NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 4-METHYLIMIDAZOLE

(CAS NO. 822-36-6)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

January 2007

NTP TR 535

NIH Publication No. 07-4471

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*) or in hardcopy upon request from the NTP Central Data Management group at *cdm@niehs.nih.gov* or (919) 541-3419.

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CONTENTS

SUMMARY

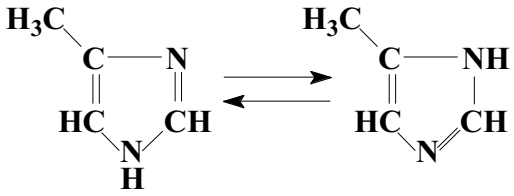
Background
4-Methylimidazole is an ingredient in a variety of chemical products including pharmaceuticals, photographic chemicals, dyes and pigments, and rubber. We studied the effects of 4-methylimidazole on male and female rats and mice to identify potential toxic or carcinogenic hazards to humans.

Methods
We gave feed containing 4-methylimidazole to groups of 50 animals for 2 years. Male and female rats received 625, 1,250, or 2,500 parts per million (ppm) 4-methylimidazole in their feed (the highest concentration corresponding to 0.25%). Male and female mice received feed containing 312, 625, or 1,250 ppm 4-methylimidazole. Groups of animals receiving untreated feed served as controls. Tissues from more than 40 sites were examined for every animal.

Results
Survival by animals exposed to 4-methylimidazole was the same as for the controls, but animals exposed to the higher concentrations weighed less than the controls. Female rats receiving 4-methylimidazole had a slightly higher rate of leukemia than the controls. Male and female mice had increased rates of adenomas and carcinomas of the lung.

Conclusions
We conclude that 4-methylimidazole caused lung cancer in male and female mice. 4-Methylimidazole may also have been associated with development of leukemia in female rats.

ABSTRACT



4-METHYLIMIDAZOLE

CAS No. 822-36-6

Chemical Formula: $C_4H_6N_2$ Molecular Weight: 82.11

Synonyms: 1H-Imidazole, 4-methyl (9Cl); imidazole, 4-methyl; 4(5)-methylglyoxaline; 4(5),4(5)-methylimidazole; 5-methylimidazole
Trade name: 4-MeI

4-Methylimidazole is used in the manufacture of pharmaceuticals, photographic chemicals, dyes and pigments, cleaning and agricultural chemicals, and rubber. It has been identified as a by-product of fermentation in foods and has been detected in mainstream and sidestream tobacco smoke. 4-Methylimidazole was nominated by the National Cancer Institute for a long-term study because of the high potential for human exposure. Male and female F344/N rats and B6C3F$_1$ mice were exposed to 4-methylimidazole (99.5% pure) in feed for 2 years. Fifteen-day and 14-week toxicity studies of 4-methylimidazole in F344/N rats and B6C3F$_1$ mice are reported in NTP Toxicity Report No. 67. Genetic toxicology studies were conducted in *Salmonella typhimurium,* rat and mouse bone marrow cells, and mouse peripheral blood.

2-Year Study in Rats

Groups of 50 male and 50 female rats were fed diets containing 0, 625, 1,250, or 2,500 ppm 4-methylimidazole (males) or 0, 1,250, 2,500, or 5,000 ppm 4-methylimidazole (females) (equivalent to average daily doses of approximately 30, 55, and 115 mg 4-methylimidazole/kg body weight to males and 60, 120, and 260 mg/kg to females) for 106 weeks. Survival of all exposed groups of male and female rats was similar to that of the control groups. Mean body weights of males in the 1,250 and 2,500 ppm groups and females in the 2,500 and 5,000 ppm groups were less than those of the control groups throughout the study; mean body weights of 1,250 ppm females were less after week 41. Feed consumption by 5,000 ppm females was less than that by the controls. Clonic seizures, excitability, hyperactivity, and impaired gait were observed primarily in 2,500 and 5,000 ppm females.

The incidence of mononuclear cell leukemia in 5,000 ppm females was significantly greater than that in the controls, and the incidence exceeded the historical range in feed study controls. The incidences of hepatic histiocytosis, chronic inflammation, and focal fatty change were generally significantly increased in all exposed groups of male and female rats. The incidences of hepatocellular eosinophilic and mixed cell focus were significantly increased in 2,500 ppm males and 5,000 ppm females.

2-Year Study in Mice

Groups of 50 male and 50 female mice were fed diets containing 0, 312, 625, or 1,250 ppm 4-methylimidazole

(equivalent to average daily doses of approximately 40, 80, and 170 mg 4-methylimidazole/kg body weight to males and females) for 106 weeks. Survival of all exposed groups of male and female mice was similar to that of the control groups. Mean body weights of males and females in the 1,250 ppm groups were less than those of the control groups after weeks 17 and 12, respectively. Mean body weights of 312 and 625 ppm females were less after weeks 85 and 65, respectively. Feed consumption by exposed groups of male and female mice was generally similar to that by the controls.

The incidences of alveolar/bronchiolar adenoma in all exposed groups of females, alveolar/bronchiolar carcinoma in 1,250 ppm males, and alveolar/bronchiolar adenoma or carcinoma (combined) in 1,250 ppm males and 625 and 1,250 ppm females were significantly greater than those in the control groups. The incidence of alveolar epithelium hyperplasia was significantly increased in 1,250 ppm females.

Genetic Toxicology

4-Methylimidazole was not mutagenic in the *S. typhimurium* mutation assay when tested in strains TA97, TA98, TA100, or TA1535, with and without hamster or rat liver metabolic activation enzymes. No consistent or significant increases in the frequencies of micronucleated erythrocytes were seen in the bone marrow of male rats or mice treated with 4-methylimidazole by intraperitoneal injection, or in peripheral blood samples from male and female mice administered the compound in dosed feed for 14 weeks.

Conclusions

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of 4-methylimidazole in male F344/N rats exposed to 625, 1,250, or 2,500 ppm. There was *equivocal evidence of carcinogenic activity* of 4-methylimidazole in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was *clear evidence of carcinogenic activity* of 4-methylimidazole in male and female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms.

Exposure to 4-methylimidazole resulted in nonneoplastic lesions in the liver of male and female rats and the lung of female mice and in clinical findings of neurotoxicity in female rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 4-Methylimidazole

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in feed	0, 625, 1,250, or 2,500 ppm	0, 1,250, 2,500, or 5,000 ppm	0, 312, 625, or 1,250 ppm	0, 312, 625, or 1,250 ppm
Body weights	1,250 and 2,500 ppm groups less than the control group	1,250, 2,500, and 5,000 ppm groups less than the control group	1,250 ppm group less than the control group	625 and 1,250 ppm groups less than the control group
Survival rates	31/50, 34/50, 33/50, 32/50	43/50, 39/50, 34/50, 35/50	45/50, 44/50, 42/50, 46/50	43/50, 40/50, 43/50, 40/50
Nonneoplastic effects	Liver: histiocytosis (38/50, 45/50, 50/50, 50/50); chronic inflammation (18/50, 32/50, 31/50, 36/50); hepatocyte, focal fatty change (21/50, 24/50, 37/50, 33/50); eosinophilic focus (4/50, 3/50, 7/50, 12/50); mixed cell focus (5/50, 7/50, 11/50, 27/50)	Liver: histiocytosis (40/50, 50/50, 48/48, 50/50); chronic inflammation (17/50, 28/50, 34/48, 35/50); hepatocyte, focal fatty change (16/50, 29/50, 29/48, 32/50); eosinophilic focus (1/50, 2/50, 5/48, 11/50); mixed cell focus (10/50, 7/50, 6/48, 18/50)	None	Lung: alveolar epithelium hyperplasia (3/50, 2/50, 3/50, 11/50)
Neoplastic effects	None	None	Lung: alveolar/bronchiolar carcinoma (2/50, 4/50, 4/50, 8/50); alveolar/bronchiolar adenoma or carcinoma (combined) (9/50, 13/50, 16/50, 22/50)	Lung: alveolar/bronchiolar adenoma (0/50, 8/50, 16/50, 8/50); alveolar/bronchiolar carcinoma (3/50, 0/50, 2/50, 7/50); alveolar/bronchiolar adenoma or carcinoma (combined) (3/50, 8/50, 17/50, 14/50)
Equivocal findings	None	Mononuclear cell leukemia: (9/50, 7/50, 16/50, 20/50)	None	None
Level of evidence of carcinogenic activity	No evidence	Equivocal evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative in strains TA97, TA98, TA100, and TA1535 with and without S9			
Micronucleated erythrocytes				
Rat bone marrow *in vivo:*	Negative when administered by intraperitoneal injection			
Mouse bone marrow *in vivo:*	Negative when administered by intraperitoneal injection			
Mouse peripheral blood *in vivo:*	Negative in males and females			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence);** one category for uncertain findings **(equivocal evidence);** one category for no observable effects **(no evidence);** and one category for experiments that cannot be evaluated because of major flaws **(inadequate study).** These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on 4-methylimidazole on September 28, 2005, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On September 28, 2005, the draft Technical Report on the toxicology and carcinogenesis studies of 4-methylimidazole received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.

Dr. P.C. Chan, NIEHS, described the nomination, design, and results of the toxicology and carcinogenesis studies of 4-methylimidazole. The proposed conclusions were *no evidence of carcinogenic activity* of 4-methylimidazole in male F344/N rats exposed to 625, 1,250, or 2,500 ppm, *equivocal evidence of carcinogenic activity* in female F344/N rats based on increased incidences of mononuclear cell leukemia, and *clear evidence of carcinogenic activity* in male and female B6C3F_1 mice based on increased incidences of alveolar/bronchiolar neoplasms.

Dr. Gasiewicz, the first principal reviewer, noted this was another study where the occurrence of mononuclear cell leukemia in female rats entered into the conclusions, and he urged explanation of how the incidences in other studies and the comparison with historical rates would ensure that conclusions were consistent. He agreed with all the other conclusions and appreciated the inclusion of toxicokinetics.

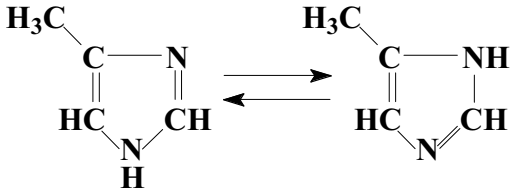
Dr. Roberts, the second reviewer, also called for consistency between studies in formulating conclusions. He felt the description of the kinetics was contradictory and thought the kinetics in rats were Michaelis-Menten rather than first-order. He found several problems with the toxicokinetic model, possibly related to assumptions made about elimination, saturation, and the time-course of absorption.

Dr. Chan explained that because the increased incidence of mononuclear cell leukemia in female rats was statistically significant but not strong, it was considered equivocal evidence. He noted also that there was an earlier onset in exposed females. Dr. Chan said there was a difference in metabolism between rats and ruminants and that metabolism may be limited in rats and absorption faster in gavage studies. Dr. J.R. Bucher, NIEHS, agreed this version of the model did not fit the data well and it would have to be revised. Dr. C.J. Portier, NIEHS, said the main discrepancy was with the chronic exposure plasma concentrations.

Dr. Crump noted this was the third study reviewed with rather similar increases in the incidences of mononuclear cell leukemia in female rats and suggested the conclusions should have been similar in all the cases, perhaps equivocal evidence. Dr. Soper agreed. Dr. Bucher explained that staff debated between some evidence and equivocal evidence and also that inhalation studies, which consistently have higher background rates for this leukemia, are considered somewhat differently from studies using other routes. Dr. Elwell said that he considered the pattern in this study at least as strong as any of the others reviewed and felt equivocal evidence was appropriate.

Dr. Gasiewicz moved, and Dr. Roberts seconded, that the conclusions be accepted as written with the addition of the word "marginally" added before the increased incidences of mononuclear cell leukemia. Dr. Crump suggested that word may not have been appropriate. Drs. Gasiewicz and Roberts agreed that the response itself was not marginal, but the basis of the equivocal conclusion seemed more about the highly variable background frequency of this lesion. Dr. Roberts moved, and Dr. Vore seconded, to remove the word marginal. The vote to accept the conclusions as originally drafted was approved unanimously with five votes.

INTRODUCTION



4-METHYLIMIDAZOLE

CAS No. 822-36-6

Chemical Formula: $C_4H_6N_2$ Molecular Weight: 82.11

Synonyms: 1H-Imidazole, 4-methyl (9Cl); imidazole, 4-methyl; 4(5)-methylglyoxaline; 4(5),4(5)-methylimidazole; 5-methylimidazole
Trade name: 4-MeI

Chemical and Physical Properties

4-Methylimidazole is a light yellow, crystalline solid that is soluble in water and alcohol. It has a melting point range from 46° to 48° C and a boiling point of 263° C (MSDS, 1996).

Production, Use, and Human Exposure

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 to synthesize the compound is by catalytic dehydrogenation of imidazoline derivatives. 4-Methylimidazole may be synthesized 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 the compound are not available (Chemical Dynamics, Corp., 1989; NCI, 1991).

4-Methylimidazole is used as a chemical intermediate, starting material, or component in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes and pigments, agricultural chemicals, and rubber. In addition, 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. The chemical 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 exchange apparatus, a component of absorbent to remove acid gases from hydrocarbon or synthesis gas, and a starting material for inks and paper dyes (Chemical Dynamics, Corp., 1989; NCI, 1991).

4-Methylimidazole, formed by interaction of ammonia with reducing sugars, has been identified as a toxic by-product of fermentation in ammoniated hay forage for livestock animals (Ray *et al.*, 1984). Ammoniation of carbohydrate-containing material including hay to increase nonprotein nitrogen content is a common farm practice. Ammonia treatment also increases digestibility of fiber components (Waagepetersen and Vestergaard, 1977). Neurologic signs had been reported in sheep and calves of nursing cows fed ammoniated hay (Weiss *et al.*, 1986; Motoi *et al.*, 1997). The disorder included febrile, hyperexcitability, abnormal circling behavior,

and epileptoid seizures. The compound responsible for causing abnormal neurologic behavior in calves was identified as 4-methylimidazole (Motoi *et al.*, 1997).

Humans may be exposed to low levels of 4-methylimidazole in food and tobacco smoke. Muller *et al.* (1998a,b) reported alkylimidazoles including 4-methylimidazole in milk, plasma, and urine in sheep and cattle fed ammoniated forage. 4-Methylimidazole has also been identified as an undesirable by-product of fermentation in several food products including caramel coloring, soy sauce, Worcestershire sauce, wine, ammoniated molasses, and caramel-colored syrups (Yoshikawa and Fujiwara, 1981; Huang *et al.*, 1983; Matyasovszky and Jeszenszky, 1985; Wong and Bernhard, 1988). However, only caramel colors (caramel color III and IV) manufactured with ammonia or its salts contain measurable levels of 4-methylimidazole (Chappel and Howell, 1992). Two batches of caramel color IV used in beverages reportedly contained 110 mg and 164 mg 4-methylimidazole per kilogram (MacKenzie *et al.*, 1992). 4-Methylimidazole has also been detected in mainstream and sidestream smoke (Moree-Testa *et al.*, 1984; Sakuma *et al.*, 1984). No quantitative data on human exposure were found in the literature.

The United States Food and Drug Administration lists caramel color as "generally recognized as safe" (Chappel and Howell, 1992). A Danish law, enacted in 1976, restricted the use of caramel coloring in food and beverages, citing a cancer risk. No standards or guidelines have been set for occupational exposures or environmental levels of 4-methylimidazole in the United States.

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

There appears to have been a species difference in 4-methylimidazole disposition in previous studies.

In rats, the uptake at 5 minutes after a single 216 mg/kg intraperitoneal injection of 4-methylimidazole was highest in the intestines, followed by blood, liver, stomach, and kidney (Hidaka, 1976a). The compound was excreted unchanged in urine, beginning approximately 30 minutes after injection, and reached approximately 90% within 8 hours.

In ewes, the absorption and elimination of a single oral dose of 4-methylimidazole followed first-order kinetics. One half of an oral dose (20 mg/kg) of 4-methylimidazole was absorbed in about 27 minutes, and the maximum plasma level was reached 5 hours after oral administration (Karangwa *et al.*, 1990). The bioavailability calculated using plasma data from three ewes was 69%, and the biological half-life was 9.03 hours. Only 0.07 mg/kg of the oral dose was recovered in urine unchanged. Metabolites of 4-methylimidazole were not detected by high-performance liquid chromatography (HPLC).

In goats and heifers, the mean residence time of 4-methylimidazole administered orally or intravenously was about 5 hours, and the volume of distribution was 0.9 L/kg body weight in both goats and heifers (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. The administered 4-methylimidazole was distributed mainly to the liver, kidney, and lung. In pregnant and postpartum cows and mice, 4-methylimidazole was found in milk following oral administration (Morgan and Edwards, 1986).

Following gavage administration of 5, 50, or 150 mg/kg 4-methylimidazole to F344/N rats, peak plasma concentration was reached between 0.5, 1.0, and 3.0 hours, respectively (Yuan and Burka, 1995). At 150 mg/kg, the plasma concentration of [^{14}C]-4-methylimidazole was almost constant during the first 5 hours after gavage; at lower doses, the decline was more rapid. The estimated terminal half-life was dose dependent. The results suggest that the elimination of parent 4-methylimidazole was saturable. Using the total urinary recovery of parent 4-methylimidazole, the estimated bioavailability was approximately 60% to 70%. Little or no metabolism of 4-methylimidazole was found. Only one minor hydrophilic metabolite was present in urine and plasma. Fecal, biliary, or respirated elimination of radioactivity was negligible.

Urinary metabolites from Long-Evans rats given 490 mg/kg by intraperitoneal injection were isolated and characterized (Cowgill, 1955). 4-Hydroxymethylimidazole and 4-imidazolecarboxylic acid were identified by comparison to authentic standards (Figure 1). The aldehyde was inferred as an intermediate because the same metabolites were observed when it was injected.

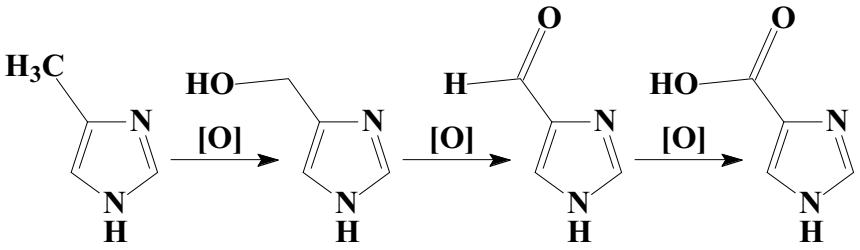


FIGURE 1
Metabolism of 4-Methylimidazole (Cowgill, 1955)

Information on absorption, distribution, metabolism, and excretion in dosed feed studies are not available.

Humans

4-Methylimidazole selectively inhibits thromboxane synthetase but shows no inhibition of arachidonic acid-induced platelet-fibrin clot retraction *in vitro* (Di Minno *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 inhibitory effects can be demonstrated by hepatic metabolism of tolbutamide (measuring plasma hydroxytolbutamide concentration by HPLC) *in vivo* in adult male Wistar rats or *in vitro* with human liver microsomes. In contrast, 2-methylimidazole does not inhibit microsomal oxidation (Back and Tjia, 1985; Back *et al.*, 1988). 4-Methylimidazole also stimulated the phosphorylation of rabbit kidney (Na^+ and K^+)-ATPase, while 2-methylimidazole did not (Schuurmans Stekhoven *et al.*, 1988).

TOXICITY

Experimental Animals

Reported LD_{50} values are 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 (Nishie *et al.*, 1969). The LD_{50} value of 4-methylimidazole orally administered in rats was 173 mg/kg (Hidaka, 1976b).

4-Methylimidazole has been associated with acute toxicity to foraging animals fed commercially ammoniated grasses or grains. Animals fed ammoniated feed 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 opisthotonos (tetanic spasms in which the spine is fixed in an extended position) and convulsion. Death may ensue (Weiss *et al.*, 1986). Neurologic signs and convulsant activity have been observed in cattle fed ammoniated molasses (Nishie *et al.*, 1970; Morgan and Edwards, 1986). Calves nursing from cows fed ammoniated hay would run 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). However, 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 induced convulsions or clonic seizure (Nielsen *et al.*, 1993).

In mice, 4-methylimidazole has been shown to induce similar toxic neurologic effects (e.g., tremor, restlessness, running, sialorrhea, opisthotonos, Straub tail, and tonic extensor seizure) terminating in death at high doses and loss of balance at lower doses. The convulsant doses (CD_{50}) were 360 mg/kg orally and 155 mg/kg intraperitoneally (Nishie *et al.*, 1970). Considering the oral LD_{50} of 370 mg/kg and the intraperitoneal LD_{50} of 165 mg/kg,

the convulsions were probably agonal rather than related to specific neurological activity. 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 (Nishie *et al.*, 1969). Convulsions were also induced in rabbits and day old chicks by 4-methylimidazole (Nishie *et al.*, 1969). The results from mice, rabbits, and chicks suggested that 4-methylimidazole was at least partly responsible for the signs of toxicity observed in cattle fed ammoniated feeds.

4-Methylimidazole at 150 mg/kg (1,827 µmol/kg) in mice induced convulsions, hyperactivity, tremor, opisthotonos, and Straub tail (Nishie *et al.*, 1969). Liver hypertrophy in mice following intraperitoneal administration of 4-methylimidazole has been reported (Hidaka, 1976c). 4-Methylimidazole given intraperitoneally induced aggressive behavior in male Wistar rats treated with lisuride; 4-methylimidazole was more potent than 2-methylimidazole (Ferrari *et al.*, 1987). MacKenzie *et al.* (1992) administered caramel color IV, which contained 110 mg 4-methylimidazole/kg, in drinking water to male and female F344 rats and B6C3F$_1$ mice at 0, 2.5, 5.0, or 10.0 g/kg for 24 months. No differences in overall survival, body weights, or tumor incidences were observed. The authors concluded that the no-observed-adverse-effect level (NOAEL) was 10.0 g caramel color IV/kg for rats and mice. Hargreaves *et al.* (1994) reported that 4-methylimidazole inhibited rat liver P450 2E1 activities.

The NTP conducted 15-day and 14-week dose-finding and toxicity studies of 4-methylimidazole in F344/N rats and B6C3F$_1$ mice (NTP, 2004a). In the 14-week toxicity study, male and female rats were administered 4-methylimidazole in dosed feed at 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm. Survival of the exposed groups of rats was not different from that of the controls. Abnormal breathing, nasal/eye discharge, ruffled fur, tremors, and ataxia were observed in the 5,000 and 10,000 ppm groups. Final body weights were significantly lower in the 5,000 ppm males (85% of the controls) and in the 10,000 ppm males (70% of the controls) and females (63% of the controls). Feed consumption was reduced in an exposure concentration-related manner. A microcytic, normochromic, nonresponsive anemia was observed in rats exposed to 2,500 ppm or greater. 4-Methylimidazole administration affected serum triiodothyronine (T_3), total thyroxine (T_4), and thyroid stimulating hormone (TSH) with no apparent pattern. Exposure concentration-related increases in relative weights were observed in the kidney and liver of males and in the liver of females. Hepatocytic vacuolation indicating lipid accumulation was observed in males exposed to 1,250 ppm or greater and in females exposed to 5,000 or 10,000 ppm. The incidences of degeneration of the seminiferous tubules of the testes were increased in males exposed to 2,500 ppm or greater. Atrophy of the prostate gland was noted in males exposed to 625 ppm or greater. The incidences of prostate gland inflammation and epididymal hypospermia were significantly increased in the 10,000 ppm males. The estimated NOAEL level of 4-methylimidazole was 1,250 ppm for males and 5,000 ppm for females.

Male and female mice in the 14-week toxicity study were administered 4-methylimidazole in dosed feed at 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm (NTP, 2004a). One of 10 males and seven of 10 females from the 10,000 ppm groups died early. Body weight gains of mice exposed to 1,250 ppm or greater were significantly reduced compared to the controls. Exposure concentration-related increases in relative liver weights were observed in exposed mice. Relative testis weights in males and relative kidney weights in females were higher in groups exposed to 2,500 ppm or greater. A minimal microcytic, normochromic, nonresponsive anemia was observed in females at all exposure concentrations. In males, there were transient increases in serum T_4 levels in the 5,000 ppm group at days 29 and 86 and exposure concentration-related increases in serum T_3 levels at days 8 and 29. In females, serum T_4 levels were lower at day 86 in the exposed groups; the levels were also significantly lower at days 8 and 29 in the 10,000 ppm group. Exposure concentration-related increases in serum T_3 levels were observed at days 29 and 86 in females. TSH levels were not assayed. Microscopic evaluation of tissues showed that no lesions were related to 4-methylimidazole exposure. No significant differences occurred in sperm motility or vaginal cytology parameters between exposed and control groups. The estimated NOAEL of 4-methylimidazole was 10,000 ppm for mice.

Humans

No information on the toxicity of 4-methylimidazole in humans was found in a review of the literature.

REPRODUCTIVE TOXICITY

Experimental Animals

Results from a 14-week toxicity study of 4-methylimidazole (625 to 10,000 ppm in feed) in F344/N rats

showed an exposure concentration-dependent degeneration of seminiferous tubules of the testes, atrophy of the prostate gland, and decreased epididymal sperm motility (NTP, 2004a). For the 2,500 ppm group, in which there were three animals with testicular degeneration and five without, both the relative and the absolute testis weights and sperm densities were correlated with this degeneration. Although decreased testis weight was associated with decreased body weight, no instances of testicular degeneration resulting from reduced body weight alone are known; therefore, the NTP concluded that 4-methylimidazole is a reproductive toxicant in male rats based on the exposure concentration-dependent testicular degeneration. Further, Adams *et al.* (1998) reported that a high dose of 4-methylimidazole (50 to 100 mg/kg) injected subcutaneously into male Sprague-Dawley rats caused decreases in luteinizing hormone secretion. 4-Methylimidazole administration also caused decreases in serum testosterone, testicular interstitial fluid testosterone concentration, and testicular interstitial fluid formation in a dose-dependent manner 2 hours after treatment. 4-Methylimidazole inhibited male fertility through suppression of testosterone secretion and testicular interstitial fluid formation. These results suggest that 4-methylimidazole disrupts pituitary luteinizing hormone secretion regulatory mechanisms.

Humans

No information on the reproductive toxicity of 4-methylimidazole in humans was found in the literature.

Carcinogenicity

No information on the carcinogenicity of 4-methylimidazole in animals or humans was found in a search of the available literature. However, in 2-year feed studies of 2-methylimidazole in male F344/N rats at exposure concentrations up to 3,000 ppm, in female F344/N rats at exposure concentrations up to 5,000 ppm, and in male and female $B6C3F_1$ mice at exposure concentrations up to 2,500 ppm, the incidences of thyroid gland follicular cell neoplasms were increased in the highest exposure concentration groups of male and female rats and male mice at the end of the studies (NTP, 2004b).

Genetic Toxicity

No information on the mutagenicity of 4-methylimidazole was found in a search of the available literature.

Study Rationale

The National Cancer Institute nominated 2- and 4-methylimidazole for study. The nomination was based on the chemical's widespread use in electronic and pharmaceutical industries, potential for widespread human exposure as contaminants in food products and in the environment, neurotoxicity in various animal species, the lack of chronic toxicity data, and a suspicion of carcinogenicity from a structure-activity standpoint. The 2-year study of 2-methylimidazole showed exposure concentration-related increases in thyroid gland follicular cell neoplasms in rats and mice.

MATERIALS AND METHODS

Procurement and Characterization of 4-Methylimidazole

4-Methylimidazole was obtained from Sigma Chemical Company (St. Louis, MO) in one lot (116H0901). Identity and purity analyses were conducted by the analytical chemistry laboratory, Battelle Columbus Operations (Columbus, OH), Galbraith Laboratories, Inc. (Knoxville, TN), and the study laboratory, Southern Research Institute (Birmingham, AL; Appendix F). Stability analyses were performed by the analytical chemistry laboratory. Reports on analyses performed in support of the 4-methylimidazole studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a white powder, was identified as 4-methylimidazole by infrared, ultraviolet/visible, and proton and carbon-13 nuclear magnetic resonance spectroscopy and melting point determination. The purity of lot 116H0901 was determined by elemental analyses, functional group titration, gas chromatography (GC), and high-performance liquid chromatography (HPLC). Karl Fischer titration indicated 0.12% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for 4-methylimidazole. Functional group titration indicated a purity of approximately 100%. GC indicated one major peak and two volatile impurities with a combined relative area of 0.7%; these impurities were not identified. HPLC detected two impurities with a combined relative area of 0.5% of the major peak. The overall purity of lot 116H0901 was determined to be greater than 99%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC. These studies indicated that 4-methylimidazole was stable as a bulk chemical for at least 14 days when stored in Teflon®-sealed amber glass vials at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at 5° C in Teflon®-sealed containers, protected from light and moisture. Stability was monitored during the 2-year studies using GC; no degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared every 2 weeks by mixing 4-methylimidazole with feed (Table F2). Homogeneity studies of 100 and 2,400 ppm or 300 and 5,000 ppm dose formulations were performed by the analytical chemistry laboratory and the study laboratory, respectively, using HPLC. Stability studies of a 100 ppm dose formulation were performed by the analytical chemistry laboratory using HPLC. Homogeneity was confirmed, and stability of the dose formulations was confirmed for at least 36 days for dose formulations stored in sealed plastic containers in a refrigerator.

Periodic analyses of the dose formulations of 4-methylimidazole were conducted by the study laboratory using HPLC. The dose formulations were analyzed at least every 12 weeks (Table F3). Of the dose formulations analyzed and used, 140 of 141 for rats and all 74 for mice were within 10% of the target concentrations.

2-Year Studies

Study Design

Groups of 50 male rats were fed diets containing 0, 625, 1,250, or 2,500 ppm 4-methylimidazole for 106 weeks. Groups of 50 female rats were fed diets containing 0, 1,250, 2,500, or 5,000 ppm 4-methylimidazole for 106 weeks. Groups of 50 male and 50 female mice were fed diets containing 0, 312, 625, or 1,250 ppm 4-methylimidazole for 106 weeks.

For rats in the 2-year study, the top exposure concentrations selected for males and females were 2,500 and 5,000 ppm, respectively. In 2,500 ppm male rats in the

14-week toxicity study, body weights were 95% of the controls', changes in hematology and clinical chemistry parameters were slight, absolute and relative liver weights were increased, and vacuolization was observed in hepatocytes; even though the no-observed-adverse-effect level was at 1,250 ppm, the hepatic histopathology at 2,500 ppm was not considered detrimental for a 2-year study (NTP, 2004a). In 5,000 ppm female rats in the 14-week toxicity study, body weights were 94% of the control group's, changes in hematology and clinical chemistry parameters were slight, and absolute and relative spleen weights were reduced compared to controls; there were no other organ weight or histopathologic changes.

Based on the reduced body weights observed in the 14-week toxicity study (NTP, 2004a), 1,250 ppm was selected as the highest exposure concentration for the 2-year study in mice. The top dose level was selected based on body weights in the 14-week toxicity study (NTP, 2004a). In the 1,250 ppm males and females, body weights were 93% and 88% of the controls', respectively. There were no changes in hematology, clinical chemistry, organ weights, or histopathology.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats and mice were quarantined for 15 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

Animal Maintenance

Male rats were housed three per cage; male mice were housed individually, and female rats and mice were housed five per cage. Feed and water were available *ad libitum*. Feed consumption was measured by cage weekly for the first 13 weeks and every 4 weeks thereafter for rats and mice. Cages and racks were rotated every two weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix H.

Clinical Examinations and Pathology

All animals were observed twice daily. Body weights were recorded initially, weekly for the first 13 weeks, every 4 weeks thereafter, and at the end of the studies. Clinical findings were recorded every 4 weeks.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (except eyes initially fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. 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 evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the bone marrow, liver, lung, pituitary gland, spleen, and thyroid gland of male and female rats; the kidney and prostate gland of male rats; the eye, heart, ovary, pancreas, and uterus of female rats; the lung, mesenteric lymph node, and thyroid gland of male and female mice; the adrenal gland, kidney, and preputial gland of male mice; and the mandibular lymph node and mammary gland of female mice. In addition, selected brain slides corresponding to neurological clinical signs exhibited by female rats were evaluated during the peer review process.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists

experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

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

Study Laboratory
Southern Research Institute (Birmingham, AL)

Strain and Species
F344/N rats
$B6C3F_1$ mice

Animal Source
Taconic Farms, Inc. (Germantown, NY)

Time Held Before Studies
15 days

Average Age When Studies Began
6 weeks

Date of First Exposure
Rats: January 20, 2000
Mice: February 3, 2000

Duration of Exposure
106 weeks

Date of Last Exposure
Rats: January 25, 2002
Mice: February 8, 2002

Necropsy Dates
Rats: January 17 to 25, 2002
Mice: January 31 to February 8, 2002

Average Age at Necropsy
111 weeks

Size of Study Groups
50 males and 50 females

Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.

