE METHODS

The 14-week studies of 2- and 4-methylimidazole were conducted in compliance with Food and Drug Administration Good Laboratory Practices Regulations (21 CFR, Part 58). The Quality Assurance Unit of Microbiological Associates, Inc., performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1988). The chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). Each chemical was incubated with the *S. typhimurium* tester strains TA97, TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of the test chemical. For the 2-methylimidazole studies and the 4-methylimidazole study conducted at SRI International, the high dose was limited to 10,000 μg/plate. For the 4-methylimidazole study conducted at Environmental Health Research and Testing, Inc., the high dose was limited to 33 μg/plate. Trials initially conducted with 10% S9 were repeated with 30% S9.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase

in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

Rat and Mouse Bone Marrow Micronucleus Test Protocol

Preliminary range-finding studies were performed; the high dose was limited by toxicity. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Groups of five male F344/N rats or B6C3F_1 mice were injected intraperitoneally three times at 24-hour intervals with 2- or 4-methylimidazole dissolved in phosphate-buffered saline. Solvent control animals were administered phosphate-buffered saline. The positive control rats and mice received injections of cyclophosphamide. The animals were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained with acridine orange; 2,000 polychromatic erythrocytes (PCEs) were scored per animal for frequency of micronucleated cells.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dose group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dose group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al*. (1990). At the end of the 14-week toxicity studies of 2- and 4-methylimidazole, peripheral blood was obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were later stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per exposure group. The frequency of micronucleated cells among NCEs was analyzed by the same software package and methods used to analyze PCEs in the bone marrow micronucleus tests.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Study Report represent a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

RATS

15-DAY STUDIES

In the 2- and 4-methylimidazole studies, all male and female rats survived to the end of the studies (Tables 2 and 3). In the 2-methylimidazole study, the final mean body weight of 10,000 ppm males and body weight gains of 3,300 and 10,000 ppm males and 10,000 ppm females were significantly less than those of the controls. In the 4-methylimidazole study, the mean body weight gain of 2,500 ppm males was significantly less than that of the controls. Feed consumption by 10,000 ppm males and females at weeks 1 and 2 in the 2-methylimidazole study and by 2,500 ppm males at week 1 in the 4-methylimidazole study was reduced. Dietary concentrations of 1,200, 3,300, or 10,000 ppm delivered average daily doses of approximately 115, 290, or 770 mg 2-methylimidazole/kg body weight to males and females. Dietary concentrations of 300, 800, or 2,500 ppm 4-methylimidazole delivered daily doses of approximately 30, 80, and 220 mg/kg to males and females. There were no exposure-related clinical findings in the 2- or 4-methylimidazole studies.

TABLE 2
Survival, Body Weights, and Feed Consumption of Rats in the 15-Day Feed Study of 2-Methylimidazole

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	123 ± 3	198 ± 4	75 ± 1	—	14.8	15.7
1,200	5/5	123 ± 4	196 ± 4	73 ± 2	99	15.0	15.8
3,300	5/5	122 ± 4	185 ± 6	63 ± 3**	94	13.5	13.6
10,000	5/5	124 ± 5	162 ± 5**	38 ± 3**	82	11.1	11.1
Female							
0	5/5	102 ± 2	136 ± 3	34 ± 2	—	11.5	11.0
1,200	5/5	103 ± 4	139 ± 6	36 ± 3	102	11.1	10.9
3,300	5/5	104 ± 3	135 ± 3	31 ± 2	99	10.4	10.6
10,000	5/5	103 ± 4	121 ± 5	18 ± 2**	89	8.4	8.6

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number of animals surviving at 15 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.

TABLE 3
Survival, Body Weights, and Feed Consumption of Rats in the 15-Day Feed Study of 4-Methylimidazole

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	123 ± 5	205 ± 6	82 ± 2	—	15.6	17.1
300	5/5	121 ± 5	200 ± 7	79 ± 3	98	15.2	16.9
800	5/5	121 ± 5	198 ± 7	76 ± 3	96	14.7	16.2
2,500	5/5	124 ± 4	190 ± 5	66 ± 2**	93	13.6	15.9
Female							
0	5/5	100 ± 3	137 ± 4	37 ± 3	—	11.5	11.3
300	5/5	101 ± 4	136 ± 4	34 ± 1	99	11.8	11.5
800	5/5	99 ± 2	139 ± 2	39 ± 3	101	11.3	11.7
2,500	5/5	102 ± 3	134 ± 3	32 ± 1	97	10.0	10.8

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number of animals surviving at 15 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.

2-Methylimidazole: The absolute heart weights of 3,300 and 10,000 ppm males, the absolute right kidney weight of 10,000 ppm males, and the heart weights 10,000 ppm females were significantly less than those of the control groups (Tables 4 and D1). The relative right kidney weight of 10,000 ppm males, however, was significantly greater than that of the controls. Relative liver weights of the 10,000 ppm males and females were also greater than controls.

TABLE 4
Selected Organ Weight Data for Rats in the 15-Day Feed Study of 2-Methylimidazole[a]

	0 ppm	1,200 ppm	3,300 ppm	10,000 ppm
n	5	5	5	5
Male				
Necropsy body wt	198 ± 4	196 ± 4	185 ± 6	162 ± 5**
Heart				
Absolute	0.687 ± 0.024	0.679 ± 0.018	0.597 ± 0.032*	0.505 ± 0.025**
Relative	3.47 ± 0.08	3.48 ± 0.09	3.22 ± 0.08	3.13 ± 0.13
R. Kidney				
Absolute	0.883 ± 0.016	0.922 ± 0.024	0.847 ± 0.032	0.772 ± 0.021**
Relative	4.46 ± 0.01	4.72 ± 0.04	4.58 ± 0.06	4.79 ± 0.12*
Liver				
Absolute	10.050 ± 0.210	10.458 ± 0.212	9.830 ± 0.394	8.656 ± 0.447*
Relative	50.82 ± 1.07	53.52 ± 0.96	53.09 ± 1.02	53.51 ± 1.35
Female				
Necropsy body wt	136 ± 3	139 ± 6	135 ± 3	121 ± 5
Heart				
Absolute	0.513 ± 0.011	0.511 ± 0.008	0.491 ± 0.014	0.378 ± 0.012**
Relative	3.77 ± 0.05	3.71 ± 0.18	3.64 ± 0.06	3.12 ± 0.05**
Liver				
Absolute	5.915 ± 0.247	6.263 ± 0.232	6.164 ± 0.127	5.656 ± 0.253
Relative	43.39 ± 1.06	45.24 ± 0.23	45.64 ± 0.28*	46.64 ± 0.74**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

At necropsy, enlarged thyroid glands were observed in 3,300 and 10,000 ppm males and females. Microscopically, the incidences of mild to moderate diffuse hyperplasia of thyroid gland follicular cells were increased in 3,300 and 10,000 ppm males and females (Table 5). The incidences of pituitary gland hypertrophy were increased in 3,300 and 10,000 ppm males and 10,000 ppm females.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 15-Day Feed Study of 2-Methylimidazole

	0 ppm	1,200 ppm	3,300 ppm	10,000 ppm
Male				
Thyroid Gland[a]	5	5	5	5
Follicular Cell, Hyperplasia, Diffuse[b]	0	0	5**	5**
Pituitary Gland	5	5	5	5
Pars Distalis, Hypertrophy	0	0	5**	5**
Female				
Thyroid Gland	5	5	5	5
Follicular Cell, Hyperplasia, Diffuse	0	0	5**	5**
Pituitary Gland	5	5	5	5
Pars Distalis, Hypertrophy	0	0	0	5**

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion

4-Methylimidazole: No biologically significant differences occurred between the organ weights of the exposed groups and the controls (Table D2). No exposure-related gross or microscopic lesions or clinical findings were observed during the study.

Exposure Concentration Selection Rationale: Based on the absence of effects on survival or body weights of exposed rats, the highest exposure concentration selected for the 14-week feed studies of 2- and 4-methylimidazole was 10,000 ppm. The body weight effects of 2-methylimidazole at 10,000 ppm and 4-methylimidazole at 2,500 ppm were not considered detrimental to the rats or serious enough to affect the dose-selection decision.

14-WEEK STUDIES

All male and female rats survived to the end of the 2-methylimidazole study (Table 6); one 10,000 ppm male died during week 1 and one 1,250 ppm female was killed moribund during week 9 in the 4-methylimidazole study (Tables 7). The final mean body weights and body weight gains of males administered 2,500 ppm or greater 2- or 4-methylimidazole, 10,000 ppm females in the 2-methylimidazole study, and 5,000 and 10,000 ppm females in the 4-methylimidazole study were significantly less than those of the controls. The mean body weight gain of females exposed to 5,000 ppm 2-methylimidazole was also significantly less than that of the controls (Tables 6 and 7 and Figures 1 and 2).

Reduced feed consumption was observed for 10,000 ppm rats in the 2-methylimidazole study and 5,000 and 10,000 ppm rats in the 4-methylimidazole study. Dietary concentrations of 625, 1,250, 2,500, 5,000, or 10,000 ppm delivered daily doses of approximately 40, 80, 160, 300, or 560 mg/kg 2- or 4-methylimidazole to males and females. Clinical findings in the 2-methylimidazole study included thinness in the 10,000 ppm females. In the 4-methylimidazole study, clinical findings included nasal/eye discharge in males and females administered 2,500 ppm or greater; ruffled fur in males and females administered 5,000 or 10,000 ppm; and thinness, ataxia (females only), and abnormal breathing in males and females in the 10,000 ppm groups.

Functional observations indicated no neurobehavioral abnormalities in groups exposed to 2-methylimidazole. In comparison, day 29 functional observations during the 4-methylimidazole study included increased respiration and mild tremors in 10,000 ppm males; walking on tiptoes, hunched posture, labored or increased respiration, and mild tremors in 10,000 ppm females; and piloerection in 5,000 and 10,000 ppm females. Day 82 observations included piloerection and increased respiration in 5,000 and 10,000 ppm males; crouching over, slight to moderate impaired coordination of movement, ataxia, walking on tiptoes, hunched posture, pupillary constriction, and mild tremors in 10,000 ppm females; and increased respiration and piloerection in 5,000 and 10,000 ppm females. These data are on file at the National Institute of Environmental Health Sciences.

TABLE 6
Survival, Body Weights, and Feed Consumption of Rats in the 14-Week Feed Study of 2-Methylimidazole

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 14
Male							
0	10/10	150 ± 3	366 ± 6	216 ± 4	—	14.9	16.3
625	10/10	150 ± 4	358 ± 7	208 ± 5	98	15.1	16.3
1,250	10/10	150 ± 3	353 ± 6	204 ± 5	97	15.1	16.6
2,500	10/10	150 ± 3	349 ± 2*	199 ± 3**	95	14.6	16.7
5,000	10/10	149 ± 3	336 ± 5**	186 ± 5**	92	14.0	14.4
10,000	10/10	148 ± 3	287 ± 5**	139 ± 4**	78	11.5	13.0
Female							
0	10/10	117 ± 2	198 ± 3	81 ± 2	—	11.2	9.6
625	10/10	118 ± 3	204 ± 2	86 ± 2	103	11.1	9.8
1,250	10/10	118 ± 2	202 ± 3	84 ± 2	102	11.1	9.4
2,500	10/10	117 ± 3	201 ± 3	84 ± 2	101	10.8	9.7
5,000	10/10	117 ± 2	190 ± 3	73 ± 2*	96	10.0	9.0
10,000	10/10	119 ± 2	176 ± 3**	57 ± 2**	89	8.7	7.8

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.

TABLE 7
Survival, Body Weights, and Feed Consumption of Rats in the 14-Week Feed Study of 4-Methylimidazole

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 14
Male							
0	10/10	144 ± 3	352 ± 6	208 ± 5	—	14.7	17.4
625	10/10	143 ± 4	362 ± 8	219 ± 7	103	14.3	16.6
1,250	10/10	146 ± 4	353 ± 6	207 ± 5	100	14.1	16.6
2,500	10/10	145 ± 4	335 ± 4*	190 ± 4**	95	13.7	16.4
5,000	10/10	144 ± 4	298 ± 4**	154 ± 2**	85	11.7	14.4
10,000	9/10[d]	144 ± 3	245 ± 4**	101 ± 3**	70	7.7	14.3
Female							
0	10/10	117 ± 2	201 ± 3	84 ± 2	—	11.6	10.6
625	10/10	116 ± 2	207 ± 3	91 ± 3	103	11.0	11.4
1,250	9/10[e]	116 ± 1	204 ± 2	88 ± 2	101	10.9	10.9
2,500	10/10	119 ± 2	198 ± 4	79 ± 3	98	10.0	9.9
5,000	10/10	117 ± 2	189 ± 6*	72 ± 5*	94	8.6	9.7
10,000	10/10	118 ± 2	127 ± 5**	9 ± 4**	63	5.0	7.6

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Feed consumption is expressed as grams per animal per day.
[d] Week of death: 1
[e] Week of death: 9

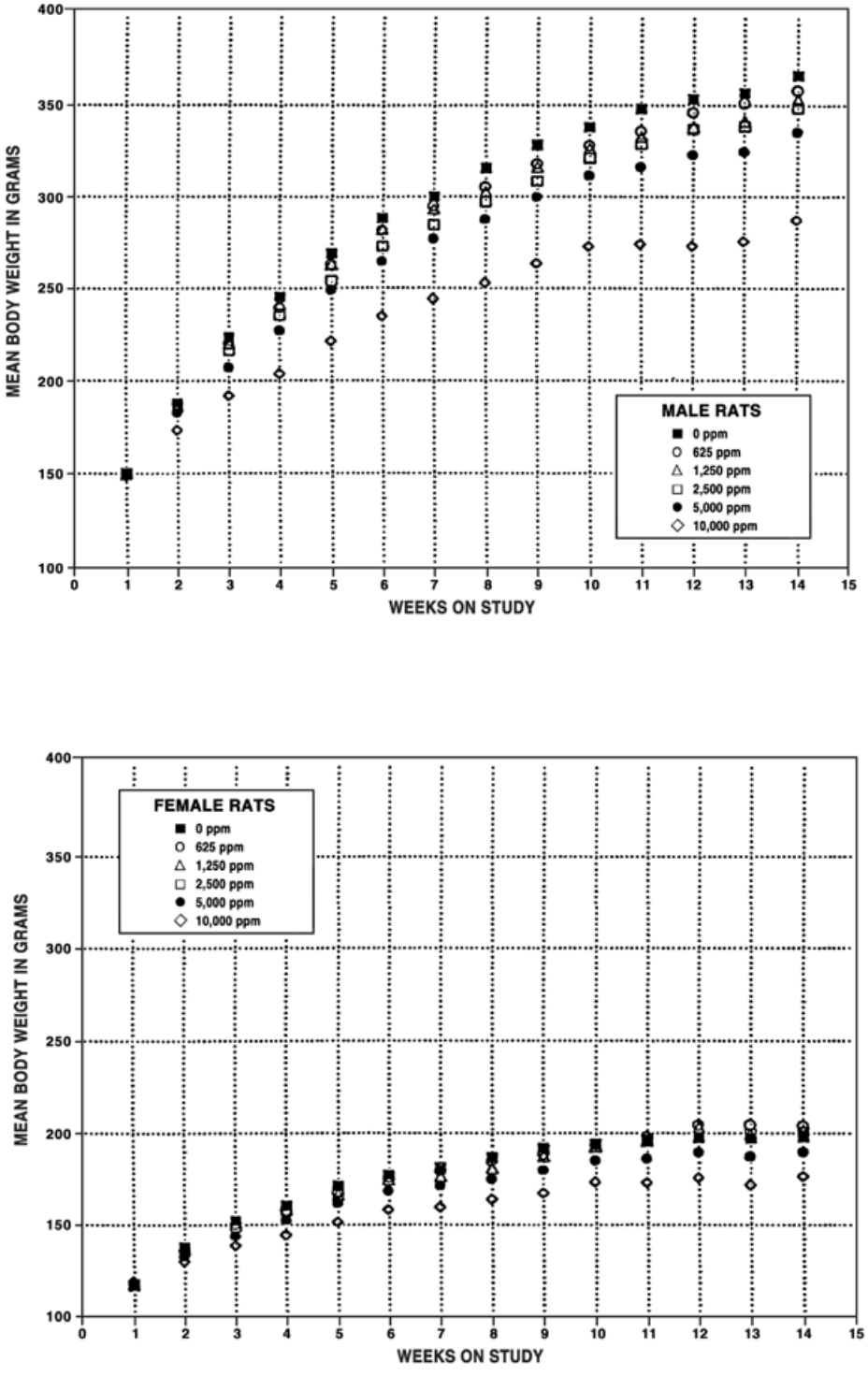


FIGURE 1
Body Weights of Rats Administered 2-Methylimidazole in Feed for 14 Weeks

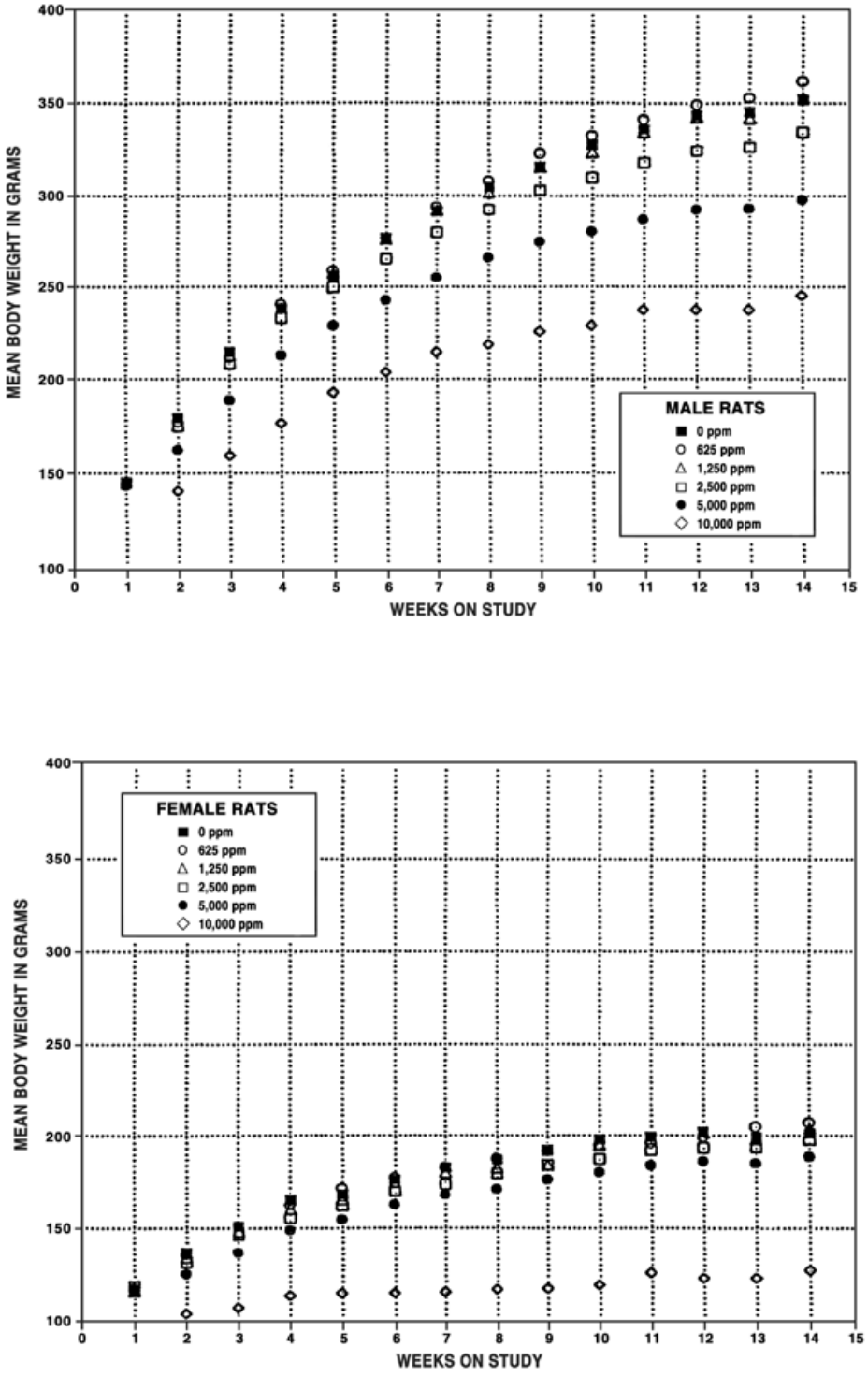


FIGURE 2
Body Weights of Rats Administered 4-Methylimidazole in Feed for 14 Weeks

2-Methylimidazole: Hematology and clinical chemistry data are presented in Tables 8 and C1. On day 8, a minimal transient erythrocytosis was evidenced by increased manual hematocrit values and erythrocyte counts of 10,000 ppm females. At this time point, there was a minimal decrease in reticulocyte counts of 5,000 and 10,000 ppm males and females. The erythrocytosis disappeared and was replaced, on day 29 and/or at week 14, by a minimal exposure concentration-related decrease in the erythron, as evidenced by decreased automated and manual hematocrit values, hemoglobin concentrations, and erythrocyte counts of 2,500 ppm females and 5,000 and 10,000 ppm males and females. At these time points, there was no change in reticulocyte counts, indicating a lack of an erythropoietic response to the decreased circulating erythroid mass. Additional evidence suggesting a treatment-related erythropoietic effect was demonstrated by decreased mean cell volumes and mean cell hemoglobin values in these groups, suggesting that the circulating erythrocytes were smaller (microcytic) than what would be expected. On day 8, a minimal increase in platelet counts occurred in 1,250 ppm males and in males and females exposed to 2,500 ppm or greater. This increase ameliorated and, at week 14, occurred only in 5,000 ppm males and 10,000 ppm males and females.

The clinical chemistry data demonstrated a marked effect of 2-methylimidazole on thyroxine, triiodothyronine, and thyroid-stimulating hormone concentrations. In general, the thyroid gland hormone effects were most pronounced on day 8, and female rats were more affected than males. On day 8, thyroxine and triiodothyronine concentrations were decreased in males and females exposed to 2,500 ppm or greater; thyroxine concentration was also decreased in 625 ppm females. These reductions ranged from 13% to 88% of control values, depending on the exposure concentration, gender, and analyte. Also on day 8, thyroid-stimulating hormone concentrations were markedly increased approximately four- to ninefold in males and females exposed to 2,500 ppm or greater. On day 29, thyroid gland hormone effects had ameliorated in males, and only thyroxine concentration was decreased in the 10,000 ppm group. In females, thyroxine concentrations were decreased in groups exposed to 2,500 ppm or greater; triiodothyronine concentration was decreased in 10,000 ppm females. Thyroid-stimulating hormone concentrations were increased in males exposed to 5,000 or 10,000 ppm and females exposed to 1,250 ppm or greater. At week 14, triiodothyronine concentrations were decreased in 10,000 ppm males and females; thyroxine concentration was decreased only in 10,000 ppm females. At week 14, increases in thyroid-stimulating hormone concentration persisted in 5,000 and 10,000 ppm males and females.

TABLE 8
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	8
Week 14	9	10	10	10	10	10
Hematology						
Automated hematocrit (%)						
Day 8	43.9 ± 0.4	44.2 ± 0.4	42.5 ± 0.3	42.3 ± 0.4	42.1 ± 0.6	43.3 ± 0.6
Day 29	45.8 ± 0.3	46.2 ± 0.4	45.7 ± 0.3	44.8 ± 0.5	43.8 ± 0.5**	41.8 ± 0.5**
Week 14	45.1 ± 0.3	45.0 ± 0.4	44.3 ± 0.5	44.1 ± 0.5	42.4 ± 0.6**	40.6 ± 0.6**
Manual hematocrit (%)						
Day 8	46.9 ± 0.5	47.6 ± 0.5	46.1 ± 0.3	45.8 ± 0.4	45.7 ± 0.5	46.6 ± 0.6
Day 29	47.8 ± 0.4	48.9 ± 0.4	48.2 ± 0.4	47.4 ± 0.5	46.6 ± 0.4	44.6 ± 0.7**
Week 14	47.6 ± 0.4	46.9 ± 0.5	46.9 ± 0.5	46.9 ± 0.5	45.3 ± 0.7**	43.7 ± 0.7**
Hemoglobin (g/dL)						
Day 8	15.2 ± 0.1	15.4 ± 0.2	14.9 ± 0.1	14.6 ± 0.1	14.8 ± 0.1	15.1 ± 0.2
Day 29	16.1 ± 0.1	16.3 ± 0.1	16.1 ± 0.1	15.7 ± 0.1	15.4 ± 0.2**	14.8 ± 0.2**
Week 14	15.8 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.4 ± 0.1*	14.8 ± 0.2**	14.1 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 8	7.28 ± 0.07	7.30 ± 0.07	7.05 ± 0.05	7.02 ± 0.06	7.04 ± 0.11	7.34 ± 0.11
Day 29	7.94 ± 0.06	7.95 ± 0.07	7.86 ± 0.05	7.77 ± 0.09	7.60 ± 0.09*	7.48 ± 0.11**
Week 14	8.76 ± 0.04	8.81 ± 0.09	8.59 ± 0.09	8.60 ± 0.08	8.40 ± 0.13*	8.11 ± 0.10**
Reticulocytes ($10^6/\mu L$)						
Day 8	0.29 ± 0.01	0.31 ± 0.02	0.28 ± 0.01	0.28 ± 0.02	0.19 ± 0.01**	0.11 ± 0.01**
Day 29	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
Week 14	0.19 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.19 ± 0.02
Mean cell volume (fL)						
Day 8	60.3 ± 0.2	60.6 ± 0.2	60.3 ± 0.2	60.2 ± 0.2	59.8 ± 0.2	59.0 ± 0.2**
Day 29	57.8 ± 0.2	58.1 ± 0.3	58.2 ± 0.3	57.7 ± 0.3	57.7 ± 0.2	55.8 ± 0.3**
Week 14	51.5 ± 0.2	51.1 ± 0.2	51.6 ± 0.3	51.2 ± 0.1	50.4 ± 0.2**	50.0 ± 0.2**
Mean cell hemoglobin (pg)						
Day 8	20.9 ± 0.1	21.1 ± 0.2	21.1 ± 0.1	20.8 ± 0.1	21.0 ± 0.2	20.6 ± 0.1
Day 29	20.3 ± 0.1	20.5 ± 0.1	20.5 ± 0.1	20.3 ± 0.1	20.3 ± 0.1	19.7 ± 0.1**
Week 14	18.0 ± 0.1[b]	17.8 ± 0.1	18.0 ± 0.1	17.9 ± 0.1	17.6 ± 0.1*	17.4 ± 0.1**
Platelets ($10^3/\mu L$)						
Day 8	927.6 ± 15.9	966.7 ± 12.2	984.9 ± 19.5*	1,042.0 ± 16.1**	1,032.8 ± 13.7**	1,043.7 ± 24.7**
Day 29	695.3 ± 10.4	684.4 ± 13.1	710.2 ± 4.0	750.3 ± 11.6**	810.8 ± 13.1**[b]	896.6 ± 13.6**
Week 14	605.3 ± 10.0	587.7 ± 14.8	597.0 ± 8.9	626.2 ± 12.5	684.9 ± 22.0*	705.7 ± 15.2**
Clinical Chemistry						
Thyroid-stimulating hormone (ng/mL)						
Day 8	1.98 ± 0.42	2.16 ± 0.28	3.05 ± 0.42	7.18 ± 0.50**	9.78 ± 0.34**	9.26 ± 0.49**
Day 29	0.52 ± 0.16	1.07 ± 0.31	0.90 ± 0.21	0.86 ± 0.23	1.40 ± 0.25*	5.41 ± 0.43**
Week 14	0.29 ± 0.12[c]	0.45 ± 0.22	0.50 ± 0.17	0.51 ± 0.24	1.92 ± 0.62*	4.19 ± 0.62**
Triiodothyronine (ng/dL)						
Day 8	164.2 ± 6.8	165.5 ± 7.1	152.8 ± 4.6	116.5 ± 2.4**	79.0 ± 1.4**	61.7 ± 2.1**
Day 29	123.4 ± 2.5	118.2 ± 3.5	126.6 ± 7.2	124.5 ± 3.7	117.0 ± 3.0	113.0 ± 2.2
Week 14	137.2 ± 4.3[c]	149.6 ± 4.1	128.5 ± 4.5	140.1 ± 3.3	124.2 ± 5.2	123.3 ± 4.2*

TABLE 8
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	8
Week 14	9	10	10	10	10	10
Clinical Chemistry (continued)						
Thyroxine (μg/dL)						
Day 8	5.44 ± 0.24	5.29 ± 0.16	5.39 ± 0.15	4.75 ± 0.13**	0.66 ± 0.04**[d]	—[e]
Day 29	3.32 ± 0.15	3.17 ± 0.19	3.27 ± 0.23	2.92 ± 0.28	2.98 ± 0.20	1.13 ± 0.13**
Week 14	2.10 ± 0.23[c]	2.27 ± 0.15	2.21 ± 0.08	2.72 ± 0.16	1.74 ± 0.23[d]	1.97 ± 0.17
Creatinine (mg/dL)						
Day 29	0.66 ± 0.02	0.62 ± 0.01	0.51 ± 0.01**	0.35 ± 0.02**	0.16 ± 0.02**[d]	—
Week 14	0.41 ± 0.02	0.27 ± 0.02**	0.18 ± 0.02**	0.04 ± 0.02**	—	—
Total protein (g/dL)						
Day 29	6.6 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.0 ± 0.1**
Week 14	6.9 ± 0.1	6.6 ± 0.0**	6.5 ± 0.1**	6.5 ± 0.0**	6.4 ± 0.1**	6.2 ± 0.1**
Albumin (g/dL)						
Day 29	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	4.7 ± 0.1*	4.7 ± 0.1**	4.3 ± 0.1**
Week 14	4.8 ± 0.0	4.8 ± 0.0	4.7 ± 0.1	4.6 ± 0.0**	4.4 ± 0.1**	4.3 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 29	44 ± 1	44 ± 2	44 ± 3	33 ± 1**	27 ± 2**	19 ± 1**
Week 14	63 ± 3	53 ± 2**	60 ± 5	42 ± 1**	44 ± 5**	30 ± 2**
Alkaline phosphatase (IU/L)						
Day 29	444 ± 12	446 ± 6	458 ± 8	438 ± 11	404 ± 12*	334 ± 7**
Week 14	269 ± 7	263 ± 4	273 ± 3	252 ± 5	252 ± 7	256 ± 5
Sorbitol dehydrogenase (IU/L)						
Day 29	20 ± 1	28 ± 2*	25 ± 2	21 ± 2	20 ± 3	15 ± 1
Week 14	27 ± 1	25 ± 1	27 ± 1	23 ± 1*	23 ± 2*	22 ± 1**
Female						
n						
Day 8	10	10	10	10	10	10
Day 29	10	8	10	10	9	9
Week 14	10	10	10	10	10	10
Hematology						
Automated hematocrit (%)						
Day 8	44.4 ± 0.7	45.4 ± 0.8	43.8 ± 0.7	45.2 ± 0.3	44.7 ± 0.4	46.6 ± 0.5
Day 29	45.6 ± 0.6	45.4 ± 0.5	44.8 ± 0.3	45.2 ± 0.3	42.7 ± 0.2**	38.7 ± 1.9**
Week 14	44.9 ± 0.5	44.6 ± 0.5	43.8 ± 0.5	42.9 ± 0.4**	41.7 ± 0.5**	39.9 ± 0.6**
Manual hematocrit (%)						
Day 8	45.3 ± 0.8	45.2 ± 0.8	45.3 ± 0.6	46.1 ± 0.3	45.1 ± 0.5	47.7 ± 0.5*
Day 29	47.2 ± 0.5	47.5 ± 0.6	47.1 ± 0.4	47.2 ± 0.5	45.6 ± 0.4*	43.1 ± 1.6**[b]
Week 14	46.3 ± 0.5	45.5 ± 0.4	45.6 ± 0.5	44.5 ± 0.4*	43.3 ± 0.4**	41.8 ± 0.6**

TABLE 8
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female						
n						
Day 8	10	10	10	10	10	10
Day 29	10	8	10	10	9	9
Week 14	10	10	10	10	10	10
Hematology (continued)						
Hemoglobin (g/dL)						
Day 8	15.2 ± 0.2	15.3 ± 0.2	14.9 ± 0.2	15.3 ± 0.1	15.1 ± 0.1	15.8 ± 0.2
Day 29	16.0 ± 0.2	15.8 ± 0.2	15.8 ± 0.1	15.8 ± 0.1	15.2 ± 0.1**	13.7 ± 0.6**
Week 14	15.5 ± 0.2	15.4 ± 0.1	15.2 ± 0.1	14.8 ± 0.1**	14.5 ± 0.1**	14.2 ± 0.2**
Erythrocytes ($10^6/\mu L$)						
Day 8	7.21 ± 0.12	7.38 ± 0.12	7.10 ± 0.12	7.37 ± 0.05	7.31 ± 0.08	7.74 ± 0.11**
Day 29	7.56 ± 0.09	7.63 ± 0.04	7.45 ± 0.07	7.56 ± 0.07	7.31 ± 0.03**	6.91 ± 0.34*
Week 14	7.89 ± 0.09	7.89 ± 0.08	7.79 ± 0.10	7.65 ± 0.07	7.71 ± 0.10	7.71 ± 0.11
Reticulocytes ($10^6/\mu L$)						
Day 8	0.20 ± 0.02	0.18 ± 0.01	0.33 ± 0.11	0.17 ± 0.02	0.15 ± 0.01*	0.10 ± 0.01**
Day 29	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.13 ± 0.01
Week 14	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.15 ± 0.02	0.14 ± 0.01	0.10 ± 0.01
Mean cell volume (fL)						
Day 8	61.6 ± 0.2	61.5 ± 0.2	61.7 ± 0.1	61.4 ± 0.2	61.2 ± 0.2	60.2 ± 0.3**
Day 29	60.4 ± 0.2	59.6 ± 0.5	60.1 ± 0.3	59.8 ± 0.2	58.5 ± 0.3**	56.1 ± 0.1**
Week 14	56.9 ± 0.1	56.5 ± 0.1*	56.3 ± 0.1**	56.1 ± 0.1**	54.1 ± 0.1**	51.8 ± 0.2**
Mean cell hemoglobin (pg)						
Day 8	21.1 ± 0.1	20.7 ± 0.1*	21.0 ± 0.1	20.8 ± 0.1	20.6 ± 0.0**	20.4 ± 0.1**
Day 29	21.2 ± 0.1	20.8 ± 0.2	21.2 ± 0.1	20.9 ± 0.1*	20.8 ± 0.1	19.9 ± 0.1**
Week 14	19.6 ± 0.1	19.5 ± 0.1	19.5 ± 0.1	19.4 ± 0.1	18.8 ± 0.1**[d]	18.4 ± 0.1**
Platelets ($10^3/\mu L$)						
Day 8	818.2 ± 20.9	872.8 ± 19.3	856.2 ± 24.1	932.3 ± 21.5**	938.9 ± 26.7**	931.0 ± 15.2**
Day 29	652.6 ± 10.2	732.4 ± 30.8**	715.9 ± 16.2**	712.8 ± 11.7**	757.1 ± 11.3**	869.6 ± 30.1**
Week 14	576.4 ± 13.0	562.6 ± 18.4	574.1 ± 13.1	603.8 ± 10.5	631.8 ± 20.4	685.0 ± 23.0**
Clinical Chemistry						
Thyroid-stimulating hormone (ng/mL)						
Day 8	1.04 ± 0.11	0.89 ± 0.15	1.74 ± 0.31	4.39 ± 0.73**	9.05 ± 0.47**	7.83 ± 0.36**
Day 29	0.38 ± 0.09	1.13 ± 0.66	0.91 ± 0.10**	0.76 ± 0.12**	2.32 ± 0.42**	8.49 ± 0.62**
Week 14	0.27 ± 0.13	0.49 ± 0.22	0.27 ± 0.16	0.52 ± 0.16	1.23 ± 0.40*[d]	7.90 ± 0.87**[d]
Triiodothyronine (ng/dL)						
Day 8	142.5 ± 6.6	130.5 ± 5.5	141.1 ± 3.1	119.9 ± 1.8**	81.6 ± 2.0**	76.3 ± 2.1**
Day 29	138.5 ± 6.0	139.6 ± 4.1	143.4 ± 4.2	135.3 ± 3.5	128.4 ± 4.1	116.4 ± 3.2**[b]
Week 14	136.5 ± 6.1	142.0 ± 6.7	139.2 ± 5.6	135.8 ± 4.7	137.9 ± 3.7	112.2 ± 4.2**
Thyroxine ($\mu g/dL$)						
Day 8	3.87 ± 0.29	3.11 ± 0.28*	3.64 ± 0.19	3.01 ± 0.16**	0.60 ± 0.07**[b]	0.74 ± 0.11**[b]
Day 29	3.44 ± 0.31	2.33 ± 0.36	2.92 ± 0.28	2.23 ± 0.24**	2.18 ± 0.25**	0.80 ± 0.08**[b]
Week 14	2.57 ± 0.28	2.17 ± 0.20	1.94 ± 0.28	2.16 ± 0.30	2.42 ± 0.22	0.79 ± 0.12**[b]

TABLE 8
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	8	10	10	9	9
Week 14	10	10	10	10	10	10
Clinical Chemistry (continued)						
Creatinine (mg/dL)						
Day 29	0.66 ± 0.02	0.53 ± 0.02**[f]	0.44 ± 0.02**	0.23 ± 0.02**	0.05 ± 0.05**[g]	—
Week 14	0.42 ± 0.01	0.32 ± 0.02**	0.27 ± 0.02**	0.10 ± 0.03**	—	—
Total protein (g/dL)						
Day 29	6.2 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.3 ± 0.2**
Week 14	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.4 ± 0.1	6.6 ± 0.1	6.3 ± 0.1**
Albumin (g/dL)						
Day 29	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.5 ± 0.1**	4.0 ± 0.1**
Week 14	5.0 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	4.6 ± 0.1**	4.3 ± 0.0**
Alanine aminotransferase IU/L)						
Day 29	35 ± 1	29 ± 2**	31 ± 1*	29 ± 1**	25 ± 2**	23 ± 4**
Week 14	59 ± 6	52 ± 4	65 ± 8	50 ± 4	36 ± 3**	25 ± 2**
Alkaline phosphatase (IU/L)						
Day 29	343 ± 7	336 ± 10	353 ± 16	329 ± 9	326 ± 7	283 ± 8**
Week 14	217 ± 8	209 ± 6	215 ± 5	204 ± 12	221 ± 6	240 ± 6
Sorbitol dehydrogenase (IU/L)						
Day 29	24 ± 2	22 ± 2	22 ± 1	21 ± 1	17 ± 2**	25 ± 6*
Week 14	27 ± 2	25 ± 2	27 ± 2	26 ± 2	23 ± 1	19 ± 2**

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=8
[c] n=10
[d] n=9
[e] Not detected
[f] n=7
[g] n=2

Other alterations in clinical chemistry parameters included decreased alanine aminotransferase, alkaline phosphatase activities, and total protein and albumin concentrations. These changes occurred at various exposure concentrations at both time points evaluated but were more consistent for males exposed to 2,500 ppm or greater and females exposed to 5,000 or 10,000 ppm. Exposure-related decreases in creatinine concentrations occurred in all exposed groups of males and females on day 29 and at week 14. On day 29, some evidence suggestive of muscle injury was demonstrated by mild increases in creatine kinase activity in 5,000 and 10,000 ppm females. This effect was sporadic and was not considered toxicologically relevant.