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

Animals per Cage
Rats: 3 (males) or 5 (females)
Mice: 1 (males) or 5 (females)

Method of Animal Identification
Tail tattoo

Diet
Irradiated NTP-2000 open formula meal (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*

Water
Tap water (Birmingham, AL, municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*

Cages
Solid bottomed polycarbonate (Lab Products, Inc., Maywood, NJ), changed once (male mice) or twice weekly

Bedding
Heat-treated, irradiated hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed once (male mice) or twice weekly

Cage Filters
Reemay® spun-bonded polyester (Andico, Birmingham, AL), changed every 2 weeks

Racks
Stainless steel (Lab Products, Inc., Maywood, NJ), changed every 2 weeks

Animal Room Environment
Temperature: 72° ± 3° F
Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: 15-30/hour

Exposure Concentrations
Rats: 0, 625, 1,250, or 2,500 ppm (males) or 0, 1,250, 2,500, or 5,000 ppm (females) in feed, available *ad libitum*
Mice: 0, 312, 625, or 1,250 ppm in feed, available *ad libitum*

Type and Frequency of Observation
Observed twice daily; animals were weighed initially, weekly for 13 weeks then every 4 weeks, and at the end of the studies; clinical findings were recorded every 4 weeks. Feed consumption was recorded weekly for 13 weeks then every 4 weeks for a 7-day period.

Method of Sacrifice
CO_2 asphyxiation

Necropsy
Necropsies were performed on all animals.

Histopathology
Complete histopathology was performed on all rats and mice at 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, eye, gallbladder (mice only), harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung with mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, spinal cord and sciatic nerve were examined in exposed female rats that displayed clinical signs of possible neurotoxicity and corresponding tissue samples for comparison from five terminally sacrificed control female rats.

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes or missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B5, C1, C5, D1, and D5 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only on the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and $B6C3F_1$ mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall exposure-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein

and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The current NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed up to the present. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

Quality Assurance Methods

The 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of 4-methylimidazole was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium,* micronucleated erythrocytes in rat and mouse bone marrow, and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.,* 1987; Zeiger *et al.,* 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 2 and in the Kaplan-Meier survival curves (Figure 2). Survival of all exposed groups of male and female rats was similar to that of the control groups.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of males in the 1,250 and 2,500 ppm groups and females in the 2,500 and 5,000 ppm groups were less than those of the control groups throughout the study; mean body weights of 1,250 ppm females were less after week 41 (Tables 3 and 4; Figure 3). Feed consumption by exposed groups of males was generally similar to that by the controls (Table G1). However, feed consumption by 5,000 ppm females was less than that by the controls (Table G2). Dietary concentrations of 625, 1,250, or 2,500 ppm for males and 1,250, 2,500, or 5,000 ppm for females resulted in average daily doses of approximately 30, 55, and 115 mg 4-methylimidazole/kg body weight to males and 60, 120, and 260 mg/kg to females.

Clonic seizures, excitability, hyperactivity, and impaired gait were observed in 5,000 ppm females; some of these clinical findings were also observed in the lower exposed groups at greater frequencies than in the controls (Table 5). The study laboratory performed a histopathologic examination of brain, spinal cord, and sciatic nerve from 82 females; 77 displayed clinical signs of possible neurotoxicity (two from the 1,250 ppm group, 25 from the 2,500 ppm group, and 50 from the 5,000 ppm group), and five control animals were evaluated for comparison. As part of the pathology peer review, a neuropathologic evaluation of 10 5,000 ppm and 10 control females was performed. FluoroJade B (a fluorescent marker for neuronal degeneration) staining was conducted on five 5,000 ppm and five control females to identify any subtle antemortem neuronal changes that may not have been readily apparent during evaluation of the standard sections stained with H&E. Positive controls used for comparison were from a previous study in which neuronal necrosis was detected (Morgan *et al.*, 2004). H&E and FluoroJade B staining did not confirm morphologic neural correlates for the neurologic signs exhibited.

TABLE 2
Survival of Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	13	11	12	10
Natural deaths	6	5	5	8
Animals surviving to study termination	31	34	33	32
Percent probability of survival at end of study[a]	62	68	66	64
Mean survival (days)[b]	701	681	695	689
Survival analysis[c]	P=0.964	P=0.842N	P=0.926N	P=1.000
	0 ppm	**1,250 ppm**	**2,500 ppm**	**5,000 ppm**
Female				
Animals initially in study	50	50	50	50
Moribund	5	5	5	9
Natural deaths	2	6	11	6
Animals surviving to study termination	43[d]	39	34	35[e]
Percent probability of survival at end of study	86	78	68	70
Mean survival (days)	697	701	684	691
Survival analysis	P=0.078	P=0.449	P=0.060	P=0.099

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A lower mortality in an exposed group is indicated by **N**.
[d] Includes three animals that died during the last week of the study
[e] Includes two animals that died during the last week of the study

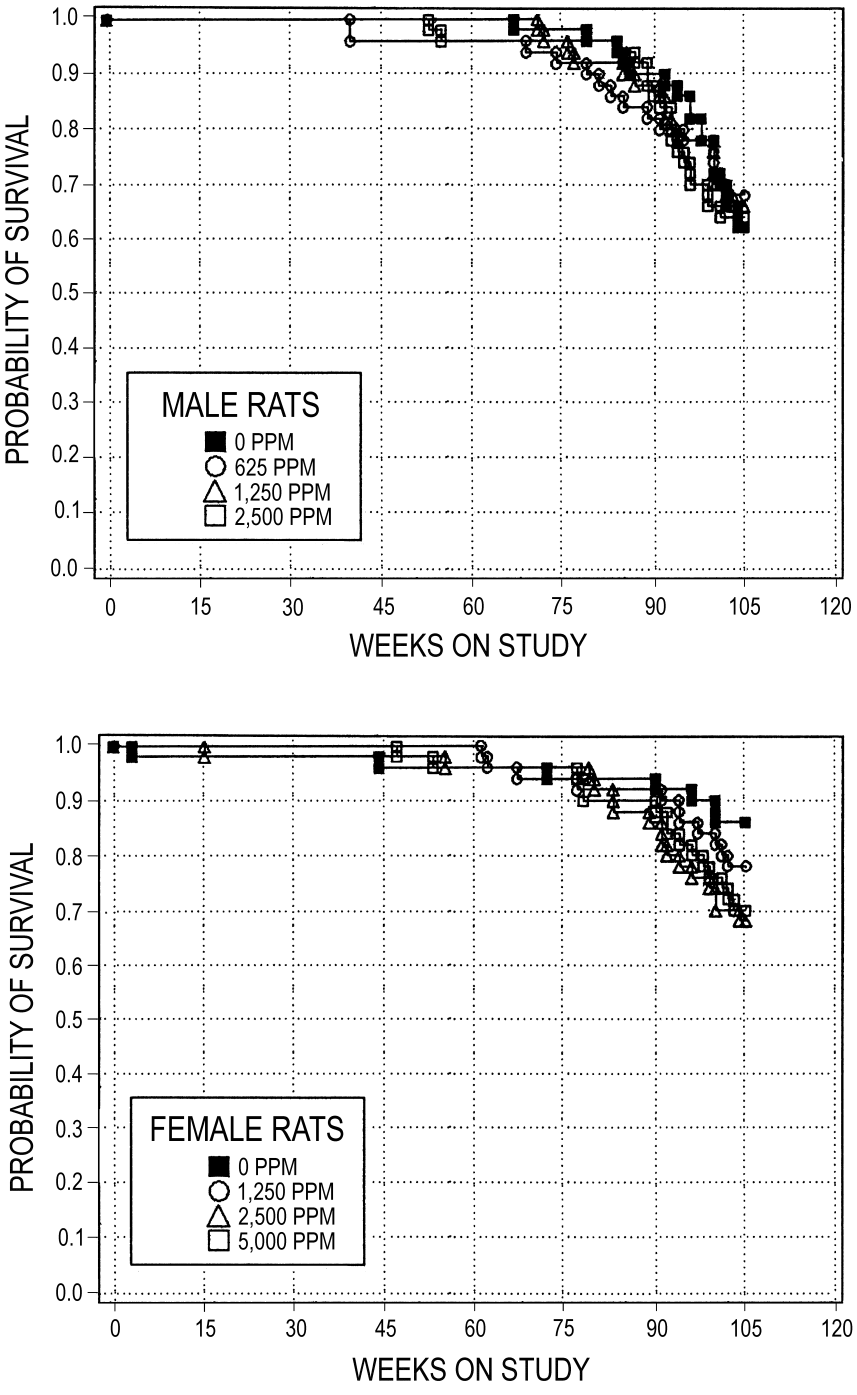


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to 4-Methylimidazole in Feed for 2 Years

TABLE 3
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of 4-Methylimidazole

Weeks on Study	0 ppm		625 ppm			1,250 ppm			2,500 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	124	50	124	100	50	123	99	50	124	100	50
2	179	50	173	97	50	165	92	50	151	84	50
3	210	50	205	98	50	193	92	50	180	86	50
4	240	50	235	98	50	221	92	50	205	85	50
5	255	50	250	98	50	237	93	50	223	87	50
6	270	50	266	99	50	252	94	50	239	89	50
7	286	50	280	98	50	265	93	50	250	88	50
8	304	50	297	98	50	280	92	50	262	86	50
9	313	50	307	98	50	288	92	50	270	86	50
10	324	50	317	98	50	295	91	50	278	86	50
11	330	50	321	97	50	297	90	50	280	85	50
12	342	50	331	97	50	304	89	50	285	83	50
13	345	50	332	96	50	303	88	50	284	82	50
17	365	50	352	97	50	322	88	50	292	80	50
21	382	50	368	96	50	340	89	50	313	82	50
25	395	50	383	97	50	351	89	50	323	82	50
29	410	50	396	96	50	363	89	50	336	82	50
33	418	50	402	96	50	370	89	50	340	81	50
37	426	50	408	96	50	379	89	50	343	81	50
41	429	50	415	97	48	380	89	50	349	81	50
45	433	50	419	97	48	384	89	50	353	82	50
49	437	50	421	96	48	385	88	50	357	82	50
53	440	50	429	98	48	386	88	50	359	82	50
57	438	50	425	97	48	390	89	50	359	82	48
61	445	50	428	96	48	396	89	50	364	82	48
65	448	50	435	97	48	396	88	50	366	82	48
69	445	49	430	97	47	402	90	50	367	82	48
73	444	49	424	96	47	395	89	48	366	83	48
77	438	49	425	97	46	396	90	47	365	83	48
81	438	48	419	96	45	386	88	46	364	83	48
85	433	46	416	96	42	388	90	46	362	84	47
89	421	45	408	97	41	387	92	44	354	84	46
93	426	44	414	97	40	383	90	41	349	82	42
97	422	41	408	97	39	381	90	39	350	83	35
101	400	36	405	101	35	378	95	36	350	87	32
Mean for weeks											
1-13	271		264	97		248	92		233	86	
14-52	411		396	96		364	89		334	81	
53-101	434		420	97		390	90		360	83	

TABLE 4
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of 4-Methylimidazole

Weeks on Study	0 ppm		1,250 ppm			2,500 ppm			5,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	100	50	98	98	50	100	99	50	99	99	50
2	127	50	124	98	50	118	93	50	96	75	50
3	138	49	137	99	50	133	96	50	109	79	50
4	149	49	148	100	50	144	97	50	118	79	50
5	156	49	155	100	50	152	98	50	126	81	50
6	163	49	162	99	50	161	99	50	132	81	50
7	169	49	166	98	50	166	99	50	137	81	50
8	176	49	172	98	50	172	98	50	142	81	50
9	177	49	173	98	50	175	99	50	143	81	50
10	182	49	176	97	50	178	98	50	145	80	50
11	186	49	178	96	50	180	97	50	146	79	50
12	187	49	180	96	50	181	97	50	149	80	50
13	190	49	182	96	50	181	95	50	152	80	50
17	194	49	189	97	50	189	97	49	154	79	50
21	204	49	194	95	50	193	95	49	158	77	50
25	212	49	202	96	50	202	95	49	164	77	50
29	217	49	209	96	50	203	93	49	167	77	50
33	223	49	215	96	50	209	94	49	172	77	50
37	229	49	218	96	50	215	94	49	174	76	50
41	228	49	220	97	50	216	95	49	178	78	50
45	238	48	223	94	50	217	91	49	179	75	50
49	242	48	227	94	50	221	91	49	183	76	49
53	251	48	232	93	50	224	89	49	187	75	48
57	255	48	234	92	50	224	88	48	187	74	48
61	270	48	239	89	49	227	84	48	193	72	48
65	275	48	244	89	48	231	84	48	195	71	48
69	278	48	248	89	47	232	83	48	192	69	48
73	284	47	248	87	47	233	82	48	195	69	48
77	291	47	254	88	46	237	82	48	197	68	48
81	296	47	256	86	46	237	80	46	200	68	45
85	297	47	258	87	46	241	81	44	197	66	45
89	301	47	258	86	46	244	81	43	200	67	45
93	305	46	259	85	45	247	81	40	202	66	42
97	300	45	265	88	42	250	84	38	203	68	40
101	311	43	264	85	40	253	81	35	202	65	37
Mean for weeks											
1-13	162		158	98		157	97		130	80	
14-52	221		211	95		207	94		170	77	
53-101	286		251	88		237	83		196	69	

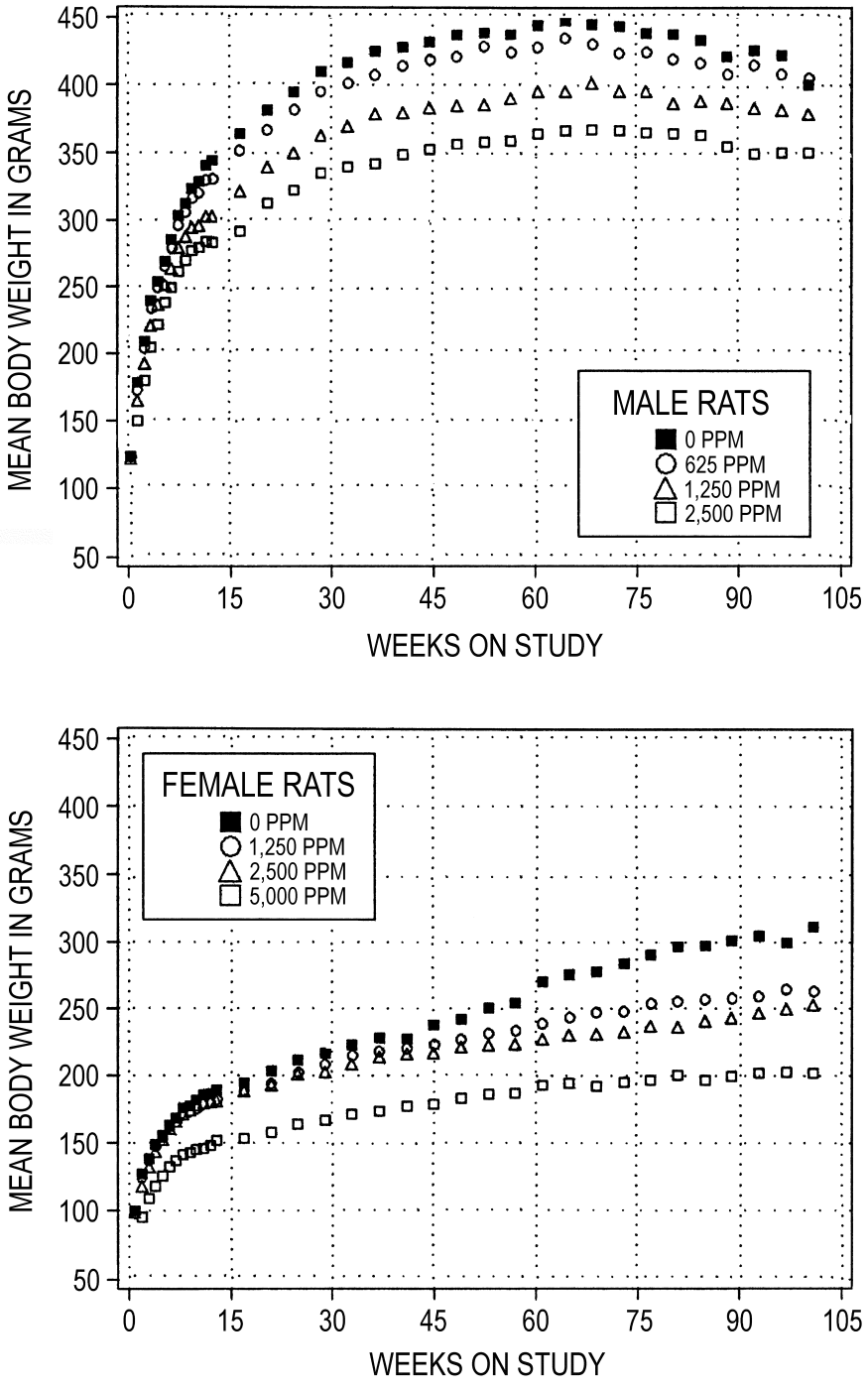


FIGURE 3
Growth Curves for Male and Female Rats Exposed to 4-Methylimidazole in Feed for 2 Years

TABLE 5
Neurological Clinical Findings in Female Rats in the 2-Year Feed Study of 4-Methylimidazole[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Clonic Seizures	0/50	0/50	21/50	36/50
Excitability	0/50	2/50	9/50	50/50
Hyperactive	0/50	0/50	0/50	5/50
Impaired Gait	1/50	0/50	4/50	49/50

[a] Number of rats with clinical finding per number of rats in the exposure group

Toxicokinetics

After a single gavage dose of 4-methylimidazole (10, 50, or 100 mg/kg) to male and female F344/N rats, the plasma concentration versus time data can be described by a one-compartment model with no lag phase and first-order absorption and elimination for both males and females (Appendix J). The absorption half-life ranged from 5 to 23 minutes and decreased with dose (Table J1). The elimination half-life ranged from 1 to 8 hours and increased with dose. The plasma concentration versus time data following intravenous administration of 10 mg/kg 4-methylimidazole (Appendix K) was described as a one-compartment model with first-order elimination. From comparisons of the area under the concentration versus time curves for the two routes of administration, bioavailability was determined to range from approximately 85% to 145%; because it increased with dose, this may indicate saturation of first-pass processes. However, plasma concentrations from the dosed feed study at 625, 1,250, or 2,500 ppm in male rats (Figures K7, K8, and K9, respectively) and at 1,250, 2,500, or 5,000 ppm in female rats (Figures K10, K11, and K12, respectively) did not show an increase with time, implying no saturation with dosed feed exposure was reached. The gavage bolus data generated from the single gavage administration did not predict the outcome of the dosed feed study. Probably, the slow absorption in the feed study would not allow the concentration to reach the saturation point. Tissue levels were not measured.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and neoplasms and/or nonneoplastic lesions of the liver, prostate gland, pituitary gland, thyroid gland, lung, heart, pancreas, adrenal medulla, clitoral gland, mammary gland, and uterus. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Mononuclear cell leukemia: The incidence of mononuclear cell leukemia in 5,000 ppm females was significantly greater than that in the controls, and the incidence exceeded the historical range in feed study controls given NTP-2000 diet (Tables 6, B1, B3, and B4). Mononuclear cell leukemia is a common finding with highly variable incidences in F344/N rats used in 2-year NTP studies and may have been exacerbated by 4-methylimidazole treatment, as the onset in 5,000 ppm females was earlier (day 368) than in control females (day 624).

Liver: The incidences of histiocytosis were significantly greater in all exposed groups of male and female rats than in the control groups, and the severities increased with increasing exposure concentration (Tables 7, A4, and B5). Histologically, this lesion in exposed animals was randomly distributed throughout the hepatic parenchyma and characterized by focal to multifocal clusters of enlarged histiocytes with prominent foamy cytoplasm often containing vacuoles and/or cleft-like

TABLE 6
Incidences of Mononuclear Cell Leukemia in Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
Mononuclear Cell Leukemia[a]				
Overall rate[b]	15/50 (30%)	18/50 (36%)	22/50 (44%)	20/50 (40%)
Adjusted rate[c]	31.9%	39.5%	46.2%	42.2%
Terminal rate[d]	5/31 (16%)	9/34 (27%)	12/33 (36%)	7/32 (22%)
First incidence (days)	582	567	493	584
Poly-3 test[e]	P=0.184	P=0.292	P=0.110	P=0.205
	0 ppm	**1,250 ppm**	**2,500 ppm**	**5,000 ppm**
Female				
Mononuclear Cell Leukemia[f]				
Overall rate	9/50 (18%)	7/50 (14%)	16/50 (32%)	20/50 (40%)
Adjusted rate	19.1%	14.8%	34.5%	41.7%
Terminal rate	7/43 (16%)	4/39 (10%)	7/34 (21%)	11/35 (31%)
First incidence (days)	624	434	578	368
Poly-3 test	P<0.001	P=0.386N	P=0.073	P=0.013

[a] Historical incidence for 2-year feed study controls given NTP-2000 diet (mean ± standard deviation): 246/510 (46.8% ± 13.0%); range, 30%-68%
[b] Number of animals with neoplasm per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposure group is indicated by **N**.
[f] Historical incidence: 121/510 (23.8% ± 9.1%); range, 12%-38%

TABLE 7
Incidences of Nonneoplastic Lesions of the Liver in Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
Number Examined Microscopically	50	50	50	50
Histiocytosis[a]	38 (1.1)[b]	45* (1.4)	50** (1.9)	50** (2.3)
Inflammation, Chronic	18 (1.1)	32** (1.2)	31** (1.3)	36** (1.3)
Hepatocyte, Fatty Change, Focal	21 (1.5)	24 (1.8)	37** (1.9)	33** (2.5)
Eosinophilic Focus	4	3	7	12*
Mixed Cell Focus	5	7	11	27**
	0 ppm	**1,250 ppm**	**2,500 ppm**	**5,000 ppm**
Female				
Number Examined Microscopically	50	50	48	50
Histiocytosis	40 (1.0)	50** (1.3)	48** (1.9)	50** (2.5)
Inflammation, Chronic	17 (1.2)	28* (1.5)	34** (1.8)	35** (1.7)
Hepatocyte, Fatty Change, Focal	16 (1.2)	29** (1.6)	29** (2.0)	32** (2.2)
Clear Cell Focus	20	32**	23	27
Eosinophilic Focus	1	2	5	11**
Mixed Cell Focus	10	7	6	18*

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

spaces (Plate 1). Occasional syncytial cells were present. In control animals, the histiocytes occurred primarily as scattered individual cells and rarely as clusters. The cytoplasm of these cells was foamy, and cleft-like spaces were absent (Plate 2). Electron microscopy confirmed the light microscopic findings. The contents of the cleft-like spaces could not be determined.

Incidences of chronic inflammation of the liver in all exposed groups of rats were significantly greater than those in the controls (Tables 7, A4, and B5). Microscopically, there were small, focal accumulations of macrophages with granular cytoplasm surrounded by variable numbers of lymphoid cells within the hepatic parenchyma (Plate 3). Foci of chronic inflammation are commonly seen in the F344/N rat liver as a background change resulting from bacterial showering from the intestinal tract; however, incidences in all exposed groups may have been increased by 4-methylimidazole treatment.

There were significant increases in the incidences of hepatocytic focal fatty change in 1,250 and 2,500 ppm males and all exposed groups of females, and the severities increased with increasing exposure concentration (Tables 7, A4, and B5). This diagnosis was made when there were distinct foci of vacuolated hepatocytes (more than 10) in which 80% or more of the cell appeared to contain lipid.

In 2,500 ppm males and 5,000 ppm females, the incidences of eosinophilic and mixed cell foci (mixture of eosinophilic and clear cells in which no one cell type exceeds 80%) were significantly increased. In females, there was a significant increase in the incidence of clear cell focus in the 1,250 ppm group. In general, these foci consisted of enlarged hepatocytes with altered tinctorial characteristics of the cytoplasm that usually did not compress the surrounding parenchyma. Occasionally, larger foci were multilobular, contained biliary structures, and compressed the adjacent liver, but there was no loss of normal hepatic architecture.

Other organs (increased incidences of nonneoplastic lesions): There were statistically significant increased incidences of nonneoplastic lesions in the prostate gland, pituitary gland (pars distalis), and thyroid gland of exposed groups of male rats (Tables 8 and A4) and in the thyroid gland, lung, heart, and pancreas of exposed groups of female rats (Tables 8 and B5). The biological significance of these increases is not certain. However, these lesions are common background changes in F344/N rats that may have been exacerbated by 4-methylimidazole exposure.

The incidence of mild chronic inflammation was significantly increased in the prostate gland of 2,500 ppm males. Interstitial and intraluminal infiltrates of small numbers of mononuclear cells characterized this lesion.

In the pituitary gland (pars distalis), the incidences of focal hypertrophy in 1,250 and 2,500 ppm males were significantly greater than those in the controls. This cellular alteration was characterized by small foci of enlarged, lightly eosinophilic cells with round vesicular nuclei.

The incidence of follicle mineralization was significantly increased in the thyroid gland of 5,000 ppm females. This lesion consisted of one to two small basophilic (mineralized) structures within the follicle lumen. In the thyroid gland of 2,500 ppm males, the

TABLE 8
Increased Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
Prostate Gland[a]	50	50	50	49
Inflammation, Chronic[b]	27 (1.7)[c]	24 (2.0)	28 (1.6)	36* (2.0)
Pituitary Gland (Pars Distalis)	49	49	48	48
Hypertrophy, Focal	8 (1.8)	9 (1.6)	20** (1.8)	22** (2.0)
Thyroid Gland	47	46	48	44
Follicle Cyst	0	3	1	5*
	0 ppm	**1,250 ppm**	**2,500 ppm**	**5,000 ppm**
Female				
Thyroid Gland	47	44	40	45
Follicle Mineralization	2 (1.0)	7 (1.0)	6 (1.0)	19** (1.0)
Lung	50	50	50	50
Inflammation, Chronic, Focal	25 (1.1)	40** (1.2)	39** (1.0)	43** (1.2)
Heart	50	50	48	50
Cardiomyopathy	30 (1.3)	43** (1.5)	38** (1.8)	44** (1.9)
Pancreas	49	49	47	47
Acinus, Atrophy, Focal	13 (1.3)	22* (1.7)	26** (1.8)	30** (1.7)

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

incidence of follicle cyst was significantly greater than that in the controls. A few dilated follicles (greater than 300 µm) lined by flattened cuboidal epithelium and distended by normal colloid characterized this lesion.

In the lung, significantly increased incidences of focal chronic inflammation occurred in all exposed groups of females. Histologically, this lesion was of minimal severity and characterized by very small, focal, subpleural accumulations of macrophages and/or mixed inflammatory cells within alveoli.

In the heart, the incidences of cardiomyopathy of generally minimal severity were significantly increased in all exposed groups of females. This lesion was characterized by small focal accumulations of mononuclear cells and occasional degenerative myocardial fibers.

In all exposed groups of females, there were significantly increased incidences of minimal pancreatic focal acinar atrophy. Microscopically, there was a slight reduction in the number of acini, which were small and lined by flattened epithelial cells with a loss of acidophilic granules.

Other organs (decreased incidences of neoplasms): There were statistically significant decreases in the incidences of neoplasms of the pituitary gland (pars distalis) and adrenal medulla in exposed groups of males (Tables 9, A1, and A3) and in the pituitary gland (pars distalis), clitoral gland, mammary gland, and uterus in exposed groups of females (Tables 9, B1, and B3). These incidences in the exposed groups were either below the historical control ranges or at the lower end of the historical control ranges in feed study controls given NTP-2000 diet (Table 9).

In the pituitary gland (pars distalis), the incidences of adenoma in 2,500 ppm males and 1,250 and 5,000 ppm females were significantly less than those in the controls. The decreased incidences of these neoplasms in males and females were probably related in part to lower body weights. Similar decreased incidences of pituitary gland (pars distalis) adenoma were reported in 5,000 ppm females in the 2-methylimidazole study (NTP, 2004b).

In the adrenal medulla, significantly decreased incidences of benign, complex, or malignant pheochromocytoma (combined) occurred in 1,250 and 2,500 ppm males.

In all exposed groups of females, the incidences of clitoral gland adenoma, mammary gland fibroadenoma, and uterine stromal polyp were significantly less than those in the control group. Similar decreased incidences of clitoral gland adenoma and mammary gland fibroadenoma were reported in 5,000 ppm females from the 2-year 2-methylimidazole carcinogenesis study (NTP, 2004b).

TABLE 9
Decreased Incidences of Neoplasms in Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
Pituitary Gland (Pars Distalis): Adenoma[a]				
Overall rate[b]	16/49 (33%)	13/49 (27%)	10/48 (21%)	7/48 (15%)
Adjusted rate[c]	34.5%	29.8%	23.3%	16.1%
Terminal rate[d]	10/31 (32%)	10/33 (30%)	10/32 (31%)	6/32 (19%)
First incidence (days)	464	477	729 (T)	384
Poly-3 test[e]	P=0.022N	P=0.402N	P=0.176N	P=0.037N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma (Combined)[f]				
Overall rate	10/50 (20%)	6/50 (12%)	3/50 (6%)	3/50 (6%)
Adjusted rate	22.0%	13.8%	6.8%	6.8%
Terminal rate	8/31 (26%)	3/34 (9%)	3/33 (9%)	2/32 (6%)
First incidence (days)	685	700	729 (T)	607
Poly-3 test	P=0.019N	P=0.234N	P=0.037N	P=0.039N

TABLE 9
Decreased Incidences of Neoplasms in Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Female				
Pituitary Gland (Pars Distalis): Adenoma[g]				
Overall rate	29/48 (60%)	19/50 (38%)	20/50 (40%)	9/50 (18%)
Adjusted rate	62.1%	40.7%	44.7%	20.0%
Terminal rate	25/42 (60%)	15/39 (39%)	17/34 (50%)	6/35 (17%)
First incidence (days)	498	636	555	642
Poly-3 test	P<0.001N	P=0.029N	P=0.068N	P<0.001N
Clitoral Gland: Adenoma[h]				
Overall rate	8/50 (16%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	17.0%	2.2%	0.0%	0.0%
Terminal rate	6/43 (14%)	0/39 (0%)	0/34 (0%)	0/35 (0%)
First incidence (days)	624	434	—[1]	—
Poly-3 test	P<0.001N	P=0.017N	P=0.005N	P=0.005N
Mammary Gland: Fibroadenoma (includes multiple)[j]				
Overall rate	24/50 (48%)	6/50 (12%)	4/50 (8%)	1/50 (2%)
Adjusted rate	51.0%	13.1%	9.1%	2.3%
Terminal rate	22/43 (51%)	6/39 (15%)	4/34 (12%)	1/35 (3%)
First incidence (days)	624	729 (T)	729 (T)	729 (T)
Poly-3 test	P<0.001N	P<0.001N	P<0.001N	P<0.001N
Uterus: Stromal Polyp[k]				
Overall rate	16/50 (32%)	5/50 (10%)	2/50 (4%)	2/50 (4%)
Adjusted rate	33.6%	10.9%	4.6%	4.5%
Terminal rate	14/43 (33%)	4/39 (10%)	2/34 (6%)	2/35 (6%)
First incidence (days)	302	677	729 (T)	729 (T)
Poly-3 test	P<0.001N	P=0.007N	P<0.001N	P<0.001N

(T) Terminal sacrifice
[a] Historical incidence for 2-year feed study controls given NTP-2000 diet (mean ± standard deviation): 110/506 (22.6% ± 6.0%); range, 17%-33%
[b] Number of animals with neoplasm per number of animals necropsied (mammary gland and uterus) or number of animals with tissue microscopically examined (pituitary gland, adrenal gland, and clitoral gland)
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[f] Historical incidence: 55/510 (11.6% ± 5.5%); range, 5%-20%
[g] Historical incidence: 190/507 (39.1% ± 10.9%); range, 29%-60%
[h] Historical incidence: 55/503 (11.0% ± 6.5%); range, 2%-20%
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence: 237/510 (44.8% ± 11.1%); range, 28%-55%
[k] Historical incidence: 90/510 (17.9% ± 6.5%); range, 12%-32%

MICE

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 10 and in the Kaplan-Meier survival curves (Figure 4). Survival of all exposed groups of male and female mice was similar to that of the control groups.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of males and females in the 1,250 ppm groups were less than those in the control groups after weeks 17 and 12, respectively (Tables 11 and 12; Figure 5). Mean body weights of 312 and 625 ppm females were less after weeks 85 and 65, respectively. Feed consumption by exposed groups of males and females was generally similar to that by the controls (Tables H3 and H4). Dietary levels of 312, 625, or 1,250 ppm resulted in average daily doses of approximately 40, 80, and 170 mg 4-methylimidazole/kg body weight to males and females. No clinical findings in exposed groups of male or female mice were considered to be related to chemical exposure.

Toxicokinetics

After a single gavage dose of 4-methylimidazole (10, 50, or 100 mg/kg) to male and female B6C3F$_1$ mice, the plasma concentration versus time data can be described by a one-compartment model with no lag phase and first-order absorption and elimination for both males and females (Appendix J). The absorption half-life ranged from 2 to 5 minutes and declined with dose (Table J2). The elimination half-life ranged from 21 to 87 minutes and increased with dose. The plasma concentration versus time data following intravenous administration of 10 mg/kg 4-methylimidazole (Appendix K) was described as a one-compartment model with first-order elimination. From comparisons of the area under the concentration versus time curves for the two routes of administration, bioavailability was determined to vary from approximately 76% to 182%, and it increased with dose. Clearance rates decreased with dose but were comparable between male and female mice.

TABLE 10
Survival of Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
Animals initially in study	50	50	50	50
Other[a]	0	0	0	1
Moribund	1	0	0	3
Natural deaths	4	6	8	0
Animals surviving to study termination	45[e]	44	42	46
Percent probability of survival at end of study[b]	90	88	84	94
Mean survival (days)[c]	717	714	700	721
Survival analysis[d]	P=0.631N	P=0.990	P=0.514	P=0.735N
Female				
Animals initially in study	50	50	50	50
Moribund	3	4	3	2
Natural deaths	4	6	4	8
Animals surviving to study termination	43[e]	40	43[f]	40[e]
Percent probability of survival at end of study	86	80	86	80
Mean survival (days)	702	716	717	703
Survival analysis	P=0.635	P=0.635	P=1.000N	P=0.601

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or a lower mortality in an exposure group is indicated by **N**.
[e] Includes one animal that died during the last week of the study
[f] Includes two animals that died during the last week of the study

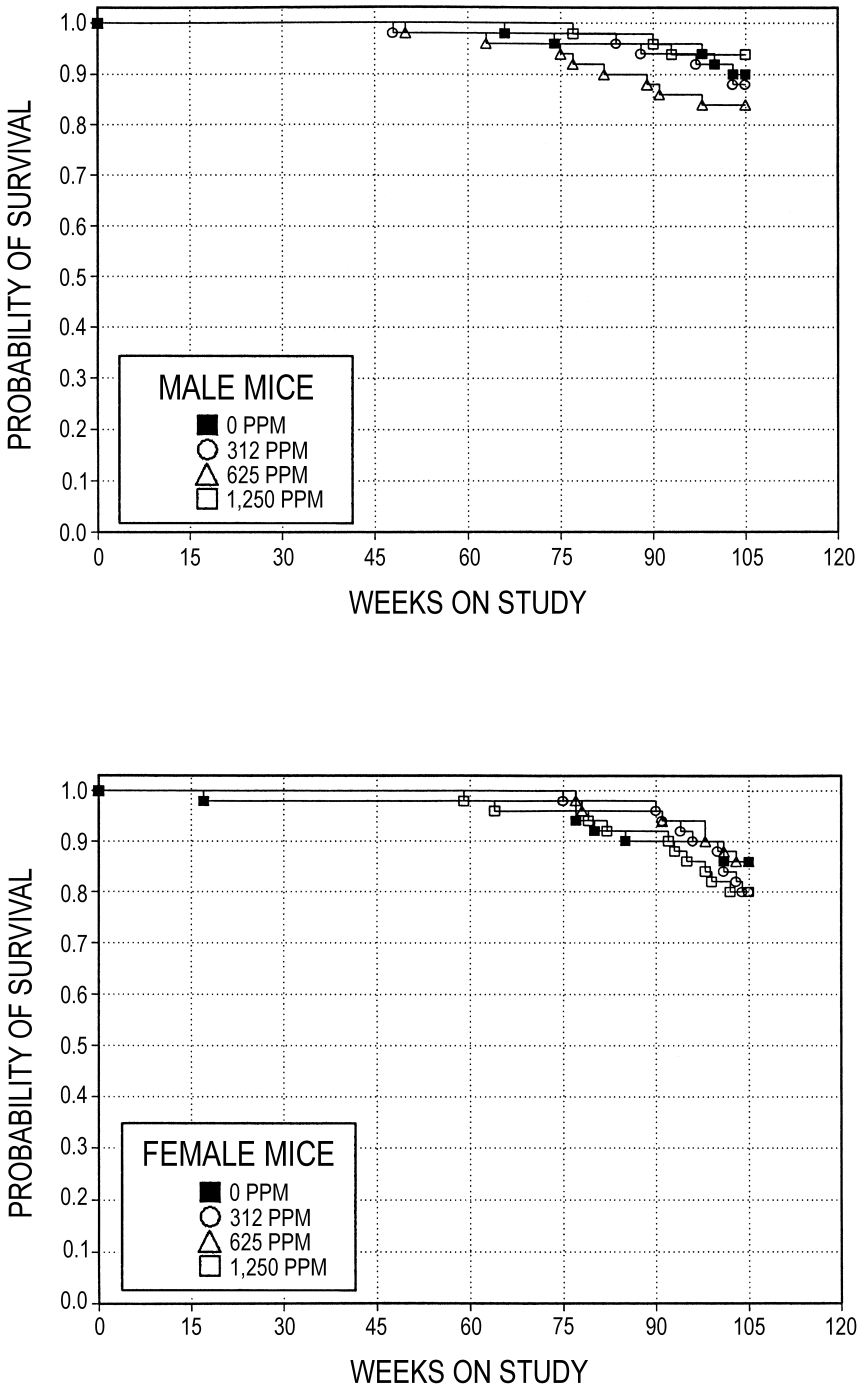


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to 4-Methylimidazole in Feed for 2 Years

TABLE 11
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of 4-Methylimidazole

Weeks on Study	0 ppm		312 ppm			625 ppm			1,250 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	21.2	50	21.3	101	50	21.1	100	50	21.2	100	50
2	22.1	50	22.3	101	50	21.9	99	50	22.0	100	50
3	23.2	50	22.8	98	50	23.1	100	50	22.8	98	50
4	24.4	50	24.7	101	50	24.3	100	50	24.0	98	50
5	25.5	50	25.7	101	50	25.3	99	50	25.0	98	50
6	26.8	50	26.7	100	50	26.6	99	50	26.2	98	50
7	27.4	50	27.4	100	50	27.1	99	50	26.8	98	50
8	28.3	50	28.3	100	50	27.8	98	50[a]	27.5	97	50
9	28.8	50	28.9	100	50	28.4	99	50	27.9	97	50
10	29.1	50	29.1	100	50	28.8	99	50	28.4	98	50
11	29.6	50	29.7	100	50	29.1	98	50	28.8	97	50
12	30.8	50	30.6	99	50	30.3	98	50	29.4	96	50
13	31.3	50	31.2	100	50	31.0	99	50	30.3	97	50
17	34.4	50	34.0	99	50	33.6	98	50	32.6	95	50
21	36.1	50	36.1	100	50	35.3	98	50	33.5	93	50
25	37.6	50	37.6	100	50	36.7	98	50	34.7	92	50
29	40.1	50	40.3	101	50	39.3	98	50	37.1	93	50
33	41.4	50	41.7	101	50	40.2	97	50	37.5	91	50
37	42.3	50	42.4	100	50	41.0	97	50	38.3	91	50
41	44.1	50	43.9	100	50	42.8	97	50	39.8	90	50
45	43.5	50	44.2	102	50	43.0	99	50	40.0	92	50
49	43.9	50	44.1	101	49	42.7	97	50	39.5	90	50
53	45.3	50	45.7	101	49	44.2	98	49	41.1	91	49
57	46.1	50	46.5	101	49	45.1	98	49	41.1	89	49
61	45.9	50	46.1	100	49	45.2	99	49	40.7	89	49
65	44.3	50	44.9	101	49	43.6	98	48	39.5	89	49
69	44.8	49	43.7	98	49	42.5	95	48	38.8	87	49
73	44.9	49	44.1	98	49	42.9	96	48	39.0	87	49
77	45.7	48	44.2	97	49	42.3	93	47	38.4	84	49
81	45.2	48	43.1	95	49	42.0	93	46	38.0	84	48
85	45.7	48	43.9	96	48	42.9	94	45	38.4	84	48
89	45.0	48	43.7	97	47	42.7	95	45	38.0	84	48
93	44.9	48	43.7	97	47	43.0	96	43	38.1	85	47
97	44.7	48	43.7	98	47	42.8	96	43	38.4	86	46
101	43.7	46	42.6	98	46	41.9	96	42	37.4	86	46
Mean for weeks											
1-13	26.8		26.8	100		26.5	99		26.2	98	
14-52	40.4		40.5	100		39.4	98		37.0	92	
53-101	45.1		44.3	98		43.2	96		39.0	87	

[a] The number of male mice weighed for this week is less than the number of male mice surviving.

TABLE 12
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of 4-Methylimidazole

Weeks on Study	0 ppm		312 ppm			625 ppm			1,250 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	17.4	50	17.3	99	50	17.4	100	50	17.4	100	50
2	18.3	50	18.0	98	50	18.6	102	50	17.7	97	50
3	19.0	50	18.8	99	50	19.1	101	50	18.9	100	50
4	20.5	50	20.3	99	50	20.1	98	50	19.6	96	50
5	21.3	50	20.4	96	50	21.2	100	50	20.0	94	50
6	22.3	50	22.0	99	50	22.2	100	50	21.2	95	50
7	23.1	50	22.5	97	50	22.5	97	50	21.6	94	50
8	23.1	50	23.3	101	50	23.2	100	50	22.2	96	50
9	23.9	50	23.7	99	50	23.6	99	50	22.9	96	50
10	24.6	50	24.4	99	50	24.3	99	50	23.5	96	50
11	24.9	50	24.7	99	50	24.7	99	50	23.7	95	50
12	25.0	50	25.3	101	50	25.1	100	50	23.8	95	50
13	25.7	50	25.5	99	50	25.1	98	50	24.1	94	50
17	28.7	49	28.5	99	50	27.6	96	50	25.9	90	50
21	30.4	49	30.1	99	50	30.0	99	50	27.5	91	50
25	32.5	49	32.1	99	50	31.2	96	50	28.3	87	50
29	35.9	49	34.5	96	50	33.9	94	50	30.1	84	50
33	36.0	49	35.1	98	50	33.1	92	50	29.3	81	50
37	37.1	49	35.8	97	50	35.5	96	50	31.1	84	50
41	37.7	49	37.6	100	50	37.4	99	50	31.8	84	50
45	39.0	49	39.0	100	50	38.1	98	50	33.4	86	50
49	40.0	49	39.5	99	50	38.9	97	50	33.1	83	50
53	41.6	49	40.6	98	50	39.9	96	50	34.5	83	50
57	43.3	49	41.8	97	50	41.2	95	50	35.0	81	50
61	43.0	49	41.1	96	50	41.2	96	50	34.5	80	49
65	42.6	49	40.7	96	50	40.3	95	50	34.5	81	48
69	43.4	49	41.1	95	50	39.6	91	50	35.1	81	48
73	42.9	49	41.0	96	50	39.8	93	50	35.4	83	48
77	42.9	49	41.5	97	49	39.3	92	50	35.4	83	48
81	42.5	46	40.5	95	49	39.1	92	48	34.7	82	47
85	43.1	46	41.4	96	49	40.0	93	48	35.5	82	46
89	44.2	45	41.0	93	49	40.2	91	48	35.6	81	46
93	45.3	45	42.4	94	47	39.9	88	47	35.4	78	45
97	45.6	45	43.2	95	45	40.3	88	47	36.3	80	43
101	44.6	45	41.8	94	44	41.5	93	45	36.2	81	41
Mean for weeks											
1-13	22.2		22.0	99		22.1	99		21.3	96	
14-52	35.3		34.7	98		34.0	96		30.1	85	
53-101	43.5		41.4	95		40.2	93		35.2	81	

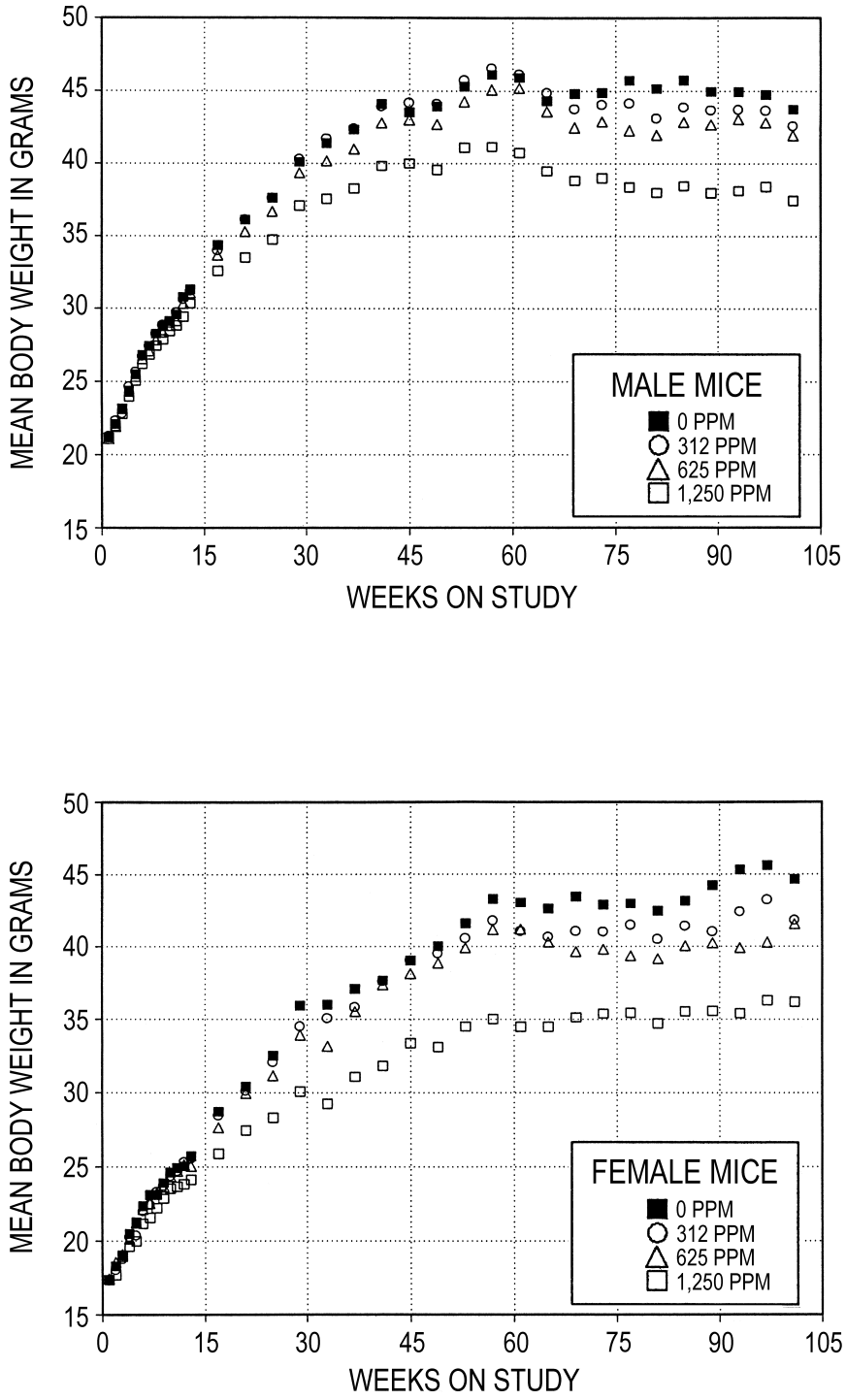


FIGURE 5
Growth Curves for Male and Female Mice Exposed to 4-Methylimidazole in Feed for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the lung, thyroid gland, and mammary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Lung: The incidences of alveolar/bronchiolar adenoma in all exposed groups of females, alveolar/bronchiolar carcinoma in 1,250 ppm males, and alveolar/bronchiolar adenoma or carcinoma (combined) in 1,250 ppm males and 625 and 1,250 ppm females were significantly greater than those in the control groups (Tables 13, C3, and D3). Histologically, adenomas were focal, generally well-demarcated nodular lesions that usually compressed the surrounding parenchyma. Adenomas were composed of increased numbers of large cuboidal to polygonal epithelial cells that were arranged in abnormal patterns, most commonly as papillary structures that distorted the alveolar architecture. Microscopically, carcinomas were generally larger (up to 1 centimeter in diameter) than adenomas and were composed of cuboidal to columnar, mildly to markedly pleomorphic epithelial cells. These neoplastic cells were often densely packed and formed multiple layers that showed a tendency toward solid growth. Most of the carcinomas were discrete masses that compressed the adjacent parenchyma, although some were locally invasive into the parenchyma and airways.

The incidence of alveolar epithelium hyperplasia in 1,250 ppm females was significantly greater than that in the controls (Tables 13 and D5). Histologically, this lesion was considered a morphologic continuum to adenoma and was characterized by increased numbers of cuboidal epithelial cells that lined alveoli; however, the septal architecture was well maintained.

The incidence of histiocytic cellular infiltration, a lesion often seen secondary to lung neoplasms, was

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Hyperplasia[a]	7 (2.0)[b]	3 (1.0)	1 (2.0)	9 (1.9)
Infiltration Cellular, Histiocyte	5 (2.2)	6 (1.7)	5 (1.8)	11 (1.7)
Alveolar/bronchiolar Adenoma (includes multiple)[c]	8	11	13	15
Alveolar/bronchiolar Carcinoma (includes multiple)[d]				
Overall rate[e]	2/50 (4%)	4/50 (8%)	4/50 (8%)	8/50 (16%)
Adjusted rate[f]	4.1%	8.3%	8.8%	16.7%
Terminal rate[g]	1/45 (2%)	3/44 (7%)	4/42 (10%)	8/46 (17%)
First incidence (days)	513	613	729 (T)	729 (T)
Poly-3 test[h]	P=0.024	P=0.332	P=0.307	P=0.042
Alveolar/bronchiolar Adenoma or Carcinoma (combined)[i]				
Overall rate	9/50 (18%)	13/50 (26%)	16/50 (32%)	22/50 (44%)
Adjusted rate	18.4%	26.9%	35.0%	46.0%
Terminal rate	8/45 (18%)	11/44 (25%)	16/42 (38%)	22/46 (48%)
First incidence (days)	513	613	729 (T)	729 (T)
Poly-3 test	P<0.001	P=0.226	P=0.053	P=0.003

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Female				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Hyperplasia	3 (1.7)	2 (2.5)	3 (1.7)	11* (1.9)
Infiltration Cellular, Histiocyte	1 (1.0)	5 (1.4)	1 (1.0)	8* (2.0)
Alveolar/bronchiolar Adenoma (includes multiple)[j]				
Overall rate	0/50 (0%)	8/50 (16%)	16/50 (32%)	8/50 (16%)
Adjusted rate	0.0%	16.6%	33.2%	17.4%
Terminal rate	0/43 (0%)	7/40 (18%)	15/43 (35%)	8/40 (20%)
First incidence (days)	—[k]	632	684	729 (T)
Poly-3 test	P=0.017	P=0.004	P<0.001	P=0.003
Alveolar/bronchiolar Carcinoma (includes multiple)[l]	3	0	2	7
Alveolar/bronchiolar Adenoma or Carcinoma (combined)[m]				
Overall rate	3/50 (6%)	8/50 (16%)	17/50 (34%)	14/50 (28%)
Adjusted rate	6.4%	16.6%	35.3%	30.3%
Terminal rate	3/43 (7%)	7/40 (18%)	16/43 (37%)	13/40 (33%)
First incidence (days)	729 (T)	632	684	687
Poly-3 test	P=0.002	P=0.109	P<0.001	P=0.002

* Significantly different (P≤0.05) from the control group by the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year feed study controls given NTP-2000 diet (mean ± standard deviation): 75/510 (15.8% ± 6.3%); range, 9%-28%
[d] Historical incidence: 40/510 (7.8% ± 3.8%); range, 4%-14%
[e] Number of animals with neoplasm per number of animals with lung examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[i] Historical incidence: 108/510 (22.2% ± 6.3%); range, 14%-32%
[j] Historical incidence: 19/509 (3.7% ± 3.8%); range, 0%-10%
[k] Not applicable; no neoplasms in animal group
[l] Historical incidence: 16/509 (2.9% ± 2.5%); range, 0%-6%
[m] Historical incidence: 35/509 (6.6% ± 4.2%); range, 0%-12%

significantly greater than that of the controls in 1,250 ppm females and slightly increased in 1,250 ppm males (Tables 13, C5, and D5). Microscopically, the infiltration was characterized by small numbers of histiocytes scattered within alveolar lumens adjacent to many of the adenomas and carcinomas.

Thyroid gland: The incidence of follicular cyst in 1,250 ppm females was significantly greater than that in the controls (0 ppm, 20/50; 312 ppm, 22/49; 625 ppm, 29/50; 1,250 ppm, 30/48; Table D5). Histologically, the affected follicles were variably dilated, filled with pale staining colloid, and often lined by flattened epithelial cells. This change ranged in severity from minimal to moderate. Minimal lesions affected a single follicle, while moderate lesions affected a cluster of adjacent follicles. In larger, more severe lesions, the walls of the follicles appeared to have ruptured and formed highly irregular cystic structures. These changes were usually focal but could be multifocal and occasionally bilateral in more severe cases.

Mammary gland: There was a significant positive trend in the incidences of hyperplasia in females (16/50, 10/50, 14/49, 24/49; P=0.013); however, none of the exposed groups differed significantly from the control group (Table D5). Microscopically, hyperplasia was usually a minimal change that was characterized by an increase in the number of mammary gland ducts with a slight increase in the degree of duct cellularity. Small cuboidal epithelial cells lined unaffected mammary ducts, while affected ducts were lined by increased numbers of larger cuboidal cells.

GENETIC TOXICOLOGY

4-Methylimidazole (10,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535, when tested with and without 10% or 30% hamster or rat liver S9 activation enzymes (Table E1). In addition, no increases in the frequencies of micronucleated erythrocytes were seen in bone marrow of male rats (Table E2) or male mice (Table E3) administered 4-methylimidazole by intraperitoneal injection three times at 24-hour intervals or in peripheral blood samples from male and female mice administered the compound in dosed feed for 14 weeks (Table E4). In the mouse bone marrow micronucleus test, two trials were conducted; a significant increase in micronucleated polychromatic erythrocytes (PCEs) was seen in the first trial, but the response was not replicated in a repeat trial (Trial 2), and the test was judged to be negative overall. No significant alterations in percent PCEs, a rough indicator of bone marrow toxicity, were seen in the mouse bone marrow or peripheral blood tests, but in bone marrow of male rats, percent PCEs declined with increasing dose of 4-methylimidazole and were significantly depressed at the highest dose.

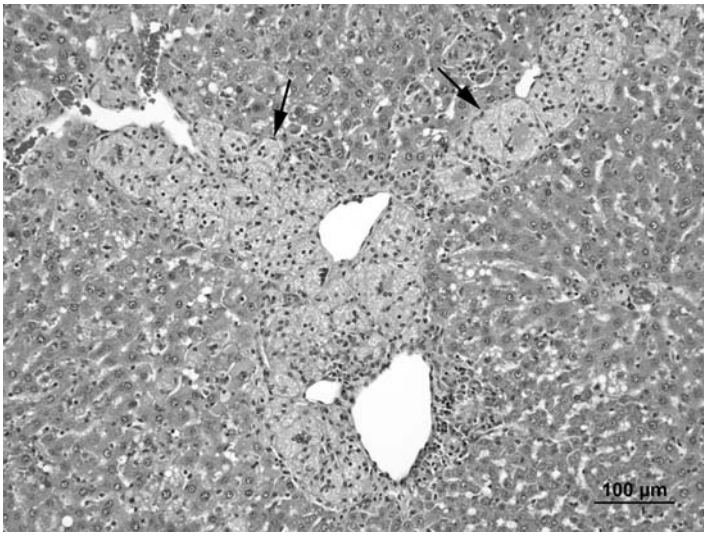


PLATE 1
Histiocytosis in the liver of a female F344/N rat exposed to 5,000 ppm 4-methylimidazole in feed for 2 years. Note the clusters of enlarged histiocytes with prominent foamy cytoplasm containing vacuoles and/or cleft-like spaces (arrows). H&E; 20×

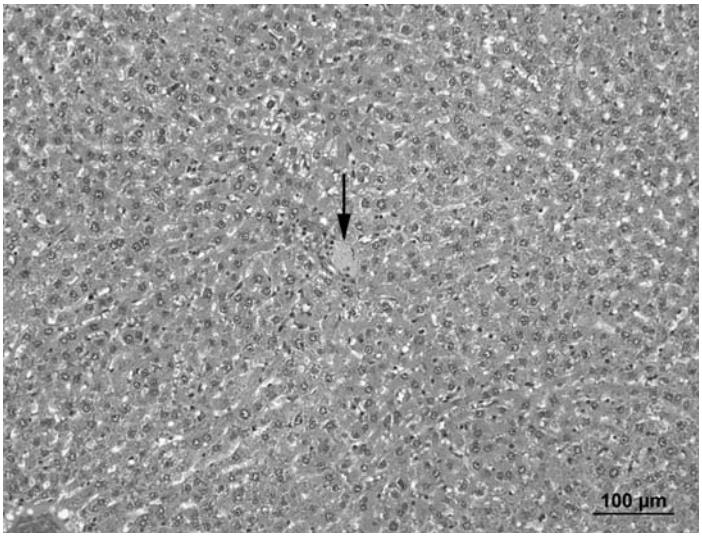


PLATE 2
Histiocytosis in the liver of a control female F344/N rat in the 2-year feed study of 4-methylimidazole. Note the individual histiocyte (arrow) containing foamy cytoplasm without vacuoles and/or cleft-like spaces. H&E; 20×

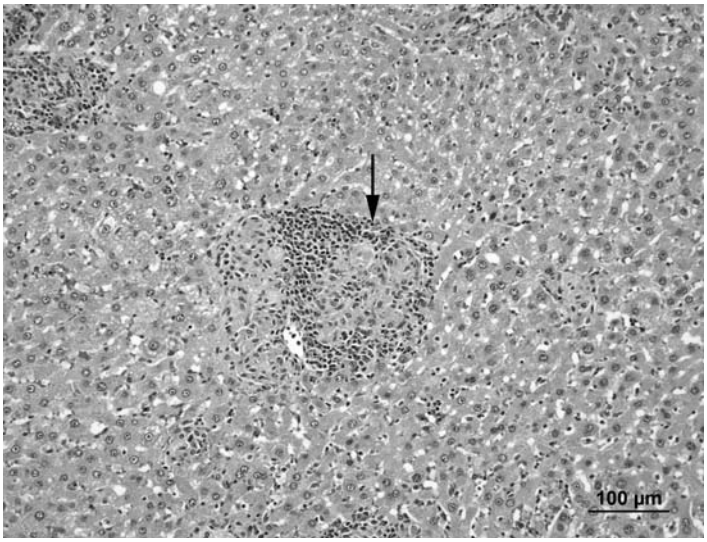


PLATE 3
Chronic inflammation in the liver of a female F344/N rat exposed to 5,000 ppm 4-methylimidazole in feed for 2 years. This focus is composed primarily of macrophages surrounded by a zone of lymphocytes (arrow). H&E; 20×

DISCUSSION AND CONCLUSIONS

4-Methylimidazole was nominated by the National Cancer Institute for study, together with 2-methylimidazole, because of its widespread use in industry, its presence in food products, and the lack of toxicity data. In carcinogenicity studies of 2-methylimidazole, the NTP reported that the chemical induced thyroid gland follicular cell adenoma and carcinoma in rats and mice (NTP, 2004b). The current report describes the toxicity, carcinogenicity, toxicokinetic, and genotoxicity studies of 4-methylimidazole. Short-term toxicity studies of 2- and 4-methylimidazole have also been reported by the NTP (2004a).

The highest exposure concentrations selected for the 2-year studies were 2,500 ppm for male rats, 5,000 ppm for female rats, and 1,250 ppm for male and female mice. These same exposure concentrations and species combinations in the 14-week toxicity studies had minimal effects on survival, hematology, clinical chemistry, organ weights, and histopathology; the final body weights of these animals relative to controls were: 95% (male rats); 94% (female rats); 93% (male mice); and 88% (female mice) (NTP, 2004a). However, these exposure concentrations markedly reduced body weight gains in rats and mice during the course of the 2-year studies; the body weight effects were seen as early as week 14 in the rat study. Feed consumption was lower only in 5,000 ppm female rats. The neurobehavioral effects observed in 5,000 ppm female rats probably influenced their feed intake. The body weight effects observed in rats and mice were likely attributable to the toxicity of 4-methylimidazole at the highest exposure concentrations, but the reduced body weight gain may be partly due to reduced water intake. MacKenzie *et al.* (1992) reported that male and female F344/N rats given caramel color IV (which contained 110 mg 4-methylimidazole per kilogram) in drinking water at 10 g/kg for 2 years had significantly lower body weights but no accompanying histopathology. Tierney (1979) reported that B6C3F_1 mice given caramel color IV at up to 63 g/kg body weight per day in drinking water for 4 weeks also had significantly reduced body weight gains. In these studies, the body weight effects were attributed to reduced fluid intake. The palatability of caramel water in the two studies was not known. Fluid intake was not measured in the current studies.

During the 2-year study period, female rats in the 2,500 and 5,000 ppm groups showed numerous clinical findings associated with 4-methylimidazole administration, whereas 2,500 ppm male rats did not. These treatment-related clinical findings included clonic seizures, unusual stance or gait, and excessive activity manifested as either hyperactivity or excitability. No microscopic lesions were observed in nervous tissues that correlated with the observed behavioral effects. The reason why female F344/N rats are more sensitive to the neurobehavioral effects of 4-methylimidazole than male F344/N rats is unclear. The neurobehavioral effects displayed by female rats are consistent with those observed in farm animals fed ammoniated hay (Wiggins, 1956; Nishie *et al.*, 1970; Morgan and Edwards, 1986; Weiss *et al.*, 1986; Perdok and Leng, 1987; Nielsen *et al.*, 1993).

In male albino mice, a single dose of 4-methylimidazole induced tremors, restlessness, running, sialorrhea, opisthotonos, Straub tail, and tonic extensor seizure (Nishie *et al.*, 1969). The estimated median convulsant dose (CD_{50}) of 4-methylimidazole for male albino mice was 155 mg/kg intraperitoneally and 360 mg/kg orally. In the current 2-year feed study, the 1,250 ppm groups of male and female B6C3F_1 mice received 4-methylimidazole equivalent to 170 mg/kg body weight per day and exhibited no convulsions. In the 14-week toxicity studies of 4-methylimidazole in feed (NTP, 2004a), the highest exposure concentration groups (10,000 ppm) of male and female mice received estimated doses of 1,840 and 3,180 mg/kg per day, respectively, and exhibited no convulsions. It is probable that the dosed feed route of administration delivered much less 4-methylimidazole at any time point compared to the bolus gavage effect shown in the study by Nishie *et al.* (1969).

Yuan and Burka (1995) showed that metabolism and renal clearance of 4-methylimidazole were saturated by a 50 mg/kg oral dose. The neurotoxic syndrome of

4-methylimidazole observed by Hidaka (1976c) occurred soon after a high gavage dose of 4-methylimidazole under conditions where both metabolism and renal clearance were saturated. Imidazoles, especially those substituted at the 4-position, have been recognized as inhibitors of cytochromes P450. Back and Tjia (1985) compared the *in vivo* competitive inhibition of tolbutamide metabolism by a series of imidazole-containing chemicals. 4-Methylimidazole was the most effective inhibitor of the three methylimidazoles used in the study. Hargreaves *et al.* (1994) reported that 4-methylimidazole was a strong inhibitor of *p*-nitrophenol hydrolase in rat liver. *p*-Nitrophenol is a cytochrome P450 2E1 substrate. 4-Methylimidazole forms complexes with heme-containing enzymes such as cytochrome P450 and results in inhibition of mixed function oxidase activity (Karangwa *et al.*, 1990). In a review of the metabolism of heterocyclic chemicals, Dalvie *et al.* (2002) outlined the mechanism of inhibition of heme-containing enzymes by imidazoles as involving coordination of the imidazole nitrogens, with the heme causing a change in the spin state of the iron atom. This change makes the heme-imidazole complex more difficult for the P450 oxidoreductase to reduce and results in inhibition. The authors also pointed out that imidazoles are metabolized primarily by cytochromes P450. 4-Methylimidazole could be characterized as a high affinity/low turnover substrate. Binding of 4-methylimidazole by heme may be partly responsible for its long half-life. The phenomenon is well illustrated by the present toxicokinetic study data in rats (Figures K7 to K12) and mice (Figures K13 to K18), which show that plasma concentrations of 4-methylimidazole increase as dose concentrations are increased. The slow clearance of 4-methylimidazole might have allowed the manifestation of 4-methylimidazole toxicity.