Organ weights are presented in Tables 9 and D3. The absolute spleen weights of all exposed groups of males and relative spleen weights of 10,000 ppm males were significantly less than those of the controls. The relative right kidney and right testis weights of all exposed groups of males and the relative lung weights of 5,000 and 10,000 ppm males were significantly greater. In females, the absolute heart weights of the 5,000 and 10,000 ppm groups and the relative heart weight of the 5,000 ppm group were significantly less.

TABLE 9
Selected Organ Weight Data for Rats in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	8	8	8	8	8	8
Male						
Necropsy body wt[b]	366 ± 6	358 ± 7	353 ± 6	349 ± 2*	336 ± 5**	287 ± 5**
R. Kidney						
Absolute	1.240 ± 0.034	1.303 ± 0.029	1.287 ± 0.029	1.283 ± 0.019	1.242 ± 0.027	1.143 ± 0.018*
Relative	3.39 ± 0.04	3.63 ± 0.06**	3.63 ± 0.07**	3.66 ± 0.06**	3.69 ± 0.05**	4.01 ± 0.05**
Lung						
Absolute	1.626 ± 0.033	1.495 ± 0.068	1.519 ± 0.055	1.485 ± 0.062	1.641 ± 0.067	1.380 ± 0.038*
Relative	4.45 ± 0.08	4.16 ± 0.14	4.27 ± 0.12	4.23 ± 0.15	4.87 ± 0.17*	4.84 ± 0.10*
Spleen						
Absolute	0.773 ± 0.018	0.726 ± 0.018*	0.706 ± 0.016*	0.737 ± 0.013*	0.682 ± 0.015**	0.543 ± 0.014**
Relative	2.11 ± 0.05	2.02 ± 0.03	1.99 ± 0.02	2.10 ± 0.03	2.03 ± 0.04	1.90 ± 0.04**
R. Testis						
Absolute	1.388 ± 0.064	1.478 ± 0.030[c]	1.466 ± 0.022	1.478 ± 0.031	1.471 ± 0.029	1.247 ± 0.029*
Relative	3.78 ± 0.13	4.12 ± 0.08*[c]	4.13 ± 0.06*	4.22 ± 0.08**	4.38 ± 0.09**	4.37 ± 0.08**
Female						
Necropsy body wt	198 ± 3	204 ± 2	202 ± 3	201 ± 3	190 ± 3	176 ± 3**
Heart						
Absolute	0.728 ± 0.038	0.702 ± 0.013	0.689 ± 0.018	0.709 ± 0.026	0.630 ± 0.019**	0.597 ± 0.015**
Relative	3.71 ± 0.18	3.47 ± 0.07	3.43 ± 0.09	3.53 ± 0.10	3.29 ± 0.08*	3.40 ± 0.05
Thymus						
Absolute	0.265 ± 0.012	0.270 ± 0.012	0.253 ± 0.009	0.269 ± 0.014	0.245 ± 0.015	0.209 ± 0.008**
Relative	1.35 ± 0.06	1.33 ± 0.07	1.26 ± 0.06	1.35 ± 0.08	1.28 ± 0.07	1.19 ± 0.04

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] For body weights n=10
[c] n=7

Sperm motility and vaginal cytology data are presented in Tables E1 and E2. Spermatid heads per testis and the mean spermatid count were significantly decreased in 10,000 ppm males compared to the controls. In females, the estrous cycle of the 10,000 ppm group was significantly increased.

Gross lesions related to 2-methylimidazole exposure included enlarged thyroid glands in 10,000 ppm males and females and small uteri and mottled adrenal glands in 10,000 ppm females. Microscopically, males exposed to 1,250 ppm or greater and females exposed to 2,500 ppm or greater had significantly increased incidences of diffuse follicular cell hyperplasia of the thyroid gland compared to the controls (Tables 10, A1, and A2). The severity of this lesion increased with increasing exposure concentration in females and was also increased in males in the 5,000 and 10,000 ppm groups. One male in the 10,000 ppm group had a follicular cell cyst, and two males in this group had follicular cell adenoma. The incidence, but not the severity, of testicular degeneration was significantly increased in 10,000 ppm males.

TABLE 10
Incidences of Neoplasms and Selected Nonneoplastic Lesions in Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
Thyroid Gland[a]	10	10	10	10	10	10
Follicular Cell, Hyperplasia, Diffuse[b]	2 (1.5)[c]	0	8* (1.1)	10** (1.1)	10** (1.9)	10** (2.9)
Follicular Cell Cyst	0	0	0	0	0	1 (2.0)
Follicular Cell Adenoma	0	0	0	0	0	2
Testes	10	10	10	10	10	10
Degeneration	2 (2.5)	2 (1.0)	1 (1.0)	2 (1.0)	2 (1.0)	9** (1.2)
Female						
Thyroid Gland	10	9	10	10	10	10
Follicular Cell, Hyperplasia, Diffuse	0	0	0	10** (1.0)	10** (2.0)	10** (3.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

4-Methylimidazole: Hematology and clinical chemistry data are listed in Tables 11 and C2. As in the 2-methylimidazole study, the hematology data for rats in the 14-week study of 4-methylimidazole demonstrated erythron effects. On day 8, there was evidence of a transient erythrocytosis, demonstrated by increased automated and manual hematocrit values, hemoglobin concentrations, and erythrocyte counts of 5,000 ppm males and 10,000 ppm males and females. On day 29, the erythrocytosis was replaced by a decrease in the erythron in males exposed to 2,500 ppm or greater. At week 14, this decrease persisted for males and was accompanied by similar decreases in 10,000 ppm females. On day 8, there was a minimal decrease in reticulocyte counts of 2,500 ppm males and 5,000 and 10,000 ppm males and females; this effect was transient and absent at the later time points. Evidence suggesting a treatment-related erythropoietic effect was demonstrated by decreases in mean cell volumes, mean cell hemoglobin values, and mean cell hemoglobin concentrations. For mean cell volume and mean cell hemoglobin, the effects consistently occurred in males and females exposed to 5,000 or 10,000 ppm at all time points; for mean cell hemoglobin concentration, the effect was most consistent for males exposed to 2,500 ppm or greater at week 14 and females exposed to 2,500 ppm or greater on day 29.

Platelet count effects also occurred but differed from those in the 2-methylimidazole study; instead of increasing, as in the 2-methylimidazole study, platelet counts decreased. On day 8, a minimal decrease in platelet counts occurred in males exposed to 2,500 ppm and males and females exposed to 5,000 or 10,000 ppm. This decrease ameliorated and, by week 14, occurred only in 10,000 ppm females.

While administration of 2-methylimidazole resulted in strong decreases in triiodothyronine and thyroxine concentrations and increases in thyroid-stimulating hormone concentrations, 4-methylimidazole induced no consistent thyroid gland hormone effects. On day 8, thyroxine concentration was decreased in 10,000 ppm males, and triiodothyronine concentrations were decreased in 5,000 and 10,000 ppm females; thyroid-stimulating hormone concentrations, however, were either unaffected or decreased. These changes were transient, and no thyroid gland hormone effects occurred in exposed males or females on day 29. At week 14, while triiodothyronine concentration (and possibly thyroxine concentration) was decreased in 10,000 ppm females, thyroxine concentration was increased in 10,000 ppm males. Thyroid-stimulating hormone concentrations were unaffected for exposed males and females.

TABLE 11
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	9	8
Week 14	10	10	10	10	10	9
Hematology						
Automated hematocrit (%)						
Day 8	40.7 ± 0.7	42.2 ± 0.6	40.5 ± 0.4	40.8 ± 0.5	43.1 ± 1.0*	47.3 ± 0.6**
Day 29	45.2 ± 0.4	45.5 ± 0.3	45.3 ± 0.4	43.8 ± 0.4*	43.5 ± 0.3**	41.6 ± 0.6**
Week 14	45.3 ± 0.7	46.1 ± 0.4	45.7 ± 0.5	44.1 ± 0.5	42.8 ± 0.4**	39.6 ± 0.3**
Manual hematocrit (%)						
Day 8	43.8 ± 0.8	45.3 ± 0.6	42.8 ± 0.5	44.7 ± 0.8	45.2 ± 1.1	49.1 ± 0.7**
Day 29	47.8 ± 0.4	47.0 ± 0.5	47.4 ± 0.2	45.9 ± 0.4**	45.0 ± 0.2**	43.3 ± 0.6**
Week 14	46.7 ± 0.7	47.8 ± 0.3	47.3 ± 0.4	45.4 ± 0.3*	44.0 ± 0.6**	41.1 ± 0.3**
Hemoglobin (g/dL)						
Day 8	14.3 ± 0.2	14.7 ± 0.2*	14.3 ± 0.1	14.3 ± 0.1	15.1 ± 0.3*	16.5 ± 0.1**
Day 29	15.9 ± 0.1	15.8 ± 0.1	15.9 ± 0.1	15.4 ± 0.1*	15.1 ± 0.1**	14.2 ± 0.2**
Week 14	15.8 ± 0.2	15.9 ± 0.1	15.9 ± 0.1	15.2 ± 0.1**	14.6 ± 0.1**	13.6 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 8	6.86 ± 0.11	7.02 ± 0.08	6.88 ± 0.06	6.95 ± 0.09	7.40 ± 0.16**	8.18 ± 0.08**
Day 29	7.81 ± 0.09	7.90 ± 0.09	7.89 ± 0.09	7.73 ± 0.08	7.76 ± 0.08	7.80 ± 0.12
Week 14	8.86 ± 0.12	8.97 ± 0.08	8.83 ± 0.08	8.53 ± 0.10*	8.44 ± 0.08**	8.03 ± 0.08**
Reticulocytes ($10^6/\mu L$)						
Day 8	0.33 ± 0.02	0.32 ± 0.02	0.27 ± 0.02	0.26 ± 0.02*	0.19 ± 0.02**	0.08 ± 0.01**
Day 29	0.16 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.15 ± 0.01
Week 14	0.16 ± 0.02	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.02	0.18 ± 0.01
Mean cell volume (fL)						
Day 8	59.3 ± 0.4	60.0 ± 0.4	58.9 ± 0.3	58.8 ± 0.3	58.3 ± 0.3*	57.8 ± 0.2**
Day 29	58.0 ± 0.3	57.6 ± 0.3	57.4 ± 0.3	56.7 ± 0.2**	56.0 ± 0.4**	53.4 ± 0.2**
Week 14	51.1 ± 0.3	51.5 ± 0.2	51.7 ± 0.2	51.7 ± 0.2	50.7 ± 0.1	49.3 ± 0.2**
Mean cell hemoglobin (pg)						
Day 8	20.9 ± 0.2	20.9 ± 0.1	20.9 ± 0.1	20.6 ± 0.1	20.4 ± 0.1**	20.2 ± 0.1**
Day 29	20.4 ± 0.1	20.1 ± 0.1	20.1 ± 0.2	19.9 ± 0.1**	19.5 ± 0.1**	18.2 ± 0.1**
Week 14	17.9 ± 0.1	17.7 ± 0.1	18.0 ± 0.2	17.8 ± 0.1	17.3 ± 0.1**	16.9 ± 0.2**
Mean cell hemoglobin concentration (g/dL)						
Day 8	35.2 ± 0.3	34.8 ± 0.3	35.4 ± 0.1	35.2 ± 0.2	35.0 ± 0.1	34.9 ± 0.2
Day 29	35.1 ± 0.2	34.8 ± 0.1	35.0 ± 0.1	35.1 ± 0.1	34.8 ± 0.1	34.2 ± 0.2**
Week 14	35.0 ± 0.1	34.5 ± 0.2	34.7 ± 0.3[b]	34.4 ± 0.2*	34.1 ± 0.1**	34.3 ± 0.2**
Platelets ($10^3/\mu L$)						
Day 8	908.3 ± 25.1	847.7 ± 31.6	915.6 ± 29.6	781.7 ± 27.9**	754.6 ± 24.8**	649.3 ± 24.2**
Day 29	639.9 ± 18.6	626.4 ± 12.5	638.9 ± 14.7	651.4 ± 12.6	613.1 ± 17.9	611.8 ± 15.0
Week 14	610.2 ± 49.4	555.3 ± 19.1	577.9 ± 12.9	586.4 ± 13.5	605.1 ± 10.2	565.3 ± 13.4

TABLE 11
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	9	8
Week 14	10	10	10	10	10	9
Clinical Chemistry						
Thyroid-stimulating hormone (ng/mL)						
Day 8	2.07 ± 0.44	2.24 ± 0.45	1.97 ± 0.50	2.20 ± 0.51	1.72 ± 0.46	1.97 ± 0.50
Day 29	0.56 ± 0.23	0.94 ± 0.17	1.15 ± 0.29	0.80 ± 0.21	1.38 ± 0.30	1.25 ± 0.25
Week 14	1.34 ± 0.45	1.19 ± 0.27	0.87 ± 0.13	0.88 ± 0.07	1.68 ± 0.46	1.69 ± 0.38
Triiodothyronine (ng/dL)						
Day 8	143.6 ± 3.0	149.7 ± 6.2	141.4 ± 4.1	144.3 ± 4.9	145.2 ± 4.4	128.8 ± 5.9
Day 29	135.3 ± 4.6	139.6 ± 6.0	137.1 ± 4.5	138.9 ± 4.4	128.2 ± 6.5	127.3 ± 5.7
Week 14	142.4 ± 5.1	151.5 ± 3.2	140.4 ± 6.0	130.4 ± 4.3	137.4 ± 5.2	141.4 ± 4.5
Thyroxine (μg/dL)						
Day 8	5.24 ± 0.22	4.82 ± 0.27	5.08 ± 0.11	5.00 ± 0.22	4.88 ± 0.16	4.12 ± 0.24**
Day 29	3.67 ± 0.15	3.35 ± 0.18	3.39 ± 0.21	3.11 ± 0.09*	3.21 ± 0.16	3.78 ± 0.10
Week 14	2.23 ± 0.22	2.78 ± 0.20	2.21 ± 0.22	2.09 ± 0.20	2.81 ± 0.32	3.74 ± 0.24**
Total protein (g/dL)						
Day 29	6.7 ± 0.1	6.5 ± 0.1	6.7 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.0 ± 0.1**
Week 14	6.9 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	7.0 ± 0.2	7.0 ± 0.1	6.6 ± 0.1
Albumin (g/dL)						
Day 29	4.9 ± 0.0	4.9 ± 0.1	5.1 ± 0.1[b]	4.8 ± 0.1	4.8 ± 0.1	4.5 ± 0.1**
Week 14	4.9 ± 0.0	5.0 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	4.8 ± 0.1
Alkaline phosphatase (IU/L)						
Day 29	464 ± 10	456 ± 10	472 ± 8	468 ± 8	492 ± 9	713 ± 27**
Week 14	251 ± 8	265 ± 7	275 ± 8	287 ± 5**	297 ± 13**	434 ± 19**
Sorbitol dehydrogenase (IU/L)						
Day 29	25 ± 1	23 ± 1	29 ± 2	41 ± 4**	29 ± 1*	34 ± 1**
Week 14	24 ± 2	28 ± 3	27 ± 1	31 ± 3*	36 ± 5*	36 ± 2**
Female						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	7
Week 14	10	10	9	10	10	10
Hematology						
Automated hematocrit (%)						
Day 8	43.8 ± 0.6	44.5 ± 0.7	45.4 ± 0.4*	43.6 ± 0.4	45.4 ± 0.9	50.2 ± 0.7**
Day 29	44.3 ± 0.6	44.7 ± 0.6	45.6 ± 0.8	45.7 ± 0.6	45.3 ± 0.5	44.9 ± 0.9
Week 14	44.0 ± 0.4	45.1 ± 0.3	44.4 ± 0.3	43.5 ± 0.5	42.8 ± 0.5	40.4 ± 0.5**
Manual hematocrit (%)						
Day 8	44.8 ± 0.6	44.9 ± 0.6	45.6 ± 0.4	44.3 ± 0.4	45.3 ± 1.0	49.6 ± 0.4**
Day 29	45.6 ± 0.5	46.0 ± 0.6	47.1 ± 0.5	46.5 ± 0.7	45.5 ± 0.4	45.4 ± 0.7
Week 14	44.8 ± 0.3	46.5 ± 0.4	45.3 ± 0.4	44.3 ± 0.4	44.4 ± 0.5	41.4 ± 0.4**

TABLE 11
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	7
Week 14	10	10	9	10	10	10
Hematology (continued)						
Hemoglobin (g/dL)						
Day 8	14.8 ± 0.2	15.0 ± 0.2	15.2 ± 0.1	14.7 ± 0.1	15.3 ± 0.3	16.9 ± 0.2**
Day 29	15.4 ± 0.2	15.5 ± 0.2	15.7 ± 0.2	15.7 ± 0.2	15.4 ± 0.1	15.3 ± 0.3
Week 14	15.2 ± 0.1	15.6 ± 0.1	15.2 ± 0.1	15.1 ± 0.1	14.6 ± 0.1**	14.0 ± 0.2**
Erythrocytes ($10^6/\mu L$)						
Day 8	7.19 ± 0.09	7.30 ± 0.11	7.53 ± 0.07*	7.20 ± 0.07	7.64 ± 0.17*	8.52 ± 0.13**
Day 29	7.28 ± 0.10	7.34 ± 0.09	7.49 ± 0.13	7.60 ± 0.10*	7.64 ± 0.07*	8.20 ± 0.17**
Week 14	7.82 ± 0.08	7.98 ± 0.06	7.85 ± 0.06	7.78 ± 0.09	7.76 ± 0.09	7.84 ± 0.10
Reticulocytes ($10^6/\mu L$)						
Day 8	0.22 ± 0.01	0.21 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.14 ± 0.01**	0.06 ± 0.01**
Day 29	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.01
Week 14	0.14 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.01	0.10 ± 0.01
Mean cell volume (fL)						
Day 8	60.9 ± 0.2	60.9 ± 0.2	60.3 ± 0.1*	60.5 ± 0.3	59.4 ± 0.2**	59.0 ± 0.2**
Day 29	60.8 ± 0.1	60.9 ± 0.2	60.8 ± 0.2	60.1 ± 0.2*	59.3 ± 0.2**	54.8 ± 0.2**
Week 14	56.3 ± 0.2	56.5 ± 0.2	56.6 ± 0.1	56.0 ± 0.1	55.1 ± 0.1**	51.5 ± 0.3**
Mean cell hemoglobin (pg)						
Day 8	20.6 ± 0.1	20.5 ± 0.1	20.2 ± 0.2	20.5 ± 0.1	20.1 ± 0.1**	19.9 ± 0.1**
Day 29	21.1 ± 0.1	21.1 ± 0.1	21.0 ± 0.2	20.7 ± 0.1**	20.1 ± 0.1**	18.7 ± 0.1**
Week 14	19.5 ± 0.1	19.6 ± 0.1	19.3 ± 0.1	19.4 ± 0.1	18.9 ± 0.1**	17.9 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 8	33.8 ± 0.2	33.7 ± 0.2	33.6 ± 0.2	33.8 ± 0.2	33.8 ± 0.1	33.7 ± 0.2
Day 29	34.8 ± 0.2	34.7 ± 0.2	34.6 ± 0.3	34.4 ± 0.1*	34.0 ± 0.2**	34.2 ± 0.2*
Week 14	34.6 ± 0.2	34.6 ± 0.2	34.2 ± 0.2	34.6 ± 0.2	34.2 ± 0.2	34.8 ± 0.2
Platelets ($10^3/\mu L$)						
Day 8	744.9 ± 18.9	775.4 ± 20.1	738.5 ± 19.9	737.8 ± 20.5	673.1 ± 18.7*	616.4 ± 25.5**
Day 29	670.7 ± 16.7	652.3 ± 18.1	622.8 ± 8.5*	631.0 ± 17.9	592.0 ± 16.7**	594.1 ± 20.1**
Week 14	600.3 ± 18.5	558.7 ± 16.5	560.0 ± 17.7	546.5 ± 19.1	535.2 ± 30.3	503.2 ± 12.3**
Clinical Chemistry						
Thyroid-stimulating hormone (ng/mL)						
Day 8	1.67 ± 0.24	1.70 ± 0.30	1.34 ± 0.18	1.34 ± 0.28	1.05 ± 0.18*	0.78 ± 0.15**
Day 29	0.49 ± 0.11	0.44 ± 0.07	0.55 ± 0.07	0.48 ± 0.08	0.30 ± 0.07	1.06 ± 0.17**
Week 14	1.07 ± 0.20	1.06 ± 0.26	1.04 ± 0.15	0.91 ± 0.11	1.05 ± 0.13	1.16 ± 0.21
Triiodothyronine (ng/dL)						
Day 8	146.2 ± 3.2	135.6 ± 6.8	134.3 ± 5.3	135.0 ± 3.2	132.1 ± 4.7*	115.4 ± 4.5**
Day 29	138.7 ± 5.5	138.3 ± 6.7	140.9 ± 6.0	136.6 ± 4.5	128.9 ± 4.1	137.1 ± 9.0
Week 14	142.9 ± 3.3	141.1 ± 6.4	143.3 ± 9.3	131.6 ± 5.3	135.5 ± 6.3	125.4 ± 4.5**
Thyroxine ($\mu g/dL$)						
Day 8	3.41 ± 0.28	2.97 ± 0.41	2.46 ± 0.28	3.38 ± 0.22	3.80 ± 0.17	3.23 ± 0.23
Day 29	3.43 ± 0.40	2.70 ± 0.41	2.53 ± 0.31	2.22 ± 0.22	2.87 ± 0.27	2.57 ± 0.45
Week 14	2.60 ± 0.29	2.48 ± 0.30	2.87 ± 0.25	2.06 ± 0.15	2.49 ± 0.30	1.70 ± 0.15

TABLE 11
Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	7
Week 14	10	10	9	10	10	10
Clinical Chemistry (continued)						
Total protein (g/dL)						
Day 29	6.1 ± 0.1	6.1 ± 0.1	6.4 ± 0.1	6.2 ± 0.0	6.3 ± 0.4	5.8 ± 0.1
Week 14	6.8 ± 0.1	6.7 ± 0.1	6.2 ± 0.5	6.4 ± 0.1*	6.3 ± 0.1**	6.0 ± 0.1**
Albumin (g/dL)						
Day 29	4.7 ± 0.1[b]	4.7 ± 0.1	4.9 ± 0.1	4.8 ± 0.0	4.5 ± 0.1	4.4 ± 0.1*
Week 14	5.0 ± 0.0	5.1 ± 0.1	4.6 ± 0.3	4.8 ± 0.1	4.7 ± 0.1**	4.5 ± 0.1**
Alkaline phosphatase (IU/L)						
Day 29	336 ± 7	320 ± 5	356 ± 10	353 ± 12	342 ± 9	398 ± 10**
Week 14	212 ± 8	219 ± 9	231 ± 9	246 ± 5**	266 ± 10**	321 ± 11**
Sorbitol dehydrogenase (IU/L)						
Day 29	19 ± 2	18 ± 2	25 ± 2	20 ± 3	17 ± 2	21 ± 2
Week 14	21 ± 2	28 ± 3	27 ± 3	28 ± 4	26 ± 3	25 ± 2

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9

Similar to the 2-methylimidazole study, exposure to 4-methylimidazole induced decreases in total protein and albumin concentrations of 10,000 ppm males and females on day 29 and 5,000 and 10,000 ppm females at week 14. While alkaline phosphatase activities were decreased in the 2-methylimidazole study, 4-methylimidazole induced increases in alkaline phosphatase activities of 10,000 ppm males and females on day 29 and of males and females exposed to 2,500 ppm or greater at week 14. Additionally, bile acid concentrations were increased in 5,000 ppm females on day 29 and 10,000 ppm males at week 14. The changes in the biomarkers of hepatocellular injury also differed between the two studies. For the 2-methylimidazole study, decreases in alanine aminotransferase and sorbitol dehydrogenase activities occurred. However, for males exposed to 2,500 ppm or greater in the 4-methylimidazole study, sorbitol dehydrogenase and, less consistently, alanine aminotransferase activities increased slightly (greater than a 50% increase). For the markers of renal function, creatinine concentration decreased and urea nitrogen concentration was unaffected in the 2-methylimidazole study. However, for 4-methylimidazole, urea nitrogen concentrations were decreased in males exposed to 2,500 ppm or greater and females exposed to 5,000 or 10,000 ppm on day 29 and/or at

week 14; creatinine concentration was unaffected for exposed males and in exposed females demonstrated a minimal increase that was not exposure concentration-related and was not considered to be clinically or toxicologically relevant.

Organ weights are presented in Tables 12 and D4. The liver weights of males exposed to 2,500 ppm or greater were significantly greater those of the controls. In females, the absolute liver weight of the 10,000 ppm group was significantly less and the relative liver weights of the 5,000 and 10,000 ppm groups were significantly greater. The spleen weights of females exposed to 2,500 ppm or greater were significantly less than those of the controls. The absolute right testis weights of 5,000 and 10,000 ppm males and the relative right testis weight of 10,000 ppm males were significantly less. The absolute right kidney weight of 10,000 ppm males was significantly less and the relative right kidney weights of 5,000 and 10,000 ppm males were significantly greater. Other differences in organ weights of exposed animals were not considered to be biologically significant.

Sperm motility and vaginal cytology data are presented in Tables E3 and E4. In 1,250 ppm males, the spermatid heads per testis and mean spermatid count were significantly greater and the epididymal spermatozoal motility was significantly less than those of the controls. The epididymal spermatozoal concentrations of 1,250 and 5,000 ppm males were significantly greater than those of the controls. No significant differences occurred in vaginal cytology parameters between exposed and control females. The estrous cycle of 5,000 ppm rats appeared to be lengthened.

TABLE 12
Selected Organ Weight Data for Rats in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	8	8	8	8	8	7
Necropsy body wt[b]	352 ± 6	362 ± 8	353 ± 6	335 ± 4*	298 ± 4**	245 ± 4**
R. Kidney						
Absolute	1.296 ± 0.031	1.282 ± 0.027	1.332 ± 0.036	1.260 ± 0.023	1.218 ± 0.035	1.165 ± 0.026**
Relative	3.70 ± 0.05	3.56 ± 0.05	3.78 ± 0.06	3.82 ± 0.06	4.12 ± 0.09**	4.78 ± 0.06**
Liver						
Absolute	11.935 ± 0.448	12.569 ± 0.272	12.644 ± 0.301	13.919 ± 0.404**	18.811 ± 0.645**	16.823 ± 0.632**
Relative	33.96 ± 0.70	34.96 ± 0.61	35.96 ± 0.82	42.12 ± 1.07**	63.73 ± 2.28**	68.92 ± 1.70**
R. Testis						
Absolute	1.436 ± 0.047	1.477 ± 0.042	1.501 ± 0.023	1.461 ± 0.027	1.275 ± 0.042**	0.511 ± 0.027**
Relative	4.10 ± 0.14	4.11 ± 0.08	4.28 ± 0.10	4.42 ± 0.09	4.32 ± 0.13	2.10 ± 0.10**
Female						
n	8	8	7	8	8	10
Necropsy body wt	201 ± 3	207 ± 3	204 ± 2	198 ± 4	189 ± 6*	127 ± 5**
Liver						
Absolute	7.062 ± 0.256	7.702 ± 0.158	7.383 ± 0.200	6.987 ± 0.243	7.152 ± 0.298	6.038 ± 0.243**
Relative	35.37 ± 1.22	37.12 ± 0.64	36.07 ± 0.93	35.56 ± 0.76	38.55 ± 0.77*	47.54 ± 1.02**
Spleen						
Absolute	0.501 ± 0.019	0.519 ± 0.019	0.506 ± 0.015	0.443 ± 0.013*	0.436 ± 0.019**	0.292 ± 0.011**
Relative	2.51 ± 0.10	2.50 ± 0.07	2.47 ± 0.08	2.26 ± 0.05*	2.35 ± 0.04*	2.30 ± 0.03*

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] For body weights n=10

Gross lesions related to 4-methylimidazole exposure included pale livers in males administered 2,500 ppm or greater and small testes and uteri in 10,000 ppm male and female rats. Microscopic liver analysis identified a significant increase in the incidences of cytoplasmic hepatocyte vacuolization in males exposed to 2,500 ppm or greater and 10,000 ppm females compared to the controls (Tables 13, A3, and A4). The incidences of epididymal hypospermia and prostate gland inflammation were significantly increased in 10,000 ppm males. The incidences of prostate gland atrophy and testicular degeneration were significantly increased in 5,000 and 10,000 ppm males.

TABLE 13
Incidences of Selected Nonneoplastic Lesions in Rats in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm		625 ppm		1,250 ppm		2,500 ppm		5,000 ppm		10,000 ppm	
Male												
Liver[a]	10		—[c]		10		10		10		9	
Hepatocyte, Vacuolization Cytoplasmic[b]	1	(1.0)[d]	—		3	(1.0)	10**	(2.2)	10**	(3.0)	9**	(3.0)
Epididymis	10		—		—		—		—		10	
Hypospermia	0		—		—		—		—		9**	
Prostate Gland	10		1		10		10		10		10	
Atrophy	0		1	(1.0)	1	(1.0)	2	(1.0)	8**	(1.1)	8**	(1.9)
Inflammation	2	(1.5)	0		3	(1.0)	0		1	(2.0)	8*	(1.5)
Testes	10		1		10		10		10		10	
Degeneration	1	(2.0)	1	(1.0)	0		4	(1.0)	9**	(1.3)	9**	(3.1)
Female												
Liver	10		1		2		10		10		10	
Hepatocyte, Vacuolization Cytoplasmic	0		0		0		0		1	(1.0)	8**	(1.4)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Not examined at this exposure concentration
[d] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

MICE

15-DAY STUDIES

In the 2- and 4-methylimidazole studies, all male and female mice survived to the end of the studies (Tables 14 and 15). The mean body weight gains of males and females exposed to 10,000 ppm 2-methylimidazole were significantly less than those of the controls; the females lost weight during the study. In the 4-methylimidazole study, the mean body weight gains of exposed groups were similar to those of the controls. No significant differences in feed consumption or clinical findings were observed during the 2- or 4-methylimidazole studies. Dietary concentrations of 1,200, 3,300, or 10,000 ppm delivered daily doses of approximately 220, 640, or 2,100 mg/kg 2-methylimidazole to males and 300, 800, and 2,400 mg/kg to females. Dietary concentrations of 300, 800, or 2,500 ppm delivered daily doses of approximately 65, 170, or 560 mg/kg 4-methylimidazole to males and 60, 165, or 440 mg/kg to females.

TABLE 14
Survival, Body Weights, and Feed Consumption of Mice in the 15-Day Feed Study of 2-Methylimidazole

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	22.1 ± 0.4	24.8 ± 0.4	2.8 ± 0.3	—	4.7	4.2
1,200	5/5	22.0 ± 0.8	24.9 ± 1.0	2.9 ± 0.2	100	4.0	4.5
3,300	5/5	21.8 ± 0.6	24.3 ± 0.4	2.5 ± 0.2	98	4.1	4.8
10,000	5/5	22.1 ± 0.6	22.8 ± 0.5	0.7 ± 0.2**	92	4.9	4.8
Female							
0	5/5	19.1 ± 0.6	21.6 ± 0.5	2.5 ± 0.2	—	4.1	4.3
1,200	5/5	19.0 ± 0.3	21.4 ± 0.5	2.4 ± 0.3	100	4.1	5.5
3,300	5/5	18.5 ± 0.7	21.0 ± 0.8	2.5 ± 0.2	97	4.1	5.4
10,000	5/5	18.7 ± 0.6	18.5 ± 0.3**	–0.2 ± 0.3**	86	3.7	5.2

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 15 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.