The incidence of mononuclear cell leukemia was significantly increased in 5,000 ppm female rats, and the effect was exposure concentration-related. Mononuclear cell leukemia in F344/N rats is a common background lesion, and the control rate in the current study was similar to those in historical controls. The increased incidence of mononuclear cell leukemia in 5,000 ppm female rats slightly exceeded the historical range in feed study controls, and therefore, it was uncertain if the effect was attributable to 4-methylimidazole exposure.

The liver was a target organ for 4-methylimidazole toxicity in rats. There were increases in the incidences and severities of several nonneoplastic hepatic lesions in both sexes; these included histiocytosis, chronic inflammation, hepatocytic focal fatty change, and eosinophilic and mixed cell foci of hepatocytes. Cytoplasmic vacuolization of hepatocytes was also observed in male and female rats in the 14-week toxicity study (NTP, 2004a). These lesions may be related to altered lipid metabolism and hepatic injury. The hepatic effects were consistent with increases in serum alanine aminotransferase, sorbitol dehydrogenase, and alkaline phosphatase activities, and with bile acid concentrations reported in the 14-week toxicity study (NTP, 2004a).

Incidences of thyroid gland follicle cyst in 2,500 ppm male rats and thyroid gland follicle mineralization in 5,000 ppm female rats were increased. In addition, the increased incidence of thyroid gland follicular cyst in 1,250 ppm female mice was statistically significant. These cysts are commonly found in aging mice, but the increased incidences may be related to 4-methylimidazole exposure. In the 14-week toxicity studies (NTP, 2004a), the 4-methylimidazole-treated male and female rats and mice exhibited no specific changes in serum triiodothyronine (T_3), total thyroxine (T_4), or thyroid stimulating hormone (TSH) levels that could be attributed to 4-methylimidazole exposure. There were also no changes in thyroid gland histopathology at terminal sacrifice in the 14-week toxicity studies compared with controls. In the 2-year feed studies of 2-methylimidazole in rats and mice, increased incidences of thyroid gland follicular cell neoplasms were observed (NTP, 2004b). The thyroid gland carcinogenesis induced by 2-methylimidazole was probably due to increased UDP-glucuronyl transferase metabolism of T_4, which in turn stimulated TSH synthesis and release, leading to neoplastic development in the thyroid gland. Sanders *et al.* (1998) reported that 2-methylimidazole enhanced, whereas 4-methylimidazole inhibited, hepatic UPD-glucuronyl transferase activity. The inhibitory effects of 4-methylimidazole on UPD-glucuronyl transferase probably did not affect serum T_4 and TSH levels and as a result, had no stimulatory effect on the thyroid gland.

The incidence of prostate gland inflammation in 2,500 ppm male rats was significantly increased. Increased incidences of chronic focal lung inflammation, heart cardiomyopathy, and focal pancreatic acinus atrophy were observed in all exposed groups of female rats. The cause of these increases was not clear.

Exposure concentration-related decreases in the incidences of benign, complex, or malignant adrenal

medulla pheochromocytoma (combined) in male rats, pituitary gland adenoma in the pars distalis in both male and female rats, and clitoral gland adenoma, mammary gland fibroadenoma, and uterine stromal polyp in female rats were probably related to exposure concentration-related body weight loss. However, using the equations of Haseman *et al.* (1997) to predict the number of neoplasms that can be explained by body weight, there appears to be a greater decrease in pituitary gland neoplasms in 5,000 ppm female rats than can be attributed to body weight differences alone (Table 14). Likewise, there was a greater decrease in mammary gland neoplasms in all exposed groups of female rats than can be attributed to body weight differences alone.

The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) were significantly increased in 1,250 ppm male and 625 and 1,250 ppm female mice; these increases were exposure concentration-related. The incidence of lung alveolar epithelium hyperplasia was significantly increased in 1,250 ppm female mice compared with controls. Hyperplasia of the alveolar epithelium is thought to be a precursor to neoplastic development. Interestingly, 4-methylimidazole had no effect on the respiratory epithelium in the 14-week toxicity study at concentrations as high as 10,000 ppm (NTP, 2004a). The mechanism of action of 4-methylimidazole in mouse lung tumorigenesis is not clear.

Clara cells in the terminal bronchiolar epithelium are a cell type from which alveolar/bronchiolar neoplasms are thought to arise. This cytochrome P450-containing cell type is capable of xenobiotic metabolism, whereas other cell types in the lung have little or no cytochrome P450. 4-Methylimidazole is structurally similar to 2- and 3-methylfuran. Both alkyl furans are metabolized in the Clara cell to reactive species, which may account for their pulmonary toxicity. Oxidative metabolism of heterocycles like 4-methylimidazole has been reviewed by Dalvie *et al.* (2002). They suggest that oxidative metabolism of imidazoles would lead to at least two reactive intermediates, an epoxide and dicarbonyl compound, and pyruvaldehyde, if their general scheme is adapted to 4-methylimidazole (Figure 6). Whether either of these intermediates is formed or is responsible for the development of alveolar/bronchiolar neoplasms is not known at this time. It is unlikely that an alkylating intermediate is involved in mouse lung carcinogenesis in view of the genetic toxicity study findings that 4-methylimidazole is not mutagenic in *Salmonella typhimurium* and does not induce micronuclei in mouse peripheral blood erythrocytes or rat or mouse bone marrow cells.

MacKenzie *et al.* (1992) administered caramel color 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 caramel color IV/kg for 2 years and reported no effects on histopathology. The exposure concentrations of 4-methylimidazole in the caramel color IV studies were lower than those in the current study.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of 4-methylimidazole in male F344/N rats exposed to 625, 1,250, or 2,500 ppm. There was *equivocal evidence of carcinogenic activity* of 4-methylimidazole in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was *clear evidence of carcinogenic activity* of 4-methylimidazole in male and female $B6C3F_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms.

Exposure to 4-methylimidazole resulted in nonneoplastic lesions in the liver of male and female rats and the lung of female mice and in clinical findings of neurotoxicity in female rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Subcommittee comments and the public discussion on this Technical Report appears on page 12.

TABLE 14
Estimated Effect of Body Weight on Incidences of Selected Neoplasms in Rats[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
Pituitary Gland				
Expected Neoplasms	14.7	13.9	11.9	10.7
Pars Distalis Adenomas Observed	16	13	10	7
Pars Distalis or Pars Intermedia Adenomas Observed	16	13	10	8
	0 ppm	**1,250 ppm**	**2,500 ppm**	**5,000 ppm**
Female				
Pituitary Gland				
Expected Neoplasms	24.1	23.8	22.4	19.9
Pars Distalis Adenomas Observed	29	19	20	9
Pars Distalis or Pars Intermedia Adenomas or Carcinomas Observed	29	20	20	9
Mammary Gland				
Expected Neoplasms	16.7	14.2	12.3	8.3
Fibroadenomas Observed	24	6	4	1
Fibroadenomas or Carcinomas Observed	26	7	6	1

[a] Number of animals with neoplasm per 50 animals

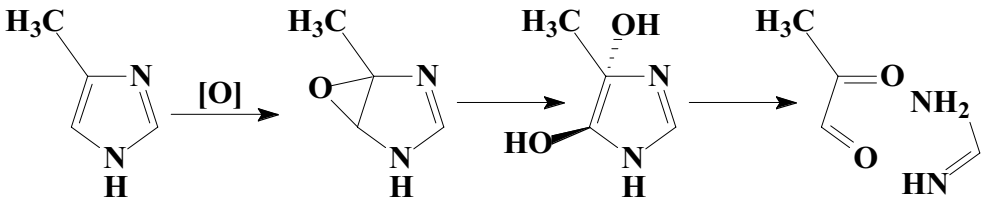

FIGURE 6
Potential Reactive Intermediates from Metabolism of 4-Methylimidazole (adapted from Dalvie *et al.*, 2002)

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF 4-METHYLIMIDAZOLE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	13	11	12	10
Natural deaths	6	5	5	8
Survivors				
Terminal sacrifice	31	34	33	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(48)	(49)	(49)	(46)
Intestine large, cecum	(46)	(46)	(46)	(46)
Intestine small, duodenum	(49)	(48)	(47)	(47)
Intestine small, jejunum	(47)	(45)	(45)	(43)
Intestine small, ileum	(46)	(45)	(45)	(44)
Liver	(50)	(50)	(50)	(50)
Basal cell carcinoma, metastatic, skin			1 (2%)	
Histiocytic sarcoma				1 (2%)
Ito cell tumor benign			1 (2%)	
Mesentery	(13)	(10)	(11)	(10)
Basal cell carcinoma, metastatic, skin			1 (9%)	
Histiocytic sarcoma				1 (10%)
Pancreas	(50)	(48)	(48)	(49)
Acinus, adenoma	1 (2%)			2 (4%)
Salivary glands	(50)	(50)	(50)	(50)
Fibrosarcoma			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(49)
Stomach, glandular	(50)	(50)	(49)	(49)
Tongue		(3)	(1)	(1)
Squamous cell carcinoma		1 (33%)		
Squamous cell papilloma		1 (33%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant		2 (4%)		
Pheochromocytoma complex	2 (4%)			
Pheochromocytoma benign	6 (12%)	3 (6%)	3 (6%)	3 (6%)
Bilateral, pheochromocytoma benign	2 (4%)	2 (4%)		
Islets, pancreatic	(50)	(49)	(49)	(49)
Adenoma	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Carcinoma			1 (2%)	
Parathyroid gland	(48)	(50)	(48)	(50)
Adenoma	1 (2%)			
Pituitary gland	(49)	(49)	(48)	(48)
Pars distalis, adenoma	16 (33%)	13 (27%)	10 (21%)	7 (15%)
Pars intermedia, adenoma				1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Endocrine System (continued)				
Thyroid gland	(47)	(46)	(48)	(44)
Chemodectoma benign				1 (2%)
Bilateral, C-cell, adenoma		1 (2%)		1 (2%)
C-cell, adenoma	11 (23%)	6 (13%)	3 (6%)	9 (20%)
C-cell, carcinoma			2 (4%)	
Follicular cell, adenoma		1 (2%)		
General Body System				
Peritoneum		(2)	(3)	(1)
Tissue NOS	(6)	(4)	(6)	(5)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(49)
Adenoma	2 (4%)	1 (2%)		
Carcinoma			1 (2%)	
Prostate	(50)	(50)	(50)	(49)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	40 (80%)	40 (80%)	34 (68%)	31 (62%)
Interstitial cell, adenoma	6 (12%)	4 (8%)	13 (26%)	14 (28%)
Hematopoietic System				
Bone marrow	(47)	(50)	(48)	(50)
Lymph node	(19)	(18)	(26)	(27)
Lymph node, mandibular		(1)	(1)	(2)
Lymph node, mesenteric	(50)	(48)	(48)	(49)
Spleen	(50)	(48)	(48)	(49)
Fibroma		1 (2%)		
Thymus	(48)	(49)	(46)	(45)
Integumentary System				
Mammary gland	(47)	(46)	(41)	(45)
Fibroadenoma	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)		1 (2%)	2 (4%)
Basal cell carcinoma			1 (2%)	
Keratoacanthoma	1 (2%)		3 (6%)	
Squamous cell carcinoma	1 (2%)			
Subcutaneous tissue, fibroma	7 (14%)	2 (4%)	4 (8%)	3 (6%)
Subcutaneous tissue, fibroma, multiple	1 (2%)			
Subcutaneous tissue, hemangioma			1 (2%)	
Subcutaneous tissue, histiocytic sarcoma			1 (2%)	
Subcutaneous tissue, lipoma				1 (2%)
Subcutaneous tissue, sarcoma			1 (2%)	
Musculoskeletal System				
Skeletal muscle		(2)	(2)	
Histiocytic sarcoma			1 (50%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant				1 (2%)
Glioma malignant			1 (2%)	1 (2%)
Pineal gland, neoplasm NOS		1 (2%)		
Spinal cord	(2)	(3)	(2)	(3)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)		1 (2%)
Alveolar/bronchiolar carcinoma			1 (2%)	
Basal cell carcinoma, metastatic, skin			1 (2%)	
Chordoma, metastatic, uncertain primary site				1 (2%)
Nose	(49)	(50)	(50)	(50)
Olfactory epithelium, carcinoma			1 (2%)	
Special Senses System				
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(3)	(1)	(2)	
Carcinoma	3 (100%)	1 (100%)	2 (100%)	
Urinary System				
Kidney	(48)	(49)	(48)	(50)
Histiocytic sarcoma				1 (2%)
Sarcoma	1 (2%)			
Urinary bladder	(49)	(50)	(50)	(50)
Transitional epithelium, papilloma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Leukemia mononuclear	15 (30%)	18 (36%)	22 (44%)	20 (40%)
Lymphoma malignant				1 (2%)
Mesothelioma malignant		2 (4%)	3 (6%)	1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	49	50	50
Total primary neoplasms	124	104	114	103
Total animals with benign neoplasms	50	49	48	47
Total benign neoplasms	102	79	76	78
Total animals with malignant neoplasms	22	21	30	23
Total malignant neoplasms	22	24	38	25
Total animals with metastatic neoplasms			2	1
Total metastatic neoplasms			19	1
Total animals with malignant neoplasms of uncertain primary site			1	1
Total animals with uncertain neoplasms benign or malignant		1		
Total uncertain neoplasms		1		

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	4 6 4	5 4 7	5 8 2	5 8 9	5 9 7	6 4 2	6 5 3	6 6 7	6 6 7	6 8 5	6 8 5	7 0 0	7 0 0	7 0 0	7 0 5	7 0 9	7 1 2	7 2 2	7 2 3	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 4 9	0 2 3	0 2 8	0 4 3	0 2 9	0 1 2	0 4 6	0 0 6	0 0 9	0 1 4	0 3 5	0 0 5	0 1 0	0 1 1	0 0 7	0 4 8	0 0 3	0 3 9	0 1 7	0 0 4	0 1 3	0 1 5	0 1 6	0 1 8	0 2 5
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery			+			+	+						+		+			+				+		
Oral mucosa	+																							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																								
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth		+																						
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma complex											X											X		
Pheochromocytoma benign																				X				
Bilateral, pheochromocytoma benign													X											
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X																	
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma	X																							
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X	X				X				X				X				X						
Thyroid gland	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
C-cell, adenoma									X										X			X		X
General Body System																								
Tissue NOS							+						+				+	+						

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 2 6	0 2 7	0 2 3 0	0 3 4 0	0 4 4 1	0 4 4 2	0 5 5 0	0 6 0 1	0 6 0 2	0 6 0 8	0 6 2 2	0 6 2 4	0 6 3 1	0 6 3 2	0 6 3 3	0 6 3 7	0 6 3 8	0 6 4 7	0 6 4 9	0 7 1 0	0 7 2 0	0 7 2 1	0 7 3 4	0 7 3 6	0 7 4 4	0 7 4 5	Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery							+	+			+				+		+					+					13
Oral mucosa																											1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma			X																								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																											1
Cardiovascular System																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma									X																		1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma complex																											2
Pheochromocytoma benign		X					X			X		X	X														6
Bilateral, pheochromocytoma benign																									X		2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X										X															3
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																											1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma		X	X	X			X		X	X					X	X	X					X					16
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
C-cell, adenoma	X											X				X		X	X			X		X			11
General Body System																											
Tissue NOS								+		+																	6

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	4 6 4	5 4 7	5 8 2	5 8 9	5 9 7	6 4 2	6 5 3	6 6 7	6 6 7	6 8 5	6 8 5	7 0 0	7 0 0	7 0 0	7 0 5	7 0 9	7 1 2	7 2 2	7 2 3	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 4 9	0 2 3	0 2 8	0 4 3	0 2 9	0 1 2	0 4 6	0 0 6	0 0 9	0 1 4	0 3 5	0 0 5	0 1 0	0 1 1	0 1 7	0 4 8	0 3 3	0 3 9	0 1 7	0 1 4	0 1 3	0 1 5	0 1 6	0 1 8	0 2 5
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X	X	X		X		X	X	X	X		X	X	X	X	X	X	X		X	X		X
Interstitial cell, adenoma								X					X											X	
Hematopoietic System																									
Bone marrow	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+	+		+	+	+	+		+	+	+	+	+	+		+	+	+						
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma																									
Squamous cell carcinoma																									
Subcutaneous tissue, fibroma				X				X			X													X	
Subcutaneous tissue, fibroma, multiple																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve					+					+															
Spinal cord					+					+															
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland		+							+	+															
Carcinoma		X							X	X															

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 2 6	0 2 7	0 3 0	0 4 0	0 4 1	0 4 2	0 5 0	0 1 0	0 0 2	0 0 8	0 2 2	0 2 4	0 3 1	0 3 2	0 3 3	0 3 7	0 3 8	0 4 7	0 1 9	0 2 0	0 2 1	0 3 4	0 3 6	0 4 4	0 4 5	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X								X										2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X		40
Interstitial cell, adenoma				X															X						X	6
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node						+									+		+						+			19
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibroadenoma													X				X								X	3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma		X																								1
Keratoacanthoma				X																						1
Squamous cell carcinoma																						X				1
Subcutaneous tissue, fibroma				X								X	X													7
Subcutaneous tissue, fibroma, multiple																							X			1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										2
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Zymbal's gland																										3
Carcinoma																										3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	4 6 4	5 4 7	5 8 2	5 8 9	5 9 7	6 4 2	6 5 3	6 6 7	6 6 7	6 8 5	6 8 5	7 0 0	7 0 0	7 0 0	7 0 5	7 0 9	7 1 2	7 2 2	7 2 3	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 4 9	0 2 3	0 2 8	0 4 3	0 2 9	0 1 2	0 4 6	0 0 6	0 0 9	0 1 4	0 3 5	0 1 5	0 1 0	0 1 1	0 1 7	0 4 8	0 3 3	0 3 9	0 1 7	0 1 4	0 1 3	0 1 5	0 1 6	0 1 8	0 2 5
Urinary System																									
Kidney	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																X									
Urinary bladder	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X		X		X					X	X	X	X		X	X	X			X			X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	730	730	730	730	730	730	730	734	734	734	734	734	734	734	734	734	734	734	735	735	735	735	735	735	735	
Carcass ID Number	02626	02727	03000	03400	04401	04712	05020	00101	00002	00008	02202	02404	03101	03202	03303	03707	03808	04707	04919	05020	05201	05314	05336	05444	05545	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear								X									X	X								15

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	2 7 5	2 7 5	4 7 7	5 1 6	5 4 7	5 6 7	5 7 7	5 9 3	6 2 1	6 3 7	6 6 5	6 9 4	6 9 5	7 0 0	7 0 0	7 1 2	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 7 1	0 9 0	0 9 7	0 7 0	0 7 3	0 5 8	0 8 4	0 5 2	0 6 7	0 7 5	0 9 3	0 6 3	0 8 0	0 7 9	0 9 9	0 8 5	0 5 4	0 5 5	0 5 6	0 5 7	0 6 9	0 6 0	0 6 1	0 6 2	0 7 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	A	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	+	+	+	A	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery			+			+		+					+			+									
Oral mucosa	+																								
Pancreas	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue					+																				
Squamous cell carcinoma					X																				
Squamous cell papilloma																									
Tooth																									
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant															X				X						
Pheochromocytoma benign																X									
Bilateral, pheochromocytoma benign														X	X										
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X					
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X				X				X									X		X		X	
Thyroid gland	+	A	+	+	+	+	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma																	X							X	
Follicular cell, adenoma																									
General Body System																									
Peritoneum						+																			
Tissue NOS								+					+		+										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 0 7 4	0 0 7 6	0 0 7 7	0 0 7 8	0 0 8 1	0 0 8 2	0 0 8 3	0 0 8 6	0 0 9 6	0 0 9 8	0 0 5 1	0 0 5 3	0 0 6 4	0 0 6 5	0 0 6 6	0 0 6 8	0 0 6 9	0 0 8 7	0 0 8 8	0 0 8 9	0 0 9 1	0 0 9 2	0 0 9 4	0 0 9 5	1 0 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery		+									+										+	+			+	10
Oral mucosa																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue				+																					+	3
Squamous cell carcinoma																										1
Squamous cell papilloma				X																						1
Tooth			+																							1
Cardiovascular System																										
Blood vessel																				+						1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										2
Pheochromocytoma benign						X								X												3
Bilateral, pheochromocytoma benign																										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																					X					2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	49
Pars distalis, adenoma		X	X					X				X			X				X						X	13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Bilateral, C-cell, adenoma																X										1
C-cell, adenoma			X	X									X						X							6
Follicular cell, adenoma													X													1
General Body System																										
Peritoneum																						+				2
Tissue NOS																									+	4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	2 7 5	2 7 5	4 7 7	5 1 6	5 4 7	5 6 7	5 7 7	5 9 3	6 2 1	6 3 7	6 6 5	6 9 4	6 9 5	7 0 0	7 0 0	7 1 2	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 7 1	0 9 0	0 9 7	0 7 0	0 7 3	0 5 8	0 8 4	0 5 2	0 6 7	0 7 5	0 9 3	0 6 3	0 8 0	0 7 9	0 9 9	0 8 5	0 5 4	0 5 5	0 5 6	0 5 7	0 5 9	0 6 0	0 6 1	0 6 2	0 7 2
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma														X											
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma				X	X	X		X	X		X	X	X	X	X	X	X	X	X		X		X	X	X
Interstitial cell, adenoma										X										X					
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+				+	+		+	A	A	+	+	+	+	+	+		+	+			+			
Lymph node, mandibular	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma															X										
Thymus	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																				X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma		X																X							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle						+																			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pineal gland, neoplasm NOS				X																					
Peripheral nerve				+		+	+																		
Spinal cord				+		+	+																		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear	+																								
Eye	+	A	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland																									
Zymbal's gland																									
Carcinoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	00774	00776	00777	00778	00881	00882	00883	00886	00896	00898	00951	00953	00954	00964	00965	00966	00968	00969	00987	00988	00989	00991	00992	00994	00995	01010	Total Tissues/ Tumors
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																											1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X		X	X	X	X	X		40
Interstitial cell, adenoma		X																		X							4
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node			+	+					+			+														+	18
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibroma																											1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+		46
Fibroadenoma																											1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																											2
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										+	2
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pineal gland, neoplasm NOS																											1
Peripheral nerve																											3
Spinal cord																											3
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X																						1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																											
Ear																											1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lacrimal gland													+														1
Zymbal's gland			+																								1
Carcinoma			X																								1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	2	2	4	5	5	5	5	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7
	7	7	7	1	4	6	7	9	2	3	6	9	9	0	0	1	3	3	3	3	3	3	3	3	3
	5	5	7	6	7	7	7	3	1	7	5	4	5	0	0	2	0	0	0	0	0	0	0	0	0
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	7	9	9	7	7	5	8	5	6	7	9	6	8	7	9	8	5	5	5	5	5	6	6	6	7
	1	0	7	0	3	8	4	2	7	5	3	3	0	9	9	5	4	5	6	7	9	0	1	2	2
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X		X	X	X	X	X	X		X	X		X	X	X			X		X
Mesothelioma malignant						X																			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 7 4	0 7 6	0 7 7	0 7 8	0 8 1	0 8 2	0 8 3	0 8 6	0 9 6	0 9 8	0 9 1	0 5 1	0 5 3	0 6 4	0 6 5	0 6 6	0 6 8	0 6 9	0 8 7	0 8 8	0 8 9	0 9 1	0 9 2	0 9 4	0 9 5	1 0 0	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X			X																				X		X	18
Mesothelioma malignant																							X				2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	4 9 3	4 9 8	5 3 1	5 3 8	5 9 4	6 0 5	6 3 5	6 4 2	6 4 2	6 5 0	6 5 3	6 5 4	6 9 0	7 0 0	7 0 0	7 0 6	7 1 2	7 1 7	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	1 1 3	1 4 5	1 5 0	1 1 0	1 0 9	1 1 4	1 3 3	1 0 5	1 1 5	1 2 6	1 2 2	1 2 3	1 0 2	1 2 7	1 4 6	1 1 6	1 2 4	1 3 4	1 0 1	1 0 2	1 1 7	1 1 8	1 1 9	1 2 0	1 2 1	1 2 8
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																					
Intestine large, rectum	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	A	A	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																					
Intestine small, jejunum	+	+	+	+	+	A	A	+	+	+	+	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	A	A	+	+	+	+	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma, metastatic, skin																		X								
Ito cell tumor benign																										
Mesothelioma malignant, metastatic, mesentery					X																					
Mesentery		+			+			+			+				+	+	+	+	+				+			
Basal cell carcinoma, metastatic, skin																		X								
Oral mucosa																										
Pancreas	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma													X													
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																					
Stomach, glandular	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																					
Tongue																										
Cardiovascular System																										
Blood vessel				+																+						
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	730	730	730	730	730	730	733	734	734	734	734	734	734	734	734	734	734	735	735	735	735	735	735	735	735	
Carcass ID Number	12029	13030	14043	14447	14478	14488	10044	10066	10067	10068	12235	12251	13311	13422	14040	14411	14422	11111	11223	13356	13367	13378	13389	14499		Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Mesothelioma malignant, metastatic, mesentery																										1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Mesothelioma malignant, metastatic, mesentery																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma, metastatic, skin																										1
Ito cell tumor benign																		X								1
Mesothelioma malignant, metastatic, mesentery																										1
Mesentery										+																11
Basal cell carcinoma, metastatic, skin																										1
Oral mucosa																+										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mesothelioma malignant, metastatic, mesentery																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, mesentery																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Mesothelioma malignant, metastatic, mesentery																										1
Tongue						+																				1
Cardiovascular System																										
Blood vessel																										2
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	4 9 3	4 9 8	5 3 1	5 3 8	5 9 4	6 0 5	6 3 5	6 4 2	6 4 2	6 5 0	6 5 3	6 9 4	7 0 0	7 0 0	7 0 6	7 1 2	7 1 7	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	1 1 3	1 4 5	1 5 0	1 1 0	1 0 9	1 1 4	1 3 3	1 0 5	1 1 5	1 2 6	1 2 2	1 0 3	1 2 7	1 4 6	1 1 6	1 2 4	1 3 4	1 3 1	1 0 2	1 1 7	1 1 8	1 1 9	1 2 0	1 2 1	1 2 8
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																				
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																				
Pheochromocytoma benign																		X							
Islets, pancreatic	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																						X	X		X
Thyroid gland	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma															X										
C-cell, carcinoma																									
General Body System																									
Peritoneum					+															+					
Tissue NOS		+						+						+											
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma				X																					
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																				
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																				
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma				X		X				X	X	X	X	X	X		X	X	X	X	X			X	X
Interstitial cell, adenoma		X	X		X		X	X	X													X	X		
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+		+	+	+	+			+	+	+	+	+					+		
Mediastinal, mesothelioma malignant, metastatic, uncertain primary site					X																				
Pancreatic, mesothelioma malignant, metastatic, mesentery					X																				
Lymph node, mandibular	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																				
Spleen	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery					X																				
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	1 2 9	1 3 0	1 4 3	1 4 4	1 4 7	1 4 8	1 0 4	1 0 6	1 0 7	1 0 8	1 2 3	1 2 5	1 3 1	1 3 2	1 4 0	1 4 1	1 4 2	1 1 1	1 1 2	1 3 5	1 3 6	1 3 7	1 3 8	1 3 9	1 4 9	Total Tissues/ Tumors
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, mesentery																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, mesentery																										1
Pheochromocytoma benign														X					X							3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma															X											1
Carcinoma										X																1
Parathyroid gland	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma	X				X		X		X	X					X		X									10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma				X											X											3
C-cell, carcinoma		X												X												2
General Body System																										
Peritoneum																	+									3
Tissue NOS			+															+		+						6
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, mesentery																										1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, mesentery																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma		X	X	X	X	X	X	X	X				X	X	X		X	X	X		X	X	X	X	X	34
Interstitial cell, adenoma										X	X	X				X				X						13
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	+	+	+				+			+				+		+		+	+			+				26
Mediastinal, mesothelioma malignant, metastatic, uncertain primary site																										1
Pancreatic, mesothelioma malignant, metastatic, mesentery																										1
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mesothelioma malignant, metastatic, mesentery																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mesothelioma malignant, metastatic, mesentery																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	I	+	+	+	+	+	46

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	4 9 3	4 9 8	5 3 1	5 3 8	5 9 4	6 0 5	6 3 5	6 4 2	6 4 2	6 5 0	6 5 3	6 9 4	7 0 0	7 0 0	7 0 6	7 1 2	7 1 7	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	1 1 3	1 4 5	1 5 0	1 1 0	1 0 9	1 1 4	1 3 3	1 0 5	1 1 5	1 2 6	1 2 2	1 0 3	1 2 7	1 4 6	1 1 6	1 2 4	1 3 4	1 3 0	1 0 2	1 1 7	1 1 8	1 1 9	1 2 0	1 2 1	1 2 8
Integumentary System																									
Mammary gland	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M	M	+	+	+
Fibroadenoma				X																					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Basal cell carcinoma																	X								
Keratoacanthoma																			X			X	X		
Subcutaneous tissue, fibroma							X						X				X								
Subcutaneous tissue, hemangioma																									
Subcutaneous tissue, histiocytic sarcoma																X									
Subcutaneous tissue, sarcoma												X													
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle					+											+									
Histiocytic sarcoma																X									
Mesothelioma malignant, metastatic, mesentery					X																				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant						X																			
Peripheral nerve																			+						
Spinal cord										+									+						
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									
Basal cell carcinoma, metastatic, skin																	X								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Olfactory epithelium, carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland			+																						
Carcinoma			X																						
Urinary System																									
Kidney	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Capsule, mesothelioma malignant, metastatic, mesentery					X																				
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma															X										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	730	730	730	730	730	730	734	734	734	734	734	734	734	734	734	734	734	735	735	735	735	735	735	735	735	
Carcass ID Number	12 9	13 0	14 3	14 4	14 7	14 8	10 4	10 6	10 7	10 8	12 3	12 5	13 1	13 2	14 0	14 1	14 2	11 1	11 2	13 5	13 6	13 7	13 8	13 9	14 9	Total Tissues/ Tumors
Integumentary System																										
Mammary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	41
Fibroadenoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																									X	1
Basal cell carcinoma																										1
Keratoacanthoma																										3
Subcutaneous tissue, fibroma		X																								4
Subcutaneous tissue, hemangioma										X																1
Subcutaneous tissue, histiocytic sarcoma																										1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Histiocytic sarcoma																										1
Mesothelioma malignant, metastatic, mesentery																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma malignant																										1
Peripheral nerve																										1
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma		X																								1
Basal cell carcinoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Olfactory epithelium, carcinoma		X																								1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Zymbal's gland		+																								2
Carcinoma		X																								2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Capsule, mesothelioma malignant, metastatic, mesentery																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	4 9 3	4 9 8	5 3 1	5 3 8	5 9 4	6 0 5	6 3 5	6 4 2	6 4 2	6 5 0	6 5 3	6 5 4	6 9 0	7 0 0	7 0 0	7 0 6	7 1 2	7 1 7	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	1 1 3	1 4 5	1 5 0	1 1 0	1 0 9	1 1 4	1 3 3	1 0 5	1 1 5	1 2 6	1 2 2	1 2 3	1 0 7	1 2 7	1 4 6	1 1 6	1 2 4	1 3 4	1 0 1	1 0 2	1 1 7	1 1 8	1 1 9	1 2 0	1 2 1	1 2 8
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																	X									
Leukemia mononuclear	X	X					X	X	X	X	X				X	X		X								X
Mesothelioma malignant					X																					

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	730	730	730	730	730	730	734	734	734	734	734	734	734	734	734	734	734	735	735	735	735	735	735	735	735	
Carcass ID Number	12 9	13 0	14 3	14 4	14 7	14 8	10 4	10 6	10 7	10 8	12 3	12 5	13 1	13 2	14 0	14 1	14 2	11 1	11 2	13 5	13 6	13 7	13 8	13 9	14 9	Total Tissues/ Tumors
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear	X	X	X							X						X		X	X	X		X	X		X	22
Mesothelioma malignant	X																X									3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	3 6 9	3 8 4	5 8 4	6 0 7	6 2 2	6 2 2	6 2 8	6 3 2	6 5 0	6 5 0	6 5 1	6 5 3	6 6 3	6 6 6	6 7 0	6 8 9	6 9 3	7 0 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0
Carcass ID Number	1 6 7	1 5 6	1 7 5	1 6 8	1 5 1	1 8 9	1 8 7	1 6 5	1 5 4	1 8 0	1 8 1	1 8 2	1 9 4	1 9 8	1 7 1	1 5 2	1 9 9	1 6 6	1 8 4	1 8 5	1 8 6	1 9 0	1 9 1	1 9 2	1 5 7
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	A	+	+	+	A	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	A	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	A	+	+	+	A	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	A	+	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	M	+	+	+	A	+	+	A	+	+	+	A	M	A	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	A	+	+	+	A	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																		
Mesentery					+	+				+			+		+						+			
Histiocytic sarcoma						X																		
Pancreas	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																							X	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																								
Cardiovascular System																								
Blood vessel																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign				X																				X
Islets, pancreatic	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+
Pars distalis, adenoma		X																					X	
Pars intermedia, adenoma																								
Thyroid gland	+	+	A	A	+	+	+	A	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+
Chemodectoma benign																								
Bilateral, C-cell, adenoma																								
C-cell, adenoma																						X	X	
General Body System																								
Peritoneum										+														
Tissue NOS				+				+					+				+							

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	
Carcass ID Number	1 5 5 8	1 5 5 9	1 8 3 3	1 9 3 3	1 9 5 5	2 0 0 0	1 5 3 3	1 5 5 5	1 6 0 0	1 6 6 1	1 6 6 2	1 6 6 3	1 6 6 4	1 6 6 9	1 7 7 0	1 7 7 2	1 7 7 3	1 7 7 4	1 7 7 8	1 7 7 9	1 9 9 6	1 9 9 7	1 7 7 6	1 7 7 7	1 7 7 8	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Mesentery							+					+	+		+											10
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinus, adenoma																						X				2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue		+																								1
Cardiovascular System																										
Blood vessel		+																								1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign					X																					3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma															X											1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma					X						X									X	X			X		7
Pars intermedia, adenoma		X																								1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Chemodectoma benign								X																		1
Bilateral, C-cell, adenoma													X													1
C-cell, adenoma	X			X								X					X					X	X	X		9
General Body System																										
Peritoneum																										1
Tissue NOS				+																						5

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	3 6 9	3 8 4	5 8 4	6 0 7	6 2 2	6 2 2	6 2 8	6 3 2	6 5 0	6 5 0	6 5 1	6 5 3	6 6 3	6 6 6	6 7 0	6 8 9	6 9 3	7 0 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0
Carcass ID Number	1 6 7	1 5 6	1 7 5	1 6 8	1 5 1	1 8 9	1 8 7	1 6 5	1 5 4	1 8 0	1 8 1	1 8 2	1 9 4	1 9 8	1 7 1	1 5 2	1 9 9	1 6 6	1 8 4	1 8 5	1 8 6	1 9 0	1 9 1	1 9 2	1 5 7
Genital System																									
Coagulating gland											+														
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma							X	X					X		X	X	X		X	X	X	X	X	X	X
Interstitial cell, adenoma				X	X				X	X	X	X		X				X							
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+	+	+	+		+	+	+	+	+	+	+	+	+	+		+			+			+
Lymph node, mandibular	M	+	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	M	+	+	M	M	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Subcutaneous tissue, fibroma							X					X							X						
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant		X																							
Glioma malignant	X																								
Peripheral nerve		+								+						+									
Spinal cord		+								+						+									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Chordoma, metastatic, uncertain primary site																								X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	A	+	+	+	A	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	
Carcass ID Number	1 5 5 8	1 5 5 9	1 8 3	1 9 3	1 9 5	2 0 0	1 5 3	1 5 5	1 6 0	1 6 1	1 6 2	1 6 3	1 6 4	1 6 9	1 7 0	1 7 2	1 7 3	1 7 4	1 7 8	1 7 9	1 9 6	1 9 7	1 7 6	1 7 7	1 8	Total Tissues/ Tumors
Genital System																											
Coagulating gland																											1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Bilateral, interstitial cell, adenoma	X	X	X	X		X	X			X		X	X	X	X	X	X	X			X	X	X	X			31
Interstitial cell, adenoma					X				X		X								X	X					X		14
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Lymph node				+	+	+		+		+		+	+			+				+				+			27
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M		2
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		45
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+		45
Fibroadenoma									X																		1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Basal cell adenoma														X										X			2
Subcutaneous tissue, fibroma																											3
Subcutaneous tissue, lipoma																			X								1
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Astrocytoma malignant																											1
Glioma malignant																											1
Peripheral nerve																											3
Spinal cord																											3
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar adenoma		X																									1
Chordoma, metastatic, uncertain primary site																											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Special Senses System																											
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		45
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	3 6 9	3 8 4	5 8 4	6 0 7	6 2 2	6 2 2	6 2 8	6 3 2	6 5 0	6 5 0	6 5 1	6 5 3	6 6 3	6 6 6	6 7 0	6 8 9	6 9 3	7 0 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0
Carcass ID Number	1 1 6 7	1 5 6	1 7 5	1 6 8	1 5 1	1 8 9	1 8 7	1 6 5	1 5 4	1 8 0	1 8 1	1 8 2	1 9 4	1 9 8	1 7 1	1 5 2	1 9 9	1 6 6	1 8 4	1 8 5	1 8 6	1 9 0	1 9 1	1 9 2	1 5 7
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Urethra											+														
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Leukemia mononuclear			X	X	X	X		X	X	X		X		X	X	X	X	X	X						
Lymphoma malignant													X												
Mesothelioma malignant										X															

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	
Carcass ID Number	1 5 8	1 5 9	1 8 3	1 9 3	1 9 5	2 0 0	1 5 3	1 5 5	1 6 0	1 6 1	1 6 2	1 6 3	1 6 4	1 6 9	1 7 0	1 7 2	1 7 3	1 7 4	1 7 8	1 7 9	1 9 6	1 9 7	1 7 6	1 7 7	1 8 8	Total Tissues/ Tumors
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																											1
Urethra																											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																											1
Leukemia mononuclear	X			X						X		X					X			X						20
Lymphoma malignant																											1
Mesothelioma malignant																											1

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	8/50 (16%)	5/50 (10%)	3/50 (6%)	3/50 (6%)
Adjusted rate[b]	17.7%	11.5%	6.8%	6.8%
Terminal rate[c]	7/31 (23%)	2/34 (6%)	3/33 (9%)	2/32 (6%)
First incidence (days)	700	700	729 (T)	607
Poly-3 test[d]	P=0.066N	P=0.303N	P=0.104N	P=0.106N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	10/50 (20%)	6/50 (12%)	3/50 (6%)	3/50 (6%)
Adjusted rate	22.0%	13.8%	6.8%	6.8%
Terminal rate	8/31 (26%)	3/34 (9%)	3/33 (9%)	2/32 (6%)
First incidence (days)	685	700	729 (T)	607
Poly-3 test	P=0.019N	P=0.234N	P=0.037N	P=0.039N
Mammary Gland: Fibroadenoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.6%	2.3%	2.2%	2.3%
Terminal rate	3/31 (10%)	1/34 (3%)	0/33 (0%)	1/32 (3%)
First incidence (days)	729 (T)	729 (T)	538	729 (T)
Poly-3 test	P=0.242N	P=0.322N	P=0.307N	P=0.318N
Pancreatic Islets: Adenoma				
Overall rate	3/50 (6%)	2/49 (4%)	1/49 (2%)	1/49 (2%)
Adjusted rate	6.6%	4.7%	2.3%	2.3%
Terminal rate	2/31 (7%)	2/34 (6%)	1/33 (3%)	1/32 (3%)
First incidence (days)	653	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.207N	P=0.533N	P=0.319N	P=0.325N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/49 (4%)	2/49 (4%)	1/49 (2%)
Adjusted rate	6.6%	4.7%	4.6%	2.3%
Terminal rate	2/31 (7%)	2/34 (6%)	2/33 (6%)	1/32 (3%)
First incidence (days)	653	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.243N	P=0.533N	P=0.518N	P=0.325N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	16/49 (33%)	13/49 (27%)	10/48 (21%)	7/48 (15%)
Adjusted rate	34.5%	29.8%	23.3%	16.1%
Terminal rate	10/31 (32%)	10/33 (30%)	10/32 (31%)	6/32 (19%)
First incidence (days)	464	477	729 (T)	384
Poly-3 test	P=0.022N	P=0.402N	P=0.176N	P=0.037N
Skin: Keratoacanthoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.2%	0.0%	6.8%	0.0%
Terminal rate	1/31 (3%)	0/34 (0%)	3/33 (9%)	0/32 (0%)
First incidence (days)	729 (T)	—[e]	729 (T)	—
Poly-3 test	P=0.507N	P=0.509N	P=0.299	P=0.507N
Skin: Keratoacanthoma or Squamous Cell Papilloma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.4%	0.0%	6.8%	0.0%
Terminal rate	2/31 (7%)	0/34 (0%)	3/33 (9%)	0/32 (0%)
First incidence (days)	729 (T)	—	729 (T)	—
Poly-3 test	P=0.298N	P=0.248N	P=0.492	P=0.245N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Skin: Keratoacanthoma, Basal Cell Adenoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	5/50 (10%)	2/50 (4%)
Adjusted rate	6.6%	0.0%	11.3%	4.6%
Terminal rate	3/31 (10%)	0/34 (0%)	4/33 (12%)	2/32 (6%)
First incidence (days)	729 (T)	—	717	729 (T)
Poly-3 test	P=0.536	P=0.127N	P=0.347	P=0.516N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	8/50 (16%)	2/50 (4%)	4/50 (8%)	3/50 (6%)
Adjusted rate	17.4%	4.5%	8.9%	6.8%
Terminal rate	5/31 (16%)	1/34 (3%)	1/33 (3%)	1/32 (3%)
First incidence (days)	589	275	635	628
Poly-3 test	P=0.124N	P=0.052N	P=0.189N	P=0.111N
Skin (Subcutaneous Tissue): Fibroma or Sarcoma				
Overall rate	8/50 (16%)	2/50 (4%)	5/50 (10%)	3/50 (6%)
Adjusted rate	17.4%	4.5%	11.1%	6.8%
Terminal rate	5/31 (16%)	1/34 (3%)	1/33 (3%)	1/32 (3%)
First incidence (days)	589	275	635	628
Poly-3 test	P=0.141N	P=0.052N	P=0.290N	P=0.111N
Testes: Adenoma				
Overall rate	46/50 (92%)	44/50 (88%)	47/50 (94%)	45/50 (90%)
Adjusted rate	95.1%	93.9%	95.5%	94.9%
Terminal rate	30/31 (97%)	32/34 (94%)	32/33 (97%)	31/32 (97%)
First incidence (days)	582	516	498	607
Poly-3 test	P=0.571	P=0.579N	P=0.670	P=0.689N
Thyroid Gland (C-cell): Adenoma				
Overall rate	11/47 (23%)	7/46 (15%)	3/48 (6%)	10/44 (23%)
Adjusted rate	25.1%	17.1%	6.9%	25.3%
Terminal rate	9/31 (29%)	7/34 (21%)	2/33 (6%)	10/32 (31%)
First incidence (days)	667	729 (T)	706	729 (T)
Poly-3 test	P=0.552N	P=0.262N	P=0.019N	P=0.593
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	11/47 (23%)	7/46 (15%)	5/48 (10%)	10/44 (23%)
Adjusted rate	25.1%	17.1%	11.6%	25.3%
Terminal rate	9/31 (29%)	7/34 (21%)	4/33 (12%)	10/32 (31%)
First incidence (days)	667	729 (T)	706	729 (T)
Poly-3 test	P=0.523	P=0.262N	P=0.085N	P=0.593
Zymbal's Gland: Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate	6.5%	2.3%	4.4%	0.0%
Terminal rate	0/31 (0%)	1/34 (3%)	1/33 (3%)	0/32 (0%)
First incidence (days)	547	729 (T)	531	—
Poly-3 test	P=0.107N	P=0.330N	P=0.512N	P=0.129N
All Organs: Mononuclear Cell Leukemia				
Overall rate	15/50 (30%)	18/50 (36%)	22/50 (44%)	20/50 (40%)
Adjusted rate	31.9%	39.5%	46.2%	42.2%
Terminal rate	5/31 (16%)	9/34 (27%)	12/33 (36%)	7/32 (22%)
First incidence (days)	582	567	493	584
Poly-3 test	P=0.184	P=0.292	P=0.110	P=0.205

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
All Organs: Malignant Mesothelioma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	4.6%	6.7%	2.3%
Terminal rate	0/31 (0%)	1/34 (3%)	2/33 (6%)	0/32 (0%)
First incidence (days)	—	567	594	650
Poly-3 test	P=0.428	P=0.229	P=0.117	P=0.494
All Organs: Benign Neoplasms				
Overall rate	50/50 (100%)	49/50 (98%)	48/50 (96%)	47/50 (94%)
Adjusted rate	100.0%	99.9%	97.5%	97.4%
Terminal rate	31/31 (100%)	34/34 (100%)	33/33 (100%)	32/32 (100%)
First incidence (days)	464	275	498	384
Poly-3 test	P=0.123N	P=1.000N	P=0.399N	P=0.362N
All Organs: Malignant Neoplasms				
Overall rate	22/50 (44%)	21/50 (42%)	30/50 (60%)	24/50 (48%)
Adjusted rate	45.6%	45.5%	60.1%	48.6%
Terminal rate	7/31 (23%)	11/34 (32%)	14/33 (42%)	8/32 (25%)
First incidence (days)	547	547	493	369
Poly-3 test	P=0.354	P=0.579N	P=0.106	P=0.462
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	49/50 (98%)	50/50 (100%)	50/50 (100%)
Adjusted rate	100.0%	99.9%	100.0%	100.0%
Terminal rate	31/31 (100%)	34/34 (100%)	33/33 (100%)	32/32 (100%)
First incidence (days)	464	275	493	369
Poly-3 test	P=1.000	P=1.000N	—[f]	—

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal medulla, pancreatic islets, pituitary gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 4-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	13	11	12	10
Natural deaths	6	5	5	8
Survivors				
Terminal sacrifice	31	34	33	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(46)	(46)	(46)	(46)
Congestion		1 (2%)		
Edema				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis, focal		1 (2%)		
Atrophy, focal			1 (2%)	
Basophilic focus	31 (62%)	26 (52%)	30 (60%)	36 (72%)
Clear cell focus	31 (62%)	28 (56%)	29 (58%)	31 (62%)
Congestion	2 (4%)			
Degeneration, cystic, focal	4 (8%)	3 (6%)	1 (2%)	7 (14%)
Eosinophilic focus	4 (8%)	3 (6%)	7 (14%)	12 (24%)
Fibrosis	1 (2%)			
Hepatodiaphragmatic nodule	5 (10%)	2 (4%)	5 (10%)	4 (8%)
Histiocytosis	38 (76%)	45 (90%)	50 (100%)	50 (100%)
Hyperplasia, focal, histiocytic	1 (2%)		1 (2%)	3 (6%)
Infiltration cellular, mixed cell	15 (30%)	5 (10%)	5 (10%)	17 (34%)
Inflammation, chronic	18 (36%)	32 (64%)	31 (62%)	36 (72%)
Inflammation, granulomatous	1 (2%)			
Mixed cell focus	5 (10%)	7 (14%)	11 (22%)	27 (54%)
Thrombosis			1 (2%)	
Bile duct, dilatation, focal	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Bile duct, hyperplasia	48 (96%)	48 (96%)	49 (98%)	42 (84%)
Hepatocyte, fatty change, focal	21 (42%)	24 (48%)	37 (74%)	33 (66%)
Hepatocyte, necrosis, focal		2 (4%)	1 (2%)	
Hepatocyte, vacuolization cytoplasmic	11 (22%)	14 (28%)	12 (24%)	18 (36%)
Serosa, inflammation, chronic, focal	1 (2%)			
Mesentery	(13)	(10)	(11)	(10)
Inflammation, chronic			1 (9%)	1 (10%)
Artery, thrombosis				1 (10%)
Fat, hemorrhage, focal	1 (8%)			
Fat, necrosis, focal	9 (69%)	4 (40%)	6 (55%)	5 (50%)
Oral mucosa	(1)	(1)	(1)	
Foreign body		1 (100%)		
Hyperplasia, focal, squamous			1 (100%)	
Inflammation, chronic, focal		1 (100%)		
Pancreas	(50)	(48)	(48)	(49)
Angiectasis			1 (2%)	
Inflammation, chronic				1 (2%)
Acinus, atrophy, diffuse			1 (2%)	
Acinus, atrophy, focal	23 (46%)	24 (50%)	24 (50%)	25 (51%)
Duct, cyst, focal, multiple	15 (30%)	12 (25%)	16 (33%)	10 (20%)

[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 Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Alimentary System (continued)				
Salivary glands	(50)	(50)	(50)	(50)
Atrophy, focal	1 (2%)			
Inflammation, chronic		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(49)
Diverticulum				1 (2%)
Edema	3 (6%)			
Hyperkeratosis, focal		1 (2%)		
Inflammation	1 (2%)		1 (2%)	3 (6%)
Ulcer	5 (10%)	2 (4%)	2 (4%)	3 (6%)
Epithelium, hyperplasia	4 (8%)	3 (6%)	2 (4%)	4 (8%)
Stomach, glandular	(50)	(50)	(49)	(49)
Cyst				1 (2%)
Edema	2 (4%)			
Erosion	3 (6%)	1 (2%)	1 (2%)	6 (12%)
Hyperplasia, focal, histiocytic			1 (2%)	
Inflammation, focal		1 (2%)		
Ulcer	1 (2%)	1 (2%)	2 (4%)	
Epithelium, hyperplasia			1 (2%)	
Tongue		(3)	(1)	(1)
Epithelium, cyst		1 (33%)		
Epithelium, hyperplasia			1 (100%)	1 (100%)
Tooth	(1)	(1)		
Peridontal tissue, inflammation	1 (100%)	1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	40 (80%)	38 (76%)	36 (72%)	39 (78%)
Thrombosis	1 (2%)	3 (6%)	1 (2%)	4 (8%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	9 (18%)	6 (12%)	5 (10%)	9 (18%)
Angiectasis		1 (2%)		
Cytoplasmic alteration, focal	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Degeneration, cystic		1 (2%)		
Hematopoietic cell proliferation	1 (2%)			
Hemorrhage, focal	1 (2%)			
Hyperplasia, focal				1 (2%)
Necrosis	1 (2%)	1 (2%)	1 (2%)	
Vacuolization cytoplasmic, focal	6 (12%)	5 (10%)	13 (26%)	7 (14%)
Capsule, hyperplasia, focal				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia, focal	7 (14%)	1 (2%)	3 (6%)	
Necrosis		1 (2%)		
Islets, pancreatic	(50)	(49)	(49)	(49)
Hyperplasia	1 (2%)			1 (2%)
Parathyroid gland	(48)	(50)	(48)	(50)
Hyperplasia, focal	1 (2%)			2 (4%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Endocrine System (continued)				
Pituitary gland	(49)	(49)	(48)	(48)
Angiectasis	1 (2%)		4 (8%)	1 (2%)
Cyst	4 (8%)	2 (4%)	6 (13%)	1 (2%)
Fibrosis, focal				1 (2%)
Hemorrhage				1 (2%)
Pars distalis, cytoplasmic alteration, focal			2 (4%)	
Pars distalis, degeneration, cystic, focal		1 (2%)		
Pars distalis, hemorrhage, focal	2 (4%)			
Pars distalis, hyperplasia	1 (2%)			
Pars distalis, hyperplasia, focal	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Pars distalis, hypertrophy, focal	8 (16%)	9 (18%)	20 (42%)	22 (46%)
Pars nervosa, inflammation	1 (2%)			
Thyroid gland	(47)	(46)	(48)	(44)
C-cell, hyperplasia	41 (87%)	28 (61%)	26 (54%)	29 (66%)
Follicle, cyst		3 (7%)	1 (2%)	5 (11%)
Follicle, degeneration, focal	6 (13%)	4 (9%)	8 (17%)	4 (9%)
Follicle, mineralization	3 (6%)	3 (7%)	5 (10%)	6 (14%)
Follicular cell, hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	
General Body System				
Peritoneum		(2)	(3)	(1)
Inflammation, chronic, focal			1 (33%)	
Tissue NOS	(6)	(4)	(6)	(5)
Inflammation, chronic				1 (20%)
Mediastinum, cyst	1 (17%)			
Genital System				
Coagulating gland				(1)
Inflammation, chronic				1 (100%)
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)			1 (2%)
Hemorrhage				1 (2%)
Inflammation, chronic			1 (2%)	
Spermatocele	1 (2%)			
Preputial gland	(50)	(50)	(50)	(49)
Degeneration, cystic	5 (10%)	3 (6%)	2 (4%)	3 (6%)
Fibrosis	1 (2%)			
Hyperplasia, cystic	3 (6%)	1 (2%)		
Inflammation, chronic	30 (60%)	28 (56%)	21 (42%)	23 (47%)
Prostate	(50)	(50)	(50)	(49)
Inflammation, chronic	27 (54%)	24 (48%)	28 (56%)	36 (73%)
Epithelium, hyperplasia, focal	4 (8%)	6 (12%)	5 (10%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation				1 (2%)
Testes	(50)	(50)	(50)	(50)
Atrophy	15 (30%)	10 (20%)	8 (16%)	10 (20%)
Cyst	1 (2%)			
Interstitial cell, hyperplasia, focal	4 (8%)		6 (12%)	7 (14%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Hematopoietic System				
Bone marrow	(47)	(50)	(48)	(50)
Angiectasis	1 (2%)			
Atrophy	1 (2%)			
Hyperplasia	2 (4%)	3 (6%)	2 (4%)	4 (8%)
Lymph node	(19)	(18)	(26)	(27)
Hyperplasia, plasma cell		1 (6%)		
Deep cervical, hyperplasia, histiocytic			1 (4%)	
Deep cervical, hyperplasia, plasma cell	1 (5%)			
Mediastinal, ectasia	2 (11%)	2 (11%)		3 (11%)
Mediastinal, hemorrhage	3 (16%)	2 (11%)	2 (8%)	3 (11%)
Mediastinal, hyperplasia		1 (6%)		
Mediastinal, hyperplasia, histiocytic	5 (26%)		2 (8%)	3 (11%)
Mediastinal, hyperplasia, plasma cell	2 (11%)	2 (11%)	2 (8%)	1 (4%)
Pancreatic, ectasia	1 (5%)			
Pancreatic, fibrosis				1 (4%)
Pancreatic, hemorrhage		1 (6%)	3 (12%)	1 (4%)
Pancreatic, hyperplasia, histiocytic	2 (11%)		1 (4%)	4 (15%)
Lymph node, mesenteric	(50)	(48)	(48)	(49)
Ectasia	1 (2%)	1 (2%)		
Hemorrhage				1 (2%)
Hyperplasia, histiocytic	2 (4%)	1 (2%)		2 (4%)
Hyperplasia, lymphoid				1 (2%)
Spleen	(50)	(48)	(48)	(49)
Accessory spleen	3 (6%)		1 (2%)	3 (6%)
Atrophy	3 (6%)			
Fibrosis, diffuse	1 (2%)			
Fibrosis, focal	2 (4%)	2 (4%)	2 (4%)	
Hematopoietic cell proliferation	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hemorrhage	1 (2%)		1 (2%)	
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Infarct		1 (2%)		2 (4%)
Inflammation, granulomatous		2 (4%)	1 (2%)	2 (4%)
Thymus	(48)	(49)	(46)	(45)
Angiectasis			1 (2%)	
Hemorrhage	2 (4%)			
Integumentary System				
Mammary gland	(47)	(46)	(41)	(45)
Dilatation	12 (26%)	7 (15%)	1 (2%)	2 (4%)
Fibrosis	1 (2%)			1 (2%)
Inflammation, chronic, focal		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Hyperkeratosis, focal	1 (2%)		1 (2%)	
Inflammation, focal				1 (2%)
Ulcer		1 (2%)		
Epidermis, hyperplasia, focal		1 (2%)	1 (2%)	
Subcutaneous tissue, edema			1 (2%)	
Subcutaneous tissue, fibrosis, focal				1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Vertebra, cyst	1 (2%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression, focal	4 (8%)	6 (12%)	1 (2%)	1 (2%)
Hemorrhage, focal	1 (2%)	4 (8%)	3 (6%)	3 (6%)
Meninges, congestion, focal		1 (2%)		
Meninges, hemorrhage, focal	1 (2%)			
Spinal cord	(2)	(3)	(2)	(3)
Hemorrhage, focal		1 (33%)		1 (33%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	4 (8%)	1 (2%)		2 (4%)
Hemorrhage, focal	4 (8%)	1 (2%)	3 (6%)	
Infiltration cellular, mixed cell	3 (6%)		1 (2%)	2 (4%)
Inflammation, chronic, focal	3 (6%)	3 (6%)	4 (8%)	2 (4%)
Inflammation, focal				1 (2%)
Alveolar epithelium, hyperplasia, focal	2 (4%)	4 (8%)	3 (6%)	
Alveolus, proteinosis			1 (2%)	
Nose	(49)	(50)	(50)	(50)
Foreign body	1 (2%)		1 (2%)	1 (2%)
Hemorrhage	1 (2%)			
Inflammation, suppurative	2 (4%)	1 (2%)	4 (8%)	2 (4%)
Nasolacrimal duct, inflammation				4 (8%)
Special Senses System				
Eye	(48)	(47)	(48)	(45)
Cataract	2 (4%)	1 (2%)		2 (4%)
Anterior chamber, exudate		1 (2%)		2 (4%)
Retina, degeneration	2 (4%)			
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal, histiocytic	1 (2%)			
Hyperplasia, focal, lymphoid			1 (2%)	
Inflammation			2 (4%)	
Inflammation, chronic				1 (2%)
Epithelium, hyperplasia, focal	1 (2%)			

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Urinary System				
Kidney	(48)	(49)	(48)	(50)
Congestion	1 (2%)			
Cyst	2 (4%)	1 (2%)		
Infarct		2 (4%)	1 (2%)	4 (8%)
Nephropathy	47 (98%)	43 (88%)	47 (98%)	46 (92%)
Pelvis, dilatation			1 (2%)	1 (2%)
Renal tubule, accumulation, hyaline droplet	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Renal tubule, hyperplasia, focal		1 (2%)		
Renal tubule, pigmentation	1 (2%)	1 (2%)	2 (4%)	
Urethra				(1)
Muscularis, inflammation, chronic				1 (100%)
Urinary bladder	(49)	(50)	(50)	(50)
Hemorrhage	1 (2%)	1 (2%)		1 (2%)
Inflammation	1 (2%)			1 (2%)
Ulcer	1 (2%)			
Muscularis, hyperplasia			1 (2%)	
Transitional epithelium, hyperplasia			1 (2%)	

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF 4-METHYLIMIDAZOLE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 4-Methylimidazole[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	5	5	9
Natural deaths	2	6	11	6
Survivors				
Died last week of study	3			2
Terminal sacrifice	40	39	34	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(49)	(48)	(45)	(46)
Histiocytic sarcoma	1 (2%)			
Intestine large, cecum	(48)	(46)	(43)	(43)
Intestine small, duodenum	(49)	(48)	(43)	(45)
Intestine small, ileum	(45)	(47)	(42)	(43)
Liver	(50)	(50)	(48)	(50)
Cholangiocarcinoma			1 (2%)	
Hepatocellular adenoma			1 (2%)	2 (4%)
Histiocytic sarcoma	2 (4%)			
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Mesentery	(9)	(7)	(11)	(8)
Fibrosarcoma	1 (11%)			
Histiocytic sarcoma	1 (11%)			
Pancreas	(49)	(49)	(47)	(47)
Histiocytic sarcoma	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(49)	(50)
Stomach, glandular	(50)	(50)	(49)	(49)
Tongue		(5)	(4)	(3)
Squamous cell papilloma		1 (20%)	1 (25%)	1 (33%)
Cardiovascular System				
Heart	(50)	(50)	(48)	(50)
Schwannoma benign	1 (2%)	1 (2%)	3 (6%)	
Schwannoma benign, multiple			1 (2%)	
Schwannoma malignant			1 (2%)	
Epicardium, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Adenoma	1 (2%)		1 (2%)	1 (2%)
Carcinoma			1 (2%)	
Adrenal medulla	(48)	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)			
Pheochromocytoma benign	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Bilateral, pheochromocytoma benign	1 (2%)			
Islets, pancreatic	(49)	(50)	(48)	(47)
Adenoma		1 (2%)		
Mixed tumor benign		1 (2%)		
Pituitary gland	(48)	(50)	(50)	(50)
Pars distalis, adenoma	29 (60%)	19 (38%)	20 (40%)	9 (18%)
Pars distalis, carcinoma		1 (2%)		
Thyroid gland	(47)	(44)	(40)	(45)
Bilateral, C-cell, adenoma			1 (3%)	
C-cell, adenoma	10 (21%)	8 (18%)	5 (13%)	8 (18%)
C-cell, carcinoma	1 (2%)		2 (5%)	
Follicular cell, adenoma	2 (4%)	3 (7%)		
General Body System				
Tissue NOS	(5)	(3)	(6)	(6)
Neoplasm NOS			1 (17%)	
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung		1 (33%)		
Mediastinum, schwannoma malignant	1 (20%)			
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	8 (16%)	1 (2%)		
Carcinoma	2 (4%)	1 (2%)		
Ovary	(50)	(50)	(48)	(50)
Granulosa-theca tumor malignant		1 (2%)		
Histiocytic sarcoma	1 (2%)			
Oviduct			(2)	(1)
Uterus	(50)	(50)	(48)	(50)
Carcinoma			1 (2%)	
Endometrium, polyp stromal	16 (32%)	5 (10%)	2 (4%)	2 (4%)
Endometrium, sarcoma stromal	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(48)	(49)	(48)
Osteosarcoma, metastatic, bone	1 (2%)			
Lymph node	(24)	(11)	(24)	(35)
Mediastinal, carcinoma, metastatic, thyroid gland	1 (4%)			
Pancreatic, histiocytic sarcoma	2 (8%)			
Lymph node, mandibular	(1)	(3)	(1)	(4)
Lymph node, mesenteric	(49)	(49)	(49)	(47)
Spleen	(50)	(48)	(47)	(48)
Histiocytic sarcoma	1 (2%)			
Thymus	(47)	(50)	(48)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Carcinoma	2 (4%)	1 (2%)	2 (4%)	
Fibroadenoma	20 (40%)	5 (10%)	3 (6%)	1 (2%)
Fibroadenoma, multiple	4 (8%)	1 (2%)	1 (2%)	
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma				1 (2%)
Squamous cell papilloma	1 (2%)			
Trichoepithelioma	1 (2%)			
Pinna, neural crest tumor				1 (2%)
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)	5 (10%)	
Subcutaneous tissue, histiocytic sarcoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)	1 (2%)		
Maxilla, sarcoma				1 (2%)
Skeletal muscle		(1)	(1)	
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (100%)		
Carcinoma, metastatic, thyroid gland			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant		1 (2%)		
Carcinoma, metastatic, pituitary gland		1 (2%)		
Spinal cord	(7)	(3)	(24)	(50)
Respiratory System				
Larynx			(1)	
Carcinoma, metastatic, thyroid gland			1 (100%)	
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma		2 (4%)		
Histiocytic sarcoma, metastatic, uncertain primary site	1 (2%)			
Osteosarcoma, metastatic, bone		1 (2%)		
Nose	(49)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland			1 (2%)	
Special Senses System				
Eye	(48)	(45)	(40)	(45)
Lids, neural crest tumor		1 (2%)	1 (3%)	
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland		(3)	(1)	
Carcinoma		1 (33%)	1 (100%)	
Carcinosarcoma		1 (33%)		
Urinary System				
Kidney	(48)	(48)	(43)	(43)
Bilateral, renal tubule, adenoma		1 (2%)		
Pelvis, transitional epithelium, papilloma	1 (2%)			
Urinary bladder	(49)	(50)	(49)	(48)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)			
Leukemia mononuclear	9 (18%)	7 (14%)	16 (32%)	20 (40%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	39	42	36
Total primary neoplasms	118	67	73	49
Total animals with benign neoplasms	48	32	29	20
Total benign neoplasms	97	49	46	27
Total animals with malignant neoplasms	19	15	24	21
Total malignant neoplasms	21	17	25	21
Total animals with metastatic neoplasms	3	3	2	
Total metastatic neoplasms	3	6	4	
Total animals with malignant neoplasms of uncertain primary site	1		1	
Total animals with uncertain neoplasms benign or malignant		1	2	1
Total uncertain neoplasms		1	2	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	0161	3029	4988	6264	6667	6994	7000	7303	7333	7334	7335	7355	7355	7355	7355	7355	7356	7366	7366	7366	7366	7366	7366	7366
Carcass ID Number	2245	2337	2448	2220	2236	2221	2444	2431	2224	2219	2116	2117	2118	2446	2447	2449	2505	2006	2007	2008	2009	2101	2111	2113
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																					
Intestine large, rectum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	A	+	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	A	+	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																					
Mesentery			+			+	+						+	+							+			
Fibrosarcoma																								
Histiocytic sarcoma			X																					
Pancreas	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth									+															
Cardiovascular System																								
Blood vessel																					+			
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma benign																								
Endocrine System																								
Adrenal cortex	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																						X		
Adrenal medulla	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																								
Pheochromocytoma benign																								
Bilateral, pheochromocytoma benign																								
Islets, pancreatic	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X		X	X	X	X	X		X		X		X		X	X	X		X		X	X
Thyroid gland	+	+	+	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma							X				X	X			X							X	X	
C-cell, carcinoma																		X						
Follicular cell, adenoma																								
General Body System																								
Tissue NOS	+								+	+						+								
Mediastinum, schwannoma malignant										X														