TABLE 15
Survival, Body Weights, and Feed Consumption of Mice in the 15-Day Feed Study of 4-Methylimidazole

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	21.9 ± 1.4	24.6 ± 1.4	2.7 ± 0.2	—	4.7	5.4
300	5/5	21.6 ± 1.1	24.0 ± 0.8	2.3 ± 0.5	97	5.2	4.6
800	5/5	21.6 ± 1.1	24.2 ± 0.8	2.6 ± 0.5	98	4.7	4.9
2500	5/5	21.6 ± 0.8	23.2 ± 0.6	1.5 ± 0.4	94	5.2	4.9
Female							
0	5/5	18.2 ± 0.5	19.8 ± 0.4	1.6 ± 0.4	—	4.7	3.8
300	5/5	18.3 ± 0.8	19.7 ± 0.8	1.4 ± 0.2	100	3.4	4.3
800	5/5	17.8 ± 0.4	20.5 ± 0.5	2.6 ± 0.2*	103	3.9	3.9
2500	5/5	18.5 ± 0.4	19.7 ± 0.5	1.2 ± 0.2	100	3.4	3.3

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Number of animals surviving at 15 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.

2-Methylimidazole: There were no biologically significant differences between the organ weights of the exposed groups and the control groups (Table D5). The incidences of thyroid gland follicular cell hypertrophy were significantly increased in all exposed groups of males and females compared to the controls, and the severity increased with increasing exposure concentration (Table 16). This lesion was characterized by an increase in cytoplasm with increased cell height and eosinophilic vacuoles. The severities of splenic hematopoietic cell proliferation generally increased with increasing exposure concentration in males and females. This lesion was characterized by increased blood cell precursors in the red pulp.

TABLE 16
Incidences of Selected Nonneoplastic Lesions in Mice in the 15-Day Feed Study of 2-Methylimidazole

	0 ppm	1,200 ppm	3,300 ppm	10,000 ppm
Male				
Thyroid Gland[a]	5	5	5	5
Follicular Cell, Hypertrophy[b]	0	2* (1.0)[c]	5** (1.4)	5** (2.2)
Spleen	5	5	5	5
Hematopoietic Cell, Proliferation	3 (1.0)	5 (1.4)	5 (2.8)	5 (2.8)
Female				
Thyroid Gland	5	5	5	5
Follicular Cell, Hypertrophy	0	3* (1.0)	5** (1.4)	5** (2.8)
Spleen	5	5	5	5
Hematopoietic Cell, Proliferation	5 (1.8)	5 (2.2)	5 (2.6)	5 (2.8)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

4-Methylimidazole: No significant differences were observed between the organ weights of the exposed groups and the controls (Table D6). No exposure-related gross or microscopic lesions were observed during the study. There were no exposure-related clinical findings.

Exposure Concentration Selection Rationale: Based on the absence of effects on survival or body weights of exposed mice, the highest exposure concentration selected for the 14-week feed studies of 2- and 4-methylimidazole was 10,000 ppm.

14-WEEK STUDIES

All male and female mice survived to the end of the 2-methylimidazole study (Table 17). During the 4-methylimidazole study, one 10,000 ppm male during week 4 and seven 10,000 ppm females during weeks 1, 2, and 3 were found dead (Table 18). In the 2-methylimidazole study, the final mean body weights and body weight gains of 5,000 and 10,000 ppm males and females were significantly less than those of the controls (Table 17 and Figure 3). In the 4-methylimidazole study, the final mean body weights and body weight gains of males exposed to 1,250 ppm or greater and all exposed groups of females were significantly less than those of the controls (Table 18 and Figure 4). Dietary concentrations of 625, 1,250, 2,500, 5,000, or 10,000 ppm delivered daily doses of approximately 100, 165, 360, 780, or 1,740 mg/kg 2-methylimidazole to males and 90, 190, 400, 800, or 1,860 mg/kg to females and approximately 100, 240, 440, 915, or 1,840 mg/kg 4-methylimidazole to males and 110, 250, 540, 1,130, or 3,180 mg/kg to females. There were no significant clinical findings during the 2-methylimidazole study; findings in the 4-methylimidazole study included ruffled fur and dull coats in the 10,000 ppm females.

TABLE 17
Survival, Body Weights, and Feed Consumption of Mice in the 14-Week Feed Study of 2-Methylimidazole

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 14
Male							
0	10/10	25.2 ± 0.3	37.4 ± 0.5	12.2 ± 0.4	—	4.6	4.1
625	10/10	24.3 ± 0.4	35.4 ± 1.0	11.1 ± 0.8	95	5.1	4.3
1250	10/10	25.5 ± 0.5	36.6 ± 0.8	11.1 ± 0.6	98	4.0	4.2
2500	10/10	25.6 ± 0.5	35.9 ± 0.7	10.3 ± 0.6*	96	4.5	4.3
5000	10/10	25.4 ± 0.8	33.7 ± 1.1**	8.3 ± 0.5**	90	4.9	4.3
10000	10/10	25.2 ± 0.7	30.0 ± 0.6**	4.9 ± 0.4**	80	5.3	4.3
Female							
0	10/10	19.7 ± 0.6	32.0 ± 1.3	12.3 ± 0.8	—	4.1	3.3
625	10/10	19.6 ± 0.5	30.3 ± 0.9	10.7 ± 0.7	95	3.9	3.6
1250	10/10	19.3 ± 0.4	30.2 ± 0.7	11.0 ± 0.6	94	3.8	3.6
2500	10/10	19.7 ± 0.4	30.1 ± 1.1	10.5 ± 0.8	94	4.3	3.5
5000	10/10	19.5 ± 0.4	26.5 ± 0.6**	7.1 ± 0.4**	83	3.7	3.7
10000	10/10	19.4 ± 0.6	23.5 ± 0.6**	4.1 ± 0.3**	73	4.2	3.8

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.

TABLE 18
Survival, Body Weights, and Feed Consumption of Mice in the 14-Week Feed Study of 4-Methylimidazole

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 14
Male							
0	10/10	22.7 ± 0.4	35.3 ± 0.6	12.6 ± 0.5	—	4.4	4.3
625	10/10	22.5 ± 0.6	33.6 ± 0.9	11.1 ± 0.7	95	4.6	4.3
1250	10/10	22.2 ± 0.6	32.6 ± 1.1*	10.4 ± 0.8**	93	5.7	4.7
2500	10/10	23.9 ± 0.6	31.8 ± 0.4**	7.8 ± 0.3**	90	5.1	4.7
5000	10/10	22.9 ± 0.5	29.6 ± 0.5**	6.8 ± 0.5**	84	5.2	4.4
10000	9/10[d]	23.0 ± 0.4	28.0 ± 0.3**	5.1 ± 0.3**	79	5.4	4.0
Female							
0	10/10	18.3 ± 0.3	29.1 ± 1.1	10.8 ± 0.9	—	4.7	3.7
625	10/10	18.8 ± 0.4	26.3 ± 0.7*	7.5 ± 0.6**	90	4.2	3.8
1250	10/10	19.0 ± 0.4	25.7 ± 1.0**	6.7 ± 0.7**	88	4.5	4.6
2500	10/10	18.8 ± 0.5	23.4 ± 0.4**	4.6 ± 0.3**	80	4.4	4.7
5000	10/10	19.0 ± 0.3	22.5 ± 0.6**	3.5 ± 0.4**	77	4.9	4.5
10000	3/10[e]	18.0 ± 0.3	21.6 ± 0.3**	3.0 ± 0.4**	74	5.5	7.1

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Feed consumption is expressed as grams per animal per day.
[d] Week of death: 4
[e] Week of death: 1, 1, 1, 1, 2, 2, 3

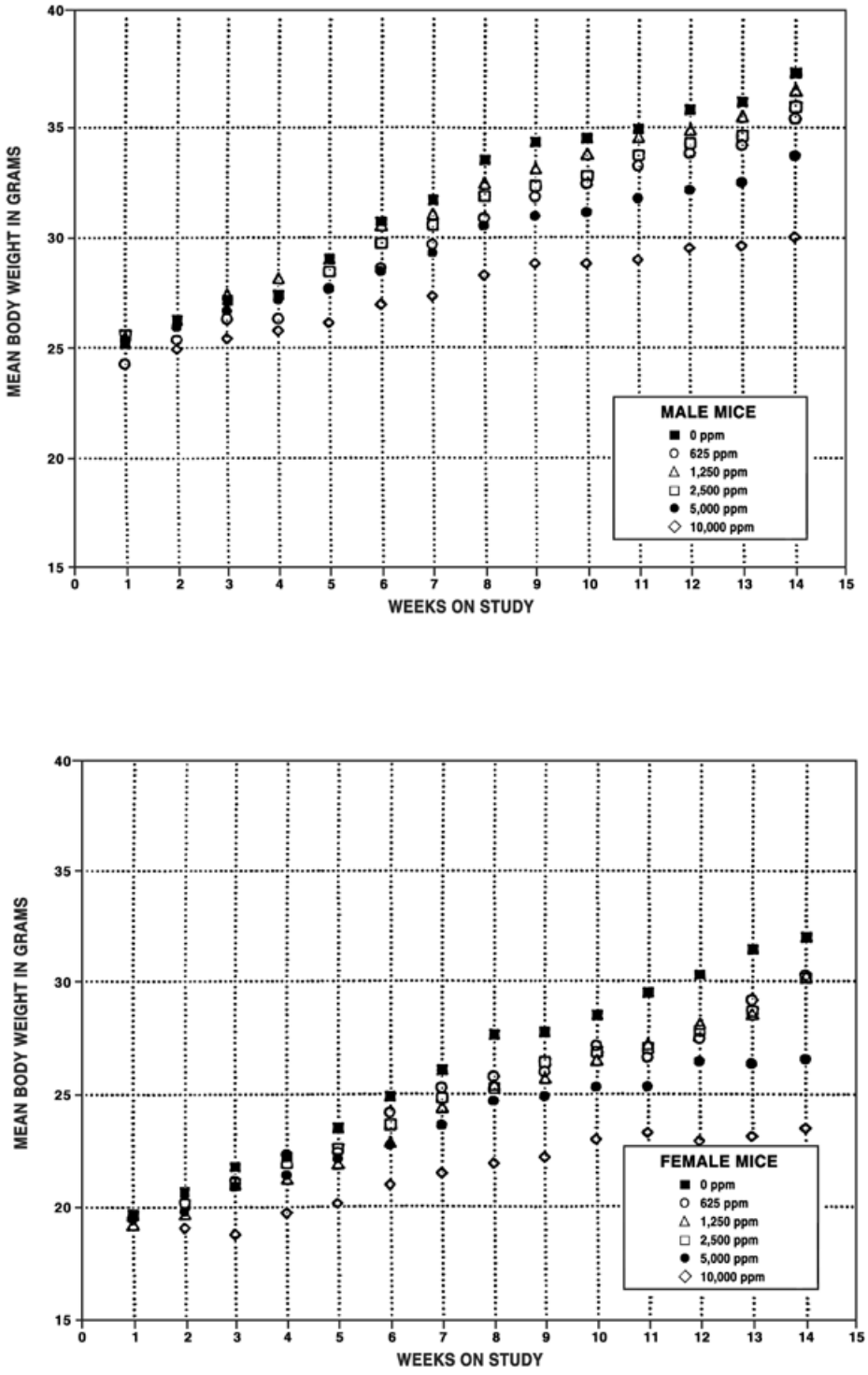


FIGURE 3
Body Weights of Mice Administered 2-Methylimidazole in Feed for 14 Weeks

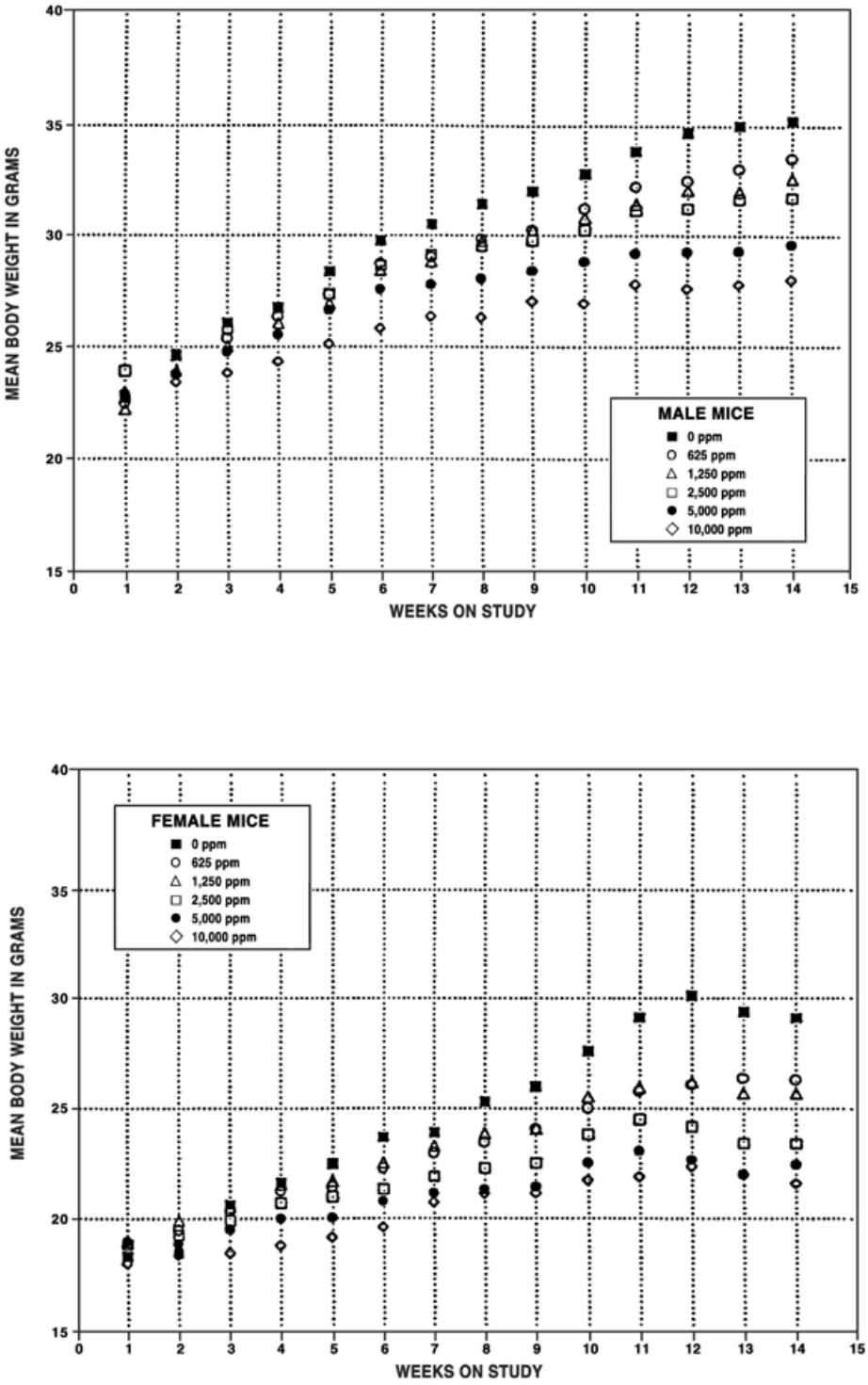


FIGURE 4
Body Weights of Mice Administered 4-Methylimidazole in Feed for 14 Weeks

2-Methylimidazole: Hematology and clinical chemistry data are listed in Tables 19 and C3. Administration of 2-methylimidazole induced a mild to moderate, exposure concentration-related, macrocytic anemia. The hyperchromic, responsive anemia was characterized by decreased automated and manual hematocrit values, hemoglobin concentrations, and erythrocyte counts in all exposed groups of males and in females exposed to 1,250 ppm or greater. In general, the erythron effects were more severe for males. A mild to marked increase in reticulocyte counts was consistent with an erythropoietic response, suggesting that the hematopoietic system was able to respond to the anemia. The increase in reticulocyte counts would account for the macrocytosis and was accompanied by increases in mean cell volumes and mean cell hemoglobin values. The hyperchromia was demonstrated by increased mean cell hemoglobin concentrations and would be consistent with a hemolytic (intra- or extravascular) process in exposed mice. Also, platelet counts were mildly to moderately increased in males and females exposed to 2,500 ppm or greater.

TABLE 19
Selected Hematology and Clinical Chemistry Data for Mice in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Male						
Hematology						
Automated hematocrit (%)	49.2 ± 0.9	46.9 ± 0.7*	41.9 ± 1.2**	36.9 ± 0.4**	34.1 ± 0.6**	34.6 ± 0.7**
Manual hematocrit (%)	50.4 ± 0.7	49.2 ± 0.3	44.8 ± 1.1**	39.9 ± 0.4**	37.2 ± 0.9**	37.5 ± 0.8**
Hemoglobin (g/dL)	15.6 ± 0.2	15.2 ± 0.1*	14.1 ± 0.4**	12.5 ± 0.1**	11.8 ± 0.2**	12.0 ± 0.2**
Erythrocytes ($10^6/\mu L$)	9.58 ± 0.15	9.04 ± 0.11*	8.02 ± 0.23**	6.83 ± 0.07**	5.97 ± 0.13**	5.90 ± 0.15**
Reticulocytes ($10^6/\mu L$)	0.17 ± 0.01	0.17 ± 0.02	0.31 ± 0.03**	0.52 ± 0.04**	0.75 ± 0.08**	0.91 ± 0.04**
Mean cell volume (fL)	51.3 ± 0.4	51.9 ± 0.3	52.3 ± 0.1*	54.0 ± 0.2**	57.1 ± 0.3**	58.7 ± 0.4**
Mean cell hemoglobin (pg)	16.3 ± 0.2	16.8 ± 0.2	17.7 ± 0.2**	18.3 ± 0.1**	19.8 ± 0.2**	20.4 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	31.9 ± 0.3	32.4 ± 0.3	33.7 ± 0.3**	34.0 ± 0.1**	34.6 ± 0.3**	34.8 ± 0.4**
Platelets ($10^3/\mu L$)	901.9 ± 30.8	934.1 ± 19.4	948.0 ± 31.1	1,183.5 ± 20.0**	1,243.5 ± 55.4**	1,247.7 ± 61.8**
Clinical Chemistry						
Triiodothyronine (ng/dL)						
Day 8	144.1 ± 2.3[b]	161.3 ± 6.2[c]	159.0 ± 5.9[d]	163.4 ± 3.9*	168.9 ± 4.0**	155.6 ± 3.1[d]
Day 29	149.3 ± 3.3[d]	163.9 ± 4.8*	173.7 ± 7.6**	164.9 ± 3.5*	172.9 ± 3.8**	178.0 ± 4.3**[c]
Day 86	—[e]	—	—	—	—	—
Thyroxine (μg/dL)						
Day 8	6.89 ± 0.21	6.19 ± 0.24[f]	6.39 ± 0.16	6.57 ± 0.15	6.98 ± 0.14	6.09 ± 0.23
Day 29	6.49 ± 0.30	6.33 ± 0.15	6.31 ± 0.19	6.48 ± 0.19	7.38 ± 0.14*	6.78 ± 0.25
Day 86	5.32 ± 0.22	4.77 ± 0.24	4.73 ± 0.11	5.13 ± 0.15[f]	6.18 ± 0.15*	5.81 ± 0.22

TABLE 19
Selected Hematology and Clinical Chemistry Data for Mice in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Female						
Hematology						
Automated hematocrit (%)	48.7 ± 0.9	47.9 ± 0.6	46.7 ± 0.6	43.8 ± 0.7**	40.4 ± 0.4**	41.5 ± 0.7**
Manual hematocrit (%)	50.3 ± 0.5	50.0 ± 0.4	48.6 ± 0.5*	46.3 ± 0.7**	43.0 ± 0.4**	43.4 ±0.6**
Hemoglobin (g/dL)	15.9 ± 0.2	15.6 ± 0.1	15.3 ± 0.1**	14.6 ± 0.1**[c]	13.8 ± 0.1**	14.1 ± 0.2**
Erythrocytes ($10^6/\mu L$)	9.61 ± 0.16	9.29 ± 0.13	9.02 ± 0.09**	8.27 ± 0.13**	7.39 ± 0.10**	7.53 ± 0.15**
Reticulocytes ($10^6/\mu L$)	0.15 ± 0.01	0.17 ± 0.02	0.21 ± 0.03	0.31 ± 0.03**	0.54 ± 0.04**	0.44 ± 0.03**
Mean cell volume (fL)	50.7 ± 0.4	51.5 ± 0.2	51.8 ± 0.3	53.0 ± 0.2**	54.6 ± 0.3**	55.1 ± 0.2**
Mean cell hemoglobin (pg)	16.6 ± 0.2	16.7 ± 0.2	16.9 ± 0.1*	17.7 ± 0.2**[c]	18.7 ± 0.1**	18.7 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.5	32.5 ± 0.3	32.7 ± 0.2	33.4 ± 0.3[c]	34.3 ± 0.1**	33.9 ± 0.1**
Platelets ($10^3/\mu L$)	846.6 ± 28.3	801.6 ± 20.3	871.5 ± 26.2	943.7 ± 25.5*	1,042.5 ± 28.1**	1,038.7 ± 31.4**
Clinical Chemistry						
Triiodothyronine (ng/dL)						
Day 8	138.4 ± 3.2	140.7 ± 4.0[c]	147.1 ± 2.5	145.8 ± 1.7	144.4 ± 3.3	126.3 ± 2.7[d]
Day 29	118.1 ± 2.7	124.8 ± 3.7	121.6 ± 3.3	139.6 ± 4.7**	140.1 ± 3.6**	131.6 ± 3.4**
Day 86	132.4 ± 2.4[d]	130.1 ± 3.0	141.8 ± 2.4*[c]	136.1 ± 2.7[c]	149.5 ± 4.0**	155.6 ± 8.3**
Thyroxine (μg/dL)						
Day 8	7.55 ± 0.36	7.71 ± 0.28	7.11 ± 0.31	7.33 ± 0.19	6.87 ± 0.20	5.56 ± 0.16**
Day 29	6.36 ± 0.25	6.05 ± 0.19	5.54 ± 0.30	5.91 ± 0.23	6.21 ± 0.27	4.94 ± 0.19**
Day 86	7.04 ± 0.34	6.36 ± 0.26	6.05 ± 0.21*	5.44 ± 0.23**	6.00 ± 0.16**	4.79 ± 0.13**

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=7
[c] n=9
[d] n=8
[e] The assay was unacceptable due to instrumentation/reagent problems.
[f] n=10

The thyroid gland hormone data demonstrated an effect on thyroxine and triiodothyronine concentrations. On day 8, thyroxine concentration was decreased in 10,000 ppm females; the decreases persisted and, on day 86, occurred in females exposed to 1,250 ppm or greater. In contrast, triiodothyronine concentrations were increased in these groups. On day 29, increases in triiodothyronine concentrations occurred in all exposed groups of males and in females exposed to 2,500 ppm or greater. On day 86, triiodothyronine concentrations were increased in females exposed to 1,250, 5,000, or 10,000 ppm.

Organ weights are presented in Tables 20 and D7. The absolute heart weights of males exposed to 2,500 ppm or greater and the relative heart weights of all exposed groups of males were significantly greater than those of the controls. The liver weights of all exposed groups of males were generally significantly greater. In females, the spleen weights of groups exposed to 2,500 ppm or greater were significantly greater.

TABLE 20
Selected Organ Weight Data for Mice in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	37.4 ± 0.5	35.4 ± 1.0	36.6 ± 0.8	35.9 ± 0.7	33.7 ± 1.1**	30.0 ± 0.6**
Heart						
Absolute	0.155 ± 0.003	0.161 ± 0.003	0.164 ± 0.003	0.176 ± 0.003**	0.171 ± 0.006**	0.166 ± 0.002**
Relative	4.15 ± 0.10	4.54 ± 0.06**	4.48 ± 0.09**	4.90 ± 0.09**	5.06 ± 0.09**	5.55 ± 0.11**
Liver						
Absolute	1.611 ± 0.021	1.658 ± 0.044	1.845 ± 0.041**	1.947 ± 0.033**	2.031 ± 0.085**	1.935 ± 0.054**
Relative	43.09 ± 0.50	46.90 ± 0.78*	50.41 ± 0.76**	54.26 ± 0.57**	60.15 ± 1.13**	64.56 ± 1.87**
Female						
Necropsy body wt	32.0 ± 1.3	30.3 ± 0.9	30.2 ± 0.7	30.1 ± 1.1	26.5 ± 0.6**	23.5 ± 0.6**
Spleen						
Absolute	0.086 ± 0.005	0.086 ± 0.001	0.094 ± 0.003	0.125 ± 0.009**	0.185 ± 0.006**	0.162 ± 0.007**
Relative	2.71 ± 0.15	2.87 ± 0.08	3.11 ± 0.14	4.22 ± 0.38**	7.00 ± 0.24**	6.90 ± 0.32**

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

No significant differences were found in sperm motility or vaginal cytology parameters between exposed and control mice (Tables E5 and E6).

Gross lesions related to 2-methylimidazole exposure included enlarged and/or darkened thyroid gland and enlarged spleen in 5,000 and 10,000 ppm males and females. Microscopic examination indicated significantly increased incidences of hematopoietic cell proliferation of the spleen in males exposed to 1,250 ppm or greater and females exposed to 2,500 ppm or greater (Tables 21, B1, and B2). The incidences of hemosiderin pigmentation in the renal tubule of males exposed to 1,250 ppm or greater and females exposed to 5,000 or 10,000 ppm were significantly increased. The incidences of thyroid gland follicular cell hypertrophy were significantly increased in males and females exposed to 2,500 ppm or greater. The severities of these lesions generally increased with increasing exposure concentration.

TABLE 21
Incidences of Selected Nonneoplastic Lesions in Mice in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
Spleen[a]	10	10	10	10	10	10
Hematopoietic Cell Proliferation[b]	0	1 (1.0)[c]	10** (1.3)	10** (2.3)	10** (2.6)	10** (3.6)
Kidney	10	10	10	10	10	10
Renal Tubule, Pigmentation, Hemosiderin	0	0	10** (1.0)	10** (2.3)	10** (2.7)	10** (3.0)
Thyroid Gland	10	9	10	9	10	10
Follicular Cell, Hypertrophy	0	0	0	9** (1.0)	10** (1.9)	10** (2.8)
Female						
Spleen	10	10	10	10	10	10
Hematopoietic Cell Proliferation	2 (1.0)	1 (1.0)	1 (2.0)	10** (1.3)	10** (2.4)	10** (2.8)
Kidney	10	10	10	10	10	10
Renal Tubule, Pigmentation, Hemosiderin	0	0	0	3 (1.0)	9** (1.4)	10** (1.5)
Thyroid Gland	10	9	8	9	10	10
Follicular Cell, Hypertrophy	0	0	0	7** (1.0)	10** (1.7)	10** (2.4)

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

4-Methylimidazole: Hematology and clinical chemistry data are presented in Tables 22 and C4. As in the 2-methylimidazole study, the hematology data for mice in the 14-week study of 4-methylimidazole demonstrated erythron effects. However, there were qualitative and quantitative differences between the two studies. While administration of 2-methylimidazole induced a mild to moderate, macrocytic, hyperchromic, responsive anemia in males and females, administration of 4-methylimidazole resulted in minimal erythron decreases only in exposed females. The erythron alteration was evidenced by decreased automated and manual hematocrit values and hemoglobin concentrations; erythrocyte counts were unaffected. Additionally, for affected females, there was a lack of an erythropoietic response, demonstrated by the absence of change in the reticulocyte counts; the only change for the erythrocyte indices was a minimally decreased mean cell volume in the 10,000 ppm group.

TABLE 22
Selected Hematology and Clinical Chemistry Data for Mice in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Male						
Hematology						
Automated hematocrit (%)	48.4 ± 0.8	47.0 ± 0.3	47.1 ± 0.5	47.9 ± 0.5	46.7 ± 1.0	46.8 ± 0.9
Manual hematocrit (%)	49.1 ± 0.6[b]	48.3 ± 0.3[b]	47.3 ± 0.5	48.9 ± 0.6[c]	48.8 ± 0.6[b]	47.4 ± 0.4[d]
Hemoglobin (g/dL)	15.4 ± 0.2	15.1 ± 0.1	15.1 ± 0.2	15.5 ± 0.1	15.0 ± 0.2	15.2 ± 0.1
Erythrocytes ($10^6/\mu$L)	9.56 ± 0.16	9.19 ± 0.09	9.37 ± 0.12	9.60 ± 0.13	9.26 ± 0.20	9.57 ± 0.16
Clinical Chemistry						
Triiodothyronine (ng/dL)						
Day 8	137.0 ± 5.4[e]	139.8 ± 3.5[f]	132.8 ± 3.7[f]	140.3 ± 3.8[g]	125.5 ± 2.5[h]	137.7 ± 5.8[g]
Day 29	142.3 ± 6.1[b]	152.5 ± 5.2[b]	141.1 ± 6.0[b]	148.0 ± 5.9[f]	163.8 ± 7.6[f]	168.0 ± 8.0[f]
Day 86	128.8 ± 3.7	130.3 ± 4.4[b]	133.4 ± 2.4	137.6 ± 4.9[b]	148.2 ± 3.8**[d]	176.7 ± 6.6**[f]
Thyroxine (μg/dL)						
Day 8	5.93 ± 0.24	6.14 ± 0.22	6.15 ± 0.27	5.55 ± 0.25	4.95 ± 0.15**	4.70 ± 0.18**[i]
Day 29	5.95 ± 0.22	6.18 ± 0.21	6.03 ± 0.14	5.57 ± 0.23	4.97 ± 0.12**	3.70 ± 0.09**
Day 86	4.47 ± 0.17	4.62 ± 0.25	4.67 ± 0.14	4.72 ± 0.09	4.62 ± 0.21	3.98 ± 0.18

TABLE 22
Selected Hematology and Clinical Chemistry Data for Mice in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Female						
Hematology						
Automated hematocrit (%)	47.9 ± 0.4	46.5 ± 0.3*	46.0 ± 0.3**	45.3 ± 0.6**	45.7 ± 0.3**	45.0 ± 0.7**
Manual hematocrit (%)	49.4 ± 0.3[b]	48.3 ± 0.5[b]	48.1 ± 0.3*[b]	47.6 ± 0.6*[c]	48.0 ± 0.4*	45.0[j]
Hemoglobin (g/dL)	15.7 ± 0.1	15.2 ± 0.1**	15.2 ± 0.1**	14.9 ± 0.1**	14.9 ± 0.1**	14.8 ± 0.2**
Erythrocytes ($10^6/\mu$L)	9.51 ± 0.12	9.25 ± 0.07	9.22 ± 0.08	9.15 ± 0.14	9.17 ± 0.09	9.32 ± 0.20
Clinical Chemistry						
Triiodothyronine (ng/dL)						
Day 8	139.0 ± 6.4[f]	134.3 ± 3.9[f]	124.0 ± 2.3[c]	132.8 ± 3.1[i]	136.0 ± 11.0[g]	—[k]
Day 29	130.8 ± 3.5	132.5 ± 5.0[b]	130.8 ± 3.0	140.9 ± 6.7[c]	150.8 ± 5.1**[b]	148.5 ± 7.5[h]
Day 86	128.1 ± 4.9[c]	116.3 ± 3.4[i]	131.0 ± 3.8	149.7 ± 8.5[g]	141.0 ± 8.1[d]	—
Thyroxine (μg/dL)						
Day 8	7.14 ± 0.41	6.99 ± 0.31	7.30 ± 0.31	7.78 ± 0.31	7.19 ± 0.42	6.56 ± 0.42[i]
Day 29	6.98 ± 0.15	6.76 ± 0.15	7.19 ± 0.18	6.80 ± 0.18	7.51 ± 0.35	5.56 ± 0.14*[d]
Day 86	6.95 ± 0.40	6.45 ± 0.16	6.53 ± 0.22	6.91 ± 0.22	5.90 ± 0.28	5.25 ± 0.55[h]

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=8
[c] n=7
[d] n=5
[e] n=4
[f] n=6
[g] n=3
[h] n=2
[i] n=9
[j] n=1; no standard error calculated
[k] Not analyzed

The thyroid gland hormone data for the 4-methylimidazole study demonstrated effects similar to but less consistent than those in the 2-methylimidazole study. On day 8, thyroxine concentrations were decreased in 5,000 and 10,000 ppm males. By day 29, thyroxine concentrations were decreased in 5,000 ppm males and 10,000 ppm males and females. The effect was, however, transient and had abrogated by day 86. On day 29, triiodothyronine concentration was increased in 5,000 ppm females; on day 86, triiodothyronine concentrations were increased in 5,000 and 10,000 ppm males.

Organ weights are presented in Tables 23 and D8. The relative liver weights of all exposed groups of males and the relative right testis weights of males exposed to 2,500 ppm or greater were significantly greater than those of the control group. In females, the absolute heart, right kidney, and liver weights of the 5,000 and 10,000 ppm groups and absolute liver weight of 2,500 ppm females were significantly less. However, the relative heart and right kidney weights of the females exposed to 2,500 ppm or greater and the relative liver weight of 10,000 ppm females were significantly greater. Other differences in organ weights of exposed animals were not considered to be biologically significant.

TABLE 23
Selected Organ Weight Data for Mice in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	10	10	10	10	10	9
Necropsy body wt	35.3 ± 0.6	33.6 ± 0.9	32.6 ± 1.1*	31.8 ± 0.4**	29.6 ± 0.5**	28.0 ± 0.3**
Liver						
Absolute	1.568 ± 0.034[b]	1.581 ± 0.050	1.558 ± 0.052	1.568 ± 0.035	1.449 ± 0.047	1.427 ± 0.032*
Relative	44.20 ± 0.62[b]	47.10 ± 0.65**	47.75 ± 0.48**	49.33 ± 0.72**	48.85 ± 0.94**	50.89 ± 0.70**
R. Testis						
Absolute	0.124 ± 0.003	0.116 ± 0.003	0.121 ± 0.003	0.126 ± 0.002	0.120 ± 0.002	0.113 ± 0.002*
Relative	3.51 ± 0.11	3.47 ± 0.12	3.72 ± 0.09	3.95 ± 0.05**	4.05 ± 0.07**	4.02 ± 0.09**
Female						
n	10	10	10	10	10	3
Necropsy body wt	29.1 ± 1.1	26.3 ± 0.7*	25.7 ± 1.0**	23.4 ± 0.4**	22.5 ± 0.6**	21.6 ± 0.3**
Heart						
Absolute	0.128 ± 0.003	0.125 ± 0.003	0.120 ± 0.002	0.121 ± 0.003	0.109 ± 0.002**	0.105 ± 0.003**
Relative	4.42 ± 0.14	4.77 ± 0.10	4.73 ± 0.17	5.16 ± 0.08**	4.86 ± 0.09**	4.85 ± 0.19*
R. Kidney						
Absolute	0.191 ± 0.005	0.189 ± 0.003	0.181 ± 0.003	0.190 ± 0.008	0.168 ± 0.003**	0.166 ± 0.004*
Relative	6.60 ± 0.21	7.19 ± 0.11	7.13 ± 0.24	8.09 ± 0.27**	7.49 ± 0.12**	7.69 ± 0.28**
Liver						
Absolute	1.154 ± 0.031	1.166 ± 0.042	1.114 ± 0.031	1.042 ± 0.028*	0.932 ± 0.036**	1.011 ± 0.026**
Relative	40.03 ± 1.51	44.32 ± 1.07*	43.65 ± 1.29	44.47 ± 0.76*	41.42 ± 0.64	46.87 ± 1.78*

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** Significantly different (P≤0.01) from the control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

Sperm motility and vaginal cytology parameters are presented in Tables E7 and E8. No significant differences occurred in sperm motility or vaginal cytology parameters between exposed and control groups.