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	2 1 4	2 1 5	2 2 6	2 2 7	2 2 8	2 2 9	2 3 0	2 3 8	2 3 9	2 4 0	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	2 2 2	2 2 3	2 2 5	2 3 2	2 3 3	2 3 4	2 3 5	2 4 1	2 4 2	2 4 3	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																X										2
Mesentery	+													+						+						9
Fibrosarcoma														X												1
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma benign																			X							1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	48
Pheochromocytoma malignant													X													1
Pheochromocytoma benign																		X								1
Bilateral, pheochromocytoma benign										X																1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma				X	X	X		X	X	X		X		X		X		X		X		X	X	X		29
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
C-cell, adenoma					X	X		X														X				10
C-cell, carcinoma																										1
Follicular cell, adenoma																		X					X			2
General Body System																										
Tissue NOS																		+								5
Mediastinum, schwannoma malignant																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	0161	3022	4928	6624	6667	6694	7040	7330	7333	7334	7335	7335	7335	7335	7335	7335	7335	7336	7336	7336	7336	7336	7336	7336	7336	7336
Carcass ID Number	2245	2337	2448	2220	2236	2221	2244	2231	2224	2191	2116	2117	2118	2446	2447	2449	2505	2006	2007	2008	2009	2101	2111	2112	2113
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X		X							X									X				
Carcinoma																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																										
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal		X			X			X		X	X		X	X		X						X		X	X
Endometrium, sarcoma stromal									X																	
Vagina					+																					
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma, metastatic, bone																					X					
Lymph node	+		+	+		A	+		+	+				+	+		+	+				+	+		+
Mediastinal, carcinoma, metastatic, thyroid gland																		X								
Pancreatic, histiocytic sarcoma			X																							
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M		M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																							
Thymus	+	+	+	+	+	I	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma						X																				
Fibroadenoma				X		X		X		X	X				X					X	X				X
Fibroadenoma, multiple									X					X					X							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																					X					
Trichoepithelioma																										
Subcutaneous tissue, fibroma																										
Subcutaneous tissue, histiocytic sarcoma			X																							
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																					X					
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+		+															+	+	+		+	+			
Spinal cord	+		+															+	+	+		+	+			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	2 1 4	2 1 5	2 2 6	2 2 7	2 2 8	2 2 9	2 3 0	2 3 8	2 3 9	2 4 0	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	2 2 2	2 2 3	2 2 5	2 3 2	2 3 3	2 3 4	2 3 5	2 4 1	2 4 2	2 4 3	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma						X	X									X						X				8
Carcinoma										X								X								2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																X										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal				X	X								X					X			X					16
Endometrium, sarcoma stromal																										1
Vagina																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, bone																										1
Lymph node	+	+		+									+	+	+	+	+	+						+	+	24
Mediastinal, carcinoma, metastatic, thyroid gland																										1
Pancreatic, histiocytic sarcoma																X										2
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																								X		2
Fibroadenoma		X	X					X	X	X	X		X		X	X				X					X	20
Fibroadenoma, multiple	X																									4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Trichoepithelioma													X													1
Subcutaneous tissue, fibroma											X															1
Subcutaneous tissue, histiocytic sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										7
Spinal cord																										7

Table B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	016	302	498	624	667	694	700	730	733	734	735	735	735	735	735	735	735	736	736	736	736	736	736	736	736
Carcass ID Number	245	237	248	220	236	221	244	231	224	219	216	217	218	246	247	249	250	206	207	208	209	210	211	212	213
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma, metastatic, uncertain primary site			X																						
Nose	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pelvis, transitional epithelium, papilloma																		X							
Urinary bladder	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Leukemia mononuclear				X		X										X						X			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	7 3 3 6	7 3 6 6	7 3 6 6	7 3 6 7	7 3 6 8	7 3 6 9	7 3 6 0	7 3 6 8	7 3 6 9	7 3 6 0	7 3 7 1	7 3 7 2	7 3 7 3	7 3 7 4	7 3 7 5	7 3 7 2	7 3 7 3	7 3 7 5	7 3 7 2	7 3 7 3	7 3 7 4	7 3 7 5	7 3 7 1	7 3 7 2	7 3 7 3	
Carcass ID Number	2 1 4	2 1 5	2 2 6	2 2 7	2 2 8	2 2 9	2 3 0	2 3 8	2 3 9	2 4 0	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	2 2 2	2 2 3	2 2 5	2 3 2	2 3 3	2 3 4	2 3 5	2 4 1	2 4 2	2 4 3	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma, metastatic, uncertain primary site																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pelvis, transitional epithelium, papilloma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																X										2
Leukemia mononuclear		X				X									X	X						X				9

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	4 2 1	4 3 4	4 6 3	5 3 6	6 3 6	6 5 3	6 5 7	6 7 7	7 0 0	7 0 5	7 1 2	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6
Carcass ID Number	2 5 6	2 7 6	2 5 7	2 7 0	2 6 5	2 5 8	2 9 6	2 8 3	2 8 4	2 9 2	2 5 5	2 5 1	2 5 2	2 5 3	2 5 4	2 5 9	2 6 0	2 6 6	2 6 7	2 6 8	2 6 9	2 7 1	2 7 2	2 7 3	2 7 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery		+	+					+																	
Pancreas	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue						+																			
Squamous cell papilloma						X																			
Tooth																									
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma benign																									
Epicardium, alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign													X												
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Mixed tumor benign																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X	X	X			X		X		X		X	X		X			X	X	X	
Pars distalis, carcinoma																					X				
Thyroid gland	A	+	A	A	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma												X	X	X	X					X					
Follicular cell, adenoma					X										X										
General Body System																									
Tissue NOS			+	+																					
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung				X																					

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	2 7 5	2 7 7	2 7 8	2 7 9	2 8 0	2 8 1	2 8 2	2 8 5	2 9 1	2 9 3	2 9 4	2 9 5	2 9 7	2 9 8	2 9 9	3 0 0	2 6 1	2 6 2	2 6 3	2 6 4	2 8 6	2 8 7	2 8 8	2 8 9	2 9 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery																					+		+	+	+	7
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue									+					+										+	+	5
Squamous cell papilloma																										1
Tooth				+																						1
Cardiovascular System																										
Blood vessel														+												1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma benign										X																1
Epicardium, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																					X					1
Mixed tumor benign																			X							1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma				X		X		X	X					X									X	X		19
Pars distalis, carcinoma																										1
Thyroid gland	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
C-cell, adenoma									X		X													X		8
Follicular cell, adenoma												X														3
General Body System																										
Tissue NOS																									+	3
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	4 2 1	4 3 4	4 6 3	5 3 6	6 3 6	6 5 3	6 5 7	6 7 7	7 0 0	7 0 5	7 1 2	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6
Carcass ID Number	2 5 6	2 7 6	2 5 7	2 7 0	2 6 5	2 5 8	2 9 6	2 8 3	2 8 4	2 9 2	2 5 5	2 5 1	2 5 2	2 5 3	2 5 4	2 5 9	2 6 0	2 6 6	2 6 7	2 6 8	2 6 9	2 7 1	2 7 2	2 7 3	2 7 4
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma		X																							
Carcinoma																					X				
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa-theca tumor malignant																							X		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal								X																	
Vagina								+																	
Hematopoietic System																									
Bone marrow	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+	+			+	+	+	+	+														+	
Lymph node, mandibular	M	M	M	M	M	M	+	M	+	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Integumentary System																									
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma										X															
Fibroadenoma																						X	X		
Fibroadenoma, multiple																								X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma									X																
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma											X														
Skeletal muscle				+																					
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant	X																								
Carcinoma, metastatic, pituitary gland																					X				
Peripheral nerve						+																			
Spinal cord						+																			
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma				X																					
Osteosarcoma, metastatic, bone											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	2 7 5	2 7 7	2 7 8	2 7 9	2 8 0	2 8 1	2 8 2	2 8 5	2 9 1	2 9 3	2 9 4	2 9 5	2 9 7	2 9 8	2 9 9	3 0 0	2 6 1	2 6 2	2 6 3	2 6 4	2 8 6	2 8 7	2 8 8	2 8 9	2 9 0	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa-theca tumor malignant																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal												X				X				X	X					5
Vagina				+																						2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node						+									+										+	11
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	3
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Fibroadenoma						X															X			X		5
Fibroadenoma, multiple																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Skeletal muscle																										1
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Carcinoma, metastatic, pituitary gland																										1
Peripheral nerve			+		+																					3
Spinal cord			+		+																					3
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma				X																						2
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	4 2 1	4 3 4	4 6 3	5 3 6	6 3 6	6 5 3	6 5 7	6 7 7	7 0 0	7 0 5	7 1 2	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6
Carcass ID Number	2 5 6	2 7 6	2 5 7	2 7 0	2 6 5	2 5 8	2 9 6	2 8 3	2 8 4	2 9 2	2 5 5	2 5 1	2 5 2	2 5 3	2 5 4	2 5 9	2 6 0	2 6 6	2 6 7	2 6 8	2 6 9	2 7 1	2 7 2	2 7 3	2 7 4
Special Senses System																									
Eye	A	+	A	+	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lids, neural crest tumor																				X					
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland					+								+												
Carcinoma													X												
Carcinosarcoma																									
Urinary System																									
Kidney	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, renal tubule, adenoma															X										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	X					X										X		X					

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	2 7 5	2 7 7	2 7 8	2 7 9	2 8 0	2 8 1	2 8 2	2 8 5	2 9 1	2 9 3	2 9 4	2 9 5	2 9 7	2 9 8	2 9 9	3 0 0	2 6 1	2 6 2	2 6 3	2 6 4	2 6 6	2 8 6	2 8 7	2 8 8	2 8 9	2 9 0	Total Tissues/ Tumors
Special Senses System																											
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Lids, neural crest tumor																											1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Zymbal's gland				+																							3
Carcinoma																											1
Carcinosarcoma				X																							1
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bilateral, renal tubule, adenoma																											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																				X						X	7

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	1 0 4	3 8 3	5 5 2	5 5 5	5 7 8	5 8 1	6 1 9	6 3 5	6 3 6	6 3 8	6 5 3	6 7 2	6 9 3	7 0 0	7 0 0	7 2 8	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 4 0 9	3 0 8	3 4 7	3 1 5	3 1 7	3 1 6	3 4 3	3 2 9	3 2 6	3 0 2	3 2 8	3 1 4	3 4 0	3 3 7	3 4 8	3 3 6	3 0 1	3 0 3	3 0 4	3 0 5	3 0 6	3 0 7	3 0 9	3 1 0	3 1 8
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	A	A	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	A	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	A	A	A	+	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	A	A	A	+	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	M	A	A	+	+	A	A	A	A	+	A	I	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	A	A	A	+	+	+	A	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Liver	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																								
Hepatocellular adenoma											X													
Osteosarcoma, metastatic, uncertain primary site													X											
Mesentery					+	+	+				+	+		+		+			+	+				
Pancreas	A	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue						+															+			
Squamous cell papilloma						X																		
Tooth		+													+									
Cardiovascular System																								
Blood vessel		+			+																			
Heart	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma benign																		X						
Schwannoma benign, multiple																							X	
Schwannoma malignant																								
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X									
Carcinoma																						X		
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign														X										
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X						X			X					X	X	X			X	
Thyroid gland	A	A	A	A	+	+	A	A	A	A	+	A	+	+	+	A	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																							X	
C-cell, adenoma														X						X		X		
C-cell, carcinoma																								
General Body System																								
Tissue NOS					+		+		+			+		+										
Neoplasm NOS																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 1 9	3 2 0	3 2 1	3 2 2	3 2 3	3 2 4	3 2 5	3 4 1	3 4 2	3 4 4	3 4 5	3 4 6	3 5 0	3 1 1	3 1 2	3 1 3	3 3 8	3 3 9	3 2 7	3 3 0	3 3 1	3 3 2	3 3 3	3 3 4	3 3 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cholangiocarcinoma										X																1
Hepatocellular adenoma																										1
Osteosarcoma, metastatic, uncertain primary site																										1
Mesentery			+										+													11
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue				+						+																4
Squamous cell papilloma																										1
Tooth																										2
Cardiovascular System																										
Blood vessel																										2
Heart	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Schwannoma benign				X																		X				3
Schwannoma benign, multiple																										1
Schwannoma malignant															X											1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																X										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X	X	X		X	X		X		X									X	X		X		X	X	20
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Bilateral, C-cell, adenoma																										1
C-cell, adenoma			X														X									5
C-cell, carcinoma													X					X								2
General Body System																										
Tissue NOS			+																							6
Neoplasm NOS			X																							1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	1 0 4	3 8 3	5 5 2	5 5 5	5 7 8	5 8 1	6 1 9	6 3 5	6 3 6	6 3 8	6 5 3	6 7 2	6 9 3	7 0 0	7 0 0	7 2 8	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 4 0 9	3 0 8	3 4 7	3 1 5	3 1 7	3 1 6	3 4 3	3 2 9	3 2 6	3 0 2	3 2 8	3 1 4	3 4 0	3 3 7	3 4 8	3 3 6	3 0 1	3 0 3	3 0 4	3 0 5	3 0 6	3 0 7	3 0 9	3 1 0	3 1 8
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oviduct					+		+																	
Uterus	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																								
Endometrium, polyp stromal																								X
Vagina						+	+			+			+							+				
Hematopoietic System																								
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+	+	+	+	+	+	+	+	+	+	+	+		+		+	+		+	
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	A	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																								
Fibroadenoma																								
Fibroadenoma, multiple																				X				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma										X										X				
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																								
Carcinoma, metastatic, thyroid gland																								
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve		+			+	+	+			+	+	+					+				+	+	+	+
Spinal cord		+			+	+	+			+	+	+					+				+	+	+	+
Respiratory System																								
Larynx																								
Carcinoma, metastatic, thyroid gland																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																								
Special Senses System																								
Eye	A	A	A	A	A	+	+	A	A	A	+	A	+	+	+	A	+	+	+	+	+	+	+	+
Lids, neural crest tumor																								
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland															+									
Carcinoma															X									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 1 9	3 2 0	3 2 1	3 2 2	3 2 3	3 2 4	3 2 5	3 4 1	3 4 2	3 4 4	3 4 5	3 4 6	3 5 0	3 1 1	3 1 2	3 1 3	3 3 8	3 3 9	3 2 7	3 3 0	3 3 1	3 3 2	3 3 3	3 3 4	3 3 5	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Oviduct																										2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma						X																				1
Endometrium, polyp stromal																							X			2
Vagina																										5
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node			+			+							+				+	+		+				+	+	24
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma		X																		X						2
Fibroadenoma			X	X					X																	3
Fibroadenoma, multiple																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma				X		X											X									5
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle													+													1
Carcinoma, metastatic, thyroid gland													X													1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve	+		+		+		+	+			+			+	+	+	+	+					+			24
Spinal cord	+		+		+		+	+			+			+	+	+	+	+					+			24
Respiratory System																										
Larynx													+													1
Carcinoma, metastatic, thyroid gland													X													1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland													X													1
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Lids, neural crest tumor																			X							1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Zymbal's gland																										1
Carcinoma																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	1	3	5	5	5	5	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7
	0	8	5	5	7	8	1	3	3	3	5	7	9	0	0	2	3	3	3	3	3	3	3	3	3
	4	3	2	5	8	1	9	5	6	8	3	2	3	0	0	8	5	5	5	5	5	5	5	5	5
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	4	0	4	1	1	1	4	2	2	0	2	1	4	3	4	3	0	0	0	0	0	0	0	1	1
	9	8	7	5	7	6	3	9	6	2	8	4	0	7	8	6	1	3	4	5	6	7	9	0	8
Urinary System																									
Kidney	A	+	A	A	+	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X	X	X	X		X	X		X		X	X	X		X		X			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 2,500 ppm

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 1 9	3 2 0	3 2 1	3 2 2	3 2 3	3 2 4	3 2 5	3 4 1	3 4 2	3 4 4	3 4 5	3 4 6	3 5 0	3 1 1	3 1 2	3 1 3	3 3 8	3 3 9	3 2 7	3 3 0	3 3 1	3 3 2	3 3 3	3 3 4	3 3 5	Total Tissues/ Tumors
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X								X				X									16

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 5,000 ppm

Number of Days on Study	3 2 3	3 6 8	5 3 8	5 4 1	5 4 1	6 2 8	6 4 2	6 4 2	6 5 4	6 6 6	6 8 6	6 8 8	7 0 5	7 1 2	7 1 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 6 4	3 8 9	3 6 7	3 5 9	3 8 0	3 7 4	3 9 9	4 0 0	3 6 3	3 9 3	3 5 2	3 7 2	3 9 4	3 8 6	3 9 1	3 8 1	3 8 2	3 8 3	3 8 4	3 8 5	3 5 3	3 6 1	3 6 2	3 6 5	3 7 9
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	A	+	+	+	A
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	A	A	A	+	A	+	+	+	+	+	A	+	+	+	A
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	A	A	+	+	A	+	+	+	+	+	A	+	+	+	A
Intestine small, jejunum	A	+	+	+	+	+	+	+	A	+	A	A	A	+	A	+	+	+	+	+	A	+	+	+	A
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	A	A	A	+	A	+	+	+	+	+	A	+	+	+	A
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																		X							
Mesentery		+	+	+			+			+			+												
Pancreas	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	A
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A
Tongue																									
Squamous cell papilloma																									
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X					
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																					X			X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	A
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X					X			X										
Thyroid gland	A	+	+	+	+	+	+	+	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	A
C-cell, adenoma								X											X		X			X	
General Body System																									
Tissue NOS		+								+	+		+	+					+						
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oviduct																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal																							X		
Vagina															+				+				+		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 5,000 ppm

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 8 8 7	3 8 8 8	3 9 0 0	3 9 9 2	3 9 9 5	3 5 1 1	3 5 5 4	3 5 5 5	3 5 5 6	3 5 5 7	3 5 5 8	3 6 0 0	3 6 6 6	3 6 6 8	3 6 6 9	3 7 0 0	3 7 7 1	3 7 7 3	3 7 7 5	3 7 7 6	3 7 7 7	3 7 7 8	3 9 9 6	3 9 9 7	3 9 9 8	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma			+					+								X										2
Mesentery			+					+																		8
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue		+				+																	+			3
Squamous cell papilloma						X																				1
Cardiovascular System																										
Blood vessel								+																		1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma							X		X					X						X	X			X		9
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
C-cell, adenoma	X			X			X									X										8
General Body System																										
Tissue NOS																										6
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oviduct																+										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal																				X						2
Vagina																				+						4

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 5,000 ppm

Number of Days on Study	3 2 3	3 6 8	5 3 8	5 4 1	5 4 1	6 2 8	6 4 2	6 4 2	6 5 4	6 6 6	6 8 6	6 8 8	7 0 5	7 1 2	7 1 7	7 2 9	7 2 9	7 2 9	7 2 9	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 6 4	3 8 9	3 6 7	3 5 9	3 8 0	3 7 4	3 9 9	4 0 0	3 6 3	3 9 3	3 5 2	3 7 2	3 9 4	3 8 6	3 9 1	3 8 1	3 8 2	3 8 3	3 8 4	3 8 5	3 5 3	3 6 1	3 6 2	3 6 5	3 7 9
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A
Lymph node		+	+	+	+	+	+	+	+	+	+			+	+	+	+		+	+	A	+	+	+	
Lymph node, mandibular	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	A
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A
Thymus	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																					X				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																			X						
Pinna, neural crest tumor						X																			
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maxilla, sarcoma																X									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	A	A	+	+	A	+	+	+	+	+	A	+	+	+	A
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	A	+	+	+	+	+	+	+	+	+	A	A	A	+	A	+	+	+	+	+	A	+	+	+	A
Urinary bladder	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	X	X	X		X			X	X		X	X					X	X				X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4-Methylimidazole: 5,000 ppm

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 8 7	3 8 8	3 9 0	3 9 2	3 9 5	3 5 1	3 5 4	3 5 5	3 5 6	3 5 7	3 5 8	3 6 0	3 6 6	3 6 8	3 6 9	3 7 0	3 7 1	3 7 3	3 7 5	3 7 6	3 7 7	3 7 8	3 9 6	3 9 7	3 9 8	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node			+		+	+	+	+	+		+		+		+	+	+	+			+	+	+	+		35
Lymph node, mandibular	M	M	M	M	M	+	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	4
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																										1
Pinna, neural crest tumor																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Maxilla, sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X	X	X	X		X			X					X					X			20

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate[a]	3/48 (6%)	1/50 (2%)	2/50 (4%)	2/50 (4%)
Adjusted rate[b]	6.7%	2.2%	4.6%	4.5%
Terminal rate[c]	3/42 (7%)	1/39 (3%)	1/34 (3%)	2/35 (6%)
First incidence (days)	729 (T)	729 (T)	700	729 (T)
Poly-3 test[d]	P=0.501N	P=0.297N	P=0.508N	P=0.503N
Clitoral Gland: Adenoma				
Overall rate	8/50 (16%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	17.0%	2.2%	0.0%	0.0%
Terminal rate	6/43 (14%)	0/39 (0%)	0/34 (0%)	0/35 (0%)
First incidence (days)	624	434	—[e]	—
Poly-3 test	P<0.001N	P=0.017N	P=0.005N	P=0.005N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	10/50 (20%)	2/50 (4%)	0/50 (0%)	0/50 (0%)
Adjusted rate	21.3%	4.3%	0.0%	0.0%
Terminal rate	8/43 (19%)	1/39 (3%)	0/34 (0%)	0/35 (0%)
First incidence (days)	624	434	—	—
Poly-3 test	P<0.001N	P=0.014N	P<0.001N	P<0.001N
Heart: Benign Schwannoma				
Overall rate	1/50 (2%)	1/50 (2%)	4/48 (8%)	0/50 (0%)
Adjusted rate	2.2%	2.2%	9.5%	0.0%
Terminal rate	1/43 (2%)	1/39 (3%)	4/33 (12%)	0/35 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	—
Poly-3 test	P=0.470N	P=0.756	P=0.150	P=0.509N
Mammary Gland: Fibroadenoma				
Overall rate	24/50 (48%)	6/50 (12%)	4/50 (8%)	1/50 (2%)
Adjusted rate	51.0%	13.1%	9.1%	2.3%
Terminal rate	22/43 (51%)	6/39 (15%)	4/34 (12%)	1/35 (3%)
First incidence (days)	624	729 (T)	729 (T)	729 (T)
Poly-3 test	P<0.001N	P<0.001N	P<0.001N	P<0.001N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	25/50 (50%)	7/50 (14%)	6/50 (12%)	1/50 (2%)
Adjusted rate	53.1%	15.3%	13.7%	2.3%
Terminal rate	23/43 (54%)	6/39 (15%)	6/34 (18%)	1/35 (3%)
First incidence (days)	624	705	729 (T)	729 (T)
Poly-3 test	P<0.001N	P<0.001N	P<0.001N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	29/48 (60%)	19/50 (38%)	20/50 (40%)	9/50 (18%)
Adjusted rate	62.1%	40.7%	44.7%	20.0%
Terminal rate	25/42 (60%)	15/39 (39%)	17/34 (50%)	6/35 (17%)
First incidence (days)	498	636	555	642
Poly-3 test	P<0.001N	P=0.029N	P=0.068N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	29/48 (60%)	20/50 (40%)	20/50 (40%)	9/50 (18%)
Adjusted rate	62.1%	42.9%	44.7%	20.0%
Terminal rate	25/42 (60%)	16/39 (41%)	17/34 (50%)	6/35 (17%)
First incidence (days)	498	636	555	642
Poly-3 test	P<0.001N	P=0.046N	P=0.068N	P<0.001N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	1/50 (2%)	1/50 (2%)	5/50 (10%)	0/50 (0%)
Adjusted rate	2.2%	2.2%	11.3%	0.0%
Terminal rate	1/43 (2%)	0/39 (0%)	4/34 (12%)	0/35 (0%)
First incidence (days)	729 (T)	700	638	—
Poly-3 test	P=0.502N	P=0.757	P=0.089	P=0.509N
Thyroid Gland (C-cell): Adenoma				
Overall rate	10/47 (21%)	8/44 (18%)	6/40 (15%)	8/45 (18%)
Adjusted rate	22.7%	19.0%	15.6%	19.5%
Terminal rate	9/42 (21%)	8/38 (21%)	5/34 (15%)	7/34 (21%)
First incidence (days)	700	729 (T)	700	642
Poly-3 test	P=0.411N	P=0.439N	P=0.299N	P=0.465N
Thyroid Gland (C-cell): Carcinoma				
Overall rate	1/47 (2%)	0/44 (0%)	2/40 (5%)	0/45 (0%)
Adjusted rate	2.3%	0.0%	5.2%	0.0%
Terminal rate	1/42 (2%)	0/38 (0%)	2/34 (6%)	0/34 (0%)
First incidence (days)	729 (T)	—	729 (T)	—
Poly-3 test	P=0.488N	P=0.509N	P=0.452	P=0.516N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	11/47 (23%)	8/44 (18%)	8/40 (20%)	8/45 (18%)
Adjusted rate	24.9%	19.0%	20.8%	19.5%
Terminal rate	10/42 (24%)	8/38 (21%)	7/34 (21%)	7/34 (21%)
First incidence (days)	700	729 (T)	700	642
Poly-3 test	P=0.363N	P=0.344N	P=0.429N	P=0.369N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	2/47 (4%)	3/44 (7%)	0/40 (0%)	0/45 (0%)
Adjusted rate	4.5%	7.1%	0.0%	0.0%
Terminal rate	2/42 (5%)	2/38 (5%)	0/34 (0%)	0/34 (0%)
First incidence (days)	729 (T)	636	—	—
Poly-3 test	P=0.082N	P=0.484	P=0.268N	P=0.255N
Uterus: Stromal Polyp				
Overall rate	16/50 (32%)	5/50 (10%)	2/50 (4%)	2/50 (4%)
Adjusted rate	33.6%	10.9%	4.6%	4.5%
Terminal rate	14/43 (33%)	4/39 (10%)	2/34 (6%)	2/35 (6%)
First incidence (days)	302	677	729 (T)	729 (T)
Poly-3 test	P<0.001N	P=0.007N	P<0.001N	P<0.001N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	17/50 (34%)	5/50 (10%)	2/50 (4%)	2/50 (4%)
Adjusted rate	35.6%	10.9%	4.6%	4.5%
Terminal rate	15/43 (35%)	4/39 (10%)	2/34 (6%)	2/35 (6%)
First incidence (days)	302	677	729 (T)	729 (T)
Poly-3 test	P<0.001N	P=0.004N	P<0.001N	P<0.001N
All Organs: Mononuclear Cell Leukemia				
Overall rate	9/50 (18%)	7/50 (14%)	16/50 (32%)	20/50 (40%)
Adjusted rate	19.1%	14.8%	34.5%	41.7%
Terminal rate	7/43 (16%)	4/39 (10%)	7/34 (21%)	11/35 (31%)
First incidence (days)	624	434	578	368
Poly-3 test	P<0.001	P=0.386N	P=0.073	P=0.013

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
All Organs: Benign Neoplasms				
Overall rate	48/50 (96%)	32/50 (64%)	29/50 (58%)	20/50 (40%)
Adjusted rate	98.0%	67.0%	63.3%	44.2%
Terminal rate	42/43 (98%)	25/39 (64%)	22/34 (65%)	16/35 (46%)
First incidence (days)	302	434	555	642
Poly-3 test	P<0.001N	P<0.001N	P<0.001N	P<0.001N
All Organs: Malignant Neoplasms				
Overall rate	19/50 (38%)	15/50 (30%)	25/50 (50%)	21/50 (42%)
Adjusted rate	39.8%	30.6%	53.5%	43.8%
Terminal rate	16/43 (37%)	8/39 (21%)	14/34 (41%)	12/35 (34%)
First incidence (days)	498	421	578	368
Poly-3 test	P=0.201	P=0.232N	P=0.128	P=0.425
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	39/50 (78%)	42/50 (84%)	36/50 (72%)
Adjusted rate	98.0%	78.0%	88.3%	73.8%
Terminal rate	42/43 (98%)	28/39 (72%)	29/34 (85%)	23/35 (66%)
First incidence (days)	302	421	555	368
Poly-3 test	P=0.004N	P=0.002N	P=0.062N	P<0.001N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal medulla, clitoral gland, heart, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE B4
Historical Incidence of Mononuclear Cell Leukemia in Control Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Feed Studies	
Benzophenone	19/50
trans-Cinnamaldehyde	21/100
Citral	24/100
p,p′-Dichlorodiphenyl sulfone	8/50
2-Methylimidazole	6/50
4-Methylimidazole	9/50
o-Nitrotoluene	21/60
p-Nitrotoluene	13/50
Overall Historical Incidence: Feed Studies	
Total (%)	121/510 (23.7%)
Mean ± standard deviation	23.8% ± 9.1%
Range	12%-38%
Overall Historical Incidence	
Total (%)	383/1,459 (26.3%)
Mean ± standard deviation	26.7% ± 10.5%
Range	12%-52%

[a] Data as of January 28, 2005

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4-Methylimidazole[a]

	0 ppm		1,250 ppm		2,500 ppm		5,000 ppm	
Disposition Summary								
Animals initially in study	50		50		50		50	
Early deaths								
Moribund	5		5		5		9	
Natural deaths	2		6		11		6	
Survivors								
Died last week of study	3						2	
Terminal sacrifice	40		39		34		33	
Animals examined microscopically	50		50		50		50	
Alimentary System								
Intestine large, cecum	(48)		(46)		(43)		(43)	
Edema	1	(2%)						
Liver	(50)		(50)		(48)		(50)	
Basophilic focus	46	(92%)	43	(86%)	31	(65%)	25	(50%)
Clear cell focus	20	(40%)	32	(64%)	23	(48%)	27	(54%)
Congestion	2	(4%)	2	(4%)			1	(2%)
Degeneration, cystic, focal	1	(2%)			1	(2%)	3	(6%)
Eosinophilic focus	1	(2%)	2	(4%)	5	(10%)	11	(22%)
Hematopoietic cell proliferation	1	(2%)						
Hemorrhage							1	(2%)
Hepatodiaphragmatic nodule	5	(10%)	4	(8%)	6	(13%)	11	(22%)
Histiocytosis	40	(80%)	50	(100%)	48	(100%)	50	(100%)
Hyperplasia, focal, histiocytic							2	(4%)
Infiltration cellular, mixed cell	5	(10%)	5	(10%)			5	(10%)
Inflammation, chronic	17	(34%)	28	(56%)	34	(71%)	35	(70%)
Inflammation, chronic, focal					1	(2%)		
Inflammation, granulomatous	1	(2%)						
Mixed cell focus	10	(20%)	7	(14%)	6	(13%)	18	(36%)
Thrombosis					1	(2%)		
Bile duct, cyst							1	(2%)
Bile duct, dilatation, focal					2	(4%)	1	(2%)
Bile duct, hyperplasia	31	(62%)	38	(76%)	43	(90%)	35	(70%)
Hepatocyte, fatty change, focal	16	(32%)	29	(58%)	29	(60%)	32	(64%)
Hepatocyte, hyperplasia, focal	1	(2%)						
Hepatocyte, necrosis, focal	1	(2%)					1	(2%)
Hepatocyte, vacuolization cytoplasmic	6	(12%)	12	(24%)	17	(35%)	11	(22%)
Hepatocyte, centrilobular, necrosis	2	(4%)			1	(2%)	1	(2%)
Oval cell, hyperplasia, focal	1	(2%)					2	(4%)
Serosa, fibrosis					1	(2%)		
Mesentery	(9)		(7)		(11)		(8)	
Inflammation, chronic	1	(11%)			1	(9%)		
Fat, necrosis, focal	8	(89%)	3	(43%)			1	(13%)
Lymphatic, angiectasis							1	(13%)
Pancreas	(49)		(49)		(47)		(47)	
Acinus, atrophy, diffuse	1	(2%)						
Acinus, atrophy, focal	13	(27%)	22	(45%)	26	(55%)	30	(64%)
Duct, cyst, focal, multiple	10	(20%)	18	(37%)	11	(23%)	9	(19%)
Salivary glands	(50)		(50)		(50)		(50)	
Atrophy, focal					2	(4%)		
Inflammation, chronic			1	(2%)	1	(2%)	1	(2%)