No exposure-related gross or microscopic lesions were identified in male mice (Table B3). In females, the significantly decreased incidence of periportal cytoplasmic vacuolization of the liver in the 10,000 ppm group (0 ppm, 6/10; 625 ppm, 9/9; 1,250 ppm, 5/6; 2,500 ppm, 5/10; 5,000 ppm, 3/10; 10,000 ppm, 0/10; Table B4) was considered to be secondary to glycogen depletion and the poor nutritional status of this group.

GENETIC TOXICOLOGY

2-Methylimidazole (100 to 10,000 μg/plate) was negative in the *Salmonella typhimurium* gene mutation assay when tested in strains TA97, TA98, TA100, and TA1535, with and without S9 metabolic activation enzymes (Table F1). The chemical was also tested in three *in vivo* assays for induction of chromosomal damage as measured by micronucleated erythrocyte frequency, and the results were mixed (Tables F3, F5, and F7). 2-Methylimidazole, administered to male mice by intraperitoneal injection three times at 24-hour intervals, produced small increases in the frequency of micronucleated polychromatic erythrocytes in bone marrow, but these increases were not significant and the results of the assay were concluded to be negative. Results of a three-injection bone marrow micronucleus test in male rats were also negative. In contrast to the results obtained in these two short-term exposure studies, results of the 14-week 2-methylimidazole study in mice showed significant exposure-related increases in the frequencies of micronucleated normochromatic erythrocytes in peripheral blood samples of males and females. The increases in frequencies of micronuclei noted in female mice were greater than those observed in male mice (the three highest doses tested in females induced micronucleated frequencies that were significantly elevated above the control frequency), but the overall magnitude of the responses in males and females was similar.

4-Methylimidazole was tested by two laboratories for induction of gene mutations in *S. typhimurium* strains TA97, TA98, TA100, and TA1535 with and without S9 activation enzymes (Table F2); negative results were obtained in both studies. The chemical was also tested in three *in vivo* assays for induction of chromosomal damage, as measured by micronucleated erythrocyte frequency, and results of these tests were negative as well (Tables F4, F6, and F8). 4-Methylimidazole, administered to male mice by intraperitoneal injection three times at 24 hour intervals, produced significant increases in the frequency of micronucleated polychromatic erythrocytes in bone marrow in the first of two trials. The second trial showed no evidence of micronucleus induction over the same dose range used in the first trial, and the mouse bone marrow micronucleus assay was concluded to be negative overall. Results of a three-injection bone marrow micronucleus test with

4-methylimidazole in male rats were also negative. In addition, no significant increases were seen in the frequencies of micronucleated normochromatic erythrocytes in peripheral blood samples of male and female mice in the 14-week 4-methylimidazole study.

DISCUSSION

Nishie *et al.* (1969) reported that 2- and 4-methylimidazole administered orally induced convulsion in the male albino mouse. The convulsion doses (CD_{50}) were 1,300 mg/kg for 2-methylimidazole and 360 mg/kg for 4-methylimidazole. In the current studies, no convulsions were observed in $B6C3F_1$ mice exposed to 2- or 4-methylimidazole in feed at concentrations up to 10,000 ppm (equivalent to 560 ng/kg 2- or 4-methylimidazole). The different results were probably due to differences in method of administration (bolus versus feed) and in mouse strains. The current 14-week studies demonstrated that 10,000 ppm 4-methylimidazole, but not 2-methylimidazole, induced tremors and ataxia in F344/N rats. Based on clinical findings, rats seemed to be more sensitive to the neurobehavioral effects of 4-methylimidazole than mice.

2-Methylimidazole induced thyroid gland follicular cell hyperplasia in rats and thyroid gland follicular cell hypertrophy in mice after 15 days of exposure. Thyroid gland follicular cell adenoma was observed in male rats after 14 weeks of exposure. It is not clear if the chemical interacts directly with cellular macromolecules in the thyroid gland, although radiolabel was concentrated actively in the rat thyroid gland 24 hours after intravenous administration of ^{14}C-labeled 2-methylimidazole (Sanders *et al.*, 1998). In the 14-week studies, 2-methylimidazole increased thyroid-stimulating hormone concentrations and decreased thyroxine and triiodothyronine concentrations in male and female rats in an exposure concentration-related manner. In the 14-week mouse study, the effects of 2-methylimidazole on thyroxine and triiodothyronine concentrations were not as clear; however, it appeared that triiodothyronine concentrations were increased in male and female mice, and thyroxine concentrations were decreased in males and females. Thyroid-stimulating hormone concentrations in mice were not affected by 2-methylimidazole. The effects of 2-methylimidazole on thyroid gland hormone synthesis (i.e., uptake of inorganic iodine), oxidation of iodide and iodinition of tyrosyl groups of thyroglobulin, and conversion of iodotyrosyl radicals to iodothyronyl radicals (Paynter *et al.*, 1988; McClain, 1992) are unknown.

Results of the current studies indicate that the effect of 2-methylimidazole on the thyroid gland is indirect. Normally, thyroxine is glucuronidated by liver uridine diphosphate glucuronosyl-transferase and excreted (Atterwill *et al.*, 1992). The decreased thyroxine concentrations observed in the current studies suggest that 2-methylimidazole elevated hepatic uridine diphosphate glucuronosyltransferase activity. The resulting decrease in thyroxine concentration caused an increase in thyroid-stimulating hormone secretion, which in turn stimulated

the development of thyroid gland follicular cell hyperplasia/hypertrophy and, eventually, neoplasms. Evidence of elevated hepatic uridine diphosphate glucuronosyltransferase activity in rats administered 2-methylimidazole has been demonstrated by Sanders *et al.* (1998). Thyroxine is also converted to triiodothyronine by a microsomal 5′-monodeiodinase in peripheral tissues and is sulfated before being excreted (Thomas and Williams, 1991). A decrease in triiodothyronine concentration also causes a compensatory increase in thyroid-stimulating hormone concentration (McClain, 1992). The effects of 2-methylimidazole on 5′-monodeiodinase and triiodothyronine sulfation have not been investigated.

The thyroxine data also suggested that elevated hepatic uridine diphosphate glucuronosyltransferase activity may have returned to normal levels by day 92 in male rats exposed to 2-methylimidazole, but the activity took longer to return to normal in female rats. However, thyroid-stimulating hormone concentrations remained increased in male and female rats by day 92, and this hormone continued to stimulate the thyroid gland follicular cells. It is interesting to note that pituitary gland pars distalis hypertrophy was observed in rats exposed to 2-methylimidazole for 15 days but not for 14 weeks, even though thyroid-stimulating hormone concentrations remained increased throughout the 14-week studies.

It is not clear if hepatic uridine diphosphate glucuronosyltransferase is the primary site of 2-methylimidazole action. Because a neurotoxic effect of 2-methylimidazole has been described in calves of nursing cows fed ammoniated hay (Weiss *et al.*, 1986), in cattle fed roughages treated with anhydrous ammonia (Perdok and Leng, 1987), and in mice after gavage administration of 2-methylimidazole (Nishie *et al.*, 1969), a neuroendocrine effect of 2-methylimidazole on the thyroid gland cannot be ruled out. Support for this pathway was provided by Sanders *et al.* (1998), who reported that radiolabel was located in the rat brain 24 hours after intravenous administration of ^{14}C-labeled 2-methylimidazole. Although the location of the radiolabel in the brain was not identified in the report, 2-methylimidazole may act on the hypothalamus to stimulate pituitary gland thyroid-stimulating hormone production.

In mice exposed to 2-methylimidazole, a mechanism of action involving higher glucuronidation of thyroxine and stimulation of thyroid-stimulating hormone, similar to that in rats, appeared likely, although hepatic uridine diphosphate glucuronosyltransferase activities were not determined. The data seemed to indicate that mice are less sensitive to the 2-methylimidazole effects on thyroid-stimulating hormone and thyroxine concentrations than rats, as the effects were less apparent in mice than in rats.

4-Methylimidazole did not cause a consistent pattern of alteration in serum thyroid-stimulating hormone, triiodothyronine, and thyroxine concentrations in rats or mice as did 2-methylimidazole. 4-Methylimidazole apparently has an effect on specific liver cytochrome P450 isoenzymes opposite to that of 2-methylimidazole. Back and Tjia (1985) reported that 4-methylimidazole inhibited P450 activity in rat liver and 2-methylimidazole did not. Hargreaves *et al.* (1994) reported that 4-methylimidazole was a strong inhibitor of rat liver P4502E1 whereas 2-methylimidazole was not.

In addition to having an effect on the thyroid gland, 2-methylimidazole exposure affected the circulating erythroid mass. 2-Methylimidazole induced a mild to moderate, exposure concentration-related, macrocytic, hyperchromic, responsive anemia in mice, as evidenced by decreased erythrocyte counts, hemoglobin concentrations, and hematocrit values and increased incidences of splenic hematopoietic cell proliferation and renal tubule hemosiderin accumulation. In rats, the effect of 2-methylimidazole on erythrocyte counts was less severe, suggesting a minimal, exposure concentration-related, microcytic, normochromic, nonresponsive anemia. 2-Methylimidazole also induced a general decrease in serum enzyme activities and total protein and albumin concentrations. 4-Methylimidazole also had an effect on erythropoiesis, serum enzyme activities, and protein concentrations. In rats and mice, microcytic characteristics of the erythrocytes were noted, although no associated abnormalities were observed in bone marrow cells. 4-Methylimidazole induced decreases in total protein and albumin concentrations. The thyroid gland effect could account for the anemia observed (Beamer *et al.*, 1981; Chu *et al.*, 1981; Hill *et al.*, 1989; Beard *et al.*, 1998). Additionally, the food consumption data suggests that animals receiving 2- or 4-methylimidazole dosed feed consumed less. It is possible that an altered nutritional status may have contributed to some of the changes observed, for example, decreases in serum enzyme activities and/or protein concentrations (Kaneko, 1989; Imai *et al.*, 1991; Jenkins and Robinson, 1975).

In rats and female mice, 4-methylimidazole induced hepatocytic vacuolization, indicating lipid accumulation and hepatic injury. The hepatic effect is consistent with increases in alanine aminotransferase, sorbitol dehydrogenase, and alkaline phosphatase activities and bile acid concentrations. 4-Methylimidazole also induced degeneration of the seminiferous tubules of the testes and atrophy of the prostate gland in male rats. Similar effects on the hepatocytes, testis, and prostate gland were not observed in animals exposed to 2-methylimidazole. MacKenzie *et al.* (1992) administered caramel IV which contained 4-methylimidazole (110 mg/kg) in drinking water to male and female F344/N rats and B6C3F$_1$ mice at doses up to 10 g/kg for 2 years and reported no effects on histopathology. The doses of 4-methylimidazole in the study by MacKenzie *et al.* (1992) were lower than those in the current study.

2-Methylimidazole and 4-methylimidazole appeared to exert no significant reproductive toxicity in rats. The depressed reproductive organ weights of male rats and the slight increase in estrous cycle in female rats in the 10,000 ppm 2-methylimidazole and the 5,000 ppm 4-methylimidazole groups were probably related to reduced body weights. However, Adams *et al*. (1998) reported that a high dose of 2-methylimidazole and 4-methylimidazole (50 to 100 mg/kg) injected subcutaneously to male Sprague-Dawley rats caused decreases in lutinizing hormone secretion. 4-Methylimidazole also caused decreases in testosterone secretion and testicular interstitial fluid formation in a dose-dependent manner.

The majority of mutagenicity tests with 2- and 4-methylimidazole gave negative results. Neither chemical induced mutations in *Salmonella typhimurium* tester strains, with or without S9 activation, and neither induced significant increases in the number of micronucleated in polychromatic erythrocytes in bone marrow of rats or mice in short-term assays. 4-Methylimidazole was also negative in a 14-week peripheral blood micronucleus test in mice. In contrast, 2-methylimidazole induced significant concentration-related increases in the frequencies of micronucleated normochromatic erythrocytes in peripheral blood of male and female mice exposed for 14 weeks. In the acute mouse bone marrow micronucleus test with 2-methylimidazole, small increases in induction of micronucleated polychromatic erythrocytes were seen at all doses tested, although these results were not statistically significant. Together, the results of these two studies may indicate that chromosomal damage, either structural or numerical in nature, may be induced by 2-methylimidazole, and the amount of damage increases with increasing duration of exposure, perhaps as a consequence of metabolic pathway overloading. It is also possible that metabolism of 2-methylimidazole to the proximate genotoxin requires time, and thus, the resultant damage from exposure to the parent compound is not observed after short-term exposure but is clearly demonstrated after longer exposures. Another possibility is that the elevated peripheral blood frequencies of micronucleated erythrocytes in mice resulted from the increased splenic erythropoiesis observed in the animals treated with 2-methylimidazole. Rapidly dividing cells in the spleen may be more prone to chromosomal damage (aneuploidy and breakage) than the more slowly cycling bone marrow cells. However, splenic erythropoietic effects were also noted in mice treated with 4-methylimidazole, yet no increase in micronucleated eryhthrocytes was noted in peripheral blood of these mice.

In summary, 2-methylimidazole induced thyroid gland follicular cell hyperplasia in male and female rats and follicular cell hypertrophy in male and female mice. At 10,000 ppm, follicular cell adenoma was observed in male rats at 14 weeks. 2-Methylimidazole also induced a responsive anemia accompanied by splenic hematopoietic cell proliferation and renal tubule hemosiderin pigmentation in mice. The estimated no-observed-adverse-effect level of 2-methylimidazole was 625 ppm for rats and 1,250 ppm for mice for thyroid gland

lesions and 625 ppm for male mice and 1,250 ppm for female mice for splenic and renal lesions. 4-Methylimidazole induced cytoplasmic vacuolization in hepatocytes in rats but no histopathologic changes in mice. The estimated no-observed-adverse-effect level of 4-methylimidazole was 1,250 ppm for male rats, 5,000 ppm for female rats, and 10,000 ppm for mice.

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APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine small, ileum	(10)					(10)
Inflammation, chronic, focal	1 (10%)					
Liver	(10)		(1)			(10)
Hepatodiaphragmatic nodule			1 (100%)			
Inflammation, focal	1 (10%)					1 (10%)
Pancreas	(10)					(10)
Acinus, degeneration, focal	1 (10%)					1 (10%)
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Cardiomyopathy	7 (70%)	7 (70%)	8 (80%)	8 (80%)	4 (40%)	4 (40%)
Endocrine System						
Thyroid gland	(10)	(10)	(10)	(10)	(10)	(10)
Follicular cell, cyst						1 (10%)
Follicular cell, hyperplasia, diffuse	2 (20%)		8 (80%)	10 (100%)	10 (100%)	10 (100%)
General Body System						
None						
Genital System						
Preputial gland	(10)					(10)
Inflammation, focal	1 (10%)					1 (10%)
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Degeneration	2 (20%)	2 (20%)	1 (10%)	2 (20%)	2 (20%)	9 (90%)
Hematopoietic System						
Lymph node, mandibular	(9)					(9)
Hemorrhage	1 (11%)					
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage					1 (10%)	
Infiltration cellular, histiocyte	1 (10%)	1 (10%)	4 (40%)	3 (30%)	4 (40%)	4 (40%)
Integumentary System						
None						

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Inflammation, focal	3 (30%)					1 (10%)
Alveolus, infiltration cellular, histiocyte	1 (10%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)				(1)	(10)
Nephropathy	6 (60%)					3 (30%)
Renal tubule, dilatation, focal						1 (10%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Died last week of study	3			3	2	5
Terminal sacrifice	7	10	10	7	8	5
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(1)		(1)	(1)	(10)
Hepatodiaphragmatic nodule		1 (100%)		1 (100%)		1 (10%)
Inflammation, focal	2 (20%)			1 (100%)	1 (100%)	4 (40%)
Mesentery	(1)	(1)	(1)			
Fat, necrosis	1 (100%)	1 (100%)	1 (100%)			
Pancreas	(10)				(1)	(10)
Acinus, degeneration, focal	2 (20%)				1 (100%)	1 (100%)
Cardiovascular System						
Heart	(10)				(1)	(10)
Cardiomyopathy	1 (10%)				1 (100%)	1 (10%)
Endocrine System						
Adrenal cortex	(10)	(1)	(1)		(10)	(10)
Necrosis, acute		1 (100%)	1 (100%)			2 (20%)
Thyroid gland	(10)	(9)	(10)	(10)	(10)	(10)
Follicular cell, hyperplasia, diffuse				10 (100%)	10 (100%)	10 (100%)
General Body System						
None						
Genital System						
Clitoral gland	(10)			(1)	(1)	(10)
Inflammation, focal	1 (10%)			1 (100%)	1 (100%)	3 (30%)
Ovary	(10)	(2)	(1)	(1)	(1)	(10)
Cyst		1 (50%)	1 (100%)	1 (100%)	1 (100%)	1 (10%)
Bilateral, cyst		1 (50%)				
Uterus	(10)	(10)	(10)	(10)	(10)	(10)
Hydrometra			1 (10%)			
Bilateral, hydrometra		2 (20%)	1 (10%)	2 (20%)	1 (10%)	

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematopoietic System						
Bone marrow	(10)					(10)
Hyperplasia						1 (10%)
Infiltration cellular, histiocyte	1 (10%)					
Lymph node, mandibular	(10)					(10)
Hemorrhage	2 (20%)					1 (10%)
Infiltration cellular, plasma cell						1 (10%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte	8 (80%)	4 (40%)	9 (90%)	5 (50%)	10 (100%)	10 (100%)
Spleen	(10)					(10)
Lymphoid follicle, necrosis	3 (30%)					1 (10%)
Thymus	(10)			(1)		(10)
Hemorrhage				1 (100%)		1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(1)	(10)
Inflammation, focal	1 (10%)				1 (100%)	4 (40%)
Nose	(10)					(10)
Respiratory epithelium, inflammation, suppurative	2 (20%)					
Special Senses System						
None						
Urinary System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early death						
Natural death						1
Survivors						
Died last week of study		1	1		7	7
Terminal sacrifice	10	9	9	10	3	2
Animals examined microscopically	10	3	10	10	10	10
Alimentary System						
Liver	(10)		(10)	(10)	(10)	(9)
Inflammation, focal				1 (10%)		
Hepatocyte, vacuolization cytoplasmic	1 (10%)		3 (30%)	10 (100%)	10 (100%)	9 (100%)
Pancreas	(10)					(9)
Acinus, degeneration, focal						2 (22%)
Cardiovascular System						
Heart	(10)					(9)
Inflammation, focal	3 (30%)					4 (44%)
Artery, inflammation	1 (10%)					1 (11%)
Endocrine System						
None						
General Body System						
None						
Genital System						
Epididymis	(10)					(10)
Hypospermia						9 (90%)
Prostate	(10)	(1)	(10)	(10)	(10)	(10)
Atrophy		1 (100%)	1 (10%)	2 (20%)	8 (80%)	8 (80%)
Inflammation	2 (20%)		3 (30%)		1 (10%)	8 (80%)
Testes	(10)	(1)	(10)	(10)	(10)	(10)
Degeneration	1 (10%)	1 (100%)		4 (40%)	9 (90%)	9 (90%)
Hypospermia						1 (10%)
Hematopoietic System						
Lymph node, mandibular	(10)					(8)
Hemorrhage	2 (20%)					
Lymph node, mesenteric	(10)					(10)
Hemorrhage						2 (20%)
Infiltration cellular, histiocyte	1 (10%)					

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematopoietic System (continued)						
Spleen	(10)				(1)	(9)
Congestion					1 (100%)	
Hematopoietic cell proliferation	1 (10%)					
Thymus	(10)					(9)
Hemorrhage						3 (33%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(2)	(4)	(1)	(6)	(9)
Inflammation, focal	4 (40%)	2 (100%)	4 (100%)	1 (100%)	6 (100%)	8 (89%)
Alveolar epithelium, hyperplasia					1 (17%)	
Nose	(10)					(9)
Respiratory epithelium, inflammation, suppurative	1 (10%)					
Special Senses System						
None						
Urinary System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early death						
Moribund			1			
Survivors						
Died last week of study	7	8	7	8	8	10
Terminal sacrifice	3	2	2	2	2	
Animals examined microscopically	10	2	5	10	10	10
Alimentary System						
Liver	(10)	(1)	(2)	(10)	(10)	(10)
Congestion			1 (50%)			
Hepatodiaphragmatic nodule	1 (10%)	1 (100%)	1 (50%)	1 (10%)		1 (10%)
Inflammation, focal					1 (10%)	1 (10%)
Hepatocyte, vacuolization cytoplasmic					1 (10%)	8 (80%)
Mesentery	(1)					
Fat, necrosis	1 (100%)					
Pancreas	(10)		(1)			(10)
Acinus, degeneration, focal	1 (10%)					4 (40%)
Cardiovascular System						
Heart	(10)		(1)			(10)
Inflammation, focal						3 (30%)
Artery, inflammation	1 (10%)					
Endocrine System						
Pituitary gland	(10)		(1)			(10)
Pars distalis, cyst	1 (10%)					
Thyroid gland	(10)		(1)			(10)
Follicle, cyst						1 (10%)
General Body System						
None						
Genital System						
Ovary	(10)		(1)	(1)		(10)
Cyst				1 (100%)		
Bilateral, cyst						1 (10%)
Bursa, cyst	1 (10%)					
Uterus	(10)		(4)	(10)	(10)	(10)
Bilateral, cyst			1 (25%)			
Bilateral, dilatation	3 (30%)		3 (75%)	1 (10%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematopoietic System						
Bone marrow	(10)		(1)			(10)
Infiltration cellular, focal, histiocyte	1 (10%)					
Lymph node, mandibular	(10)		(1)			(9)
Hemorrhage						2 (22%)
Lymph node, mesenteric	(10)		(1)			(10)
Infiltration cellular, histiocyte	1 (10%)					
Spleen	(10)		(1)			(10)
Hematopoietic cell proliferation	1 (10%)					
Pigmentation, diffuse	2 (20%)		1 (100%)			
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)	(1)		(5)	(10)
Congestion			1 (100%)			
Inflammation, focal	5 (50%)	1 (100%)			5 (100%)	2 (20%)
Nose	(10)		(1)			(10)
Olfactory epithelium, inflammation, suppurative						1 (10%)
Respiratory epithelium, inflammation, suppurative			1 (100%)			
Special Senses System						
None						
Urinary System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

APPENDIX B
SUMMARY OF NONNEOPLASTIC LESIONS IN MICE

TABLE B1
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Died last week of study		1	1			1
Terminal sacrifice	10	9	9	10	10	9
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(1)	(1)			(10)
Necrosis, acute						1 (10%)
Periportal, vacuolization cytoplasmic	9 (90%)		1 (100%)			10 (100%)
Cardiovascular System						
None						
Endocrine System						
Thyroid gland	(10)	(9)	(10)	(9)	(10)	(10)
Follicular cell, hypertrophy				9 (100%)	10 (100%)	10 (100%)
General Body System						
None						
Genital System						
Preputial gland	(10)	(1)	(1)			(10)
Cyst	7 (70%)					3 (30%)
Hematopoietic System						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		1 (10%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

TABLE B1
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Respiratory System						
Nose	(10)	(1)	(1)			(10)
Respiratory epithelium, infiltration cellular, mixed cell						1 (10%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Cyst	1 (10%)					
Renal tubule, pigmentation, hemosiderin			10 (100%)	10 (100%)	10 (100%)	10 (100%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Died last week of study			1			2
Terminal sacrifice	10	10	9	10	10	8
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)		(1)			(10)
Infiltration cellular, lymphocyte	1 (10%)					
Necrosis, acute						1 (10%)
Vacuolization cytoplasmic, diffuse	1 (10%)					
Periportal, vacuolization cytoplasmic	6 (60%)					8 (80%)
Stomach, glandular	(10)		(1)			(10)
Infiltration cellular, mixed cell						1 (10%)
Cardiovascular System						
None						
Endocrine System						
Thyroid gland	(10)	(9)	(8)	(9)	(10)	(10)
Follicular cell, hypertrophy				7 (78%)	10 (100%)	10 (100%)
General Body System						
None						
Genital System						
Clitoral gland	(9)		(1)			(6)
Cyst	6 (67%)					2 (33%)
Hematopoietic System						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	2 (20%)	1 (10%)	1 (10%)	10 (100%)	10 (100%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Nervous System						
None						
Respiratory System						
Lung	(10)		(1)			(10)
Perivascular, inflammation, chronic active	1 (10%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Pelvis, infiltration cellular, lymphocyte	1 (10%)					
Renal tubule, pigmentation, hemosiderin				3 (30%)	9 (90%)	10 (100%)
Urinary bladder	(10)		(1)			(10)
Infiltration cellular, lymphocyte	2 (20%)					

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early death						
Natural death						1
Survivors						
Terminal sacrifice	10	10	10	10	10	9
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, mixed cell				3 (30%)		
Centrilobular, vacuolization cytoplasmic					1 (10%)	2 (20%)
Periportal, vacuolization cytoplasmic	10 (100%)	10 (100%)	10 (100%)	10 (100%)	9 (90%)	7 (70%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Preputial gland	(10)		(1)			(10)
Cyst	6 (60%)		1 (100%)			8 (80%)
Hematopoietic System						
Lymph node, mandibular	(10)					(10)
Hemorrhage	1 (10%)					1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Natural deaths						7
Survivors						
Died last week of study	1	1	2	1		1
Terminal sacrifice	9	9	8	9	10	2
Animals examined microscopically	10	9	6	10	10	10
Alimentary System						
Liver	(10)	(9)	(6)	(10)	(10)	(10)
Infiltration cellular, mixed cell	1 (10%)		1 (17%)		1 (10%)	
Centrilobular, vacuolization cytoplasmic						3 (30%)
Periportal, vacuolization cytoplasmic	6 (60%)	9 (100%)	5 (83%)	5 (50%)	3 (30%)	
Salivary glands	(10)				(10)	(7)
Infiltration cellular, lymphocyte	2 (20%)					
Cardiovascular System						
Heart	(10)				(10)	(9)
Valve, pigmentation	1 (10%)					
Endocrine System						
None						
General Body System						
None						
Genital System						
Clitoral gland	(8)				(7)	(5)
Cyst	1 (13%)					
Hematopoietic System						
Lymph node, mandibular	(10)				(10)	(7)
Hemorrhage	1 (10%)					
Hyperplasia, lymphoid					1 (10%)	
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Nervous System						
None						
Respiratory System						
Lung	(10)				(10)	(7)
Infiltration cellular, mixed cell					1 (10%)	
Perivascular, infiltration cellular, mixed cell	2 (20%)					1 (14%)
Special Senses System						
None						
Urinary System						
Urinary bladder	(10)				(10)	(10)
Infiltration cellular, mixed cell					1 (10%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

APPENDIX C
CLINICAL PATHOLOGY RESULTS

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	8
Week 14	9	10	10	10	10	10
Hematology						
Automated hematocrit (%)						
Day 8	43.9 ± 0.4	44.2 ± 0.4	42.5 ± 0.3	42.3 ± 0.4	42.1 ± 0.6	43.3 ± 0.6
Day 29	45.8 ± 0.3	46.2 ± 0.4	45.7 ± 0.3	44.8 ± 0.5	43.8 ± 0.5**	41.8 ± 0.5**
Week 14	45.1 ± 0.3	45.0 ± 0.4	44.3 ± 0.5	44.1 ± 0.5	42.4 ± 0.6**	40.6 ± 0.6**
Manual hematocrit (%)						
Day 8	46.9 ± 0.5	47.6 ± 0.5	46.1 ± 0.3	45.8 ± 0.4	45.7 ± 0.5	46.6 ± 0.6
Day 29	47.8 ± 0.4	48.9 ± 0.4	48.2 ± 0.4	47.4 ± 0.5	46.6 ± 0.4	44.6 ± 0.7**
Week 14	47.6 ± 0.4	46.9 ± 0.5	46.9 ± 0.5	46.9 ± 0.5	45.3 ± 0.7**	43.7 ± 0.7**
Hemoglobin (g/dL)						
Day 8	15.2 ± 0.1	15.4 ± 0.2	14.9 ± 0.1	14.6 ± 0.1	14.8 ± 0.1	15.1 ± 0.2
Day 29	16.1 ± 0.1	16.3 ± 0.1	16.1 ± 0.1	15.7 ± 0.1	15.4 ± 0.2**	14.8 ± 0.2**
Week 14	15.8 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.4 ± 0.1*	14.8 ± 0.2**	14.1 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 8	7.28 ± 0.07	7.30 ± 0.07	7.05 ± 0.05	7.02 ± 0.06	7.04 ± 0.11	7.34 ± 0.11
Day 29	7.94 ± 0.06	7.95 ± 0.07	7.86 ± 0.05	7.77 ± 0.09	7.60 ± 0.09*	7.48 ± 0.11**
Week 14	8.76 ± 0.04	8.81 ± 0.09	8.59 ± 0.09	8.60 ± 0.08	8.40 ± 0.13*	8.11 ± 0.10**
Reticulocytes ($10^6/\mu L$)						
Day 8	0.29 ± 0.01	0.31 ± 0.02	0.28 ± 0.01	0.28 ± 0.02	0.19 ± 0.01**	0.11 ± 0.01**
Day 29	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
Week 14	0.19 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.19 ± 0.02
Nucleated erythrocytes ($10^3/\mu L$)						
Day 8	0.30 ± 0.15	0.50 ± 0.22	0.80 ± 0.33	0.80 ± 0.25	0.30 ± 0.21	0.10 ± 0.10
Week 14	0.22 ± 0.15	0.00 ± 0.00	0.20 ± 0.13	0.10 ± 0.10	0.00 ± 0.00[b]	0.10 ± 0.10
Mean cell volume (fL)						
Day 8	60.3 ± 0.2	60.6 ± 0.2	60.3 ± 0.2	60.2 ± 0.2	59.8 ± 0.2	59.0 ± 0.2**
Day 29	57.8 ± 0.2	58.1 ± 0.3	58.2 ± 0.3	57.7 ± 0.3	57.7 ± 0.2	55.8 ± 0.3**
Week 14	51.5 ± 0.2	51.1 ± 0.2	51.6 ± 0.3	51.2 ± 0.1	50.4 ± 0.2**	50.0 ± 0.2**
Mean cell hemoglobin (pg)						
Day 8	20.9 ± 0.1	21.1 ± 0.2	21.1 ± 0.1	20.8 ± 0.1	21.0 ± 0.2	20.6 ± 0.1
Day 29	20.3 ± 0.1	20.5 ± 0.1	20.5 ± 0.1	20.3 ± 0.1	20.3 ± 0.1	19.7 ± 0.1**
Week 14	18.0 ± 0.1[c]	17.8 ± 0.1	18.0 ± 0.1	17.9 ± 0.1	17.6 ± 0.1*	17.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 8	34.6 ± 0.2	34.8 ± 0.2	35.0 ± 0.1	34.6 ± 0.1	35.1 ± 0.3	34.9 ± 0.2
Day 29	35.2 ± 0.2	35.2 ± 0.1	35.2 ± 0.2	35.2 ± 0.1	35.2 ± 0.1	35.3 ± 0.1
Week 14	35.0 ± 0.2	34.7 ± 0.2	34.9 ± 0.2	35.0 ± 0.2	34.9 ± 0.2	34.8 ± 0.2
Platelets ($10^3/\mu L$)						
Day 8	927.6 ± 15.9	966.7 ± 12.2	984.9 ± 19.5*	1,042.0 ± 16.1**	1,032.8 ± 13.7**	1,043.7 ± 24.7**
Day 29	695.3 ± 10.4	684.4 ± 13.1	710.2 ± 4.0	750.3 ± 11.6**	810.8 ± 13.1**[c]	896.6 ± 13.6**
Week 14	605.3 ± 10.0	587.7 ± 14.8	597.0 ± 8.9	626.2 ± 12.5	684.9 ± 22.0*	705.7 ± 15.2**
Leukocytes ($10^3/\mu L$)						
Day 8	12.19 ± 0.30	11.91 ± 0.39	11.05 ± 0.41	11.45 ± 0.38	11.82 ± 0.38	10.80 ± 0.84
Day 29	11.7 ± 0.4	12.5 ± 0.4	11.6 ± 0.7	12.3 ± 0.3	11.8 ± 0.4	12.4 ± 1.1
Week 14	12.87 ± 0.74	13.13 ± 0.65	11.12 ± 1.14	12.67 ± 0.67	12.48 ± 0.93[b]	13.15 ± 0.64

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	8
Week 14	9	10	10	10	10	10
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
Day 8	1.22 ± 0.19	1.01 ± 0.09	0.10 ± 0.14	0.85 ± 0.12	0.10 ± 0.10	1.23 ± 0.21
Day 29	1.33 ± 0.12	1.67 ± 0.21	1.10 ± 0.08	1.33 ± 0.18	1.50 ± 0.22	1.68 ± 0.31
Week 14	2.11 ± 0.23	2.24 ± 0.23	1.87 ± 0.21	2.26 ± 0.19	2.38 ± 0.24[b]	2.46 ± 0.18
Lymphocytes ($10^3/\mu L$)						
Day 8	10.03 ± 0.16	10.05 ± 0.36	9.32 ± 0.39	9.73 ± 0.34	10.15 ± 0.32	8.98 ± 0.73
Day 29	9.95 ± 0.34	10.47 ± 0.38	10.03 ± 0.63	10.41 ± 0.33	9.93 ± 0.36	10.30 ± 0.82
Week 14	10.26 ± 0.79	10.23 ± 0.52	8.86 ± 1.01	9.99 ± 0.61	9.72 ± 0.80[b]	10.25 ± 0.53
Monocytes ($10^3/\mu L$)						
Day 8	0.59 ± 0.10	0.57 ± 0.10	0.42 ± 0.08	0.55 ± 0.10	0.42 ± 0.07	0.32 ± 0.08
Day 29	0.37 ± 0.05	0.33 ± 0.05	0.41 ± 0.06	0.43 ± 0.06	0.32 ± 0.06	0.36 ± 0.07
Week 14	0.36 ± 0.03	0.39 ± 0.06	0.25 ± 0.05	0.34 ± 0.10	0.28 ± 0.06[b]	0.36 ± 0.06
Eosinophils ($10^3/\mu L$)						
Day 8	0.05 ± 0.02	0.09 ± 0.04	0.05 ± 0.03	0.11 ± 0.04	0.01 ± 0.01	0.00 ± 0.00
Day 29	0.01 ± 0.01	0.05 ± 0.03	0.02 ± 0.01	0.06 ± 0.03	0.04 ± 0.03	0.04 ± 0.02
Week 14	0.12 ± 0.04	0.17 ± 0.04	0.13 ± 0.04	0.09 ± 0.04	0.08 ± 0.04[b]	0.02 ± 0.02
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 29	21.4 ± 0.3	21.6 ± 0.3	21.9 ± 0.6	21.2 ± 0.7	20.6 ± 0.4	18.9 ± 0.5**
Week 14	19.1 ± 0.4[b]	20.1 ± 0.4	19.5 ± 0.3	19.5 ± 0.3	18.7 ± 0.3	18.2 ± 0.3
Creatinine (mg/dL)						
Day 29	0.66 ± 0.02	0.62 ± 0.01	0.51 ± 0.01**	0.35 ± 0.02**	0.16 ± 0.02**[b]	—[d]
Week 14	0.41 ± 0.02	0.27 ± 0.02**	0.18 ± 0.02**	0.04 ± 0.02**	—	—
Total protein (g/dL)						
Day 29	6.6 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.0 ± 0.1**
Week 14	6.9 ± 0.1	6.6 ± 0.0**	6.5 ± 0.1**	6.5 ± 0.0**	6.4 ± 0.1**	6.2 ± 0.1**
Albumin (g/dL)						
Day 29	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	4.7 ± 0.1*	4.7 ± 0.1**	4.3 ± 0.1**
Week 14	4.8 ± 0.0	4.8 ± 0.0	4.7 ± 0.1	4.6 ± 0.0**	4.4 ± 0.1**	4.3 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 29	44 ± 1	44 ± 2	44 ± 3	33 ± 1**	27 ± 2**	19 ± 1**
Week 14	63 ± 3	53 ± 2**	60 ± 5	42 ± 1**	44 ± 5**	30 ± 2**
Alkaline phosphatase (IU/L)						
Day 29	444 ± 12	446 ± 6	458 ± 8	438 ± 11	404 ± 12*	334 ± 7**
Week 14	269 ± 7	263 ± 4	273 ± 3	252 ± 5	252 ± 7	256 ± 5
Creatine kinase (IU/L)						
Day 29	639 ± 49	479 ± 35	573 ± 46	593 ± 82	618 ± 75	633 ± 37
Week 14	202 ± 27	215 ± 32	195 ± 26	173 ± 24	213 ± 35	224 ± 40
Sorbitol dehydrogenase (IU/L)						
Day 29	20 ± 1	28 ± 2*	25 ± 2	21 ± 2	20 ± 3	15 ± 1
Week 14	27 ± 1	25 ± 1	27 ± 1	23 ± 1*	23 ± 2*	22 ± 1**
Bile acids (μmol/L)						
Day 29	25.9 ± 4.4	20.4 ± 2.3	19.8 ± 1.8	18.5 ± 2.2	26.1 ± 2.7	33.5 ± 6.8
Week 14	16.0 ± 0.8[c]	12.3 ± 0.6	13.9 ± 0.9	17.5 ± 2.0	17.8 ± 3.1	34.1 ± 6.5