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

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm		1,250 ppm		2,500 ppm		5,000 ppm	
Alimentary System (continued)								
Stomach, forestomach	(50)		(50)		(49)		(50)	
Diverticulum					1	(2%)		
Edema	1	(2%)	1	(2%)	2	(4%)	1	(2%)
Inflammation					2	(4%)		
Ulcer	1	(2%)	1	(2%)	3	(6%)	2	(4%)
Epithelium, hyperplasia			1	(2%)	6	(12%)	1	(2%)
Stomach, glandular	(50)		(50)		(49)		(49)	
Erosion	2	(4%)	1	(2%)	1	(2%)	4	(8%)
Ulcer			1	(2%)				
Tongue			(5)		(4)		(3)	
Epithelium, hyperplasia			1	(20%)	1	(25%)		
Tooth	(1)		(1)		(2)			
Malformation			1	(100%)	2	(100%)		
Peridontal tissue, inflammation	1	(100%)	1	(100%)				
Cardiovascular System								
Heart	(50)		(50)		(48)		(50)	
Cardiomyopathy	30	(60%)	43	(86%)	38	(79%)	44	(88%)
Inflammation, focal, suppurative			1	(2%)				
Thrombosis					2	(4%)	5	(10%)
Artery, inflammation, chronic					1	(2%)		
Epicardium, inflammation	1	(2%)						
Myocardium, fibrosis, focal	1	(2%)						
Pericardium, infiltration cellular, mixed cell	1	(2%)						
Endocrine System								
Adrenal cortex	(49)		(50)		(50)		(50)	
Accessory adrenal cortical nodule	8	(16%)	6	(12%)	3	(6%)	2	(4%)
Angiectasis	4	(8%)	2	(4%)	7	(14%)	4	(8%)
Cytoplasmic alteration, focal	1	(2%)	2	(4%)	2	(4%)	2	(4%)
Hematopoietic cell proliferation			1	(2%)				
Hemorrhage, focal					2	(4%)	1	(2%)
Vacuolization cytoplasmic, focal	8	(16%)	9	(18%)	13	(26%)	12	(24%)
Adrenal medulla	(48)		(50)		(50)		(50)	
Angiectasis	1	(2%)					1	(2%)
Atrophy					1	(2%)		
Hyperplasia, focal	2	(4%)			2	(4%)		
Hyperplasia, lymphoid			1	(2%)				
Islets, pancreatic	(49)		(50)		(48)		(47)	
Atrophy					1	(2%)		
Parathyroid gland	(50)		(49)		(48)		(49)	
Hyperplasia, focal							1	(2%)
Pituitary gland	(48)		(50)		(50)		(50)	
Angiectasis	6	(13%)	4	(8%)	3	(6%)	4	(8%)
Cyst			4	(8%)	2	(4%)	5	(10%)
Hyperplasia, focal, histiocytic			1	(2%)				
Pars distalis, angiectasis	1	(2%)	2	(4%)	3	(6%)		
Pars distalis, degeneration, cystic, focal	9	(19%)	6	(12%)	5	(10%)	3	(6%)
Pars distalis, hemorrhage, focal					1	(2%)		
Pars distalis, hyperplasia, focal	4	(8%)	4	(8%)	5	(10%)	4	(8%)
Pars distalis, hypertrophy, focal	1	(2%)			1	(2%)	4	(8%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Endocrine System (continued)				
Thyroid gland	(47)	(44)	(40)	(45)
C-cell, hyperplasia	45 (96%)	42 (95%)	36 (90%)	35 (78%)
Follicle, cyst		1 (2%)		
Follicle, degeneration, focal			1 (3%)	1 (2%)
Follicle, mineralization	2 (4%)	7 (16%)	6 (15%)	19 (42%)
Follicular cell, hyperplasia, focal	1 (2%)	1 (2%)	1 (3%)	2 (4%)
General Body System				
Tissue NOS	(5)	(3)	(6)	(6)
Fibrosis, focal	1 (20%)			
Mediastinum, cyst	1 (20%)			
Mediastinum, infiltration cellular, mixed cell	1 (20%)			
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Degeneration, cystic	4 (8%)		5 (10%)	3 (6%)
Hyperplasia, cystic	7 (14%)	4 (8%)	1 (2%)	1 (2%)
Hyperplasia, focal, histiocytic			1 (2%)	
Inflammation, chronic	2 (4%)	2 (4%)		3 (6%)
Ovary	(50)	(50)	(48)	(50)
Angiectasis				2 (4%)
Cyst	7 (14%)	3 (6%)	5 (10%)	
Periovarian tissue, hemorrhage				1 (2%)
Uterus	(50)	(50)	(48)	(50)
Inflammation, chronic, granulomatous	1 (2%)			
Ulcer			1 (2%)	
Cervix, hypertrophy	1 (2%)			
Endometrium, hyperplasia, cystic	25 (50%)	26 (52%)	18 (38%)	26 (52%)
Vagina	(1)	(2)	(5)	(4)
Angiectasis				1 (25%)
Infiltration cellular, polymorphonuclear		1 (50%)		1 (25%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Hematopoietic System				
Bone marrow	(50)	(48)	(49)	(48)
Atrophy				2 (4%)
Fibrosis		1 (2%)	1 (2%)	
Hyperplasia	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Hyperplasia, focal, histiocytic		1 (2%)	2 (4%)	3 (6%)
Erythroid cell, hyperplasia		1 (2%)		
Lymph node	(24)	(11)	(24)	(35)
Hyperplasia, plasma cell				1 (3%)
Deep cervical, hemorrhage	3 (13%)			
Mediastinal, congestion		1 (9%)		
Mediastinal, ectasia	3 (13%)	1 (9%)		1 (3%)
Mediastinal, hemorrhage	5 (21%)	3 (27%)	5 (21%)	3 (9%)
Mediastinal, hyperplasia, histiocytic	4 (17%)	1 (9%)	1 (4%)	6 (17%)
Mediastinal, hyperplasia, lymphoid	1 (4%)			1 (3%)
Mediastinal, hyperplasia, plasma cell			1 (4%)	
Mediastinal, pigmentation	1 (4%)			
Pancreatic, hemorrhage	1 (4%)		2 (8%)	2 (6%)
Pancreatic, hyperplasia, histiocytic	6 (25%)	1 (9%)	7 (29%)	7 (20%)
Pancreatic, hyperplasia, lymphoid				1 (3%)
Pancreatic, hyperplasia, plasma cell	1 (4%)			
Pancreatic, pigmentation	1 (4%)			
Renal, hyperplasia, histiocytic				1 (3%)
Lymph node, mandibular	(1)	(3)	(1)	(4)
Ectasia		1 (33%)		
Hyperplasia, histiocytic				1 (25%)
Hyperplasia, lymphoid			1 (100%)	
Lymph node, mesenteric	(49)	(49)	(49)	(47)
Ectasia			2 (4%)	
Hemorrhage				1 (2%)
Hyperplasia, histiocytic	7 (14%)		1 (2%)	2 (4%)
Hyperplasia, lymphoid				1 (2%)
Spleen	(50)	(48)	(47)	(48)
Accessory spleen	3 (6%)		1 (2%)	1 (2%)
Atrophy			2 (4%)	
Congestion			1 (2%)	
Fibrosis, focal	1 (2%)		1 (2%)	1 (2%)
Hematopoietic cell proliferation	6 (12%)	2 (4%)		
Inflammation, granulomatous		3 (6%)	10 (21%)	3 (6%)
Stromal hyperplasia		1 (2%)		
Capsule, infiltration cellular, focal, mixed cell			2 (4%)	1 (2%)
Thymus	(47)	(50)	(48)	(48)
Angiectasis			1 (2%)	
Cyst	2 (4%)			
Hemorrhage		1 (2%)		
Hyperplasia, lymphoid	1 (2%)		1 (2%)	1 (2%)
Epithelial cell, hyperplasia	1 (2%)			

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Dilatation	37 (74%)	16 (33%)	8 (16%)	1 (2%)
Fibrosis	1 (2%)			
Hyperplasia	6 (12%)	3 (6%)	3 (6%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Inflammation, focal			1 (2%)	
Ulcer	1 (2%)	1 (2%)		1 (2%)
Epidermis, hyperplasia, focal			1 (2%)	2 (4%)
Lip, cyst epithelial inclusion				1 (2%)
Pinna, inflammation, chronic, focal		1 (2%)		
Subcutaneous tissue, edema	1 (2%)	1 (2%)		1 (2%)
Subcutaneous tissue, fibrosis, focal		1 (2%)		
Subcutaneous tissue, hemorrhage, focal			1 (2%)	
Subcutaneous tissue, necrosis, fatty, focal			1 (2%)	
Musculoskeletal System				
None				
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression, focal	13 (26%)	8 (16%)	8 (16%)	2 (4%)
Hemorrhage, focal	1 (2%)	2 (4%)	4 (8%)	2 (4%)
Meninges, hemorrhage, focal				1 (2%)
Thalamus, mineralization, focal			1 (2%)	1 (2%)
Spinal cord	(7)	(3)	(24)	(50)
Hemorrhage, focal				2 (4%)
Necrosis, focal				1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)	5 (10%)	3 (6%)	5 (10%)
Hemorrhage, focal	1 (2%)		2 (4%)	2 (4%)
Hyperplasia, focal, histiocytic		1 (2%)		2 (4%)
Infiltration cellular, mixed cell	3 (6%)	1 (2%)		1 (2%)
Inflammation, chronic, focal	25 (50%)	40 (80%)	39 (78%)	43 (86%)
Alveolar epithelium, hyperplasia, focal	4 (8%)	3 (6%)		2 (4%)
Interstitium, edema	1 (2%)		1 (2%)	
Mediastinum, congestion			1 (2%)	
Mediastinum, edema	1 (2%)	1 (2%)	1 (2%)	
Mediastinum, mineralization, focal			1 (2%)	
Nose	(49)	(50)	(50)	(50)
Foreign body		2 (4%)		
Inflammation, suppurative		3 (6%)	1 (2%)	
Nasolacrimal duct, hemorrhage				1 (2%)
Nasolacrimal duct, inflammation	2 (4%)	5 (10%)	4 (8%)	5 (10%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Special Senses System				
Eye	(48)	(45)	(40)	(45)
Atrophy			3 (8%)	4 (9%)
Cataract	4 (8%)		1 (3%)	1 (2%)
Inflammation, chronic		1 (2%)		1 (2%)
Synechia	1 (2%)			2 (4%)
Anterior chamber, exudate		1 (2%)		1 (2%)
Cornea, inflammation, chronic		1 (2%)		
Cornea, necrosis, focal		1 (2%)	1 (3%)	2 (4%)
Retina, degeneration	4 (8%)	1 (2%)		3 (7%)
Harderian gland	(50)	(50)	(50)	(50)
Cytoplasmic alteration, focal			1 (2%)	
Hyperplasia, focal, histiocytic	1 (2%)	4 (8%)	5 (10%)	
Inflammation	1 (2%)	1 (2%)		
Epithelium, hyperplasia, focal			1 (2%)	
Zymbal's gland		(3)	(1)	
Cyst		1 (33%)		
Urinary System				
Kidney	(48)	(48)	(43)	(43)
Cyst			2 (5%)	
Hydronephrosis				1 (2%)
Infarct	1 (2%)	1 (2%)	1 (2%)	2 (5%)
Mineralization, focal		1 (2%)		
Nephropathy	45 (94%)	37 (77%)	36 (84%)	38 (88%)
Pelvis, dilatation	1 (2%)			
Renal tubule, accumulation, hyaline droplet	3 (6%)		2 (5%)	1 (2%)
Renal tubule, hyperplasia, focal				1 (2%)
Renal tubule, pigmentation				2 (5%)
Urinary bladder	(49)	(50)	(49)	(48)
Hemorrhage			1 (2%)	2 (4%)
Inflammation			1 (2%)	1 (2%)
Transitional epithelium, hyperplasia			1 (2%)	

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF 4-METHYLIMIDAZOLE

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 4-Methylimidazole[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	1			3
Natural deaths	4	6	8	
Other				1
Survivors				
Died last week of study	1			
Terminal sacrifice	44	44	42	46
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine small, duodenum	(50)	(49)	(48)	(50)
Carcinoma		2 (4%)		
Polyp adenomatous		1 (2%)		
Intestine small, jejunum	(48)	(47)	(48)	(50)
Carcinoma		1 (2%)		
Intestine small, ileum	(49)	(46)	(48)	(49)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	1 (2%)
Hemangiosarcoma, multiple			1 (2%)	
Hepatocellular carcinoma	8 (16%)	11 (22%)	10 (20%)	7 (14%)
Hepatocellular carcinoma, multiple	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Hepatocellular adenoma	14 (28%)	7 (14%)	10 (20%)	11 (22%)
Hepatocellular adenoma, multiple	3 (6%)	2 (4%)	1 (2%)	
Mesentery	(4)	(7)	(2)	(3)
Hemangiosarcoma		1 (14%)		1 (33%)
Hepatocellular carcinoma, metastatic, liver		1 (14%)		
Sarcoma, poorly differentiated		1 (14%)		
Oral mucosa			(1)	
Squamous cell carcinoma			1 (100%)	
Pancreas	(49)	(49)	(50)	(50)
Sarcoma, metastatic, mesentery		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma		2 (4%)		3 (6%)
Stomach, glandular	(49)	(50)	(49)	(50)
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Capsule, adenoma	3 (6%)	1 (2%)	1 (2%)	
Capsule, carcinoma		1 (2%)		
Islets, pancreatic	(49)	(49)	(50)	(50)
Adenoma		1 (2%)		1 (2%)
Pituitary gland	(49)	(48)	(49)	(50)
Pars distalis, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	1 (2%)	1 (2%)		
General Body System				
Tissue NOS	(1)			
Schwannoma malignant	1 (100%)			
Genital System				
Prostate	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	1 (2%)
Interstitial cell, adenoma		2 (4%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)		
Lymph node	(2)	(2)	(1)	(2)
Lymph node, mandibular	(48)	(50)	(48)	(45)
Lymph node, mesenteric	(49)	(48)	(48)	(50)
Spleen	(49)	(49)	(50)	(50)
Hemangiosarcoma		1 (2%)		1 (2%)
Thymus	(48)	(49)	(45)	(45)
Integumentary System				
Skin	(50)	(50)	(50)	(49)
Squamous cell carcinoma		1 (2%)		
Sebaceous gland, adenoma				1 (2%)
Musculoskeletal System				
Skeletal muscle	(1)	(2)	(1)	(1)
Carcinoma, metastatic, prostate			1 (100%)	
Hemangiosarcoma	1 (100%)			
Sarcoma, metastatic, mesentery		1 (50%)		
Nervous System				
None				

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	6 (12%)	10 (20%)	10 (20%)	14 (28%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Alveolar/bronchiolar carcinoma	2 (4%)	3 (6%)	4 (8%)	7 (14%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		1 (2%)
Carcinoma, metastatic, prostate			1 (2%)	
Hepatocellular carcinoma, metastatic, liver	3 (6%)	3 (6%)	3 (6%)	3 (6%)
Schwannoma malignant, metastatic, tissue NOS	1 (2%)			
Mediastinum, hemangiosarcoma	1 (2%)			
Special Senses System				
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	8 (16%)	4 (8%)	6 (12%)
Adenoma, multiple	1 (2%)			1 (2%)
Carcinoma	1 (2%)			1 (2%)
Bilateral, adenoma		1 (2%)		
Bilateral, carcinoma		1 (2%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Renal tubule, adenoma			2 (4%)	
Renal tubule, carcinoma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Lymphoma malignant	3 (6%)	3 (6%)	4 (8%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	34	38	36	39
Total primary neoplasms	55	68	57	63
Total animals with benign neoplasms	26	29	23	27
Total benign neoplasms	34	38	31	38
Total animals with malignant neoplasms	15	25	21	22
Total malignant neoplasms	21	30	26	25
Total animals with metastatic neoplasms	4	3	4	3
Total metastatic neoplasms	5	6	6	3

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	4 5 9	5 1 3	6 8 5	6 9 5	7 1 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 3 3	0 3 9	0 3 5	0 5 0	0 0 1	0 0 3	0 0 5	0 0 6	0 0 7	0 1 7	0 1 9	0 2 1	0 2 2	0 2 9	0 3 6	0 4 2	0 4 3	0 4 8	0 4 4	0 0 8	0 1 0	0 1 2	0 1 4	0 1 5	0 1 6
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	M	A	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma	X				X											X									
Hepatocellular carcinoma, multiple				X																					
Hepatocellular adenoma					X				X	X		X		X	X								X		
Hepatocellular adenoma, multiple								X									X								
Mesentery								+									+				+	+			
Pancreas	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Tooth																							+		
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Capsule, adenoma																	X								
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma							X																		
General Body System																									
Tissue NOS					+																				
Schwannoma malignant					X																				
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	00220	00223	00224	00226	00232	00238	00240	00241	00244	00246	00002	00009	00111	00113	00118	00125	00227	00228	00230	00301	00334	00337	00345	00347	00349	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	44
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma								X				X			X	X							X			8
Hepatocellular carcinoma, multiple																		X								2
Hepatocellular adenoma							X	X							X		X			X				X		X	14
Hepatocellular adenoma, multiple																		X								3
Mesentery																										4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell carcinoma												X														1
Stomach, glandular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue												+			+						+			+		4
Tooth	+															+			+							4
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X																								1
Capsule, adenoma													X					X								3
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
General Body System																										
Tissue NOS																										1
Schwannoma malignant																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	4 5 9	5 1 3	6 8 5	6 9 5	7 1 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 3 3	0 3 9	0 3 5	0 5 0	0 0 1	0 0 3	0 0 5	0 0 6	0 0 7	0 0 7	0 1 7	0 1 9	0 2 1	0 2 2	0 2 9	0 3 6	0 4 2	0 4 3	0 4 8	0 4 4	0 0 8	0 1 0	0 1 2	0 1 4	0 1 5	0 1 6
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																										
Lymph node			+																							
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																										
Hemangiosarcoma																										
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma													X													
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma		X																								
Hepatocellular carcinoma, metastatic, liver				X																						
Schwannoma malignant, metastatic, tissue NOS					X																					
Mediastinum, hemangiosarcoma																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X											
Adenoma, multiple																										
Carcinoma					X																					
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver				X																						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 2 0	0 2 3	0 2 4	0 2 6	0 3 2	0 3 8	0 4 0	0 4 1	0 4 4	0 4 6	0 0 2	0 0 9	0 1 1	0 1 3	0 1 8	0 2 5	0 2 7	0 2 8	0 3 0	0 3 1	0 3 4	0 3 7	0 4 5	0 4 7	0 4 9	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma											X															1
Lymph node										+																2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle											+															1
Hemangiosarcoma											X															1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																X	X	X	X					X		6
Alveolar/bronchiolar adenoma, multiple		X					X																			2
Alveolar/bronchiolar carcinoma																		X								2
Hepatocellular carcinoma, metastatic, liver															X							X				3
Schwannoma malignant, metastatic, tissue NOS																										1
Mediastinum, hemangiosarcoma											X															1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma						X					X															3
Adenoma, multiple			X																							1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic, liver																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	4 5 9	5 1 3	6 8 5	6 9 5	7 1 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 3 3	0 3 9	0 3 5	0 5 0	0 0 1	0 0 3	0 0 5	0 0 6	0 0 7	0 1 7	0 1 9	0 2 1	0 2 2	0 2 9	0 3 6	0 4 2	0 4 3	0 4 8	0 4 4	0 0 8	0 1 0	0 1 2	0 1 4	0 1 5	0 1 6
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 2 0	0 2 3	0 2 4	0 2 6	0 3 2	0 3 8	0 4 0	0 4 1	0 4 4	0 4 6	0 4 2	0 0 9	0 1 1	0 1 3	0 1 8	0 2 5	0 2 7	0 2 8	0 3 0	0 3 1	0 3 4	0 3 7	0 4 5	0 4 7	0 4 9	Total Tissues/ Tumors
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Lymphoma malignant	X									X													X				3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	3 3 5	5 8 6	6 1 3	6 7 6	7 1 5	7 1 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 5 1	0 7 1	0 8 2	0 9 9	0 9 7	0 7 0	0 5 2	0 5 3	0 5 6	0 6 1	0 6 5	0 6 6	0 6 7	0 7 4	0 7 6	0 7 8	0 8 4	0 8 6	0 8 9	0 9 0	0 9 8	1 0 0	0 5 5	0 5 7	0 6 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma													X												
Polyp adenomatous																									
Intestine small, jejunum	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, ileum	A	A	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma		X	X	X	X					X							X								
Hepatocellular carcinoma, multiple																									X
Hepatocellular adenoma							X										X								X
Hepatocellular adenoma, multiple																									
Mesentery				+	+																	+			
Hemangiosarcoma																									
Hepatocellular carcinoma, metastatic, liver					X																				
Sarcoma, poorly differentiated				X																					
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, mesentery				X																					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma											X		X												
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue															+										
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma												X													
Capsule, carcinoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X																						
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																			X						
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma									X																
General Body System																									
None																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 6 3	0 6 4	0 6 8	0 7 5	0 7 7	0 7 9	0 8 0	0 8 8	0 9 1	0 9 3	0 9 4	0 9 5	0 9 6	0 5 4	0 5 8	0 5 9	0 6 0	0 6 9	0 7 2	0 7 3	0 8 1	0 8 3	0 8 5	0 8 7	0 9 2	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma							X																			2
Polyp adenomatous										X																1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Carcinoma					X																					1
Intestine small, ileum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma						X	X				X								X						X	11
Hepatocellular carcinoma, multiple																								X		2
Hepatocellular adenoma				X								X									X			X		7
Hepatocellular adenoma, multiple							X		X																	2
Mesentery	+								+		+			+												7
Hemangiosarcoma											X															1
Hepatocellular carcinoma, metastatic, liver																										1
Sarcoma, poorly differentiated																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma, metastatic, mesentery																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma																										1
Capsule, carcinoma																				X						1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	3 3 5	5 8 6	6 1 3	6 7 6	7 1 5	7 1 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 5 1	0 7 1	0 8 2	0 9 9	0 9 7	0 7 0	0 5 2	0 5 3	0 5 6	0 6 1	0 6 5	0 6 6	0 6 7	0 7 4	0 7 6	0 7 8	0 8 4	0 8 6	0 8 9	0 9 0	0 9 8	1 0 0	0 5 5	0 5 7	0 6 2
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis	+																								
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																						X			
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Lymph node						+		+																	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma															X										
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma														X											
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle				+	+																				
Sarcoma, metastatic, mesentery				X																					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X								X						X	X		X		
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma			X																			X			
Alveolar/bronchiolar carcinoma, multiple																					X				
Hepatocellular carcinoma, metastatic, liver				X	X												X								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 6 3	0 6 4	0 6 8	0 7 5	0 7 7	0 7 9	0 8 0	0 8 8	0 9 1	0 9 3	0 9 4	0 9 5	0 9 6	0 5 4	0 5 8	0 5 9	0 6 0	0 6 9	0 7 2	0 7 3	0 8 1	0 8 3	0 8 5	0 8 7	0 9 2	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma	X																									2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma											X															1
Lymph node																										2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Sarcoma, metastatic, mesentery																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X			X	X													X			X				10
Alveolar/bronchiolar adenoma, multiple										X																1
Alveolar/bronchiolar carcinoma		X																								3
Alveolar/bronchiolar carcinoma, multiple																										1
Hepatocellular carcinoma, metastatic, liver																										3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	335	358	361	667	715	716	722	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730
Carcass ID Number	05 51	07 11	08 12	09 29	09 97	07 70	05 02	05 53	05 56	06 01	06 65	06 66	06 67	07 74	07 76	07 78	08 84	08 86	08 89	09 00	09 98	10 08	05 05	05 57	06 62
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X							X			X								X			
Bilateral, adenoma																									
Bilateral, carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant						X		X																X	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
Number of Days on Study	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Total
Carcass ID Number	6	6	6	7	7	7	8	8	9	9	9	9	9	5	5	5	6	6	7	7	8	8	8	8	9	Tissues/
	3	4	8	5	7	9	0	8	1	3	4	5	6	4	8	9	0	9	2	3	1	3	5	7	2	Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X			X																X			X		8
Bilateral, adenoma							X																			1
Bilateral, carcinoma										X																1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																										3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	3 4 8	4 4 1	5 2 2	5 3 5	5 6 9	6 2 3	6 3 2	6 8 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0
Carcass ID Number	1 1 3 8	1 1 3 2	1 1 2 7	1 1 2 3	1 1 4 6	1 1 4 4	1 1 0 4	1 1 4 5	1 1 0 7	1 1 0 9	1 1 1 0	1 1 1 1	1 1 1 2	1 1 1 4	1 1 1 5	1 1 1 6	1 1 1 8	1 1 1 9	1 1 3 7	1 1 3 9	1 1 4 0	1 1 4 1	1 1 4 8	1 1 1	1 1 0 2
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Intestine large, cecum	+	+	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma		X																								
Hemangiosarcoma, multiple																										
Hepatocellular carcinoma				X	X			X	X		X	X	X													
Hepatocellular carcinoma, multiple																										
Hepatocellular adenoma												X														X
Hepatocellular adenoma, multiple													X													
Mesentery																										
Oral mucosa			+																							
Squamous cell carcinoma			X																							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver																										
Tongue																		+						+		
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																										
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																										
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	733	733	733	733	733	733	733	733	733	733	733	733	733	
Carcass ID Number	10003	10005	10113	11117	12020	12201	12214	12225	12238	13035	14043	14477	15070	10606	10808	12222	12626	12909	13030	13311	13313	13334	13346	14292	14449	Total Tissues/ Tumors
Alimentary System																													
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																													1
Hemangiosarcoma, multiple																		X											1
Hepatocellular carcinoma						X			X								X												10
Hepatocellular carcinoma, multiple																									X				1
Hepatocellular adenoma							X		X			X	X	X	X							X		X					10
Hepatocellular adenoma, multiple																													1
Mesentery												+													+				2
Oral mucosa																													1
Squamous cell carcinoma																													1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma, metastatic, liver																									X				1
Tongue																													2
Cardiovascular System																													
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																						X							1
Endocrine System																													
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma																												X	1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																													
None																													

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	3 4 8	4 4 1	5 2 2	5 3 5	5 6 9	6 2 3	6 3 2	6 8 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0
Carcass ID Number	1 1 3 8	1 1 3 2	1 1 2 7	1 1 2 3	1 1 4 6	1 1 4 4	1 1 0 4	1 1 4 5	1 1 0 7	1 1 0 9	1 1 1 0	1 1 1 1	1 1 1 2	1 1 1 4	1 1 1 5	1 1 1 6	1 1 1 8	1 1 1 9	1 1 3 7	1 1 3 9	1 1 4 0	1 1 4 1	1 1 4 8	1 1 0 1	1 1 0 2
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma								X																		
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma		X																								
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																							+			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	
Lymph node, mesenteric	+	+	M	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Thymus	+	+	M	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Skeletal muscle								+																		
Carcinoma, metastatic, prostate								X																		
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma									X			X		X						X			X	X		
Alveolar/bronchiolar adenoma, multiple															X	X										
Alveolar/bronchiolar carcinoma											X													X		
Carcinoma, metastatic, prostate								X																		
Hepatocellular carcinoma, metastatic, liver					X																					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma																X										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	733	733	733	733	733	733	733	733	733	733	733	733	
Carcass ID Number	10003	10005	10113	11117	11220	12201	12214	12225	12238	13035	14043	14477	15070	10606	10808	11222	12026	12209	13030	13311	13334	13616	14242	14449	Total Tissues/ Tumors
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																											1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																											1
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																											1
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	45
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																											1
Carcinoma, metastatic, prostate																											1
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X			X				X							X												10
Alveolar/bronchiolar adenoma, multiple														X													3
Alveolar/bronchiolar carcinoma					X														X								4
Carcinoma, metastatic, prostate																											1
Hepatocellular carcinoma, metastatic, liver						X																		X			3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																											
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma													X	X										X			4

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	3 4 8	4 4 1	5 2 2	5 3 5	5 6 9	6 2 3	6 3 2	6 8 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0
Carcass ID Number	1 3 8	1 3 2	1 2 7	1 2 3	1 4 6	1 4 4	1 0 4	1 4 5	1 0 7	1 0 9	1 1 0	1 1 1	1 1 2	1 1 4	1 1 5	1 1 6	1 1 8	1 1 9	1 3 7	1 3 9	1 4 0	1 4 1	1 4 8	1 1 1	1 0 2	
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																										
Renal tubule, carcinoma																										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant						X																	X			

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	10003	10005	10013	10017	10020	10021	10024	10025	10028	10035	10043	10044	10047	10050	10060	10068	10022	10026	10029	10030	10031	10033	10034	10042	10049	10046	10062	Total Tissues/ Tumors
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma							X																		X			2
Renal tubule, carcinoma																						X						1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+		49
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant	X																						X					4

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	3 5 5	5 3 7	6 2 8	6 4 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	1 8 8	1 5 8	1 7 1	1 7 8	1 6 2	1 6 4	1 6 5	1 6 7	1 7 2	1 8 3	1 8 5	1 9 1	1 9 3	1 5 6	1 5 7	1 5 9	1 6 0	1 6 6	1 6 8	1 6 9	1 7 0	1 7 6	1 7 9	1 8 2	1 8 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma											X														
Hepatocellular carcinoma			X	X					X																X
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma	X					X								X									X		X
Mesentery										+															
Hemangiosarcoma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma										X															
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																			+	+					
Tooth																		+			+				
Cardiovascular System																									
Blood vessel			+																						
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma											X														

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	730	730	730	730	730	730	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	
Carcass ID Number	18486	18487	18489	19090	19094	19098	15151	15152	15153	15154	15155	16161	16163	17733	17734	17735	17737	17740	18181	19012	19025	19026	19027	19029	20200		Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M		48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Intestine small, ileum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Hemangiosarcoma																											1
Hepatocellular carcinoma					X		X															X					7
Hepatocellular carcinoma, multiple															X									X	X		3
Hepatocellular adenoma	X							X			X	X							X				X				11
Mesentery				+																+							3
Hemangiosarcoma																				X							1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Squamous cell papilloma																			X		X						3
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Tongue						+															+						4
Tooth													+														3
Cardiovascular System																											
Blood vessel																											1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Adenoma	X																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
General Body System																											
None																											
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Hemangiosarcoma																											1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	3 5 5	5 3 7	6 2 8	6 4 5	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	1 8 8	1 5 8	1 7 1	1 7 8	1 6 2	1 6 4	1 6 5	1 6 7	1 7 2	1 8 3	1 8 5	1 9 1	1 9 3	1 5 6	1 5 7	1 5 9	1 6 0	1 6 6	1 6 8	1 6 9	1 7 0	1 7 6	1 7 9	1 8 2	1 8 4
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+													+							
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sebaceous gland, adenoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																							+		
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve		+																					+		
Spinal cord		+																					+		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X	X	X		X										X		X	X
Alveolar/bronchiolar adenoma, multiple															X										
Alveolar/bronchiolar carcinoma					X		X								X										
Alveolar/bronchiolar carcinoma, multiple																									
Hepatocellular carcinoma, metastatic, liver				X																					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X	X								X			X		
Adenoma, multiple																	X								
Carcinoma						X																			
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant													X				X								

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	730	730	730	730	730	730	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	
Carcass ID Number	18486	18487	18489	19090	19094	19098	19551	19552	19553	19554	19555	19561	19663	19673	19774	19775	19777	19780	19781	19782	19795	19796	19797	19799	19800		Total Tissues/ Tumors
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Lymph node																											2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+		45
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Hemangiosarcoma								X																			1
Thymus	+	+	+	+	+	M	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	I		45
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+		49
Sebaceous gland, adenoma																X											1
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Skeletal muscle																											1
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Peripheral nerve																											2
Spinal cord																											2
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar adenoma	X			X			X		X	X	X	X															14
Alveolar/bronchiolar adenoma, multiple																											1
Alveolar/bronchiolar carcinoma								X											X	X	X						7
Alveolar/bronchiolar carcinoma, multiple		X																									1
Hepatocellular carcinoma, metastatic, liver															X									X			3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Special Senses System																											
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Adenoma																X			X								6
Adenoma, multiple																											1
Carcinoma																											1
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Lymphoma malignant																											2