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	8
Week 14	9	10	10	10	10	10
Clinical Chemistry (continued)						
Thyroid-stimulating hormone (ng/mL)						
Day 8	1.98 ± 0.42	2.16 ± 0.28	3.05 ± 0.42	7.18 ± 0.50**	9.78 ± 0.34**	9.26 ± 0.49**
Day 29	0.52 ± 0.16	1.07 ± 0.31	0.90 ± 0.21	0.86 ± 0.23	1.40 ± 0.25*	5.41 ± 0.43**
Week 14	0.29 ± 0.12[e]	0.45 ± 0.22	0.50 ± 0.17	0.51 ± 0.24	1.92 ± 0.62*	4.19 ± 0.62**
Triiodothyronine (ng/dL)						
Day 8	164.2 ± 6.8	165.5 ± 7.1	152.8 ± 4.6	116.5 ± 2.4**	79.0 ± 1.4**	61.7 ± 2.1**
Day 29	123.4 ± 2.5	118.2 ± 3.5	126.6 ± 7.2	124.5 ± 3.7	117.0 ± 3.0	113.0 ± 2.2
Week 14	137.2 ± 4.3[e]	149.6 ± 4.1	128.5 ± 4.5	140.1 ± 3.3	124.2 ± 5.2	123.3 ± 4.2*
Thyroxine (μg/dL)						
Day 8	5.44 ± 0.24	5.29 ± 0.16	5.39 ± 0.15	3.75 ± 0.13**	0.66 ± 0.04**[b]	—
Day 29	3.32 ± 0.15	3.17 ± 0.19	3.27 ± 0.23	2.92 ± 0.28	2.98 ± 0.20	1.13 ± 0.13**
Week 14	2.10 ± 0.23[e]	2.27 ± 0.15	2.21 ± 0.08	2.72 ± 0.16	1.74 ± 0.23[b]	1.97 ± 0.17
Female						
n						
Day 8	10	10	10	10	10	10
Day 29	10	8	10	10	9	9
Week 14	10	10	10	10	10	10
Hematology						
Automated hematocrit (%)						
Day 8	44.4 ± 0.7	45.4 ± 0.8	43.8 ± 0.7	45.2 ± 0.3	44.7 ± 0.4	46.6 ± 0.5
Day 29	45.6 ± 0.6	45.4 ± 0.5	44.8 ± 0.3	45.2 ± 0.3	42.7 ± 0.2**	38.7 ± 1.9**
Week 14	44.9 ± 0.5	44.6 ± 0.5	43.8 ± 0.5	42.9 ± 0.4**	41.7 ± 0.5**	39.9 ± 0.6**
Manual hematocrit (%)						
Day 8	45.3 ± 0.8	45.2 ± 0.8	45.3 ± 0.6	46.1 ± 0.3	45.1 ± 0.5	47.7 ± 0.5*
Day 29	47.2 ± 0.5	47.5 ± 0.6	47.1 ± 0.4	47.2 ± 0.5	45.6 ± 0.4*	43.1 ± 1.6**[c]
Week 14	46.3 ± 0.5	45.5 ± 0.4	45.6 ± 0.5	44.5 ± 0.4*	43.3 ± 0.4**	41.8 ± 0.6**
Hemoglobin (g/dL)						
Day 8	15.2 ± 0.2	15.3 ± 0.2	14.9 ± 0.2	15.3 ± 0.1	15.1 ± 0.1	15.8 ± 0.2
Day 29	16.0 ± 0.2	15.8 ± 0.2	15.8 ± 0.1	15.8 ± 0.1	15.2 ± 0.1**	13.7 ± 0.6**
Week 14	15.5 ± 0.2	15.4 ± 0.1	15.2 ± 0.1	14.8 ± 0.1**	14.5 ± 0.1**	14.2 ± 0.2**
Erythrocytes (10^6/μL)						
Day 8	7.21 ± 0.12	7.38 ± 0.12	7.10 ± 0.12	7.37 ± 0.05	7.31 ± 0.08	7.74 ± 0.11**
Day 29	7.56 ± 0.09	7.63 ± 0.04	7.45 ± 0.07	7.56 ± 0.07	7.31 ± 0.03**	6.91 ± 0.34*
Week 14	7.89 ± 0.09	7.89 ± 0.08	7.79 ± 0.10	7.65 ± 0.07	7.71 ± 0.10	7.71 ± 0.11
Reticulocytes (10^6/μL)						
Day 8	0.20 ± 0.02	0.18 ± 0.01	0.33 ± 0.11	0.17 ± 0.02	0.15 ± 0.01*	0.10 ± 0.01**
Day 29	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.13 ± 0.01
Week 14	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.15 ± 0.02	0.14 ± 0.01	0.10 ± 0.01
Nucleated erythrocytes (10^3/μL)						
Day 8	0.00 ± 0.00	0.40 ± 0.22	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.20 ± 0.13	0.20 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	8	10	10	9	9
Week 14	10	10	10	10	10	10
Hematology (continued)						
Mean cell volume (fL)						
Day 8	61.6 ± 0.2	61.5 ± 0.2	61.7 ± 0.1	61.4 ± 0.2	61.2 ± 0.2	60.2 ± 0.3**
Day 29	60.4 ± 0.2	59.6 ± 0.5	60.1 ± 0.3	59.8 ± 0.2	58.5 ± 0.3**	56.1 ± 0.1**
Week 14	56.9 ± 0.1	56.5 ± 0.1*	56.3 ± 0.1**	56.1 ± 0.1**	54.1 ± 0.1**	51.8 ± 0.2**
Mean cell hemoglobin (pg)						
Day 8	21.1 ± 0.1	20.7 ± 0.1*	21.0 ± 0.1	20.8 ± 0.1	20.6 ± 0.0**	20.4 ± 0.1**
Day 29	21.2 ± 0.1	20.8 ± 0.2	21.2 ± 0.1	20.9 ± 0.1*	20.8 ± 0.1	19.9 ± 0.1**
Week 14	19.6 ± 0.1	19.5 ± 0.1	19.5 ± 0.1	19.4 ± 0.1	18.8 ± 0.1**[b]	18.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 8	34.2 ± 0.2	33.7 ± 0.2	34.0 ± 0.2	33.9 ± 0.1	33.7 ± 0.1	33.9 ± 0.2
Day 29	35.1 ± 0.1	34.9 ± 0.3	35.3 ± 0.2	34.9 ± 0.1	35.6 ± 0.2	35.5 ± 0.3
Week 14	34.5 ± 0.2	34.5 ± 0.2	34.7 ± 0.2	34.6 ± 0.2	34.8 ± 0.1	35.5 ± 0.2**
Platelets ($10^3/\mu L$)						
Day 8	818.2 ± 20.9	872.8 ± 19.3	856.2 ± 24.1	932.3 ± 21.5**	938.9 ± 26.7**	931.0 ± 15.2**
Day 29	652.6 ± 10.2	732.4 ± 30.8**	715.9 ± 16.2**	712.8 ± 11.7**	757.1 ± 11.3**	869.6 ± 30.1**
Week 14	576.4 ± 13.0	562.6 ± 18.4	574.1 ± 13.1	603.8 ± 10.5	631.8 ± 20.4	685.0 ± 23.0**
Leukocytes ($10^3/\mu L$)						
Day 8	9.48 ± 0.56	8.87 ± 0.79	9.90 ± 0.51	11.28 ± 0.51	10.35 ± 0.47	10.91 ± 0.36
Day 29	10.9 ± 0.3	11.7 ± 0.3	11.6 ± 0.3	11.5 ± 0.4	10.9 ± 0.7	13.5 ± 0.8**
Week 14	11.51 ± 1.09	10.68 ± 0.93	9.80 ± 0.74	9.79 ± 0.76	11.37 ± 0.79	9.58 ± 1.0
Segmented neutrophils ($10^3/\mu L$)						
Day 8	1.06 ± 0.12	0.60 ± 0.07*	1.01 ± 0.09	0.96 ± 0.15	0.83 ± 0.12	0.80 ± 0.12
Day 29	1.19 ± 0.13	1.57 ± 0.15	1.37 ± 0.12	1.07 ± 0.13	1.25 ± 0.30	1.23 ± 0.28
Week 14	2.21 ± 0.34	2.30 ± 0.33	1.92 ± 0.23	1.90 ± 0.35	1.81 ± 0.24	1.69 ± 0.19
Lymphocytes ($10^3/\mu L$)						
Day 8	8.17 ± 0.53	8.02 ± 0.77	8.49 ± 0.47	10.07 ± 0.46*	9.25 ± 0.40	9.75 ± 0.32*
Day 29	9.31 ± 0.27	9.66 ± 0.28	9.72 ± 0.29	9.92 ± 0.31	9.22 ± 0.67	11.82 ± 0.58**
Week 14	8.96 ± 0.86	7.92 ± 0.76	7.37 ± 0.62	7.62 ± 0.62	9.27 ± 0.73	7.64 ± 0.94
Monocytes ($10^3/\mu L$)						
Day 8	0.11 ± 0.03	0.17 ± 0.06	0.27 ± 0.07	0.20 ± 0.05	0.16 ± 0.07	0.29 ± 0.06
Day 29	0.13 ± 0.07	0.12 ± 0.06	0.12 ± 0.07	0.18 ± 0.07	0.13 ± 0.07	0.09 ± 0.04
Week 14	0.17 ± 0.06	0.39 ± 0.07	0.36 ± 0.16	0.19 ± 0.06	0.21 ± 0.06	0.15 ± 0.06
Eosinophils ($10^3/\mu L$)						
Day 8	0.05 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.05 ± 0.03	0.06 ± 0.02	0.05 ± 0.02
Day 29	0.09 ± 0.02	0.10 ± 0.02	0.13 ± 0.04	0.07 ± 0.04	0.03 ± 0.02	0.07 ± 0.03
Week 14	0.10 ± 0.05	0.05 ± 0.04	0.08 ± 0.03	0.06 ± 0.02	0.06 ± 0.03	0.06 ± 0.03
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 29	22.2 ± 0.6	21.8 ± 0.4	20.7 ± 0.5	21.5 ± 0.5	20.6 ± 0.6	22.0 ± 1.3
Week 14	19.4 ± 0.5	20.7 ± 0.6	19.7 ± 0.5	19.3 ± 0.5	19.0 ± 0.4	19.6 ± 0.6
Creatinine (mg/dL)						
Day 29	0.66 ± 0.02	0.53 ± 0.02**[f]	0.44 ± 0.02**	0.23 ± 0.02**	0.05 ± 0.05**[g]	—
Week 14	0.42 ± 0.01	0.32 ± 0.02**	0.27 ± 0.02**	0.10 ± 0.03**	—	—

TABLE C1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	8	10	10	9	9
Week 14	10	10	10	10	10	10
Clinical Chemistry (continued)						
Total protein (g/dL)						
Day 29	6.2 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.3 ± 0.2**
Week 14	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.4 ± 0.1	6.6 ± 0.1	6.3 ± 0.1**
Albumin (g/dL)						
Day 29	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.5 ± 0.1**	4.0 ± 0.1**
Week 14	5.0 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	4.6 ± 0.1**	4.3 ± 0.0**
Alanine aminotransferase IU/L)						
Day 29	35 ± 1	29 ± 2**	31 ± 1*	29 ± 1**	25 ± 2**	23 ± 4**
Week 14	59 ± 6	52 ± 4	65 ± 8	50 ± 4	36 ± 3**	25 ± 2**
Alkaline phosphatase (IU/L)						
Day 29	343 ± 7	336 ± 10	353 ± 16	329 ± 9	326 ± 7	283 ± 8**
Week 14	217 ± 8	209 ± 6	215 ± 5	204 ± 12	221 ± 6	240 ± 6
Creatine kinase (IU/L)						
Day 29	429 ± 42	425 ± 70	467 ± 44	502 ± 56	643 ± 40**	685 ± 113*
Week 14	177 ± 20	212 ± 32	151 ± 14	202 ± 24	183 ± 25	171 ± 26
Sorbitol dehydrogenase (IU/L)						
Day 29	24 ± 2	22 ± 2	22 ± 1	21 ± 1	17 ± 2**	25 ± 6*
Week 14	27 ± 2	25 ± 2	27 ± 2	26 ± 2	23 ± 1	19 ± 2**
Bile acids (μmol/L)						
Day 29	23.7 ± 5.9	22.6 ± 6.3	20.0 ± 4.2	18.1 ± 3.3	24.2 ± 4.3	35.9 ± 5.6
Week 14	36.0 ± 6.2	27.7 ± 3.3	31.7 ± 5.9	21.1 ± 3.7	46.4 ± 6.7	48.5 ± 5.6
Thyroid-stimulating hormone (ng/mL)						
Day 8	1.04 ± 0.11	0.89 ± 0.15	1.74 ± 0.31	4.39 ± 0.73**	9.05 ± 0.47**	7.83 ± 0.36**
Day 29	0.38 ± 0.09	1.13 ± 0.66	0.91 ± 0.10**	0.76 ± 0.12**	2.32 ± 0.42**	8.49 ± 0.62**
Week 14	0.27 ± 0.13	0.49 ± 0.22	0.27 ± 0.16	0.52 ± 0.16	1.23 ± 0.40*[b]	7.90 ± 0.87**[b]
Triiodothyronine (ng/dL)						
Day 8	142.5 ± 6.6	130.5 ± 5.5	141.1 ± 3.1	119.9 ± 1.8**	81.6 ± 2.0**	76.3 ± 2.1**
Day 29	138.5 ± 6.0	139.6 ± 4.1	143.4 ± 4.2	135.3 ± 3.5	128.4 ± 4.1	116.4 ± 3.2**[c]
Week 14	136.5 ± 6.1	142.0 ± 6.7	139.2 ± 5.6	135.8 ± 4.7	137.9 ± 3.7	112.2 ± 4.2**
Thyroxine (μg/dL)						
Day 8	3.87 ± 0.29	3.11 ± 0.28*	3.64 ± 0.19	3.01 ± 0.16**	0.60 ± 0.07**[c]	0.74 ± 0.11**[c]
Day 29	3.44 ± 0.31	2.33 ± 0.36	2.92 ± 0.28	2.23 ± 0.24**	2.18 ± 0.25**	0.80 ± 0.08**[c]
Week 14	2.57 ± 0.28	2.17 ± 0.20	1.94 ± 0.28	2.16 ± 0.30	2.42 ± 0.22	0.79 ± 0.12**[c]

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=8
[d] Not detected
[e] n=10
[f] n=7
[g] n=2

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	9	8
Week 14	10	10	10	10	10	9
Hematology						
Automated hematocrit (%)						
Day 8	40.7 ± 0.7	42.2 ± 0.6	40.5 ± 0.4	40.8 ± 0.5	43.1 ± 1.0*	47.3 ± 0.6**
Day 29	45.2 ± 0.4	45.5 ± 0.3	45.3 ± 0.4	43.8 ± 0.4*	43.5 ± 0.3**	41.6 ± 0.6**
Week 14	45.3 ± 0.7	46.1 ± 0.4	45.7 ± 0.5	44.1 ± 0.5	42.8 ± 0.4**	39.6 ± 0.3**
Manual hematocrit (%)						
Day 8	43.8 ± 0.8	45.3 ± 0.6	42.8 ± 0.5	44.7 ± 0.8	45.2 ± 1.1	49.1 ± 0.7**
Day 29	47.8 ± 0.4	47.0 ± 0.5	47.4 ± 0.2	45.9 ± 0.4**	45.0 ± 0.2**	43.3 ± 0.6**
Week 14	46.7 ± 0.7	47.8 ± 0.3	47.3 ± 0.4	45.4 ± 0.3*	44.0 ± 0.6**	41.1 ± 0.3**
Hemoglobin (g/dL)						
Day 8	14.3 ± 0.2	14.7 ± 0.2*	14.3 ± 0.1	14.3 ± 0.1	15.1 ± 0.3*	16.5 ± 0.1**
Day 29	15.9 ± 0.1	15.8 ± 0.1	15.9 ± 0.1	15.4 ± 0.1*	15.1 ± 0.1**	14.2 ± 0.2**
Week 14	15.8 ± 0.2	15.9 ± 0.1	15.9 ± 0.1	15.2 ± 0.1**	14.6 ± 0.1**	13.6 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 8	6.86 ± 0.11	7.02 ± 0.08	6.88 ± 0.06	6.95 ± 0.09	7.40 ± 0.16**	8.18 ± 0.08**
Day 29	7.81 ± 0.09	7.90 ± 0.09	7.89 ± 0.09	7.73 ± 0.08	7.76 ± 0.08	7.80 ± 0.12
Week 14	8.86 ± 0.12	8.97 ± 0.08	8.83 ± 0.08	8.53 ± 0.10*	8.44 ± 0.08**	8.03 ± 0.08**
Reticulocytes ($10^6/\mu L$)						
Day 8	0.33 ± 0.02	0.32 ± 0.02	0.27 ± 0.02	0.26 ± 0.02*	0.19 ± 0.02**	0.08 ± 0.01**
Day 29	0.16 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.15 ± 0.01
Week 14	0.16 ± 0.02	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.02	0.18 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)						
Day 8	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.04 ± 0.03
Day 29	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.01 ± 0.01	0.03 ± 0.02	0.04 ± 0.03	0.01 ± 0.01	0.03 ± 0.02	0.01 ± 0.01
Mean cell volume (fL)						
Day 8	59.3 ± 0.4	60.0 ± 0.4	58.9 ± 0.3	58.8 ± 0.3	58.3 ± 0.3*	57.8 ± 0.2**
Day 29	58.0 ± 0.3	57.6 ± 0.3	57.4 ± 0.3	56.7 ± 0.2**	56.0 ± 0.4**	53.4 ± 0.2**
Week 14	51.1 ± 0.3	51.5 ± 0.2	51.7 ± 0.2	51.7 ± 0.2	50.7 ± 0.1	49.3 ± 0.2**
Mean cell hemoglobin (pg)						
Day 8	20.9 ± 0.2	20.9 ± 0.1	20.9 ± 0.1	20.6 ± 0.1	20.4 ± 0.1**	20.2 ± 0.1**
Day 29	20.4 ± 0.1	20.1 ± 0.1	20.1 ± 0.2	19.9 ± 0.1**	19.5 ± 0.1**	18.2 ± 0.1**
Week 14	17.9 ± 0.1	17.7 ± 0.1	18.0 ± 0.2	17.8 ± 0.1	17.3 ± 0.1**	16.9 ± 0.2**
Mean cell hemoglobin concentration (g/dL)						
Day 8	35.2 ± 0.3	34.8 ± 0.3	35.4 ± 0.1	35.2 ± 0.2	35.0 ± 0.1	34.9 ± 0.2
Day 29	35.1 ± 0.2	34.8 ± 0.1	35.0 ± 0.1	35.1 ± 0.1	34.8 ± 0.1	34.2 ± 0.2**
Week 14	35.0 ± 0.1	34.5 ± 0.2	34.7 ± 0.3[b]	34.4 ± 0.2*	34.1 ± 0.1**	34.3 ± 0.2**
Platelets ($10^3/\mu L$)						
Day 8	908.3 ± 25.1	847.7 ± 31.6	915.6 ± 29.6	781.7 ± 27.9**	754.6 ± 24.8**	649.3 ± 24.2**
Day 29	639.9 ± 18.6	626.4 ± 12.5	638.9 ± 14.7	651.4 ± 12.6	613.1 ± 17.9	611.8 ± 15.0
Week 14	610.2 ± 49.4	555.3 ± 19.1	577.9 ± 12.9	586.4 ± 13.5	605.1 ± 10.2	565.3 ± 13.4
Leukocytes ($10^3/\mu L$)						
Day 8	9.83 ± 0.59	9.47 ± 0.54	9.54 ± 0.50	8.79 ± 0.53	8.36 ± 0.32	9.06 ± 0.39
Day 29	12.12 ± 0.76	10.42 ± 0.47	10.24 ± 0.52	9.89 ± 0.38	9.82 ± 0.38	10.21 ± 0.35
Week 14	13.47 ± 0.85	11.49 ± 0.49	11.40 ± 0.44	11.19 ± 0.52	12.56 ± 0.57	11.93 ± 0.73

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	9	8
Week 14	10	10	10	10	10	9
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
Day 8	1.14 ± 0.15	1.04 ± 0.09	0.95 ± 0.16	0.97 ± 0.18	0.67 ± 0.10	0.87 ± 0.08
Day 29	1.10 ± 0.15	1.21 ± 0.13	0.92 ± 0.09	1.15 ± 0.14	1.15 ± 0.11	1.61 ± 0.27
Week 14	1.97 ± 0.34	1.67 ± 0.16	1.79 ± 0.17	1.78 ± 0.12	1.91 ± 0.17	2.22 ± 0.29
Lymphocytes ($10^3/\mu L$)						
Day 8	8.28 ± 0.43	8.11 ± 0.46	8.27 ± 0.43	7.50 ± 0.37	7.46 ± 0.30	7.86 ± 0.41
Day 29	10.39 ± 0.64	8.68 ± 0.42*	8.82 ± 0.47	8.31 ± 0.28*	8.34 ± 0.30*	8.24 ± 0.26*
Week 14	11.08 ± 0.59	9.39 ± 0.47	9.06 ± 0.36	9.04 ± 0.47	10.08 ± 0.59	9.15 ± 0.63
Atypical lymphocytes ($10^3/\mu L$)						
Day 8	0.00 ± 0.00	0.03 ± 0.03	0.02 ± 0.02	0.00 ± 0.00	0.04 ± 0.04	0.14 ± 0.13
Day 29	0.21 ± 0.06	0.09 ± 0.05	0.02 ± 0.02*	0.07 ± 0.05	0.05 ± 0.02	0.10 ± 0.05
Week 14	0.01 ± 0.01	0.02 ± 0.02	0.05 ± 0.03	0.01 ± 0.01	0.06 ± 0.03	0.12 ± 0.05*
Monocytes ($10^3/\mu L$)						
Day 8	0.39 ± 0.07	0.25 ± 0.05	0.25 ± 0.05	0.31 ± 0.05	0.19 ± 0.06	0.19 ± 0.04
Day 29	0.14 ± 0.04	0.26 ± 0.10	0.27 ± 0.13	0.16 ± 0.09	0.10 ± 0.06	0.15 ± 0.06
Week 14	0.35 ± 0.10	0.33 ± 0.11	0.31 ± 0.05	0.32 ± 0.10	0.44 ± 0.11	0.36 ± 0.06
Eosinophils ($10^3/\mu L$)						
Day 8	0.03 ± 0.02	0.02 ± 0.02	0.04 ± 0.02	0.02 ± 0.02	0.01 ± 0.01	0.00 ± 0.00
Day 29	0.10 ± 0.04	0.05 ± 0.02	0.06 ± 0.03	0.09 ± 0.03	0.06 ± 0.03	0.01 ± 0.01
Week 14	0.04 ± 0.02	0.07 ± 0.03	0.13 ± 0.04	0.03 ± 0.03	0.04 ± 0.02	0.08 ± 0.04
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 29	23.1 ± 0.5	23.4 ± 0.7	23.1 ± 0.5	23.1 ± 0.6	20.2 ± 0.5**	20.8 ± 0.6**
Week 14	22.8 ± 0.5	22.1 ± 0.5	21.9 ± 0.3	21.6 ± 0.3*	20.7 ± 0.4**	21.6 ± 0.6**
Creatinine (mg/dL)						
Day 29	0.68 ± 0.01	0.69 ± 0.02	0.70 ± 0.02	0.70 ± 0.00	0.72 ± 0.02	0.70 ± 0.02
Week 14	0.43 ± 0.02	0.45 ± 0.02	0.47 ± 0.02	0.50 ± 0.02	0.53 ± 0.03*	0.42 ± 0.06
Total protein (g/dL)						
Day 29	6.7 ± 0.1	6.5 ± 0.1	6.7 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.0 ± 0.1**
Week 14	6.9 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	7.0 ± 0.2	7.0 ± 0.1	6.6 ± 0.1
Albumin (g/dL)						
Day 29	4.9 ± 0.0	4.9 ± 0.1	5.1 ± 0.1[b]	4.8 ± 0.1	4.8 ± 0.1	4.5 ± 0.1**
Week 14	4.9 ± 0.0	5.0 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	4.8 ± 0.1
Alanine aminotransferase (IU/L)						
Day 29	51 ± 3	49 ± 2	61 ± 4	95 ± 7**	51 ± 2	39 ± 1*
Week 14	62 ± 3	71 ± 4	68 ± 2	85 ± 6*	82 ± 6*	70 ± 4
Alkaline phosphatase (IU/L)						
Day 29	464 ± 10	456 ± 10	472 ± 8	468 ± 8	492 ± 9	713 ± 27**
Week 14	251 ± 8	265 ± 7	275 ± 8	287 ± 5**	297 ± 13**	434 ± 19**
Creatine kinase (IU/L)						
Day 29	375 ± 50	403 ± 33	427 ± 50	457 ± 63	403 ± 44	390 ± 38
Week 14	292 ± 26	239 ± 35	298 ± 38	305 ± 25	393 ± 44	283 ± 53

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	9	8
Week 14	10	10	10	10	10	9
Clinical Chemistry (continued)						
Sorbitol dehydrogenase (IU/L)						
Day 29	25 ± 1	23 ± 1	29 ± 2	41 ± 4**	29 ± 1*	34 ± 1**
Week 14	24 ± 2	28 ± 3	27 ± 1	31 ± 3*	36 ± 5*	36 ± 2**
Bile acids (μmol/L)						
Day 29	24.5 ± 3.6	31.7 ± 4.5	34.8 ± 4.7	31.3 ± 3.7	26.7 ± 2.5	42.0 ± 9.3
Week 14	21.1 ± 3.1	19.2 ± 1.1	23.7 ± 3.1	31.2 ± 6.5	26.2 ± 4.5	44.5 ± 5.6*
Thyroid-stimulating hormone (ng/mL)						
Day 8	2.07 ± 0.44	2.24 ± 0.45	1.97 ± 0.50	2.20 ± 0.51	1.72 ± 0.46	1.97 ± 0.50
Day 29	0.56 ± 0.23	0.94 ± 0.17	1.15 ± 0.29	0.80 ± 0.21	1.38 ± 0.30	1.25 ± 0.25
Week 14	1.34 ± 0.45	1.19 ± 0.27	0.87 ± 0.13	0.88 ± 0.07	1.68 ± 0.46	1.69 ± 0.38
Triiodothyronine (ng/dL)						
Day 8	143.6 ± 3.0	149.7 ± 6.2	141.4 ± 4.1	144.3 ± 4.9	145.2 ± 4.4	128.8 ± 5.9
Day 29	135.3 ± 4.6	139.6 ± 6.0	137.1 ± 4.5	138.9 ± 4.4	128.2 ± 6.5	127.3 ± 5.7
Week 14	142.4 ± 5.1	151.5 ± 3.2	140.4 ± 6.0	130.4 ± 4.3	137.4 ± 5.2	141.4 ± 4.5
Thyroxine (μg/dL)						
Day 8	5.24 ± 0.22	4.82 ± 0.27	5.08 ± 0.11	5.00 ± 0.22	4.88 ± 0.16	4.12 ± 0.24**
Day 29	3.67 ± 0.15	3.35 ± 0.18	3.39 ± 0.21	3.11 ± 0.09*	3.21 ± 0.16	3.78 ± 0.10
Week 14	2.23 ± 0.22	2.78 ± 0.20	2.21 ± 0.22	2.09 ± 0.20	2.81 ± 0.32	3.74 ± 0.24**
Female						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	7
Week 14	10	10	9	10	10	10
Hematology						
Automated hematocrit (%)						
Day 8	43.8 ± 0.6	44.5 ± 0.7	45.4 ± 0.4*	43.6 ± 0.4	45.4 ± 0.9	50.2 ± 0.7**
Day 29	44.3 ± 0.6	44.7 ± 0.6	45.6 ± 0.8	45.7 ± 0.6	45.3 ± 0.5	44.9 ± 0.9
Week 14	44.0 ± 0.4	45.1 ± 0.3	44.4 ± 0.3	43.5 ± 0.5	42.8 ± 0.5	40.4 ± 0.5**
Manual hematocrit (%)						
Day 8	44.8 ± 0.6	44.9 ± 0.6	45.6 ± 0.4	44.3 ± 0.4	45.3 ± 1.0	49.6 ± 0.4**
Day 29	45.6 ± 0.5	46.0 ± 0.6	47.1 ± 0.5	46.5 ± 0.7	45.5 ± 0.4	45.4 ± 0.7
Week 14	44.8 ± 0.3	46.5 ± 0.4	45.3 ± 0.4	44.3 ± 0.4	44.4 ± 0.5	41.4 ± 0.4**
Hemoglobin (g/dL)						
Day 8	14.8 ± 0.2	15.0 ± 0.2	15.2 ± 0.1	14.7 ± 0.1	15.3 ± 0.3	16.9 ± 0.2**
Day 29	15.4 ± 0.2	15.5 ± 0.2	15.7 ± 0.2	15.7 ± 0.2	15.4 ± 0.1	15.3 ± 0.3
Week 14	15.2 ± 0.1	15.6 ± 0.1	15.2 ± 0.1	15.1 ± 0.1	14.6 ± 0.1**	14.0 ± 0.2**
Erythrocytes ($10^6/\mu$L)						
Day 8	7.19 ± 0.09	7.30 ± 0.11	7.53 ± 0.07*	7.20 ± 0.07	7.64 ± 0.17*	8.52 ± 0.13**
Day 29	7.28 ± 0.10	7.34 ± 0.09	7.49 ± 0.13	7.60 ± 0.10*	7.64 ± 0.07*	8.20 ± 0.17**
Week 14	7.82 ± 0.08	7.98 ± 0.06	7.85 ± 0.06	7.78 ± 0.09	7.76 ± 0.09	7.84 ± 0.10

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	7
Week 14	10	10	9	10	10	10
Hematology (continued)						
Reticulocytes ($10^6/\mu L$)						
Day 8	0.22 ± 0.01	0.21 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.14 ± 0.01**	0.06 ± 0.01**
Day 29	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.01
Week 14	0.14 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.01	0.10 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)						
Day 8	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Day 29	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.02	0.03 ± 0.02
Mean cell volume (fL)						
Day 8	60.9 ± 0.2	60.9 ± 0.2	60.3 ± 0.1*	60.5 ± 0.3	59.4 ± 0.2**	59.0 ± 0.2**
Day 29	60.8 ± 0.1	60.9 ± 0.2	60.8 ± 0.2	60.1 ± 0.2*	59.3 ± 0.2**	54.8 ± 0.2**
Week 14	56.3 ± 0.2	56.5 ± 0.2	56.6 ± 0.1	56.0 ± 0.1	55.1 ± 0.1**	51.5 ± 0.3**
Mean cell hemoglobin (pg)						
Day 8	20.6 ± 0.1	20.5 ± 0.1	20.2 ± 0.2	20.5 ± 0.1	20.1 ± 0.1**	19.9 ± 0.1**
Day 29	21.1 ± 0.1	21.1 ± 0.1	21.0 ± 0.2	20.7 ± 0.1**	20.1 ± 0.1**	18.7 ± 0.1**
Week 14	19.5 ± 0.1	19.6 ± 0.1	19.3 ± 0.1	19.4 ± 0.1	18.9 ± 0.1**	17.9 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 8	33.8 ± 0.2	33.7 ± 0.2	33.6 ± 0.2	33.8 ± 0.2	33.8 ± 0.1	33.7 ± 0.2
Day 29	34.8 ± 0.2	34.7 ± 0.2	34.6 ± 0.3	34.4 ± 0.1*	34.0 ± 0.2**	34.2 ± 0.2*
Week 14	34.6 ± 0.2	34.6 ± 0.2	34.2 ± 0.2	34.6 ± 0.2	34.2 ± 0.2	34.8 ± 0.2
Platelets ($10^3/\mu L$)						
Day 8	744.9 ± 18.9	775.4 ± 20.1	738.5 ± 19.9	737.8 ± 20.5	673.1 ± 18.7*	616.4 ± 25.5**
Day 29	670.7 ± 16.7	652.3 ± 18.1	622.8 ± 8.5*	631.0 ± 17.9	592.0 ± 16.7**	594.1 ± 20.1**
Week 14	600.3 ± 18.5	558.7 ± 16.5	560.0 ± 17.7	546.5 ± 19.1	535.2 ± 30.3	503.2 ± 12.3**
Leukocytes ($10^3/\mu L$)						
Day 8	10.17 ± 0.45	9.89 ± 0.55	9.53 ± 0.52	8.88 ± 0.36	9.14 ± 0.37	8.96 ± 0.50
Day 29	8.84 ± 0.48	9.31 ± 0.39	8.62 ± 0.43	8.57 ± 0.42	8.02 ± 0.44	9.10 ± 0.72
Week 14	9.81 ± 0.56	9.00 ± 0.33	9.16 ± 0.46	8.20 ± 0.33	10.22 ± 0.71	9.75 ± 0.48
Segmented neutrophils ($10^3/\mu L$)						
Day 8	1.16 ± 0.09	0.99 ± 0.12	0.84 ± 0.07*	0.89 ± 0.09	0.83 ± 0.10*	0.54 ± 0.07**
Day 29	0.96 ± 0.13	0.89 ± 0.12	0.83 ± 0.11	0.65 ± 0.07	0.59 ± 0.10	1.67 ± 0.39
Week 14	1.89 ± 0.36	1.60 ± 0.17	2.09 ± 0.32	1.37 ± 0.16	1.67 ± 0.17	2.00 ± 0.17
Lymphocytes ($10^3/\mu L$)						
Day 8	8.59 ± 0.40	8.48 ± 0.45	8.15 ± 0.49	7.61 ± 0.34	8.00 ± 0.38	8.08 ± 0.41
Day 29	7.61 ± 0.44	7.99 ± 0.29	7.30 ± 0.34	7.48 ± 0.34	7.11 ± 0.40	7.05 ± 0.67
Week 14	7.43 ± 0.33	7.00 ± 0.40	6.66 ± 0.28	6.34 ± 0.32	8.24 ± 0.60	7.42 ± 0.47
Atypical lymphocytes ($10^3/\mu L$)						
Day 8	0.14 ± 0.06	0.10 ± 0.05	0.16 ± 0.05	0.09 ± 0.03	0.06 ± 0.03	0.10 ± 0.04
Day 29	0.04 ± 0.03	0.04 ± 0.02	0.09 ± 0.03	0.03 ± 0.02	0.05 ± 0.04	0.13 ± 0.07
Week 14	0.04 ± 0.02	0.04 ± 0.03	0.05 ± 0.03	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.02
Monocytes ($10^3/\mu L$)						
Day 8	0.16 ± 0.05	0.18 ± 0.07	0.31 ± 0.08	0.20 ± 0.07	0.18 ± 0.05	0.17 ± 0.06
Day 29	0.15 ± 0.04	0.27 ± 0.09	0.29 ± 0.08	0.29 ± 0.08	0.11 ± 0.04	0.10 ± 0.05
Week 14	0.35 ± 0.08	0.29 ± 0.06	0.24 ± 0.06	0.37 ± 0.08	0.20 ± 0.06	0.25 ± 0.06

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	7
Week 14	10	10	9	10	10	10
Hematology (continued)						
Eosinophils ($10^3/\mu L$)						
Day 8	0.07 ± 0.02	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
Day 29	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.03	0.06 ± 0.03	0.04 ± 0.02
Week 14	0.08 ± 0.02	0.08 ± 0.02	0.09 ± 0.03	0.11 ± 0.03	0.07 ± 0.02	0.04 ± 0.02
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 29	24.6 ± 0.7	23.9 ± 0.4	24.8 ± 0.4	23.8 ± 0.5	20.7 ± 0.5**	20.1 ± 0.7**
Week 14	22.5 ± 1.0	21.9 ± 1.0	21.3 ± 0.6	21.9 ± 0.4	21.4 ± 0.7	23.7 ± 0.7
Creatinine (mg/dL)						
Day 29	0.67 ± 0.02	0.68 ± 0.01	0.74 ± 0.02*	0.73 ± 0.02	0.72 ± 0.02	0.66 ± 0.02
Week 14	0.47 ± 0.02	0.55 ± 0.02**	0.54 ± 0.04*	0.58 ± 0.02**	0.61 ± 0.02**	0.53 ± 0.02**
Total protein (g/dL)						
Day 29	6.1 ± 0.1	6.1 ± 0.1	6.4 ± 0.1	6.2 ± 0.0	6.3 ± 0.4	5.8 ± 0.1
Week 14	6.8 ± 0.1	6.7 ± 0.1	6.2 ± 0.5	6.4 ± 0.1*	6.3 ± 0.1**	6.0 ± 0.1**
Albumin (g/dL)						
Day 29	4.7 ± 0.1[b]	4.7 ± 0.1	4.9 ± 0.1	4.8 ± 0.0	4.5 ± 0.1	4.4 ± 0.1*
Week 14	5.0 ± 0.0	5.1 ± 0.1	4.6 ± 0.3	4.8 ± 0.1	4.7 ± 0.1**	4.5 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 29	32 ± 2	28 ± 1*	30 ± 2	28 ± 1	24 ± 1**	28 ± 2**
Week 14	55 ± 5	74 ± 6	51 ± 7	73 ± 8	54 ± 4	43 ± 2
Alkaline phosphatase (IU/L)						
Day 29	336 ± 7	320 ± 5	356 ± 10	353 ± 12	342 ± 9	398 ± 10**
Week 14	212 ± 8	219 ± 9	231 ± 9	246 ± 5**	266 ± 10**	321 ± 11**
Creatine kinase (IU/L)						
Day 29	547 ± 46[b]	572 ± 52	476 ± 37	580 ± 55	723 ± 114	713 ± 76
Week 14	401 ± 58	420 ± 59	320 ± 44	405 ± 39	420 ± 53	487 ± 47
Sorbitol dehydrogenase (IU/L)						
Day 29	19 ± 2	18 ± 2	25 ± 2	20 ± 3	17 ± 2	21 ± 2
Week 14	21 ± 2	28 ± 3	27 ± 3	28 ± 4	26 ± 3	25 ± 2
Bile acids (μmol/L)						
Day 29	31.9 ± 7.4	31.2 ± 5.0	31.5 ± 3.3	45.8 ± 8.2	48.7 ± 5.7*	32.1 ± 6.1
Week 14	25.9 ± 3.9	38.7 ± 6.2	47.0 ± 10.0	41.8 ± 8.0	46.8 ± 6.3	23.9 ± 2.7

TABLE C2
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female (continued)						
n						
Day 8	10	10	10	10	10	10
Day 29	10	10	10	10	10	7
Week 14	10	10	9	10	10	10
Clinical Chemistry (continued)						
Thyroid-stimulating hormone (ng/mL)						
Day 8	1.67 ± 0.24	1.70 ± 0.30	1.34 ± 0.18	1.34 ± 0.28	1.05 ± 0.18*	0.78 ± 0.15**
Day 29	0.49 ± 0.11	0.44 ± 0.07	0.55 ± 0.07	0.48 ± 0.08	0.30 ± 0.07	1.06 ± 0.17
Week 14	1.07 ± 0.20	1.06 ± 0.26	1.04 ± 0.15	0.91 ± 0.11	1.05 ± 0.13	1.16 ± 0.21
Triiodothyronine (ng/dL)						
Day 8	146.2 ± 3.2	135.6 ± 6.8	134.3 ± 5.3	135.0 ± 3.2	132.1 ± 4.7*	115.4 ± 4.5**
Day 29	138.7 ± 5.5	138.3 ± 6.7	140.9 ± 6.0	136.6 ± 4.5	128.9 ± 4.1	137.1 ± 9.0
Week 14	142.9 ± 3.3	141.1 ± 6.4	143.3 ± 9.3	131.6 ± 5.3	135.5 ± 6.3	125.4 ± 4.5**
Thyroxine (μg/dL)						
Day 8	3.41 ± 0.28	2.97 ± 0.41	2.46 ± 0.28	3.38 ± 0.22	3.80 ± 0.17	3.23 ± 0.23
Day 29	3.43 ± 0.40	2.70 ± 0.41	2.53 ± 0.31	2.22 ± 0.22	2.87 ± 0.27	2.57 ± 0.45
Week 14	2.60 ± 0.29	2.48 ± 0.30	2.87 ± 0.25	2.06 ± 0.15	2.49 ± 0.30	1.70 ± 0.15

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9

TABLE C3
Hematology and Clinical Chemistry Data for Mice in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
Hematology						
n	10	10	10	10	10	10
Automated hematocrit (%)	49.2 ± 0.9	46.9 ± 0.7*	41.9 ± 1.2**	36.9 ± 0.4**	34.1 ± 0.6**	34.6 ± 0.7**
Manual hematocrit (%)	50.4 ± 0.7	49.2 ± 0.3	44.8 ± 1.1**	39.9 ± 0.4**	37.2 ± 0.9**	37.5 ± 0.8**
Hemoglobin (g/dL)	15.6 ± 0.2	15.2 ± 0.1*	14.1 ± 0.4**	12.5 ± 0.1**	11.8 ± 0.2**	12.0 ± 0.2**
Erythrocytes ($10^6/\mu L$)	9.58 ± 0.15	9.04 ± 0.11*	8.02 ± 0.23**	6.83 ± 0.07**	5.97 ± 0.13**	5.90 ± 0.15**
Reticulocytes ($10^6/\mu L$)	0.17 ± 0.01	0.17 ± 0.02	0.31 ± 0.03**	0.52 ± 0.04**	0.75 ± 0.08**	0.91 ± 0.04**
Nucleated erythrocytes ($10^3/\mu L$)	0.10 ± 0.10	0.20 ± 0.13	0.10 ± 0.10	0.00 ± 0.00	0.20 ± 0.13	0.00 ± 0.00
Mean cell volume (fL)	51.3 ± 0.4	51.9 ± 0.3	52.3 ± 0.1*	54.0 ± 0.2**	57.1 ± 0.3**	58.7 ± 0.4**
Mean cell hemoglobin (pg)	16.3 ± 0.2	16.8 ± 0.2	17.7 ± 0.2**	18.3 ± 0.1**	19.8 ± 0.2**	20.4 ± 0.3**
Mean cell hemoglobin concentration (g/dL)	31.9 ± 0.3	32.4 ± 0.3	33.7 ± 0.3**	34.0 ± 0.1**	34.6 ± 0.3**	34.8 ± 0.4**
Platelets ($10^3/\mu L$)	901.9 ± 30.8	934.1 ± 19.4	948.0 ± 31.1	1,183.5 ± 20.0**	1,243.5 ± 55.4**	1,247.7 ± 61.8**
Leukocytes ($10^3/\mu L$)	5.03 ± 0.67	2.94 ± 0.27	4.13 ± 0.56	4.16 ± 0.34	7.41 ± 0.77	11.24 ± 2.01*
Segmented neutrophils ($10^3/\mu L$)	0.57 ± 0.06	0.36 ± 0.06	1.00 ± 0.48	0.63 ± 0.15	0.95 ± 0.25	1.35 ± 0.40
Lymphocytes ($10^3/\mu L$)	4.12 ± 0.59	2.49 ± 0.26	2.88 ± 0.24	3.33 ± 0.23	6.25 ± 0.72*	9.45 ± 1.69*
Monocytes ($10^3/\mu L$)	0.19 ± 0.05	0.07 ± 0.02	0.19 ± 0.06	0.16 ± 0.06	0.18 ± 0.07	0.35 ± 0.10
Eosinophils ($10^3/\mu L$)	0.05 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.01*	0.04 ± 0.03
Clinical Chemistry						
n						
Day 8	10	9	10	10	10	10
Day 29	10	10	10	10	10	10
Day 86	10	10	10	9	10	10
Thyroid-stimulating hormone (ng/mL)						
Day 8	0.31 ± 0.04	0.25 ± 0.04	0.30 ± 0.05	0.28 ± 0.06[b]	0.29 ± 0.05	0.39 ± 0.06
Day 29	0.02 ± 0.02	0.02 ± 0.01	0.03 ± 0.03	0.01 ± 0.00	0.04 ± 0.01	0.10 ± 0.02**
Day 86	0.28 ± 0.02	0.34 ± 0.03[b]	0.26 ± 0.02	0.30 ± 0.02	0.33 ± 0.04	0.37 ± 0.04
Triiodothyronine (ng/dL)						
Day 8	144.1 ± 2.3[c]	161.3 ± 6.2[b]	159.0 ± 5.9[d]	163.4 ± 3.9*	168.9 ± 4.0**	155.6 ± 3.1[d]
Day 29	149.3 ± 3.3[d]	163.9 ± 4.8*	173.7 ± 7.6**	164.9 ± 3.5*	172.9 ± 3.8**	178.0 ± 4.3**[b]
Day 86	—[e]	—	—	—	—	—
Thyroxine ($\mu g/dL$)						
Day 8	6.89 ± 0.21	6.19 ± 0.24[f]	6.39 ± 0.16	6.57 ± 0.15	6.98 ± 0.14	6.09 ± 0.23
Day 29	6.49 ± 0.30	6.33 ± 0.15	6.31 ± 0.19	6.48 ± 0.19	7.38 ± 0.14*	6.78 ± 0.25
Day 86	5.32 ± 0.22	4.77 ± 0.24	4.73 ± 0.11	5.13 ± 0.15[f]	6.18 ± 0.15*	5.81 ± 0.22

TABLE C3
Hematology and Clinical Chemistry Data for Mice in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female						
n	10	10	10	10	10	10
Hematology						
Automated hematocrit (%)	48.7 ± 0.9	47.9 ± 0.6	46.7 ± 0.6	43.8 ± 0.7**	40.4 ± 0.4**	41.5 ± 0.7**
Manual hematocrit (%)	50.3 ± 0.5	50.0 ± 0.4	48.6 ± 0.5*	46.3 ± 0.7**	43.0 ± 0.4**	43.4 ± 0.6**
Hemoglobin (g/dL)	15.9 ± 0.2	15.6 ± 0.1	15.3 ± 0.1**	14.6 ± 0.1**[b]	13.8 ± 0.1**	14.1 ± 0.2**
Erythrocytes ($10^6/\mu L$)	9.61 ± 0.16	9.29 ± 0.13	9.02 ± 0.09**	8.27 ± 0.13**	7.39 ± 0.10**	7.53 ± 0.15**
Reticulocytes ($10^6/\mu L$)	0.15 ± 0.01	0.17 ± 0.02	0.21 ± 0.03	0.31 ± 0.03**	0.54 ± 0.04**	0.44 ± 0.03**
Nucleated erythrocytes ($10^3/\mu L$)	0.20 ± 0.13	0.00 ± 0.00[b]	0.40 ± 0.22	0.20 ± 0.20	0.60 ± 0.27	0.30 ± 0.21
Mean cell volume (fL)	50.7 ± 0.4	51.5 ± 0.2	51.8 ± 0.3	53.0 ± 0.2**	54.6 ± 0.3**	55.1 ± 0.2**
Mean cell hemoglobin (pg)	16.6 ± 0.2	16.7 ± 0.2	16.9 ± 0.1*	17.7 ± 0.2**[b]	18.7 ± 0.1**	18.7 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.5	32.5 ± 0.3	32.7 ± 0.2	33.4 ± 0.3[b]	34.3 ± 0.1**	33.9 ± 0.1**
Platelets ($10^3/\mu L$)	846.6 ± 28.3	801.6 ± 20.3	871.5 ± 26.2	943.7 ± 25.5*	1,042.5 ± 28.1**	1,038.7 ± 31.4**
Leukocytes ($10^3/\mu L$)	3.72 ± 0.30	3.63 ± 0.19	4.06 ± 0.28	4.03 ± 0.46	4.00 ± 0.25	4.07 ± 0.52
Segmented neutrophils ($10^3/\mu L$)	0.52 ± 0.06	0.37 ± 0.06[b]	0.59 ± 0.09	0.51 ± 0.05	0.58 ± 0.06	0.64 ± 0.17
Lymphocytes ($10^3/\mu L$)	2.96 ± 0.25	3.11 ± 0.18[b]	3.30 ± 0.20	3.30 ± 0.40	3.26 ± 0.20	3.25 ± 0.40
Monocytes ($10^3/\mu L$)	0.20 ± 0.05	0.10 ± 0.02[b]	0.15 ± 0.04	0.17 ± 0.07	0.14 ± 0.02	0.12 ± 0.02
Eosinophils ($10^3/\mu L$)	0.03 ± 0.01	0.05 ± 0.02[b]	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Clinical Chemistry						
Thyroid-stimulating hormone (ng/mL)						
Day 8	0.15 ± 0.07[b]	0.14 ± 0.06	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.18 ± 0.06
Day 29	0.13 ± 0.07	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.14 ± 0.03
Day 86	0.23 ± 0.01	0.22 ± 0.02	0.24 ± 0.03	0.21 ± 0.03	0.25 ± 0.02[b]	0.33 ± 0.05
Triiodothyronine (ng/dL)						
Day 8	138.4 ± 3.2	140.7 ± 4.0[b]	147.1 ± 2.5	145.8 ± 1.7	144.4 ± 3.3	126.3 ± 2.7[d]
Day 29	118.1 ± 2.7	124.8 ± 3.7	121.6 ± 3.3	139.6 ± 4.7**	140.1 ± 3.6**	131.6 ± 3.4**
Day 86	132.4 ± 2.4[d]	130.1 ± 3.0	141.8 ± 2.4*[b]	136.1 ± 2.7[b]	149.5 ± 4.0**	155.6 ± 8.3**
Thyroxine (μg/dL)						
Day 8	7.55 ± 0.36	7.71 ± 0.28	7.11 ± 0.31	7.33 ± 0.19	6.87 ± 0.20	5.56 ± 0.16**
Day 29	6.36 ± 0.25	6.05 ± 0.19	5.54 ± 0.30	5.91 ± 0.23	6.21 ± 0.27	4.94 ± 0.19**
Day 86	7.04 ± 0.34	6.36 ± 0.26	6.05 ± 0.21*	5.44 ± 0.23**	6.00 ± 0.16**	4.79 ± 0.13**

* Significantly different ($P \le 0.05$) from the control group by Dunn's or Shirley's test
** $P \le 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=7
[d] n=8
[e] The assay was unacceptable due to instrumentation/reagent problems.
[f] n=10

TABLE C4
Hematology and Clinical Chemistry Data for Mice in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
Hematology						
n	9	10	10	9	9	8
Automated hematocrit (%)	48.4 ± 0.8	47.0 ± 0.3	47.1 ± 0.5	47.9 ± 0.5	46.7 ± 1.0	46.8 ± 0.9
Manual hematocrit (%)	49.1 ± 0.6[b]	48.3 ± 0.3[b]	47.3 ± 0.5	48.9 ± 0.6[c]	48.8 ± 0.6[b]	47.4 ± 0.4[d]
Hemoglobin (g/dL)	15.4 ± 0.2	15.1 ± 0.1	15.1 ± 0.2	15.5 ± 0.1	15.0 ± 0.2	15.2 ± 0.1
Erythrocytes ($10^6/\mu L$)	9.56 ± 0.16	9.19 ± 0.09	9.37 ± 0.12	9.60 ± 0.13	9.26 ± 0.20	9.57 ± 0.16
Reticulocytes ($10^6/\mu L$)	0.16 ± 0.01	0.13 ± 0.02	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.02
Nucleated erythrocytes ($10^3/\mu L$)	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	50.6 ± 0.3	51.1 ± 0.3	50.3 ± 0.2	49.9 ± 0.3	50.5 ± 0.3	48.9 ± 0.2**
Mean cell hemoglobin (pg)	16.1 ± 0.2	16.4 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.2 ± 0.2	15.9 ± 0.2
Mean cell hemoglobin concentration (g/dL)	31.9 ± 0.3	32.1 ± 0.1	32.0 ± 0.2	32.5 ± 0.2	32.2 ± 0.3	32.5 ± 0.5
Platelets ($10^3/\mu L$)	859.8 ± 15.3	902.6 ± 22.7	902.3 ± 22.4	893.2 ± 24.2	842.2 ± 22.8	805.9 ± 17.6
Leukocytes ($10^3/\mu L$)	4.54 ± 0.91	2.33 ± 0.17	2.66 ± 0.40	3.33 ± 0.63	2.90 ± 0.42	3.08 ± 0.35
Segmented neutrophils ($10^3/\mu L$)	0.65 ± 0.20	0.24 ± 0.06	0.25 ± 0.05	0.49 ± 0.15	0.29 ± 0.07	0.62 ± 0.24
Lymphocytes ($10^3/\mu L$)	3.73 ± 0.74	2.03 ± 0.15	2.35 ± 0.38	2.65 ± 0.46	2.55 ± 0.42	2.29 ± 0.19
Atypical lymphocytes ($10^3/\mu L$)	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00	0.02 ± 0.01
Monocytes ($10^3/\mu L$)	0.12 ± 0.03	0.04 ± 0.02	0.05 ± 0.02	0.11 ± 0.04	0.03 ± 0.01	0.10 ± 0.03
Eosinophils ($10^3/\mu L$)	0.05 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
Clinical Chemistry						
n	10	10	10	10	10	10
Thyroid-stimulating hormone (ng/mL)						
Day 8	0.86 ± 0.46[c]	0.24 ± 0.04	0.31 ± 0.06[b]	0.36 ± 0.12[c]	0.54 ± 0.13[e]	0.39 ± 0.07[b]
Day 29	0.06 ± 0.04	0.03 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.16 ± 0.06	0.11 ± 0.06
Day 86	0.35 ± 0.03	0.36 ± 0.05	0.39 ± 0.05	0.37 ± 0.05	0.28 ± 0.04	0.24 ± 0.06
Triiodothyronine (ng/dL)						
Day 8	137.0 ± 5.4[f]	139.8 ± 3.5[g]	132.8 ± 3.7[g]	140.3 ± 3.8[h]	125.5 ± 2.5[i]	137.7 ± 5.8[h]
Day 29	142.3 ± 6.1[b]	152.5 ± 5.2[b]	141.1 ± 6.0[b]	148.0 ± 5.9[g]	163.8 ± 7.6[g]	168.0 ± 8.0[g]
Day 86	128.8 ± 3.7	130.3 ± 4.4[b]	133.4 ± 2.4	137.6 ± 4.9[b]	148.2 ± 3.8**[d]	176.7 ± 6.6**[g]
Thyroxine ($\mu g/dL$)						
Day 8	5.93 ± 0.24	6.14 ± 0.22	6.15 ± 0.27	5.55 ± 0.25	4.95 ± 0.15**	4.70 ± 0.18**[e]
Day 29	5.95 ± 0.22	6.18 ± 0.21	6.03 ± 0.14	5.57 ± 0.23	4.97 ± 0.12**	3.70 ± 0.09**
Day 86	4.47 ± 0.17	4.62 ± 0.25	4.67 ± 0.14	4.72 ± 0.09	4.62 ± 0.21	3.98 ± 0.18

TABLE C4
Hematology and Clinical Chemistry Data for Mice in the 14-Week Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female						
Hematology						
n	10	10	10	10	10	3
Automated hematocrit (%)	47.9 ± 0.4	46.5 ± 0.3*	46.0 ± 0.3**	45.3 ± 0.6**	45.7 ± 0.3**	45.0 ± 0.7**
Manual hematocrit (%)	49.4 ± 0.3[b]	48.3 ± 0.5[b]	48.1 ± 0.3*[b]	47.6 ± 0.6*[c]	48.0 ± 0.4*	45.0[j]
Hemoglobin (g/dL)	15.7 ± 0.1	15.2 ± 0.1**	15.2 ± 0.1**	14.9 ± 0.1**	14.9 ± 0.1**	14.8 ± 0.2**
Erythrocytes ($10^6/\mu L$)	9.51 ± 0.12	9.25 ± 0.07	9.22 ± 0.08	9.15 ± 0.14	9.17 ± 0.09	9.32 ± 0.20
Reticulocytes ($10^6/\mu L$)	0.17 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.02	0.19 ± 0.03
Mean cell volume (fL)	50.4 ± 0.3	50.2 ± 0.3	49.9 ± 0.3	49.5 ± 0.3	49.9 ± 0.3	48.3 ± 0.5**
Mean cell hemoglobin (pg)	16.5 ± 0.1	16.4 ± 0.1	16.5 ± 0.1	16.3 ± 0.1	16.2 ± 0.1	15.9 ± 0.2
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.3	32.6 ± 0.3	33.0 ± 0.1	32.8 ± 0.3	32.5 ± 0.2	32.9 ± 0.3
Platelets ($10^3/\mu L$)	823.7 ± 23.4	862.6 ± 14.7	890.9 ± 13.8	852.5 ± 21.2	799.9 ± 21.8	721.0 ± 57.1
Leukocytes ($10^3/\mu L$)	3.51 ± 0.24	3.05 ± 0.25	3.29 ± 0.39	2.51 ± 0.24	2.29 ± 0.24*	4.00 ± 0.71
Segmented neutrophils ($10^3/\mu L$)	0.45 ± 0.07	0.43 ± 0.08	0.65 ± 0.18	0.54 ± 0.09	0.58 ± 0.13	0.48 ± 0.14
Lymphocytes ($10^3/\mu L$)	2.92 ± 0.27	2.50 ± 0.16	2.51 ± 0.31	1.89 ± 0.17**	1.65 ± 0.12**	3.35 ± 0.49
Atypical lymphocytes ($10^3/\mu L$)	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes ($10^3/\mu L$)	0.10 ± 0.01	0.08 ± 0.03	0.07 ± 0.03	0.07 ± 0.02	0.05 ± 0.01	0.08 ± 0.01
Eosinophils ($10^3/\mu L$)	0.02 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.07 ± 0.07
Clinical Chemistry						
n						
Day 8	10	10	10	10	10	9
Day 29	10	10	10	10	10	5
Day 86	10	10	10	10	10	2
Thyroid-stimulating hormone (ng/mL)						
Day 8	0.39 ± 0.22	0.17 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	0.54 ± 0.40	0.16 ± 0.02
Day 29	0.28 ± 0.11	0.17 ± 0.09	0.12 ± 0.06	0.10 ± 0.06	0.32 ± 0.09	0.20 ± 0.18
Day 86	0.16 ± 0.03	0.16 ± 0.03	0.14 ± 0.02	0.14 ± 0.03	0.19 ± 0.05	0.28 ± 0.14
Triiodothyronine (ng/dL)						
Day 8	139.0 ± 6.4[g]	134.3 ± 3.9[g]	124.0 ± 2.3[c]	132.8 ± 3.1[e]	136.0 ± 11.0[h]	—[k]
Day 29	130.8 ± 3.5	132.5 ± 5.0[b]	130.8 ± 3.0	140.9 ± 6.7[c]	150.8 ± 5.1**[b]	148.5 ± 7.5[i]
Day 86	128.1 ± 4.9[c]	116.3 ± 3.4[e]	131.0 ± 3.8	149.7 ± 8.5[h]	141.0 ± 8.1[d]	—
Thyroxine (μg/dL)						
Day 8	7.14 ± 0.41	6.99 ± 0.31	7.30 ± 0.31	7.78 ± 0.31	7.19 ± 0.42	6.56 ± 0.42[e]
Day 29	6.98 ± 0.15	6.76 ± 0.15	7.19 ± 0.18	6.80 ± 0.18	7.51 ± 0.35	5.56 ± 0.14*[d]
Day 86	6.95 ± 0.40	6.45 ± 0.16	6.53 ± 0.22	6.91 ± 0.22	5.90 ± 0.28	5.25 ± 0.55[i]

* Significantly different ($P \le 0.05$) from the control group by Dunn's or Shirley's test
** Significantly different ($P \le 0.01$) from the control group by Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=8
[c] n=7
[d] n=5
[e] n=9
[f] n=4
[g] n=6
[h] n=3
[i] n=2
[j] n=1; no standard error calculated
[k] Not analyzed

APPENDIX D
ORGAN WEIGHTS AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Rats in the 15-Day Feed Study of 2-Methylimidazole[a]

	0 ppm	1,200 ppm	3,300 ppm	10,000 ppm
n	5			5
Male				
Necropsy body wt	198 ± 4	196 ± 4	185 ± 6	162 ± 5**
Heart				
Absolute	0.687 ± 0.024	0.679 ± 0.018	0.597 ± 0.032*	0.505 ± 0.025**
Relative	3.47 ± 0.08	3.48 ± 0.09	3.22 ± 0.08	3.13 ± 0.13
R. Kidney				
Absolute	0.883 ± 0.016	0.922 ± 0.024	0.847 ± 0.032	0.772 ± 0.021**
Relative	4.46 ± 0.01	4.72 ± 0.04	4.58 ± 0.06	4.79 ± 0.12*
Liver				
Absolute	10.050 ± 0.210	10.458 ± 0.212	9.830 ± 0.394	8.656 ± 0.447*
Relative	50.82 ± 1.07	53.52 ± 0.96	53.09 ± 1.02	53.51 ± 1.35
Lung				
Absolute	1.179 ± 0.106	1.117 ± 0.051	1.017 ± 0.049	0.844 ± 0.024**
Relative	5.98 ± 0.60	5.73 ± 0.32	5.49 ± 0.12	5.24 ± 0.16
R. Testis				
Absolute	1.101 ± 0.029	1.058 ± 0.020	1.089 ± 0.017	1.127 ± 0.029
Relative	5.56 ± 0.06	5.41 ± 0.03	5.90 ± 0.15*	6.99 ± 0.13**
Thymus				
Absolute	0.457 ± 0.021	0.507 ± 0.020	0.451 ± 0.026	0.399 ± 0.008
Relative	2.32 ± 0.13	2.60 ± 0.15	2.44 ± 0.10	2.48 ± 0.10
Female				
Necropsy body wt	136 ± 3	139 ± 6	135 ± 3	121 ± 5
Heart				
Absolute	0.513 ± 0.011	0.511 ± 0.008	0.491 ± 0.014	0.378 ± 0.012**
Relative	3.77 ± 0.05	3.71 ± 0.18	3.64 ± 0.06	3.12 ± 0.05**
R. Kidney				
Absolute	0.601 ± 0.019	0.628 ± 0.018	0.622 ± 0.019	0.557 ± 0.021
Relative	4.41 ± 0.05	4.54 ± 0.08	4.60 ± 0.09	4.60 ± 0.05
Liver				
Absolute	5.915 ± 0.247	6.263 ± 0.232	6.164 ± 0.127	5.656 ± 0.253
Relative	43.39 ± 1.06	45.24 ± 0.23	45.64 ± 0.28*	46.64 ± 0.74**
Lung				
Absolute	0.829 ± 0.025	0.828 ± 0.038	0.800 ± 0.030	0.708 ± 0.024*
Relative	6.09 ± 0.11	5.99 ± 0.19	5.92 ± 0.20	5.85 ± 0.15
Thymus				
Absolute	0.351 ± 0.015	0.366 ± 0.012	0.358 ± 0.021	0.307 ± 0.012
Relative	2.58 ± 0.08	2.66 ± 0.14	2.65 ± 0.15	2.55 ± 0.14

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE D2
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Rats in the 15-Day Feed Study of 4-Methylimidazole[a]

	0 ppm	300 ppm	800 ppm	2,500 ppm
n	5	5	5	5
Male				
Necropsy body wt	205 ± 6	200 ± 7	198 ± 7	190 ± 5
Heart				
Absolute	0.725 ± 0.021	0.713 ± 0.022	0.695 ± 0.030	0.669 ± 0.010
Relative	3.54 ± 0.04	3.56 ± 0.04	3.52 ± 0.08	3.52 ± 0.04
R. Kidney				
Absolute	0.927 ± 0.034	0.951 ± 0.035	0.949 ± 0.041	0.982 ± 0.027
Relative	4.52 ± 0.05	4.75 ± 0.04*	4.81 ± 0.10**	5.16 ± 0.03**
Liver				
Absolute	11.025 ± 0.456	11.421 ± 0.430	11.257 ± 0.467	10.909 ± 0.306
Relative	53.72 ± 1.17	57.01 ± 1.05	57.04 ± 1.64	57.30 ± 0.48
Lung				
Absolute	1.250 ± 0.102	1.149 ± 0.072	1.082 ± 0.059	1.053 ± 0.025
Relative	6.09 ± 0.46	5.72 ± 0.21	5.48 ± 0.19	5.55 ± 0.22
R. Testis				
Absolute	1.123 ± 0.028	1.087 ± 0.044	1.138 ± 0.043	1.144 ± 0.033
Relative	5.48 ± 0.07	5.42 ± 0.08	5.76 ± 0.13	6.01 ± 0.10**
Thymus				
Absolute	0.491 ± 0.008	0.478 ± 0.026	0.477 ± 0.015	0.490 ± 0.017
Relative	2.40 ± 0.07	2.40 ± 0.17	2.43 ± 0.14	2.58 ± 0.11
Female				
Necropsy body wt	137 ± 4	136 ± 4	139 ± 2	134 ± 3
Heart				
Absolute	0.524 ± 0.014	0.521 ± 0.014	0.543 ± 0.005	0.515 ± 0.007
Relative	3.82 ± 0.04	3.85 ± 0.06	3.93 ± 0.07	3.86 ± 0.04
R. Kidney				
Absolute	0.638 ± 0.020	0.721 ± 0.068	0.684 ± 0.015	0.693 ± 0.024
Relative	4.66 ± 0.17	5.37 ± 0.64	4.95 ± 0.14	5.18 ± 0.09
Liver				
Absolute	6.429 ± 0.132	6.509 ± 0.316	6.909 ± 0.101	6.911 ± 0.142
Relative	47.00 ± 1.53	47.97 ± 1.23	49.92 ± 0.67	51.74 ± 0.94*
Lung				
Absolute	0.908 ± 0.051	0.946 ± 0.031	0.903 ± 0.021	0.876 ± 0.015
Relative	6.62 ± 0.31	6.99 ± 0.18	6.52 ± 0.14	6.56 ± 0.13
Thymus				
Absolute	0.385 ± 0.027	0.400 ± 0.010	0.411 ± 0.014	0.362 ± 0.013
Relative	2.80 ± 0.15	2.96 ± 0.08	2.97 ± 0.11	2.71 ± 0.10

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE D3
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Rats in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	8	8	8	8	8	8
Male						
Necropsy body wt [b]	366 ± 6	358 ± 7	353 ± 6	349 ± 2*	336 ± 5**	287 ± 5**
Heart						
Absolute	1.075 ± 0.022	1.069 ± 0.032	1.059 ± 0.025	1.068 ± 0.020	0.961 ± 0.016**	0.828 ± 0.015**
Relative	2.94 ± 0.03	2.98 ± 0.04	2.98 ± 0.06	3.05 ± 0.06	2.86 ± 0.07	2.90 ± 0.03
R. Kidney						
Absolute	1.240 ± 0.034	1.303 ± 0.029	1.287 ± 0.029	1.283 ± 0.019	1.242 ± 0.027	1.143 ± 0.018*
Relative	3.39 ± 0.04	3.63 ± 0.06**	3.63 ± 0.07**	3.66 ± 0.06**	3.69 ± 0.05**	4.01 ± 0.05**
Liver						
Absolute	12.160 ± 0.279	12.682 ± 0.245	12.452 ± 0.360	12.064 ± 0.158	11.621 ± 0.291	9.828 ± 0.317**
Relative	33.21 ± 0.22	35.34 ± 0.45**	35.01 ± 0.52*	34.42 ± 0.31	34.51 ± 0.49	34.40 ± 0.60
Lung						
Absolute	1.626 ± 0.033	1.495 ± 0.068	1.519 ± 0.055	1.485 ± 0.062	1.641 ± 0.067	1.380 ± 0.038*
Relative	4.45 ± 0.08	4.16 ± 0.14	4.27 ± 0.12	4.23 ± 0.15	4.87 ± 0.17*	4.84 ± 0.10*
Spleen						
Absolute	0.773 ± 0.018	0.726 ± 0.018*	0.706 ± 0.016*	0.737 ± 0.013*	0.682 ± 0.015**	0.543 ± 0.014**
Relative	2.11 ± 0.05	2.02 ± 0.03	1.99 ± 0.02	2.10 ± 0.03	2.03 ± 0.04	1.90 ± 0.04**
R. Testis						
Absolute	1.388 ± 0.064	1.478 ± 0.030[c]	1.466 ± 0.022	1.478 ± 0.031	1.471 ± 0.029	1.247 ± 0.029*
Relative	3.78 ± 0.13	4.12 ± 0.08*[c]	4.13 ± 0.06*	4.22 ± 0.08**	4.38 ± 0.09**	4.37 ± 0.08**
Thymus						
Absolute	0.340 ± 0.022	0.305 ± 0.012	0.332 ± 0.021	0.301 ± 0.016	0.305 ± 0.027	0.290 ± 0.022
Relative	0.93 ± 0.06	0.85 ± 0.03	0.94 ± 0.06	0.86 ± 0.04	0.90 ± 0.07	1.02 ± 0.09
Female						
Necropsy body wt	198 ± 3	204 ± 2	202 ± 3	201 ± 3	190 ± 3	176 ± 3**
Heart						
Absolute	0.728 ± 0.038	0.702 ± 0.013	0.689 ± 0.018	0.709 ± 0.026	0.630 ± 0.019**	0.597 ± 0.015**
Relative	3.71 ± 0.18	3.47 ± 0.07	3.43 ± 0.09	3.53 ± 0.10	3.29 ± 0.08*	3.40 ± 0.05
R. Kidney						
Absolute	0.714 ± 0.019	0.732 ± 0.012	0.703 ± 0.015	0.752 ± 0.015	0.746 ± 0.012	0.712 ± 0.021
Relative	3.64 ± 0.09	3.61 ± 0.06	3.49 ± 0.05	3.76 ± 0.07	3.90 ± 0.07*	4.05 ± 0.06**
Liver						
Absolute	6.274 ± 0.159	6.634 ± 0.082	6.527 ± 0.150	7.322 ± 0.299**	6.734 ± 0.185	6.663 ± 0.262
Relative	31.96 ± 0.67	32.75 ± 0.36	32.46 ± 0.55	36.48 ± 1.20**	35.17 ± 0.86**	37.93 ± 1.30**
Lung						
Absolute	1.101 ± 0.037	1.096 ± 0.030	1.153 ± 0.035	1.118 ± 0.037	1.131 ± 0.027	1.098 ± 0.045
Relative	5.61 ± 0.16	5.40 ± 0.10	5.73 ± 0.17	5.58 ± 0.18	5.90 ± 0.10	6.27 ± 0.29*
Spleen						
Absolute	0.458 ± 0.014	0.465 ± 0.013	0.455 ± 0.013	0.507 ± 0.018	0.448 ± 0.016	0.412 ± 0.015
Relative	2.33 ± 0.05	2.29 ± 0.04	2.26 ± 0.05	2.53 ± 0.07	2.33 ± 0.05	2.34 ± 0.07
Thymus						
Absolute	0.265 ± 0.012	0.270 ± 0.012	0.253 ± 0.009	0.269 ± 0.014	0.245 ± 0.015	0.209 ± 0.008**
Relative	1.35 ± 0.06	1.33 ± 0.07	1.26 ± 0.06	1.35 ± 0.08	1.28 ± 0.07	1.19 ± 0.04

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] For body weights n=10
[c] n=7

TABLE D4
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Rats in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	8	8	8	8	8	7
Necropsy body wt[b]	352 ± 6	362 ± 8	353 ± 6	335 ± 4*	298 ± 4**	245 ± 4**
Heart						
Absolute	1.096 ± 0.045	1.091 ± 0.036	1.078 ± 0.029	1.001 ± 0.018	1.035 ± 0.026	0.828 ± 0.028**
Relative	3.12 ± 0.09	3.03 ± 0.08	3.06 ± 0.07	3.03 ± 0.07	3.51 ± 0.11**	3.39 ± 0.07**
R. Kidney						
Absolute	1.296 ± 0.031	1.282 ± 0.027	1.332 ± 0.036	1.260 ± 0.023	1.218 ± 0.035	1.165 ± 0.026**
Relative	3.70 ± 0.05	3.56 ± 0.05	3.78 ± 0.06	3.82 ± 0.06	4.12 ± 0.09**	4.78 ± 0.06**
Liver						
Absolute	11.935 ± 0.448	12.569 ± 0.272	12.644 ± 0.301	13.919 ± 0.404**	18.811 ± 0.645**	16.823 ± 0.632**
Relative	33.96 ± 0.70	34.96 ± 0.61	35.96 ± 0.82	42.12 ± 1.07**	63.73 ± 2.28**	68.92 ± 1.70**
Lung						
Absolute	1.510 ± 0.036	1.587 ± 0.052	1.589 ± 0.081	1.429 ± 0.024	1.530 ± 0.072	1.336 ± 0.056*
Relative	4.31 ± 0.12	4.42 ± 0.16	4.53 ± 0.27	4.33 ± 0.08	5.17 ± 0.22**	5.48 ± 0.21**
Spleen						
Absolute	0.671 ± 0.015	0.703 ± 0.017	0.681 ± 0.022	0.587 ± 0.018*	0.619 ± 0.025*	0.477 ± 0.012**
Relative	1.92 ± 0.06	1.96 ± 0.06	1.93 ± 0.05	1.78 ± 0.05	2.09 ± 0.08	1.96 ± 0.06
R. Testis						
Absolute	1.436 ± 0.047	1.477 ± 0.042	1.501 ± 0.023	1.461 ± 0.027	1.275 ± 0.042**	0.511 ± 0.027**
Relative	4.10 ± 0.14	4.11 ± 0.08	4.28 ± 0.10	4.42 ± 0.09	4.32 ± 0.13	2.10 ± 0.10**
Thymus						
Absolute	0.281 ± 0.025	0.325 ± 0.029	0.303 ± 0.018	0.282 ± 0.024	0.295 ± 0.013	0.256 ± 0.020
Relative	0.80 ± 0.07	0.91 ± 0.09	0.86 ± 0.05	0.85 ± 0.07	1.00 ± 0.04	1.05 ± 0.08*
Female						
n	8	8	7	8	8	10
Necropsy body wt	201 ± 3	207 ± 3	204 ± 2	198 ± 4	189 ± 6*	127 ± 5**
Heart						
Absolute	0.725 ± 0.027	0.801 ± 0.030	0.789 ± 0.052	0.740 ± 0.049	0.631 ± 0.021	0.511 ± 0.024**
Relative	3.63 ± 0.12	3.86 ± 0.14	3.85 ± 0.24	3.76 ± 0.21	3.43 ± 0.15	4.05 ± 0.22
R. Kidney						
Absolute	0.734 ± 0.018	0.768 ± 0.023	0.807 ± 0.029	0.747 ± 0.026	0.766 ± 0.020	0.695 ± 0.017
Relative	3.67 ± 0.08	3.70 ± 0.10	3.94 ± 0.14	3.80 ± 0.08	4.17 ± 0.17*	5.51 ± 0.17**
Liver						
Absolute	7.062 ± 0.256	7.702 ± 0.158	7.383 ± 0.200	6.987 ± 0.243	7.152 ± 0.298	6.038 ± 0.243**
Relative	35.37 ± 1.22	37.12 ± 0.64	36.07 ± 0.93	35.56 ± 0.76	38.55 ± 0.77*	47.54 ± 1.02**
Lung						
Absolute	1.224 ± 0.059	1.274 ± 0.055	1.336 ± 0.058	1.238 ± 0.063	1.331 ± 0.026	0.931 ± 0.031**
Relative	6.12 ± 0.24	6.14 ± 0.25	6.53 ± 0.30	6.29 ± 0.22	7.26 ± 0.37**	7.37 ± 0.23**
Spleen						
Absolute	0.501 ± 0.019	0.519 ± 0.019	0.506 ± 0.015	0.443 ± 0.013*	0.436 ± 0.019**	0.292 ± 0.011**
Relative	2.51 ± 0.10	2.50 ± 0.07	2.47 ± 0.08	2.26 ± 0.05*	2.35 ± 0.04*	2.30 ± 0.03*
Thymus						
Absolute	0.252 ± 0.015	0.244 ± 0.014	0.262 ± 0.008	0.269 ± 0.015	0.272 ± 0.017	0.177 ± 0.012**
Relative	1.26 ± 0.08	1.17 ± 0.06	1.28 ± 0.04	1.37 ± 0.07	1.47 ± 0.09	1.38 ± 0.07

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] For body weights n=10

TABLE D5
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Mice in the 15-Day Feed Study of 2-Methylimidazole[a]

	0 ppm	1,200 ppm	3,300 ppm	10,000 ppm
n	5	5	5	5
Male				
Necropsy body wt	24.8 ± 0.4	24.9 ± 1.0	24.3 ± 0.4	22.8 ± 0.5
Heart				
Absolute	0.125 ± 0.002	0.122 ± 0.003	0.120 ± 0.003	0.120 ± 0.004
Relative	5.03 ± 0.13	4.91 ± 0.14	4.93 ± 0.07	5.29 ± 0.15
R. Kidney				
Absolute	0.260 ± 0.009	0.252 ± 0.014	0.241 ± 0.006	0.227 ± 0.008
Relative	10.48 ± 0.41	10.11 ± 0.25	9.92 ± 0.13	9.96 ± 0.16
Liver				
Absolute	1.458 ± 0.060	1.572 ± 0.052	1.575 ± 0.047	1.566 ± 0.019
Relative	58.63 ± 1.82	63.21 ± 0.90*	64.84 ± 1.68**	68.79 ± 0.88**
Lung				
Absolute	0.168 ± 0.005	0.180 ± 0.016	0.185 ± 0.011	0.180 ± 0.010
Relative	6.76 ± 0.28	7.18 ± 0.41	7.60 ± 0.36	7.91 ± 0.37
R. Testis				
Absolute	0.106 ± 0.002	0.106 ± 0.003	0.102 ± 0.004	0.097 ± 0.005
Relative	4.28 ± 0.15	4.27 ± 0.08	4.20 ± 0.12	4.23 ± 0.16
Thymus				
Absolute	0.055 ± 0.007	0.061 ± 0.002	0.054 ± 0.004	0.058 ± 0.002
Relative	2.22 ± 0.26	2.46 ± 0.15	2.21 ± 0.14	2.54 ± 0.13
Female				
Necropsy body wt	21.6 ± 0.5	21.4 ± 0.5	21.0 ± 0.8	18.5 ± 0.3**
Heart				
Absolute	0.110 ± 0.003	0.111 ± 0.001	0.109 ± 0.005	0.092 ± 0.002**
Relative	5.11 ± 0.08	5.21 ± 0.12	5.21 ± 0.13	4.99 ± 0.14
R. Kidney				
Absolute	0.175 ± 0.004	0.186 ± 0.002	0.175 ± 0.006	0.155 ± 0.004*
Relative	8.10 ± 0.05	8.69 ± 0.12**	8.32 ± 0.08	8.37 ± 0.16
Liver				
Absolute	1.145 ± 0.042	1.228 ± 0.034	1.314 ± 0.104	1.108 ± 0.037
Relative	53.07 ± 1.20	57.28 ± 0.61	62.23 ± 3.05*	59.96 ± 1.97
Lung				
Absolute	0.169 ± 0.010	0.161 ± 0.007	0.167 ± 0.009	0.164 ± 0.012
Relative	7.81 ± 0.31	7.49 ± 0.32	7.94 ± 0.18	8.86 ± 0.56
Thymus				
Absolute	0.070 ± 0.005	0.078 ± 0.005	0.069 ± 0.007	0.057 ± 0.005
Relative	3.25 ± 0.23	3.64 ± 0.21	3.26 ± 0.30	3.10 ± 0.34

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE D6
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Mice in the 15-Day Feed Study of 4-Methylimidazole[a]

	0 ppm	300 ppm	800 ppm	2,500 ppm
n	5	5	5	5
Male				
Necropsy body wt	24.6 ± 1.4	24.0 ± 0.8	24.2 ± 0.8	23.2 ± 0.6
Heart				
Absolute	0.119 ± 0.006	0.120 ± 0.003	0.120 ± 0.003	0.123 ± 0.003
Relative	4.86 ± 0.05	5.03 ± 0.11	4.96 ± 0.09	5.31 ± 0.05**
R. Kidney				
Absolute	0.255 ± 0.020	0.241 ± 0.005	0.249 ± 0.010	0.242 ± 0.006
Relative	10.31 ± 0.37	10.08 ± 0.18	10.28 ± 0.24	10.43 ± 0.20
Liver				
Absolute	1.403 ± 0.089	1.377 ± 0.039	1.400 ± 0.044	1.362 ± 0.045
Relative	56.99 ± 1.62	57.70 ± 2.61	57.96 ± 1.61	58.76 ± 1.22
Lung				
Absolute	0.172 ± 0.007	0.178 ± 0.004	0.188 ± 0.023	0.157 ± 0.016
Relative	7.01 ± 0.13	7.45 ± 0.24	7.82 ± 0.99	6.73 ± 0.62
R. Testis				
Absolute	0.104 ± 0.003	0.103 ± 0.003	0.097 ± 0.006	0.098 ± 0.004
Relative	4.26 ± 0.14	4.31 ± 0.09	4.00 ± 0.20	4.24 ± 0.14
Thymus				
Absolute	0.050 ± 0.007	0.047 ± 0.003	0.052 ± 0.003	0.052 ± 0.003
Relative	2.03 ± 0.21	1.96 ± 0.14	2.15 ± 0.19	2.26 ± 0.17
Female				
Necropsy body wt	20.0 ± 0.4	19.7 ± 0.8	20.5 ± 0.5	19.7 ± 0.5
Heart				
Absolute	0.102 ± 0.002	0.106 ± 0.006	0.105 ± 0.003	0.099 ± 0.003
Relative	5.13 ± 0.12	5.38 ± 0.14	5.14 ± 0.08	5.01 ± 0.10
R. Kidney				
Absolute	0.167 ± 0.006	0.169 ± 0.012	0.168 ± 0.005	0.165 ± 0.005
Relative	8.40 ± 0.30	8.54 ± 0.39	8.20 ± 0.16	8.39 ± 0.23
Liver				
Absolute	1.008 ± 0.023	1.019 ± 0.060	1.121 ± 0.040	1.063 ± 0.030
Relative	50.57 ± 1.38	51.51 ± 1.13	54.71 ± 1.32	53.94 ± 1.60
Lung				
Absolute	0.147 ± 0.005	0.146 ± 0.002	0.139 ± 0.005	0.150 ± 0.004
Relative	7.36 ± 0.24	7.48 ± 0.38	6.79 ± 0.19	7.59 ± 0.17
Thymus				
Absolute	0.057 ± 0.004	0.067 ± 0.003	0.064 ± 0.001	0.065 ± 0.002
Relative	2.86 ± 0.20	3.39 ± 0.20	3.13 ± 0.10	3.30 ± 0.14

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE D7
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Mice in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	37.4 ± 0.5	35.4 ± 1.0	36.6 ± 0.8	35.9 ± 0.7	33.7 ± 1.1**	30.0 ± 0.6**
Heart						
Absolute	0.155 ± 0.003	0.161 ± 0.003	0.164 ± 0.003	0.176 ± 0.003**	0.171 ± 0.006**	0.166 ± 0.002**
Relative	4.15 ± 0.10	4.54 ± 0.06**	4.48 ± 0.09**	4.90 ± 0.09**	5.06 ± 0.09**	5.55 ± 0.11**
R. Kidney						
Absolute	0.295 ± 0.008	0.303 ± 0.007	0.320 ± 0.007	0.317 ± 0.006	0.306 ± 0.011	0.291 ± 0.007
Relative	7.88 ± 0.14	8.58 ± 0.14*	8.77 ± 0.23**	8.86 ± 0.19**	9.07 ± 0.11**	9.72 ± 0.31**
Liver						
Absolute	1.611 ± 0.021	1.658 ± 0.044	1.845 ± 0.041**	1.947 ± 0.033**	2.031 ± 0.085**	1.935 ± 0.054**
Relative	43.09 ± 0.50	46.90 ± 0.78*	50.41 ± 0.76**	54.26 ± 0.57**	60.15 ± 1.13**	64.56 ± 1.87**
Lung						
Absolute	0.197 ± 0.008	0.211 ± 0.012	0.214 ± 0.013	0.215 ± 0.012	0.205 ± 0.008	0.206 ± 0.012
Relative	5.26 ± 0.21	5.94 ± 0.25	5.83 ± 0.27	5.96 ± 0.25	6.09 ± 0.19	6.90 ± 0.46**
R. Testis						
Absolute	0.121 ± 0.003	0.120 ± 0.004	0.126 ± 0.003	0.129 ± 0.002	0.127 ± 0.004	0.129 ± 0.003
Relative	3.24 ± 0.06	3.39 ± 0.11	3.44 ± 0.05	3.60 ± 0.10**	3.78 ± 0.09**	4.28 ± 0.10**
Thymus						
Absolute	0.052 ± 0.004	0.044 ± 0.003	0.046 ± 0.003	0.043 ± 0.003	0.043 ± 0.003	0.039 ± 0.003*
Relative	1.38 ± 0.11	1.23 ± 0.05	1.26 ± 0.07	1.20 ± 0.07	1.28 ± 0.08	1.31 ± 0.11
Female						
Necropsy body wt	32.0 ± 1.3	30.3 ± 0.9	30.2 ± 0.7	30.1 ± 1.1	26.5 ± 0.6**	23.5 ± 0.6**
Heart						
Absolute	0.124 ± 0.003	0.135 ± 0.003	0.134 ± 0.003	0.132 ± 0.002	0.131 ± 0.003	0.122 ± 0.005
Relative	3.92 ± 0.10	4.49 ± 0.17**	4.46 ± 0.12**	4.41 ± 0.16**	4.95 ± 0.12**	5.17 ± 0.09**
R. Kidney						
Absolute	0.176 ± 0.005	0.190 ± 0.004	0.188 ± 0.004	0.184 ± 0.005	0.184 ± 0.006	0.186 ± 0.006
Relative	5.55 ± 0.14	6.30 ± 0.14**	6.24 ± 0.12**	6.12 ± 0.17**	6.91 ± 0.12**	7.93 ± 0.18**
Liver						
Absolute	1.239 ± 0.053	1.326 ± 0.040	1.416 ± 0.042*	1.473 ± 0.059**	1.362 ± 0.042	1.298 ± 0.035
Relative	38.84 ± 1.11	43.83 ± 0.68**	46.91 ± 1.12**	48.96 ± 1.41**	51.26 ± 0.75**	55.28 ± 0.84**
Lung						
Absolute	0.204 ± 0.015	0.224 ± 0.006	0.217 ± 0.011	0.197 ± 0.012	0.196 ± 0.008	0.198 ± 0.012
Relative	6.43 ± 0.46	7.42 ± 0.25	7.19 ± 0.34	6.57 ± 0.41	7.40 ± 0.29	8.39 ± 0.43**
Spleen						
Absolute	0.086 ± 0.005	0.086 ± 0.001	0.094 ± 0.003	0.125 ± 0.009**	0.185 ± 0.006**	0.162 ± 0.007**
Relative	2.71 ± 0.15	2.87 ± 0.08	3.11 ± 0.14	4.22 ± 0.38**	7.00 ± 0.24**	6.90 ± 0.32**
Thymus						
Absolute	0.059 ± 0.004	0.054 ± 0.005	0.048 ± 0.005	0.056 ± 0.004	0.053 ± 0.003	0.048 ± 0.003
Relative	1.85 ± 0.13	1.75 ± 0.14	1.59 ± 0.17	1.85 ± 0.13	1.98 ± 0.11	2.03 ± 0.14

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE D8
Organ Weights and Organ-Weight-to-Body-Weight Ratios of Mice in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	10	10	10	10	10	9
Necropsy body wt	35.3 ± 0.6	33.6 ± 0.9	32.6 ± 1.1*	31.8 ± 0.4**	29.6 ± 0.5**	28.0 ± 0.3**
Heart						
Absolute	0.168 ± 0.006	0.162 ± 0.002	0.160 ± 0.004	0.153 ± 0.003*	0.151 ± 0.003**	0.136 ± 0.003**
Relative	4.73 ± 0.15	4.86 ± 0.11	4.92 ± 0.12	4.83 ± 0.09	5.12 ± 0.10	4.84 ± 0.11
R. Kidney						
Absolute	0.309 ± 0.008	0.295 ± 0.006	0.284 ± 0.008*	0.283 ± 0.007**	0.272 ± 0.007**	0.243 ± 0.003**
Relative	8.79 ± 0.27	8.85 ± 0.21	8.73 ± 0.19	8.91 ± 0.15	9.19 ± 0.12	8.68 ± 0.13
Liver						
Absolute	1.568 ± 0.034[b]	1.581 ± 0.050	1.558 ± 0.052	1.568 ± 0.035	1.449 ± 0.047	1.427 ± 0.032*
Relative	44.20 ± 0.62[b]	47.10 ± 0.65**	47.75 ± 0.48**	49.33 ± 0.72**	48.85 ± 0.94**	50.89 ± 0.70**
Lung						
Absolute	0.189 ± 0.004	0.180 ± 0.005	0.190 ± 0.007	0.188 ± 0.011	0.172 ± 0.004[b]	0.177 ± 0.008
Relative	5.35 ± 0.12	5.40 ± 0.14	5.85 ± 0.23	5.92 ± 0.31	5.77 ± 0.10[b]	6.29 ± 0.26**
Spleen						
Absolute	0.070 ± 0.002	0.071 ± 0.002	0.069 ± 0.003	0.069 ± 0.002	0.064 ± 0.002	0.054 ± 0.001**
Relative	1.96 ± 0.06	2.11 ± 0.04	2.14 ± 0.07	2.17 ± 0.06	2.15 ± 0.05	1.95 ± 0.06
R. Testis						
Absolute	0.124 ± 0.003	0.116 ± 0.003	0.121 ± 0.003	0.126 ± 0.002	0.120 ± 0.002	0.113 ± 0.002*
Relative	3.51 ± 0.11	3.47 ± 0.12	3.72 ± 0.09	3.95 ± 0.05**	4.05 ± 0.07**	4.02 ± 0.09**
Thymus						
Absolute	0.038 ± 0.002	0.044 ± 0.002	0.039 ± 0.004	0.040 ± 0.001	0.037 ± 0.001	0.035 ± 0.003
Relative	1.07 ± 0.05	1.31 ± 0.07	1.19 ± 0.12	1.25 ± 0.04	1.26 ± 0.04	1.26 ± 0.12
Female						
n	10	10	10	10	10	3
Necropsy body wt	29.1 ± 1.1	26.3 ± 0.7*	25.7 ± 1.0**	23.4 ± 0.4**	22.5 ± 0.6**	21.6 ± 0.3**
Heart						
Absolute	0.128 ± 0.003	0.125 ± 0.003	0.120 ± 0.002	0.121 ± 0.003	0.109 ± 0.002**	0.105 ± 0.003**
Relative	4.42 ± 0.14	4.77 ± 0.10	4.73 ± 0.17	5.16 ± 0.08**	4.86 ± 0.09**	4.85 ± 0.19*
R. Kidney						
Absolute	0.191 ± 0.005	0.189 ± 0.003	0.181 ± 0.003	0.190 ± 0.008	0.168 ± 0.003**	0.166 ± 0.004*
Relative	6.60 ± 0.21	7.19 ± 0.11	7.13 ± 0.24	8.09 ± 0.27**	7.49 ± 0.12**	7.69 ± 0.28**
Liver						
Absolute	1.154 ± 0.031	1.166 ± 0.042	1.114 ± 0.031	1.042 ± 0.028*	0.932 ± 0.036**	1.011 ± 0.026**
Relative	40.03 ± 1.51	44.32 ± 1.07*	43.65 ± 1.29	44.47 ± 0.76*	41.42 ± 0.64	46.87 ± 1.78*
Lung						
Absolute	0.176 ± 0.005	0.174 ± 0.007	0.162 ± 0.004	0.165 ± 0.007	0.155 ± 0.008	0.179 ± 0.013
Relative	6.09 ± 0.24	6.63 ± 0.25	6.38 ± 0.25	7.05 ± 0.27*	6.90 ± 0.27*	8.30 ± 0.65**
Spleen						
Absolute	0.082 ± 0.002	0.081 ± 0.002	0.078 ± 0.003	0.073 ± 0.002*	0.062 ± 0.003**	0.059 ± 0.000**
Relative	2.84 ± 0.12	3.09 ± 0.07	3.07 ± 0.11	3.09 ± 0.06	2.77 ± 0.15	2.72 ± 0.05
Thymus						
Absolute	0.050 ± 0.003	0.051 ± 0.003	0.050 ± 0.002	0.046 ± 0.003	0.040 ± 0.002	0.048 ± 0.003
Relative	1.72 ± 0.10	1.93 ± 0.10	1.94 ± 0.10	1.93 ± 0.12	1.80 ± 0.09	2.22 ± 0.09*

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

APPENDIX E
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE E1
Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	8	8	8	8
Weights (g)				
Necropsy body wt	366 ± 7	350 ± 2	336 ± 5*	294 ± 12**
L. cauda epididymis	0.1774 ± 0.0073	0.1798 ± 0.0062	0.1651 ± 0.0076	0.1250 ± 0.0036**
L. epididymis	0.4987 ± 0.0201	0.4965 ± 0.0108	0.4852 ± 0.0157	0.4341 ± 0.0097**
L. testis	1.4983 ± 0.0533	1.5389 ± 0.0353	1.5181 ± 0.0272	1.2899 ± 0.0292**
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.63 ± 0.32	8.74 ± 0.29	8.70 ± 0.30	8.63 ± 0.24
Spermatid heads (10^7/testis)	13.02 ± 0.83	13.44 ± 0.52	13.22 ± 0.53	11.13 ± 0.42*
Spermatid count (mean/10^{-4} mL suspension)	65.09 ± 4.17	67.22 ± 2.59	66.09 ± 2.64	55.66 ± 2.09*
Epididymal spermatozoal measurements				
Motility (%)	87.67 ± 0.36	86.88 ± 0.70	87.91 ± 0.51	87.46 ± 0.62
Concentration (10^6/g cauda epididymal tissue)	439 ± 25	378 ± 44	399 ± 38	487 ± 72

* Significantly different ($P \leq 0.05$) from the control group by Williams' test (body weight) or Dunn's test (spermatid measurements)
** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Data are presented as mean ± standard error. Differences from the control group for spermatid heads per g testis and epididymal spermatozoal measurements are not significant by Dunn's test.

TABLE E2
Summary of Estrous Cycle Characterization for Female Rats in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10
Necropsy body wt (g)	198 ± 3	200 ± 3	190 ± 3	176 ± 3**
Estrous cycle length (days)	4.30 ± 0.15	4.61 ± 0.14[b]	4.65 ± 0.15	5.56 ± 0.41**[b]
Estrous stages (% of cycle)				
Diestrus	30.0	36.7	32.5	41.7
Proestrus	21.7	20.8	24.2	19.2
Estrus	26.7	22.5	24.2	23.3
Metestrus	21.7	20.0	19.2	15.8

** Significantly different ($P \leq 0.01$) from the control group by Williams' test (body weight) or Shirley's test (estrous cycle length)
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

TABLE E3
Summary of Reproductive Tissue Evaluation for Male Rats in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	8	8	8	8
Weights (g)				
Necropsy body wt	351 ± 8	352 ± 8	330 ± 3*	296 ± 5**
L. cauda epididymis	0.1873 ± 0.0067	0.1763 ± 0.0100	0.1742 ± 0.0077	0.1544 ± 0.0043**
L. epididymis	0.5079 ± 0.0172	0.5241 ± 0.0116	0.5111 ± 0.0191	0.4381 ± 0.0175**
L. testis	1.5100 ± 0.0427	1.5605 ± 0.0324	1.4801 ± 0.0268	1.2914 ± 0.0407**
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.17 ± 0.24	9.81 ± 0.22	9.72 ± 0.34	9.97 ± 0.48
Spermatid heads (10^7/ testis)	13.78 ± 0.24	15.30 ± 0.40*	14.38 ± 0.50	12.81 ± 0.55
Spermatid count (mean/10^{-4} mL suspension)	68.91 ± 1.18	76.50 ± 2.02*	71.88 ± 2.51	64.03 ± 2.76
Epididymal spermatozoal measurements				
Motility (%)	91.34 ± 0.22	90.56 ± 0.21*[b]	90.63 ± 0.20	90.00[c]
Concentration (10^6/g cauda epididymal tissue)	406 ± 19	498 ± 41*	477 ± 21	504 ± 22*

* Significantly different (P≤0.05) from the control group by Williams' test (body weight) or Dunn's test (spermatid and epididymal spermatozoal measurements)
** Significantly different (P≤0.01) from the control group by Williams' test
[a] Data are presented as mean ± standard error. Differences from the control group for spermatid heads per testis were not significant by Dunn's test.
[b] n=7
[c] n=1; no standard error calculated

TABLE E4
Summary of Estrous Cycle Characterization for Female Rats in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	10	9	10	10
Necropsy body weight (g)	201 ± 2	204 ± 2	198 ± 4	189 ± 6
Estrous cycle length (days)	4.70 ± 0.15	5.14 ± 0.24[b]	5.40 ± 0.34	5.38 ± 0.24[c]
Estrous stages (% of cycle)				
Diestrus	41.7	53.7	47.5	58.3
Proestrus	15.0	11.1	16.7	15.0
Estrus	23.3	19.4	19.2	15.0
Metestrus	20.0	15.7	16.7	11.7

[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in two of nine animals.
[c] Estrous cycle was longer than 12 days or unclear in 6 of 10 animals.

TABLE E5
Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	37.4 ± 0.5	35.9 ± 0.7	33.7 ± 1.1**	29.5 ± 0.5**
L. cauda epididymis	0.0193 ± 0.0015	0.0175 ± 0.0007	0.0181 ± 0.0009	0.0183 ± 0.0007
L. epididymis	0.0556 ± 0.0025	0.0565 ± 0.0023	0.0527 ± 0.0022	0.0550 ± 0.0019
L. testis	0.1193 ± 0.0028	0.1271 ± 0.0027	0.1273 ± 0.0040	0.1279 ± 0.0028
Spermatid measurements				
Spermatid heads (10^7/g testis)	16.51 ± 0.46	15.40 ± 0.69	15.87 ± 0.45	15.75 ± 0.53
Spermatid heads (10^7/testis)	1.97 ± 0.07	1.95 ± 0.08	2.01 ± 0.04	2.01 ± 0.06
Spermatid count (mean/10^{-4} mL suspension)	61.53 ± 2.19	61.08 ± 2.66	62.68 ± 1.17	62.70 ± 1.87
Epididymal spermatozoal measurements				
Motility (%)	89.03 ± 0.27	88.93 ± 0.37	85.48 ± 3.48	89.45 ± 0.42[b]
Concentration (10^6/g cauda epididymal tissue)	800 ± 79	893 ± 36	870 ± 58	831 ± 38

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).
[b] n=8

TABLE E6
Summary of Estrous Cycle Characterization for Female Mice in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10
Necropsy body wt (g)	32.0 ± 1.3	30.1 ± 1.1	26.5 ± 0.6**	23.5 ± 0.6**
Estrous cycle length (days)	4.31 ± 0.16[b]	4.19 ± 0.13[b]	4.83 ± 0.54[c]	4.30 ± 0.13
Estrous stages (% of cycle)				
Diestrus	42.5	39.2	33.3	27.5
Proestrus	15.0	19.2	18.3	23.3
Estrus	24.2	23.3	27.5	26.7
Metestrus	18.3	18.3	20.8	22.5

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.
[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

TABLE E7
Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	9
Weights (g)				
Necropsy body wt	35.3 ± 0.6	31.8 ± 0.4**	29.6 ± 0.5**	28.0 ± 0.3**
L. cauda epididymis	0.0176 ± 0.0008	0.0173 ± 0.0005	0.0176 ± 0.0005	0.0152 ± 0.0010
L. epididymis	0.0515 ± 0.0018	0.0476 ± 0.0009	0.0487 ± 0.0015	0.0439 ± 0.0019**
L. testis	0.1181 ± 0.0016	0.1198 ± 0.0018	0.1163 ± 0.0023	0.1077 ± 0.0020**
Spermatid measurements				
Spermatid heads (10^7/g testis)	16.89 ± 0.47	15.67 ± 0.50	16.25 ± 0.61	17.14 ± 0.70
Spermatid heads (10^7/testis)	2.00 ± 0.07	1.88 ± 0.06	1.89 ± 0.06	1.84 ± 0.07
Spermatid count (10^{-4} mL suspension)	62.43 ± 2.31	58.60 ± 1.84	58.88 ± 1.87	57.58 ± 2.27
Epididymal spermatozoal measurements				
Motility (%)	90.36 ± 0.24	90.55 ± 0.34	90.00 ± 0.40	89.60 ± 0.27
Concentration (10^6/g cauda epididymal tissue)	894 ± 44	957 ± 49	899 ± 23	1,007 ± 55

** Significantly different ($P \leq 0.01$) from the control group by Williams' test (body and testis weight) or Dunnett's test (l. epididymis weight)
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (l. cauda epididymis weight) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE E8
Summary of Estrous Cycle Characterization for Female Mice in the 14-Week Feed Study of 4-Methylimidazole[a]

	0 mg/kg	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
n	10	10	10	10
Necropsy body wt (g)	29.1 ± 1.1	25.7 ± 1.0**	23.4 ± 0.4**	22.5 ± 0.6**
Estrous cycle length (days)	4.60 ± 0.49	4.28 ± 0.12[b]	4.55 ± 0.50	4.75 ± 0.27
Estrous stages (% of cycle)				
Diestrus	34.2	29.2	30.0	28.3
Proestrus	12.5	20.0	26.7	24.2
Estrus	31.7	27.5	22.5	25.8
Metestrus	21.7	23.3	20.8	21.7

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

APPENDIX F
GENETIC TOXICOLOGY

TABLE F1
Mutagenicity of 2-Methylimidazole in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b]				
		−S9	+ hamster S9		+ rat S9	
			10%	30%	10%	30%
TA100	0	125 ± 2.6	140 ± 2.6	139 ± 2.0	141 ± 2.6	108 ± 3.2
	100	126 ± 3.5	142 ± 3.0	136 ± 2.6	137 ± 3.2	113 ± 3.8
	333	131 ± 2.3	139 ± 3.2	132 ± 3.8	143 ± 2.3	122 ± 2.1
	1,000	129 ± 2.6	142 ± 2.3	134 ± 2.7	133 ± 3.8	120 ± 4.3
	3,333	122 ± 2.6	138 ± 4.1	138 ± 2.9	135 ± 3.2	108 ± 4.8
	10,000	103 ± 3.5[d]	118 ± 1.8[d]	113 ± 3.2[d]	138 ± 3.2	137 ± 3.5
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control[c]		890 ± 12.5	1,009 ± 7.5	766 ± 17.0	61 ± 36.1	783 ± 9.3
TA1535	0	14 ± 1.2	12 ± 1.5	17 ± 2.4	10 ± 1.8	16 ± 2.0
	100	16 ± 2.7	11 ± 1.5	15 ± 2.6	11 ± 2.0	17 ± 1.8
	333	15 ± 1.5	12 ± 1.2	16 ± 1.8	11 ± 1.8	16 ± 2.2
	1,000	12 ± 0.9	9 ± 1.8	14 ± 1.5	12 ± 1.7	15 ± 2.6
	3,333	12 ± 0.7	10 ± 1.2	17 ± 1.5	9 ± 1.5	13 ± 1.5
	10,000	11 ± 1.9	8 ± 2.2	14 ± 1.9	10 ± 2.5	12 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		535 ± 7.2	367 ± 4.3	372 ± 4.6	346 ± 6.8	351 ± 4.8
TA97	0	93 ± 2.8	99 ± 1.8	112 ± 2.3	125 ± 2.2	120 ± 4.0
	100	97 ± 2.0	100 ± 2.1	105 ± 3.5	123 ± 4.1	124 ± 2.3
	333	95 ± 2.0	104 ± 2.6	112 ± 3.2	123 ± 3.4	119 ± 3.5
	1,000	94 ± 2.4	97 ± 3.5	106 ± 5.8	125 ± 2.7	121 ± 3.5
	3,333	94 ± 1.5	106 ± 2.0	106 ± 7.3	122 ± 2.1	121 ± 2.3
	10,000	94 ± 2.9	105 ± 0.9	99 ± 4.1	120 ± 2.6	116 ± 2.6
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		625 ± 26.1	938 ± 5.5	572 ± 20.4	537 ± 6.4	604 ± 15.3
TA98	0	18 ± 0.9	46 ± 1.5	41 ± 2.1	43 ± 2.4	38 ± 1.7
	100	20 ± 2.2	46 ± 3.5	36 ± 0.7	42 ± 1.5	36 ± 1.7
	333	19 ± 1.8	45 ± 2.6	36 ± 0.3	41 ± 1.8	32 ± 2.0
	1,000	19 ± 2.6	45 ± 1.9	40 ± 3.5	47 ± 2.2	47 ± 0.7
	3,333	24 ± 1.8	48 ± 2.3	39 ± 2.3	44 ± 2.5	40 ± 2.3
	10,000	21 ± 2.9	48 ± 1.5	33 ± 3.8	45 ± 0.9	36 ± 3.2
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		429 ± 5.5	980 ± 3.8	954 ± 15.2	931 ± 3.5	577 ± 6.1

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Zeiger *et al.* (1988). 0 μg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d] Slight toxicity

TABLE F2
Mutagenicity of 4-Methylimidazole in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b]					
		−S9		+ hamster S9		+rat S9	
		Trial 1	Trial 2	10%	30%	10%	30%
Study performed at SRI International							
TA100	0	136 ± 2.9	131 ± 3.5	131 ± 3.0	160 ± 4.7	135 ± 5.3	155 ± 3.5
	100	153 ± 7.0	119 ± 4.3	133 ± 9.5	166 ± 4.0	133 ± 2.8	163 ± 10.2
	333	143 ± 4.7	127 ± 0.0	129 ± 2.3	164 ± 4.4	125 ± 1.2	156 ± 5.2
	1,000	152 ± 14.4	121 ± 6.4	133 ± 2.6	171 ± 4.1	149 ± 5.5	158 ± 2.6
	3,333	149 ± 0.3	121 ± 8.4	131 ± 1.8	169 ± 5.2	128 ± 7.6	157 ± 4.1
	10,000	144 ± 8.4	115 ± 3.2	126 ± 1.2	151 ± 7.8	123 ± 3.0	150 ± 12.4
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		959 ± 5.8	991 ± 51.4	426 ± 13.1	562 ± 16.6	334 ± 16.9	817 ± 34.8
TA1535	0	11 ± 1.8	14 ± 2.9	13 ± 1.0	13 ± 0.9	13 ± 2.4	11 ± 1.0
	100	13 ± 0.6	15 ± 0.7	13 ± 2.1	15 ± 1.5	13 ± 0.3	11 ± 0.3
	333	15 ± 1.7	16 ± 1.5	12 ± 0.3	11 ± 0.9	12 ± 1.5	13 ± 3.2
	1,000	12 ± 2.5	13 ± 2.5	16 ± 2.8	13 ± 1.5	9 ± 0.3	12 ± 1.5
	3,333	14 ± 0.9	17 ± 0.3	9 ± 0.3	13 ± 0.6	13 ± 2.4	12 ± 1.5
	10,000	12 ± 1.2	9 ± 0.6	13 ± 1.7	12 ± 2.5	13 ± 1.9	15 ± 2.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		858 ± 15.0	830 ± 12.6	136 ± 5.8	145 ± 4.4	137 ± 6.4	143 ± 11.1
TA97	0	177 ± 9.1	151 ± 3.7	170 ± 9.4	176 ± 9.5	143 ± 3.3	168 ± 9.3
	100	158 ± 6.1	156 ± 2.8	153 ± 3.3	168 ± 10.3	160 ± 9.2	155 ± 7.0
	333	177 ± 10.1	156 ± 1.5	160 ± 4.7	172 ± 2.2	167 ± 11.1	149 ± 10.4
	1,000	186 ± 5.6	155 ± 12.9	162 ± 9.0	178 ± 4.7	170 ± 3.0	165 ± 4.3
	3,333	165 ± 14.6	163 ± 1.2	149 ± 13.9	165 ± 9.3	168 ± 12.5	152 ± 12.8
	10,000	151 ± 11.7	168 ± 6.2	133 ± 14.0	169 ± 8.7	145 ± 4.4	176 ± 4.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		361 ± 21.4	461 ± 29.7	453 ± 13.3	487 ± 12.2	363 ± 8.9	466 ± 18.8
TA98	0	15 ± 0.9	19 ± 0.9	24 ± 1.7	20 ± 0.7	22 ± 1.8	16 ± 1.9
	100	15 ± 1.8	21 ± 2.0	18 ± 1.8	19 ± 3.3	21 ± 3.1	16 ± 2.4
	333	20 ± 0.7	23 ± 4.3	20 ± 0.7	19 ± 3.6	18 ± 1.8	18 ± 0.6
	1,000	19 ± 1.2	22 ± 2.9	23 ± 3.0	20 ± 0.3	23 ± 2.3	21 ± 2.6
	3,333	18 ± 1.5	20 ± 2.4	22 ± 0.3	17 ± 0.3	23 ± 1.2	18 ± 0.6
	10,000	15 ± 1.2	22 ± 2.0	18 ± 0.7	17 ± 1.2	21 ± 1.7	14 ± 0.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		337 ± 25.3	349 ± 26.6	333 ± 23.3	424 ± 22.5	321 ± 16.8	407 ± 3.4

TABLE F2
Mutagenicity of 4-Methylimidazole in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/Plate					
		−S9		+hamster S9		+rat S9	
		Trial 1	Trial 2	10%	30%	10%	30%
Study performed at Environmental Health Research and Testing, Inc.							
TA100	0	127 ± 0.9	128 ± 2.1	128 ± 1.2	151 ± 1.5	136 ± 2.3	137 ± 1.5
	1	138 ± 1.7	130 ± 1.8	135 ± 1.8	149 ± 2.0	133 ± 2.3	139 ± 1.5
	3.3	133 ± 1.5	132 ± 1.5	138 ± 1.8	148 ± 1.3	139 ± 1.5	138 ± 1.5
	10	131 ± 2.1	135 ± 0.9	145 ± 2.4	143 ± 1.5	128 ± 1.5	140 ± 2.0
	20	136 ± 1.5	137 ± 2.3	139 ± 2.1	153 ± 0.9	131 ± 2.7	141 ± 2.1
	33	134 ± 2.1	134 ± 2.7	134 ± 2.3	151 ± 1.8	136 ± 2.1	137 ± 1.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		531 ± 5.2	863 ± 14.3	985 ± 2.0	729 ± 3.5	900 ± 5.5	882 ± 4.6
TA1535	0	18 ± 0.9	15 ± 1.5	19 ± 0.6	18 ± 1.2	16 ± 0.9	20 ± 0.7
	1	17 ± 1.2	13 ± 0.9	19 ± 0.9	18 ± 1.5	16 ± 0.9	22 ± 1.2
	3.3	19 ± 0.7	13 ± 1.3	17 ± 1.2	20 ± 2.3	17 ± 1.5	18 ± 0.6
	10	17 ± 1.5	16 ± 1.0	17 ± 1.5	18 ± 1.5	18 ± 1.2	18 ± 1.5
	20	18 ± 2.1	13 ± 1.5	18 ± 1.0	18 ± 0.6	17 ± 1.5	20 ± 1.2
	33	20 ± 2.0	15 ± 1.5	18 ± 1.9	19 ± 0.9	17 ± 0.6	19 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		804 ± 18.2	511 ± 5.4	241 ± 2.3	152 ± 2.3	190 ± 3.5	202 ± 6.7
TA97	0	117 ± 1.5	129 ± 1.8	139 ± 3.8	125 ± 1.5	138 ± 2.4	143 ± 0.3
	1	121 ± 1.8	133 ± 2.0	147 ± 4.4	139 ± 0.9	129 ± 2.0	156 ± 3.2
	3.3	123 ± 2.0	138 ± 1.5	146 ± 2.7	138 ± 1.2	135 ± 2.0	160 ± 1.5
	10	125 ± 1.5	127 ± 2.0	149 ± 4.6	141 ± 1.7	136 ± 1.8	158 ± 1.5
	20	126 ± 1.7	126 ± 1.7	140 ± 2.3	137 ± 1.5	142 ± 1.2	149 ± 2.3
	33	127 ± 1.3	128 ± 1.8	136 ± 3.5	141 ± 1.8	141 ± 1.9	148 ± 1.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		348 ± 6.7	296 ± 4.6	814 ± 14.8	708 ± 17.3	795 ± 4.9	535 ± 7.8
TA98	0	47 ± 0.9	22 ± 1.2	28 ± 1.5	29 ± 0.6	41 ± 1.5	35 ± 2.0
	1	47 ± 0.9	24 ± 1.2	32 ± 2.4	36 ± 0.9	41 ± 1.5	39 ± 1.2
	3.3	50 ± 2.1	29 ± 1.8	37 ± 1.3	39 ± 1.5	39 ± 1.8	40 ± 2.0
	10	50 ± 2.1	29 ± 1.5	40 ± 0.3	39 ± 0.6	40 ± 2.4	41 ± 0.7
	20	50 ± 1.0	27 ± 1.8	42 ± 1.2	40 ± 0.9	44 ± 2.1	44 ± 1.5
	33	46 ± 1.8	27 ± 1.0	32 ± 1.2	38 ± 1.2	45 ± 0.9	39 ± 2.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		285 ± 3.8	345 ± 4.2	849 ± 9.5	829 ± 2.6	460 ± 4.1	442 ± 3.8

[a] The detailed protocol is presented by Zeiger *et al.* (1988). 0 μg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE F3
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with 2-Methylimidazole by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]
Phosphate-buffered saline[c]		5	1.7 ± 0.3
Cyclophosphamide[d]	7.5	5	22.3 ± 1.6
2-Methylimidazole	25	5	1.3 ± 0.4
	50	5	1.2 ± 0.3
	100	5	0.8 ± 0.2
	200	4	1.3 ± 0.4
	400	0	Lethal
			P=0.813[e]

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al*. (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error; differences of 2-methylimidazole groups versus the solvent control not significant by pairwise comparison ($P \leq 0.005$) (ILS, 1990)
[c] Solvent control
[d] Positive control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at $P \leq 0.025$ (ILS, 1990); 400 mg/kg group excluded from statistical analysis due to 100% mortality

TABLE F4
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with 4-Methylimidazole by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]
Phosphate-buffered saline[c]		5	1.7 ± 0.3
Cyclophosphamide[d]	7.5	5	22.3 ± 1.6
4-Methylimidazole	25	5	1.6 ± 0.2
	50	5	1.4 ± 0.3
	100	4	0.9 ± 0.2
	200	0	Lethal
			P=0.939[e]

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al*. (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error; differences of 4-methylimidazole groups versus the solvent control not significant by pairwise comparison (P≤0.006) (ILS, 1990)
[c] Solvent control
[d] Positive control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990); 200 mg/kg group excluded from statistical analysis due to 100% mortality

TABLE F5
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with 2-Methylimidazole by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]
Phosphate-buffered saline[d]		5	1.2 ± 0.3	
Cyclophosphamide[e]	25	5	23.8 ± 0.7	
2-Methylimidazole	200	5	2.5 ± 0.4	0.0174
	300	5	1.9 ± 0.3	0.0949
	400	5	2.2 ± 0.4	0.0420
	500	1	3.5	0.0087
			P=0.068[f]	

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al*. (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the solvent group; significant at P≤0.006 (ILS, 1990)
[d] Solvent control
[e] Positive control
[f] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990); 500 mg/kg group excluded from statistical analysis due to poor survival (minimum of three animals required for a valid data point)

TABLE F6
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with 4-Methylimidazole by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]
Trial 1				
Phosphate-buffered saline[d]		5	2.2 ± 0.4	
Cyclophosphamide[e]	25	5	31.3 ± 1.8	
4-Methylimidazole	25	5	2.5 ± 0.2	0.3307
	50	5	4.3 ± 1.1	0.0045
	100	5	4.1 ± 0.6	0.0083
			P=0.003[f]	
Trial 2				
Phosphate-buffered saline		5	2.5 ± 0.2	
Cyclophosphamide	10	5	12.9 ± 1.3	
4-Methylimidazole	25	5	3.0 ± 0.3	0.2498
	50	5	3.1 ± 0.7	0.2110
	100	5	2.4 ± 0.6	0.5569
	200	0	Lethal	
			P=0.614[g]	

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al*. (1993).
PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the solvent control; significant at P≤0.008 (ILS, 1990)
[d] Solvent control
[e] Positive control
[f] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)
[g] 200 mg/kg group excluded from statistical analysis due to 100% mortality

TABLE F7
Frequency of Micronuclei in Mouse Peripheral Blood Normochromatic Erythrocytes Following Treatment with 2-Methylimidazole in Feed for 14 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]
Male				
NIH-07 Feed[d]		5	2.5 ± 0.3	
2-Methylimidazole	625	5	2.6 ± 0.3	0.4442
	1,250	5	3.0 ± 0.4	0.2498
	2,500	5	3.8 ± 0.5	0.0505
	5,000	5	4.0 ± 0.6	0.0312
	10,000	5	4.6 ± 0.6	0.0063
			P=0.001[e]	
Female				
NIH-07 Feed		5	1.7 ± 0.3	
2-Methylimidazole	625	5	1.8 ± 0.4	0.4328
	1,250	5	2.2 ± 0.3	0.2114
	2,500	5	3.6 ± 0.3	0.0045
	5,000	5	3.7 ± 0.3	0.0032
	10,000	5	4.9 ± 0.4	0.0000
			P≤0.001	

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by MacGregor *et al*. (1990). NCE=normochromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the solvent control; significant at P≤0.005 (ILS, 1990)
[d] Control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE F8
Frequency of Micronuclei in Mouse Peripheral Blood Normochromatic Erythrocytes Following Treatment with 4-Methylimidazole in Feed for 14 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]
Male				
NIH-07 Feed[d]		5	2.3 ± 0.3	
4-Methylimidazole	625	5	2.4 ± 0.4	0.4419
	1,250	5	2.5 ± 0.4	0.3863
	2,500	5	1.7 ± 0.4	0.8289
	5,000	5	2.5 ± 0.3	0.3863
	10,000	5	2.9 ± 0.7	0.2024
			P=0.153[e]	
Female				
NIH-07 Feed		5	1.9 ± 0.6	
4-Methylimidazole	625	5	1.7 ± 0.2	0.6307
	1,250	5	1.9 ± 0.3	0.5000
	2,500	5	2.1 ± 0.2	0.3758
	5,000	5	2.5 ± 0.6	0.1826
	10,000	3	1.8 ± 0.3	0.5376
			P=0.326	

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by MacGregor *et al*. (1990). NCE=normochromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the solvent control; significant at P≤0.005 (ILS, 1990)
[d] Control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX G
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF 2- AND 4-METHYLIMIDAZOLE

2-Methylimidazole (lot 323734/1 193) was supplied by Fluka Chemie AG (Buchs, Switzerland), and 4-methylimidazole (lot 08302BF) was supplied by Aldrich Chemical Company (Milwaukee, WI); these lots were used throughout the 15-day and 14-week studies. Identity and purity analyses were performed by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO); chemical identities were confirmed by the study laboratory. Reports on analyses performed in support of the 2- and 4-methylimidazole studies are on file at the National Institute of Environmental Health Sciences.

2-Methylimidazole, a white crystalline solid, and 4-methylimidazole, a light yellow powder, were identified by the analytical chemistry laboratory using proton nuclear magnetic resonance spectroscopy. The study laboratory confirmed the identity of 2- and 4-methylimidazole by infrared spectroscopy. All spectra were consistent with the literature spectra (*Aldrich*, 1981) of 2- and 4-methylimidazole. The infrared spectrum for 2-methylimidazole and the nuclear magnetic resonance spectrum for 4-methylimidazole are presented in Figures G1 and G2**.**

The purity of lots 323734/1 193 and 08302BF was determined by Karl Fischer water analysis and high-performance liquid chromatography (HPLC). HPLC was performed with a Phenomenex Ultracarb ODS 30 250 mm $\times$ 4.6 mm, 5 μm column (Phenomenex, Torrance, CA) using ultraviolet detection at 215 nm and a solvent system of: A) water and 0.025 M heptane sulfonic acid adjusted to pH 2.5 with phosphoric acid and B) methanol the flow rate was 1 mL/minute. The solvent program was 75:25 A:B (isocratic) for 35 minutes, then to 100% B in 55 minutes. Theophylline and caffeine were used as internal standards.

For lot 323734/1 193, Karl Fischer water analysis indicated 0.03% $\pm$ 0.02% water. HPLC indicated a purity of 100.3% $\pm$ 0.2% and no impurity peak with an area greater than or equal to 0.1% relative to the major peak. The manufacturer indicated a purity of 100.8% for 2-methylimidazole using nonaqueous titration.

For lot 08302BF, Karl Fischer water analysis indicated 0.13% $\pm$ 0.03% water. HPLC indicated a purity of 99.0% $\pm$ 0.1% and one impurity peak with area equal to 0.1% relative to the major peak. The manufacturer indicated a purity of 101.7% for 4-methylimidazole using nonaqueous titration and 99.1% using gas chromatography.

Based on the manufacturers' recommendations, the bulk chemicals were stored at room temperature in the dark, protected from strong oxidizers.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

For the 15-day studies, a single set of dose formulations for each chemical was prepared 6 days before the studies began. For the 14-week studies, dose formulations were prepared at the beginning of the studies, weekly for the first 4 weeks of the studies, and every 2 weeks thereafter.

Premixes were prepared by mixing 2- or 4-methylimidazole with feed (Table G1). Final dose formulations were obtained by blending additional feed with the premixes in a twin-shell blender for 15 minutes, using an intensifier bar for the first 5 minutes. Dose formulations were stored in double plastic bags at a temperature of 4° $\pm$ 2° C and used within 4 weeks of preparation.

Homogeneity and stability studies of 2-methylimidazole (625, 666, 6,000, and 10,000 ppm) and 4-methylimidazole formulations (167, 300, 625, 1,500, 2,500, and 10,000 ppm) were performed by the study laboratory using high-performance liquid chromatography (HPLC) with an Alltech Nucleosil C_8 column (150 mm × 4.6 mm) and ultraviolet detection (215 nm). The solvent system was 32.5% aqueous methanol containing 0.005M sodium dodecyl sulfate and 0.05M sodium dihydrogen phosphate. The flow rate was 1 mL/minute; a solution of imidazole in aqueous methanol was added as an internal standard in the 4-methylimidazole studies. Samples were initially extracted with 1% methanolic phosphoric acid. Due to low initial homogeneity results, the analyses were repeated with 4% methanolic phosphoric acid. Homogeneity was verified. Stability was confirmed for up to 28 days for formulations stored at up to 5° C.

Periodic analyses of 2- and 4-methylimidazole dose formulations were conducted by the study laboratory using HPLC with the system described for the homogeneity and stability studies. The dose formulations for the 15-day studies were analyzed once; the initial, midpoint, and final dose formulations for the 14-week studies were analyzed. Animal room samples of the same dose formulations were also analyzed. All dose formulations and animal room samples in the 15-day 2-methylimidazole studies were within 10% of the target concentrations (Table G2). For the 15-day 4-methylimidazole studies, all dose formulations were within 10% of the target concentrations; one of three animal room samples for rats and two of three for mice were more than 10% below the target concentrations (Table G3). In the 14-week 2-methylimidazole studies, 14 of 15 of the dose formulations were within 10% of the target concentrations (Table G4). One initial dose formulation used was 111% of the target concentration; the error was not discovered until after study completion. All animal room samples for rats and 13 of 15 for mice were within 10% of the target concentrations. In the 14-week 4-methylimidazole studies, all dose formulations were within 10% of the target concentrations; 14 of 15 of the animal room samples for rats and 12 of 15 for mice were also within 10% of the target concentrations (Table G5).

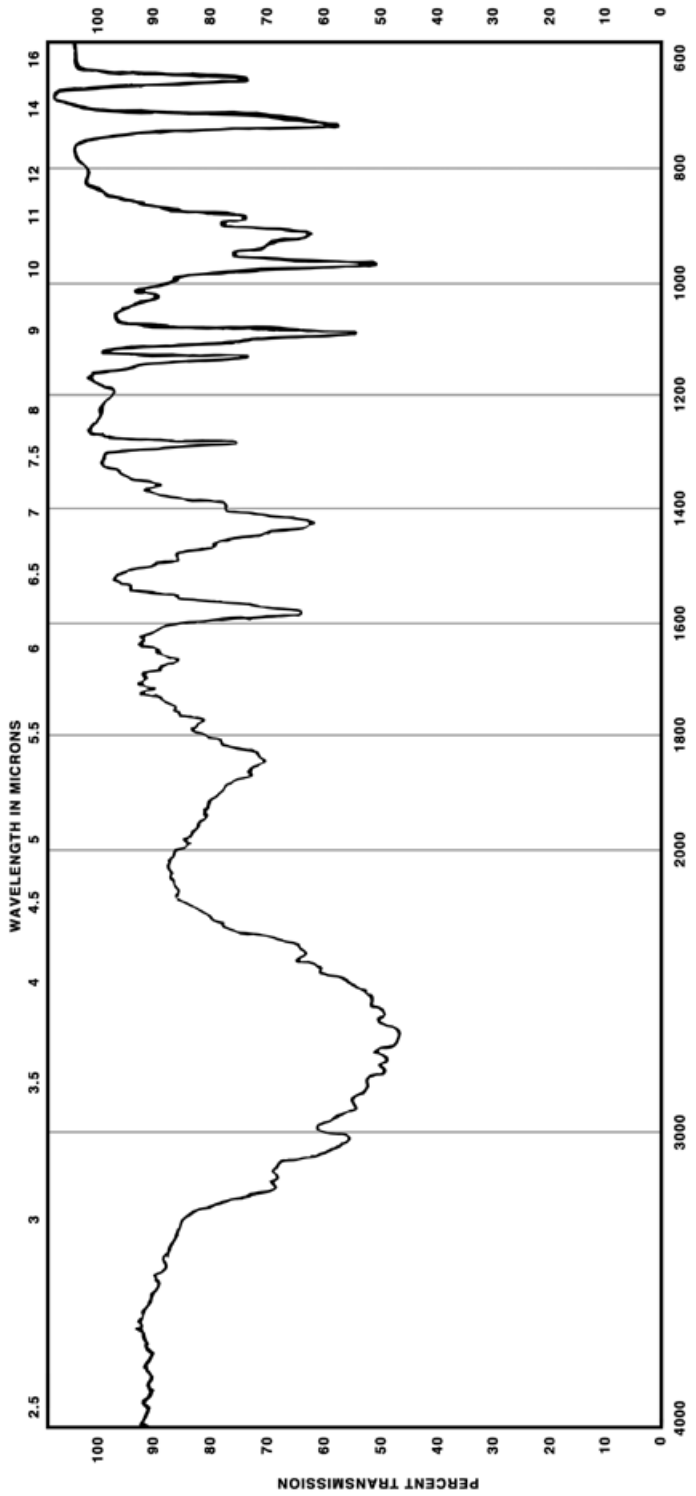

FIGURE G1
Infrared Absorption Spectrum of 2-Methylimidazole

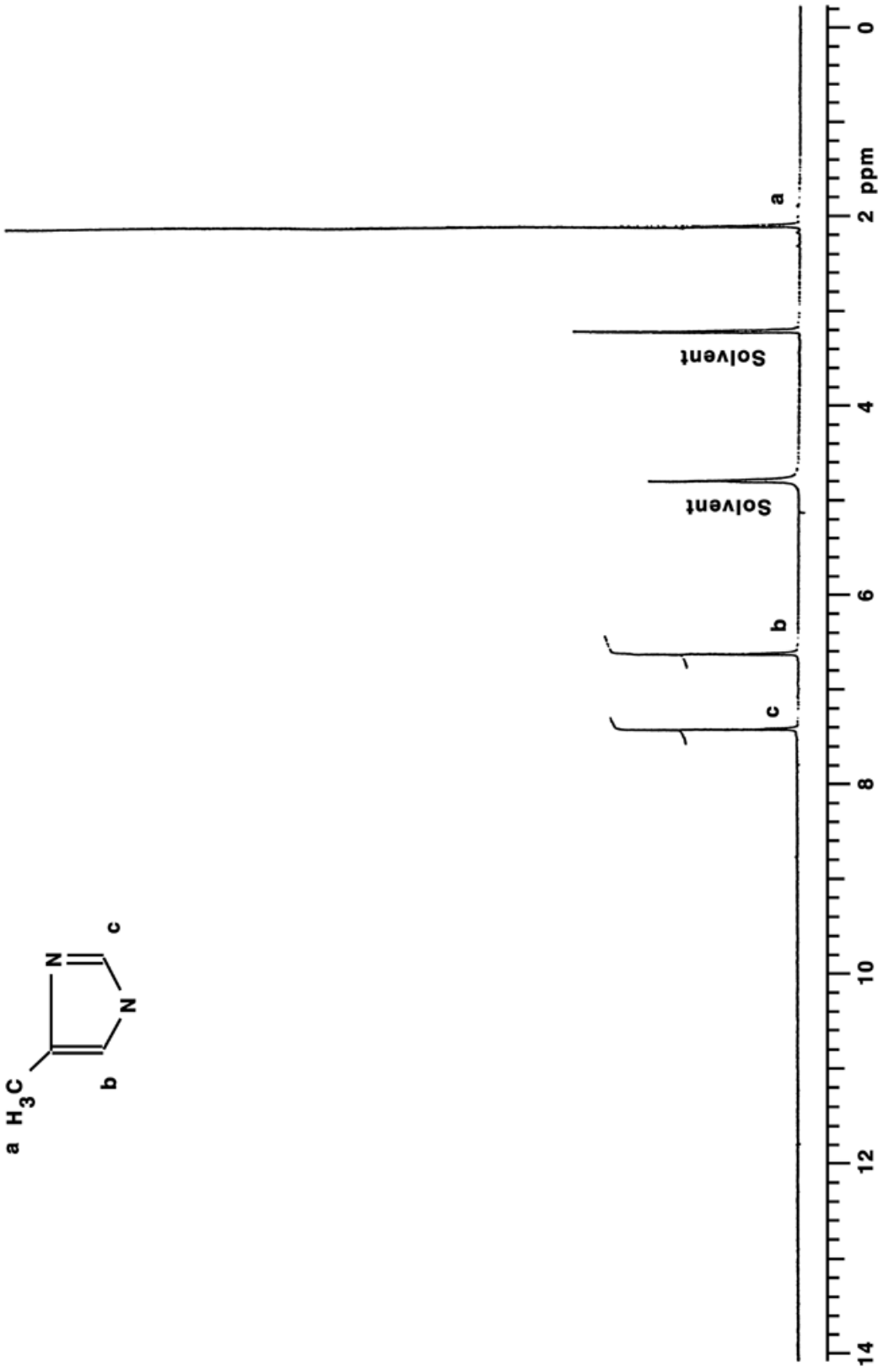

FIGURE G2
Nuclear Magnetic Resonance Spectrum of 4-Methylimidazole

TABLE G1
Preparation and Storage of Dose Formulations in the Feed Studies of 2- and 4-Methylimidazole

15-Day Studies	14-Week Studies
Preparation	
Premixes were prepared by mixing 2- or 4-methylimidazole with feed. Final dose formulations were obtained by blending additional feed with the premixes in a twin-shell blender for 15 minutes, using an intensifier bar for the first 5 minutes. Dose formulations for each chemical were prepared once.	Same as the 15-day studies; dose formulations were prepared at the beginning of the studies, weekly for the first 4 weeks, and every 2 weeks thereafter.
Chemical Lot Number	
2-Methylimidazole: 323734/1 193 4-Methylimidazole: 08302BF	2-Methylimidazole: 323734/1 193 4-Methylimidazole: 08302BF
Maximum Storage Time	
4 weeks	4 weeks
Storage Conditions	
Stored in double, labeled plastic bags at 4° ± 2° C	Same as 15-day studies
Study Laboratory	
Microbiological Associates, Inc. (Bethesda, MD)	Microbiological Associates, Inc. (Bethesda, MD)

TABLE G2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 15-Day Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
September 7, 1993	September 8, 1993	1,200	1,220	+2
		3,300	3,440	+4
		10,000	10,300	+3
	September 29, 1993[b]	1,200	1,210	+1
		3,300	3,360	+2
		10,000	10,000	0
Mice				
September 7, 1993	September 8, 1993	1,200	1,220	+2
		3,300	3,440	+4
		10,000	10,300	+3
	September 29, 1993[b]	1,200	1,210	+1
		3,300	3,280	−1
		10,000	10,100	+1

[a] Results of duplicate analyses
[b] Animal room samples

TABLE G3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 15-Day Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
September 14, 1993	September 14, 1993	300	290	–3
		800	728	–9
		2,500	2,610	+4
	October 7, 1993[b]	300	281	–6
		800	706	–12
		2,500	2,450	–2
Mice				
September 14, 1993	September 14, 1993	300	290	–3
		800	728	–9
		2,500	2,610	+4
	October 7, 1993[b]	300	260	–13
		800	683	–15
		2,500	2,530	+1

[a] Results of duplicate analyses
[b] Animal room samples

TABLE G4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
January 12, 1994	January 13, 1994	625	660	+6
		1,250	1,310	+5
		2,500	2,560	+2
		5,000	5,180	+4
		10,000	11,100	+11
	January 31, 1994[b]	625	644	+3
		1,250	1,350	+8
		2,500	2,620	+5
		5,000	5,050	+1
		10,000	9,970	0
February 23, 1994	February 23, 1994	625	652	+4
		1,250	1,280	+2
		2,500	2,590	+4
		5,000	5,330	+7
		10,000	10,600	+6
	March 21, 1994[b]	625	638	+2
		1,250	1,300	+4
		2,500	2,550	+2
		5,000	5,140	+3
		10,000	10,200	+2
April 6, 1994	April 6, 1994	625	622	0
		1,250	1,280	+2
		2,500	2,590	+4
		5,000	4,990	0
		10,000	10,100	+1
	April 29, 1994[b]	625	653	+4
		1,250	1,300	+4
		2,500	2,550	+2
		5,000	5,400	+8
		10,000	10,600	+6

TABLE G4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice				
January 12, 1994	January 13, 1994	625	660	+6
		1,250	1,310	+5
		2,500	2,560	+2
		5,000	5,180	+4
		10,000	11,100	+11
	January 31, 1994[b]	625	593	–5
		1,250	1,280	+2
		2,500	2,380	–5
		5,000	4,640	–7
		10,000	9,610	–4
February 23, 1994	February 23, 1994	625	652	+4
		1,250	1,280	+2
		2,500	2,590	+4
		5,000	5,330	+7
		10,000	10,600	+6
	March 21, 1994[b]	625	640	+2
		1,250	1,290	+3
		2,500	2,630	+5
		5,000	4,940	–1
		10,000	10,300	+3
April 6, 1994	April 6, 1994	625	622	0
		1,250	1,280	+2
		2,500	2,590	+4
		5,000	4,990	0
		10,000	10,100	+1
	April 29, 1994[b]	625	695	+11
		1,250	1,330	+6
		2,500	2,660	+6
		5,000	5,470	+9
		10,000	11,300	+13

[a] Results of duplicate analyses
[b] Animal room samples

TABLE G5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
January 24, 1994	January 24, 1994	625	642	+3
		1,250	1,280	+2
		2,500	2,450	–2
		5,000	5,250	+5
		10,000	9,980	0
	February 14, 1994[b]	625	652	+4
		1,250	1,260	+1
		2,500	2,470	–1
		5,000	4,910	–2
		10,000	9,760	–2
March 7, 1994	March 7, 1994	625	655	+5
		1,250	1,330	+6
		2,500	2,700	+8
		5,000	5,260	+5
		10,000	10,400	+4
	April 10, 1994[b]	625	584	–7
		1,250	1,160	–7
		2,500	2,300	–8
		5,000	4,810	–4
		10,000	8,730	–13
April 18, 1994	April 18, 1994	625	680	+9
		1,250	1,310	+5
		2,500	2,760	+10
		5,000	5,260	+5
		10,000	10,500	+5
	May 13, 1994[b]	625	637	+2
		1,250	1,300	+4
		2,500	2,530	+1
		5,000	4,970	–1
		10,000	9,990	0

TABLE G5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice				
January 24, 1994	January 24, 1994	625	642	+3
		1,250	1,280	+2
		2,500	2,450	−2
		5,000	5,250	+5
		10,000	9,980	0
	February 14, 1994[b]	625	640	+2
		1,250	1,230	−2
		2,500	2,480	−1
		5,000	4,920	−2
		10,000	8,990	−10
March 7, 1994	March 7, 1994	625	655	+5
		1,250	1,330	+6
		2,500	2,700	+8
		5,000	5,260	+5
		10,000	10,400	+4
	April 10, 1994[b]	625	550	−12
		1,250	1,140	−9
		2,500	1,950	−22
		5,000	4,180	−16
		10,000	9,040	−10
April 18, 1994	April 18, 1994	625	680	+9
		1,250	1,310	+5
		2,500	2,760	+10
		5,000	5,260	+5
		10,000	10,500	+5
	May 13, 1994[b]	625	645	+3
		1,250	1,280	+2
		2,500	2,510	0
		5,000	4,750	−5
		10,000	10,100	+1

[a] Results of duplicate analyses
[b] Animal room samples

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Chemical	TOX No.
Hexachloro-1,3-butadiene	1
n-Hexane	2
Acetone	3
1,2-Dichloroethane	4
Cobalt Sulfate Heptahydrate	5
Pentachlorobenzene	6
1,2,4,5-Tetrachlorobenzene	7
D & C Yellow No. 11	8
o-Cresol, *m*-Cresol, and *p*-Cresol	9
Ethylbenzene	10
Antimony Potassium Tartrate	11
Castor Oil	12
Trinitrofluorenone	13
p-Chloro-α,α,α-trifluorotoluene	14
t-Butyl Perbenzoate	15
Glyphosate	16
Black Newsprint Ink	17
Methyl Ethyl Ketone Peroxide	18
Formic Acid	19
Diethanolamine	20
2-Hydroxy-4-methoxybenzophenone	21
N, N-Dimethylformamide	22
o-Nitrotoluene, *m*-Nitrotoluene, and *p*-Nitrotoluene	23
1,6-Hexanediamine	24
Glutaraldehyde	25
Ethylene Glycol Ethers	26
Riddelliine	27
Tetrachlorophthalic Anhydride	28
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Dibutyl Phthalate	30
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1,1,1-Trichloroethane	41
1,3-Diphenylguanidine	42
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Methapyrilene Hydrochloride	46
Methacrylonitrile	47
1,1,2,2-Tetrachloroethane	49
Cyclohexanone Oxime	50
Methyl Ethyl Ketoxime	51
Urethane	52
t-Butyl Alcohol	53
1,4-Butanediol	54
trans-1,2-Dichloroethylene	55
Carisoprodol	56
Benzyltrimethylammonium Chloride	57
60-Hz Magnetic Fields	58
Chloral Hydrate	59
Benzophenone	61
3,3′,4,4′-Tetrachloroazobenzene	65
3,3′,4,4′-Tetrachloroazoxybenzene	66
2- and 4-Methylimidazole	67
Butanal Oxime	69
p-tert-Butylcatechol	70
Diazoaminobenzene	73

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