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	4/50 (8%)	1/50 (2%)	1/50 (2%)	0/50 (0%)
Adjusted rate[b]	8.3%	2.1%	2.2%	0.0%
Terminal rate[c]	4/45 (9%)	1/44 (2%)	1/42 (2%)	0/46 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	—[e]
Poly-3 test[d]	P=0.034N	P=0.181N	P=0.196N	P=0.062N
Harderian Gland: Adenoma				
Overall rate	4/50 (8%)	9/50 (18%)	4/50 (8%)	7/50 (14%)
Adjusted rate	8.3%	18.7%	8.8%	14.6%
Terminal rate	4/45 (9%)	8/44 (18%)	4/42 (10%)	7/46 (15%)
First incidence (days)	729 (T)	676	729 (T)	729 (T)
Poly-3 test	P=0.373	P=0.115	P=0.613	P=0.257
Harderian Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	10/50 (20%)	4/50 (8%)	8/50 (16%)
Adjusted rate	10.4%	20.8%	8.8%	16.7%
Terminal rate	4/45 (9%)	9/44 (21%)	4/42 (10%)	8/46 (17%)
First incidence (days)	715	676	729 (T)	729 (T)
Poly-3 test	P=0.390	P=0.128	P=0.535N	P=0.270
Kidney (Renal Tubule): Adenoma or Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	6.6%	0.0%
Terminal rate	0/45 (0%)	0/44 (0%)	3/42 (7%)	0/46 (0%)
First incidence (days)	—	—[f]	729 (T)	—
Poly-3 test	P=0.540		P=0.109	—
Liver: Hepatocellular Adenoma				
Overall rate	17/50 (34%)	9/50 (18%)	11/50 (22%)	11/50 (22%)
Adjusted rate	35.2%	18.8%	24.1%	22.6%
Terminal rate	16/45 (36%)	9/44 (21%)	11/42 (26%)	10/46 (22%)
First incidence (days)	715	729 (T)	729 (T)	355
Poly-3 test	P=0.179N	P=0.055N	P=0.170N	P=0.125N
Liver: Hepatocellular Carcinoma				
Overall rate	10/50 (20%)	13/50 (26%)	11/50 (22%)	10/50 (20%)
Adjusted rate	20.3%	26.5%	23.4%	20.6%
Terminal rate	7/45 (16%)	9/44 (21%)	8/42 (19%)	8/46 (17%)
First incidence (days)	459	586	535	628
Poly-3 test	P=0.475N	P=0.315	P=0.453	P=0.585
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	23/50 (46%)	18/50 (36%)	18/50 (36%)	20/50 (40%)
Adjusted rate	46.8%	36.7%	38.3%	40.5%
Terminal rate	20/45 (44%)	14/44 (32%)	15/42 (36%)	17/46 (37%)
First incidence (days)	459	586	535	355
Poly-3 test	P=0.375N	P=0.211N	P=0.264N	P=0.336N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	8/50 (16%)	11/50 (22%)	13/50 (26%)	15/50 (30%)
Adjusted rate	16.6%	22.9%	28.5%	31.4%
Terminal rate	8/45 (18%)	10/44 (23%)	13/42 (31%)	15/46 (33%)
First incidence (days)	729 (T)	716	729 (T)	729 (T)
Poly-3 test	P=0.055	P=0.300	P=0.128	P=0.071

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	4/50 (8%)	8/50 (16%)
Adjusted rate	4.1%	8.3%	8.8%	16.7%
Terminal rate	1/45 (2%)	3/44 (7%)	4/42 (10%)	8/46 (17%)
First incidence (days)	513	613	729 (T)	729 (T)
Poly-3 test	P=0.024	P=0.332	P=0.307	P=0.042
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	9/50 (18%)	13/50 (26%)	16/50 (32%)	22/50 (44%)
Adjusted rate	18.4%	26.9%	35.0%	46.0%
Terminal rate	8/45 (18%)	11/44 (25%)	16/42 (38%)	22/46 (48%)
First incidence (days)	513	613	729 (T)	729 (T)
Poly-3 test	P<0.001	P=0.226	P=0.053	P=0.003
Small Intestine (Duodenum or Jejunum): Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	6.3%	0.0%	0.0%
Terminal rate	0/45 (0%)	3/44 (7%)	0/42 (0%)	0/46 (0%)
First incidence (days)	—	729 (T)	—	—
Poly-3 test	P=0.315N	P=0.118	—	—
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	0/50 (0%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	4.2%	0.0%	6.3%
Terminal rate	0/45 (0%)	2/44 (5%)	0/42 (0%)	3/46 (7%)
First incidence (days)	—	729 (T)	—	729 (T)
Poly-3 test	P=0.093	P=0.236	—	P=0.118
Stomach (Forestomach): Squamous Cell Papilloma or Carcinoma				
Overall rate	1/50 (2%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.1%	4.2%	0.0%	6.3%
Terminal rate	1/45 (2%)	2/44 (5%)	0/42 (0%)	3/46 (7%)
First incidence (days)	729 (T)	729 (T)	—	729 (T)
Poly-3 test	P=0.237	P=0.498	P=0.511N	P=0.303
All Organs: Hemangiosarcoma				
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	3/50 (6%)
Adjusted rate	2.1%	4.2%	6.5%	6.3%
Terminal rate	1/45 (2%)	2/44 (5%)	2/42 (5%)	3/46 (7%)
First incidence (days)	729 (T)	729 (T)	441	729 (T)
Poly-3 test	P=0.224	P=0.498	P=0.292	P=0.303
All Organs: Malignant Lymphoma				
Overall rate	3/50 (6%)	3/50 (6%)	4/50 (8%)	2/50 (4%)
Adjusted rate	6.2%	6.3%	8.7%	4.2%
Terminal rate	3/45 (7%)	2/44 (5%)	3/42 (7%)	2/46 (4%)
First incidence (days)	729 (T)	716	623	729 (T)
Poly-3 test	P=0.426N	P=0.659	P=0.475	P=0.504N
All Organs: Benign Neoplasms				
Overall rate	26/50 (52%)	29/50 (58%)	23/50 (46%)	27/50 (54%)
Adjusted rate	53.8%	59.7%	50.4%	55.4%
Terminal rate	25/45 (56%)	26/44 (59%)	23/42 (55%)	26/46 (57%)
First incidence (days)	715	613	729 (T)	355
Poly-3 test	P=0.508N	P=0.352	P=0.448N	P=0.519

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
All Organs: Malignant Neoplasms				
Overall rate	15/50 (30%)	25/50 (50%)	21/50 (42%)	22/50 (44%)
Adjusted rate	30.1%	50.9%	43.1%	45.4%
Terminal rate	11/45 (24%)	20/44 (46%)	15/42 (36%)	20/46 (44%)
First incidence (days)	459	586	441	628
Poly-3 test	P=0.163	P=0.027	P=0.130	P=0.087
All Organs: Benign or Malignant Neoplasms				
Overall rate	34/50 (68%)	38/50 (76%)	36/50 (72%)	39/50 (78%)
Adjusted rate	68.2%	77.4%	73.8%	79.0%
Terminal rate	30/45 (67%)	33/44 (75%)	30/42 (71%)	36/46 (78%)
First incidence (days)	459	586	441	355
Poly-3 test	P=0.179	P=0.213	P=0.348	P=0.162

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal cortex, kidney, liver, and lung; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE C4
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Feed Studies			
Benzophenone	14/50	2/50	16/50
trans-Cinnamaldehyde	9/100	7/100	14/100
Citral	12/100	9/100	20/100
p,p′-Dichlorodiphenyl sulfone	6/50	7/50	13/50
2-Methylimidazole	11/50	6/50	14/50
4-Methylimidazole	8/50	2/50	9/50
o-Nitrotoluene	9/60	5/60	14/60
p-Nitrotoluene	6/50	2/50	8/50
Overall Historical Incidence: Feed Studies			
Total (%)	75/510 (14.7%)	40/510 (7.8%)	108/510 (21.2%)
Mean ± standard deviation	15.8% ± 6.3%	7.8% ± 3.8%	22.2% ± 6.3%
Range	9%-28%	4%-14%	14%-32%
Overall Historical Incidence			
Total (%)	258/1,507 (17.1%)	151/1,507 (10.0%)	385/1,507 (25.6%)
Mean ± standard deviation	16.7% ± 7.3%	9.9% ± 5.0%	25.1% ± 9.4%
Range	4%-28%	4%-24%	12%-44%

[a] Data as of January 28, 2005

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 4-Methylimidazole[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	1			3
Natural deaths	4	6	8	
Other				1
Survivors				
Died last week of study	1			
Terminal sacrifice	44	44	42	46
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(49)	(47)	(50)
Edema	3 (6%)	1 (2%)	3 (6%)	4 (8%)
Hyperplasia, lymphoid			1 (2%)	
Inflammation			1 (2%)	
Ulcer	1 (2%)		1 (2%)	
Intestine small, jejunum	(48)	(47)	(48)	(50)
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Intestine small, ileum	(49)	(46)	(48)	(49)
Hyperplasia, lymphoid		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Basophilic focus	1 (2%)		1 (2%)	1 (2%)
Clear cell focus	3 (6%)	3 (6%)	2 (4%)	3 (6%)
Cyst		1 (2%)		1 (2%)
Eosinophilic focus	5 (10%)	2 (4%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid				1 (2%)
Infiltration cellular, mixed cell			3 (6%)	1 (2%)
Mixed cell focus	3 (6%)	7 (14%)	2 (4%)	
Necrosis, focal	2 (4%)	3 (6%)	2 (4%)	
Tension lipidosis	1 (2%)		1 (2%)	
Centrilobular, necrosis			1 (2%)	
Hepatocyte, hypertrophy		1 (2%)		1 (2%)
Hepatocyte, vacuolization cytoplasmic	4 (8%)	5 (10%)	2 (4%)	
Mesentery	(4)	(7)	(2)	(3)
Hemorrhage	1 (25%)			
Fat, necrosis	3 (75%)	3 (43%)	2 (100%)	2 (67%)
Pancreas	(49)	(49)	(50)	(50)
Cyst			1 (2%)	
Acinus, hyperplasia, focal				1 (2%)
Stomach, forestomach	(49)	(50)	(50)	(50)
Diverticulum		2 (4%)	1 (2%)	1 (2%)
Erosion			1 (2%)	
Hyperplasia				1 (2%)
Inflammation, chronic			2 (4%)	
Ulcer		1 (2%)	1 (2%)	
Epithelium, hyperplasia	3 (6%)	2 (4%)	4 (8%)	
Stomach, glandular	(49)	(50)	(49)	(50)
Erosion	2 (4%)		1 (2%)	1 (2%)
Glands, hyperplasia				1 (2%)
Tooth	(4)			(3)
Inflammation, chronic	3 (75%)			2 (67%)
Malformation	1 (25%)			

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

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Inflammation, chronic	2 (4%)		2 (4%)	1 (2%)
Mineralization			2 (4%)	
Thrombosis				1 (2%)
Myocardium, necrosis			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	2 (4%)	4 (8%)	6 (12%)	8 (16%)
Hyperplasia, focal	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Hypertrophy, focal	17 (34%)	11 (22%)	10 (20%)	14 (28%)
Capsule, hyperplasia	1 (2%)	1 (2%)	2 (4%)	5 (10%)
Islets, pancreatic	(49)	(49)	(50)	(50)
Hyperplasia	4 (8%)	2 (4%)		1 (2%)
Parathyroid gland	(47)	(49)	(49)	(49)
Cyst	1 (2%)		1 (2%)	1 (2%)
Pituitary gland	(49)	(48)	(49)	(50)
Pars distalis, cyst	1 (2%)	3 (6%)	3 (6%)	2 (4%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicle, cyst	13 (26%)	13 (26%)	13 (26%)	14 (28%)
Follicular cell, hyperplasia		1 (2%)		
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Atypia cellular	1 (2%)	1 (2%)		
Spermatocele	1 (2%)			1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Cyst	29 (58%)	27 (54%)	31 (62%)	36 (72%)
Inflammation, chronic	17 (34%)	19 (38%)	24 (48%)	23 (46%)
Seminal vesicle	(50)	(50)	(50)	(50)
Degeneration	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, atrophy		3 (6%)	1 (2%)	3 (6%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	13 (26%)	15 (30%)	14 (28%)	15 (30%)
Lymph node	(2)	(2)	(1)	(2)
Inguinal, hyperplasia, lymphoid				1 (50%)
Inguinal, pigmentation	1 (50%)			
Pancreatic, hyperplasia, lymphoid				1 (50%)
Lymph node, mandibular	(48)	(50)	(48)	(45)
Hematopoietic cell proliferation		2 (4%)		1 (2%)
Hyperplasia, lymphoid	10 (21%)	8 (16%)	9 (19%)	12 (27%)
Pigmentation	10 (21%)	5 (10%)	7 (15%)	12 (27%)
Lymph node, mesenteric	(49)	(48)	(48)	(50)
Hematopoietic cell proliferation				1 (2%)
Hemorrhage	4 (8%)	7 (15%)	4 (8%)	14 (28%)
Hyperplasia, lymphoid	7 (14%)	4 (8%)	4 (8%)	7 (14%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Hematopoietic System (continued)				
Spleen	(49)	(49)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)		1 (2%)
Hematopoietic cell proliferation	10 (20%)	14 (29%)	9 (18%)	12 (24%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Lymphoid follicle, atrophy		1 (2%)	1 (2%)	1 (2%)
Lymphoid follicle, hyperplasia	10 (20%)	8 (16%)	2 (4%)	7 (14%)
Thymus	(48)	(49)	(45)	(45)
Atrophy	7 (15%)	7 (14%)	5 (11%)	8 (18%)
Integumentary System				
Skin	(50)	(50)	(50)	(49)
Cyst epithelial inclusion				1 (2%)
Edema		2 (4%)		
Ulcer			1 (2%)	
Epidermis, hyperplasia	2 (4%)		2 (4%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy		1 (2%)		1 (2%)
Skeletal muscle	(1)	(2)	(1)	(1)
Atrophy				1 (100%)
Necrosis		1 (50%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Inflammation, chronic	1 (2%)			
Peripheral nerve				(2)
Atrophy				1 (50%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion			1 (2%)	1 (2%)
Hemorrhage	3 (6%)	2 (4%)	1 (2%)	5 (10%)
Hyperplasia, lymphoid	3 (6%)	2 (4%)	6 (12%)	2 (4%)
Infiltration cellular, histiocyte	5 (10%)	6 (12%)	5 (10%)	11 (22%)
Metaplasia, osseous			1 (2%)	
Thrombosis	1 (2%)			
Alveolar epithelium, hyperplasia	7 (14%)	3 (6%)	1 (2%)	9 (18%)
Bronchiole, epithelium, hyperplasia			1 (2%)	1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		2 (4%)	
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	1 (2%)			2 (4%)
Inflammation, chronic	1 (2%)			1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	3 (6%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	13 (26%)	12 (24%)	12 (24%)	7 (14%)
Hydronephrosis			1 (2%)	1 (2%)
Hyperplasia, lymphoid	2 (4%)	2 (4%)		2 (4%)
Inflammation, chronic active		1 (2%)		
Metaplasia, osseous	2 (4%)	3 (6%)		2 (4%)
Mineralization			2 (4%)	
Nephropathy	34 (68%)	40 (80%)	38 (76%)	35 (70%)
Renal tubule, necrosis			1 (2%)	
Urinary bladder	(50)	(49)	(49)	(50)
Edema	1 (2%)	1 (2%)		

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF 4-METHYLIMIDAZOLE

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 4-Methylimidazole[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	3	4	3	2
Natural deaths	4	6	4	8
Survivors				
Died last week of study	1		2	1
Terminal sacrifice	42	40	41	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(48)	(50)	(50)	(49)
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (2%)	
Intestine large, cecum	(49)	(48)	(46)	(46)
Histiocytic sarcoma				1 (2%)
Intestine small, duodenum	(48)	(47)	(49)	(48)
Carcinoma			2 (4%)	
Histiocytic sarcoma				1 (2%)
Polyp adenomatous	1 (2%)			
Intestine small, jejunum	(50)	(48)	(47)	(47)
Intestine small, ileum	(48)	(45)	(48)	(48)
Liver	(50)	(50)	(50)	(50)
Hepatocellular carcinoma	1 (2%)	2 (4%)		1 (2%)
Hepatocellular adenoma	4 (8%)	5 (10%)		1 (2%)
Histiocytic sarcoma		1 (2%)	1 (2%)	2 (4%)
Mast cell tumor malignant				1 (2%)
Sarcoma, metastatic, skin	1 (2%)			
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (2%)	
Mesentery	(3)	(7)	(7)	(4)
Carcinoma, metastatic, mammary gland		1 (14%)		
Histiocytic sarcoma		1 (14%)		
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (14%)	
Pancreas	(49)	(48)	(48)	(49)
Sarcoma, metastatic, skin	1 (2%)			
Salivary glands	(50)	(48)	(50)	(49)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma			2 (4%)	
Squamous cell papilloma	2 (4%)		1 (2%)	2 (4%)
Stomach, glandular	(49)	(49)	(50)	(49)
Adenoma				1 (2%)
Sarcoma, metastatic, skin	1 (2%)			
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (2%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, mammary gland		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Carcinoma, metastatic, mammary gland		1 (2%)		
Capsule, adenoma		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)			1 (2%)
Pheochromocytoma benign		1 (2%)		
Islets, pancreatic	(49)	(48)	(47)	(49)
Adenoma		3 (6%)		
Carcinoma		1 (2%)	1 (2%)	
Pituitary gland	(49)	(46)	(49)	(50)
Pars distalis, adenoma	5 (10%)	4 (9%)	3 (6%)	2 (4%)
Thyroid gland	(50)	(49)	(50)	(48)
Bilateral, adenoma	1 (2%)			
Follicular cell, adenoma			1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Ovary	(48)	(49)	(49)	(50)
Cystadenoma	2 (4%)		1 (2%)	1 (2%)
Granulosa cell tumor benign	2 (4%)			
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma			1 (2%)	
Luteoma		1 (2%)	2 (4%)	2 (4%)
Tubulostromal adenoma		1 (2%)		1 (2%)
Uterus	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Hemangiosarcoma		1 (2%)		1 (2%)
Histiocytic sarcoma	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Leiomyoma			1 (2%)	
Polyp stromal		2 (4%)		2 (4%)
Sarcoma, metastatic, skin	1 (2%)			
Sarcoma stromal			1 (2%)	
Schwannoma malignant	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		1 (2%)
Histiocytic sarcoma				1 (2%)
Mast cell tumor malignant				1 (2%)
Lymph node	(3)	(9)	(12)	(7)
Iliac, histiocytic sarcoma		1 (11%)		
Mediastinal, histiocytic sarcoma				1 (14%)
Pancreatic, squamous cell carcinoma, metastatic, stomach, forestomach			1 (8%)	
Lymph node, mandibular	(47)	(47)	(48)	(48)
Histiocytic sarcoma		1 (2%)		
Lymph node, mesenteric	(49)	(47)	(49)	(50)
Histiocytic sarcoma				1 (2%)
Mast cell tumor malignant				1 (2%)
Sarcoma, metastatic, skin	1 (2%)			
Spleen	(49)	(49)	(49)	(49)
Fibrosarcoma, metastatic, skin	1 (2%)			
Hemangiosarcoma	2 (4%)	2 (4%)		
Histiocytic sarcoma			1 (2%)	
Mast cell tumor malignant				1 (2%)
Thymus	(49)	(49)	(50)	(48)
Integumentary System				
Mammary gland	(50)	(50)	(49)	(49)
Adenoma				1 (2%)
Carcinoma	1 (2%)	1 (2%)		1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma			1 (2%)	
Subcutaneous tissue, fibrosarcoma	1 (2%)			1 (2%)
Subcutaneous tissue, hemangiosarcoma	2 (4%)			1 (2%)
Subcutaneous tissue, melanoma benign		1 (2%)		
Subcutaneous tissue, sarcoma, poorly differentiated	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma				1 (2%)
Skeletal muscle	(1)	(1)	(2)	(2)
Sarcoma, metastatic, skin	1 (100%)			
Squamous cell carcinoma, metastatic, stomach, forestomach			2 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, mammary gland		1 (2%)		
Histiocytic sarcoma		1 (2%)		
Mast cell tumor malignant				1 (2%)
Spinal cord		(2)		(1)
Osteosarcoma, metastatic, bone				1 (100%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		8 (16%)	12 (24%)	6 (12%)
Alveolar/bronchiolar adenoma, multiple			4 (8%)	2 (4%)
Alveolar/bronchiolar carcinoma	3 (6%)		2 (4%)	6 (12%)
Alveolar/bronchiolar carcinoma, multiple				1 (2%)
Carcinoma, metastatic, mammary gland		1 (2%)		
Histiocytic sarcoma				2 (4%)
Mast cell tumor malignant				1 (2%)
Osteosarcoma, metastatic, bone				1 (2%)
Sarcoma, metastatic, skin	1 (2%)			
Nose	(50)	(49)	(50)	(50)
Special Senses System				
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	1 (2%)	5 (10%)	6 (12%)
Carcinoma	1 (2%)	1 (2%)		2 (4%)
Zymbal's gland	(1)		(1)	
Carcinoma	1 (100%)		1 (100%)	
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Carcinoma, metastatic, mammary gland		1 (2%)		
Histiocytic sarcoma			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Sarcoma, metastatic, skin	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Lymphoma malignant	7 (14%)	10 (20%)	12 (24%)	13 (26%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	29	36	37	40
Total primary neoplasms	46	50	55	66
Total animals with benign neoplasms	18	23	23	21
Total benign neoplasms	23	28	30	28
Total animals with malignant neoplasms	20	20	23	29
Total malignant neoplasms	23	22	25	38
Total animals with metastatic neoplasms	2	1	2	1
Total metastatic neoplasms	9	6	7	2

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	1 1 4	5 3 9	5 3 9	5 5 5	5 9 3	7 0 6	7 0 7	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	2 3 7	2 0 1	2 4 8	2 0 6	2 2 2	2 4 7	2 4 1	2 0 4	2 0 2	2 0 3	2 0 5	2 1 1	2 1 2	2 1 3	2 1 4	2 1 5	2 1 6	2 1 7	2 1 8	2 1 9	2 2 0	2 3 1	2 3 2	2 3 3	2 3 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular adenoma										X															X
Sarcoma, metastatic, skin							X																		
Mesentery														+					+						
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin							X																		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																	X								
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin							X																		
Tongue																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant										X															
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma														X								X	X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, adenoma										X															
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	2 3 5	2 3 6	2 3 8	2 3 9	2 4 0	2 4 2	2 4 3	2 4 4	2 4 5	2 4 6	2 4 9	2 5 0	2 0 7	2 0 8	2 0 9	2 1 0	2 2 1	2 2 3	2 2 4	2 2 5	2 2 6	2 2 7	2 2 8	2 2 9	2 3 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Polyp adenomatous			X																							1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																X										1
Hepatocellular adenoma	X												X													4
Sarcoma, metastatic, skin																										1
Mesentery																							+			3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma, metastatic, skin																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																			X							2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma, metastatic, skin																										1
Tongue						+		+											+							3
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma																X							X			5
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, adenoma																										1
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	1 1 4	5 3 9	5 3 9	5 5 5	5 9 3	7 0 6	7 0 7	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	2 3 7	2 0 1	2 4 8	2 0 6	2 2 2	2 4 7	2 4 1	2 0 4	2 0 2	2 0 3	2 0 5	2 1 1	2 1 2	2 1 3	2 1 4	2 1 5	2 1 6	2 1 7	2 1 8	2 1 9	2 2 0	2 3 1	2 3 2	2 3 3	2 3 4
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																										
Granulosa cell tumor benign																										X
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																										
Sarcoma, metastatic, skin							X																			
Schwannoma malignant																										
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																										
Lymph node, mandibular	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin							X																			
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin				X																						
Hemangiosarcoma						X			X																	
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma												X														
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma				X																						
Subcutaneous tissue, hemangiosarcoma									X																	X
Subcutaneous tissue, sarcoma, poorly differentiated							X																			
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle							+																			
Sarcoma, metastatic, skin							X																			
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma															X											
Sarcoma, metastatic, skin							X																			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	2 3 5	2 3 6	2 3 8	2 3 9	2 4 0	2 4 2	2 4 3	2 4 4	2 4 5	2 4 6	2 4 9	2 5 0	2 0 7	2 0 8	2 0 9	2 1 0	2 2 1	2 2 3	2 2 4	2 2 5	2 2 6	2 2 7	2 2 8	2 2 9	2 3 0	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	M	+	+	+	+	+	+	+	+	48
Cystadenoma												X	X													2
Granulosa cell tumor benign																							X			2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma													X													1
Sarcoma, metastatic, skin																										1
Schwannoma malignant																							X			1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+												+						+							3
Lymph node, mandibular	+	+	I	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma, metastatic, skin																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrosarcoma, metastatic, skin																										1
Hemangiosarcoma																										2
Thymus	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, hemangiosarcoma																										2
Subcutaneous tissue, sarcoma, poorly differentiated																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Sarcoma, metastatic, skin																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma			X														X									3
Sarcoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	1 1 4	5 3 9	5 3 9	5 5 5	5 9 3	7 0 6	7 0 7	7 3 0	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	2 3 7	2 0 1	2 4 8	2 0 6	2 2 2	2 4 7	2 4 1	2 0 4	2 0 2	2 0 3	2 0 5	2 1 1	2 1 2	2 1 3	2 1 4	2 1 5	2 1 6	2 1 7	2 1 8	2 1 9	2 2 0	2 3 1	2 3 2	2 3 3	2 3 4
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X										X				X					X		
Carcinoma																										
Zymbal's gland			+																							
Carcinoma			X																							
Urinary System																										
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin							X																			
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																										
Lymphoma malignant													X													

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 0 ppm

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	2 3 5	2 3 6	2 3 8	2 3 9	2 4 0	2 4 2	2 4 3	2 4 4	2 4 5	2 4 6	2 4 9	2 5 0	2 0 7	2 0 8	2 0 9	2 1 0	2 2 1	2 2 3	2 2 4	2 2 5	2 2 6	2 2 7	2 2 8	2 2 9	2 3 0	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X																					X			6
Carcinoma							X																			1
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, skin																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma													X													1
Lymphoma malignant	X		X							X			X	X					X							7

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	5 2 5	6 2 9	6 3 2	6 5 6	6 6 8	6 9 7	7 0 2	7 0 5	7 2 1	7 2 7	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5
Carcass ID Number	2 5 4	2 6 3	2 6 8 1	2 6 8 5	2 6 6 8	2 6 8 4	2 6 9 3	2 6 9 6	2 6 9 2	2 6 8 7	2 8 5 1	2 5 5 2	2 5 5 3	2 5 5 5	2 6 6 6	2 6 6 7	2 6 6 9	2 7 7 0	2 7 7 1	2 7 7 2	2 7 7 3	2 7 7 4	2 7 7 5	2 5 6 6	2 5 6 7
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	A	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	I	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	A	+	A	+	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma				X																					
Hepatocellular adenoma												X													
Histiocytic sarcoma	X																								
Mesentery	+					+		+										+							
Carcinoma, metastatic, mammary gland						X																			
Histiocytic sarcoma	X																								
Pancreas	+	+	A	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	M	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue											+														
Tooth										+															
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland						X																			
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland						X																			
Capsule, adenoma																	X								
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	A	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X																			X	
Carcinoma																									X
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma													X										X		
Thyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	2 5 8	2 5 9	2 6 0	2 6 1	2 6 2	2 6 4	2 6 5	2 8 2	2 8 3	2 8 6	2 8 8	2 8 9	2 9 0	2 7 6	2 7 7	2 7 8	2 7 9	2 8 0	2 9 1	2 9 4	2 9 5	2 9 7	2 9 8	2 9 9	3 0 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																						X				2
Hepatocellular adenoma									X				X								X	X				5
Histiocytic sarcoma																										1
Mesentery													+	+			+									7
Carcinoma, metastatic, mammary gland																										1
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue																+			+							3
Tooth																										1
Cardiovascular System																										
Blood vessel																						+				1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, mammary gland																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, mammary gland																										1
Capsule, adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign														X												1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma									X																	3
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pars distalis, adenoma																				X					X	4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	5 2 5	6 2 9	6 3 2	6 5 6	6 6 8	6 9 7	7 0 2	7 0 5	7 2 1	7 2 7	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5
Carcass ID Number	2 5 4	2 6 3	2 8 1	2 8 5	2 6 8	2 8 4	2 9 3	2 9 6	2 9 2	2 8 7	2 5 1	2 5 2	2 5 3	2 5 5	2 6 6	2 6 7	2 6 9	2 7 0	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 5 6	2 5 7
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Luteoma								X																	
Tubulostromal adenoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma							X																		
Hemangiosarcoma									X																
Histiocytic sarcoma	X																								
Polyp stromal																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Lymph node	+	+	+			+		+		+						+									
Iliac, histiocytic sarcoma	X																								
Lymph node, mandibular	+	M	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Lymph node, mesenteric	+	M	A	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																			X						
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma						X																			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, melanoma benign																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle								+																	
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland						X																			
Histiocytic sarcoma	X																								
Peripheral nerve	+						+																		
Spinal cord	+						+																		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma			X														X								
Carcinoma, metastatic, mammary gland						X																			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	2 5 8	2 5 9	2 6 0	2 6 1	2 6 2	2 6 4	2 6 5	2 8 2	2 8 3	2 8 6	2 8 8	2 8 9	2 9 0	2 7 6	2 7 7	2 7 8	2 7 9	2 8 0	2 9 1	2 9 4	2 9 5	2 9 7	2 9 8	2 9 9	3 0 0	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	49
Hemangiosarcoma		X																								1
Luteoma																										1
Tubulostromal adenoma	X																									1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Hemangiosarcoma																										1
Histiocytic sarcoma																										1
Polyp stromal								X			X															2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma							X																			1
Lymph node															+								+			9
Iliac, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma							X																			2
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, melanoma benign															X											1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, mammary gland																										1
Histiocytic sarcoma																										1
Peripheral nerve																										2
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X						X			X					X			X		X		8
Carcinoma, metastatic, mammary gland																										1
Nose	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	5 2 5	6 2 9	6 3 2	6 5 6	6 6 8	6 9 7	7 0 2	7 0 5	7 2 1	7 2 7	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5
Carcass ID Number	2 5 4	2 6 3	2 8 1	2 8 5	2 6 8	2 8 4	2 9 3	2 9 6	2 9 2	2 8 7	2 5 1	2 5 2	2 5 3	2 5 5	2 6 6	2 6 7	2 6 9	2 7 0	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 5 6	2 5 7
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X														
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland						X																			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Lymphoma malignant		X			X			X	X					X		X						X			

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 312 ppm

Number of Days on Study	735	735	735	735	735	735	735	735	735	735	735	735	735	735	736	736	736	736	736	736	736	736	737	737	737	737	
Carcass ID Number	25 8	25 9	26 0	26 1	26 2	26 4	26 5	28 2	28 3	28 6	28 8	28 9	29 0	27 6	27 7	27 8	27 9	28 0	29 1	29 4	29 5	29 7	29 8	29 9	30 0	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma																			X							1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, mammary gland																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant	X														X						X					10

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	5 3 9	5 4 1	6 3 5	6 8 4	6 8 5	7 0 7	7 2 0	7 3 2	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 2 4	3 3 3	3 3 2	3 4 7	3 4 1	3 1 8	3 0 2	3 1 0	3 0 6	3 0 7	3 0 8	3 0 9	3 1 6	3 1 7	3 1 9	3 2 0	3 4 0	3 1 1	3 1 2	3 1 3	3 1 4	3 1 5	3 2 1	3 2 2	3 2 3
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, stomach, forestomach								X																	
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	M	+	+	+	+	A	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma										X															
Intestine small, jejunum	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Squamous cell carcinoma, metastatic, stomach, forestomach																	X								
Mesentery					+	+	+									+	+								+
Squamous cell carcinoma, metastatic, stomach, forestomach																	X								
Pancreas	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma								X									X								
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, stomach, forestomach								X																	
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	M	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Carcinoma																									X
Parathyroid gland	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
None																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 2 5	3 2 6	3 2 7	3 2 8	3 2 9	3 3 0	3 3 6	3 3 7	3 3 8	3 3 9	3 4 2	3 4 3	3 4 4	3 4 5	3 4 6	3 4 8	3 4 9	3 5 0	3 5 1	3 3 4	3 3 5	3 0 1	3 0 3	3 0 4	3 0 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																									X	2
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Mesentery																				+						7
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										2
Squamous cell papilloma		X																								1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma			X	X							X															3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma													X													1
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	539	541	635	684	685	707	720	732	733	734	734	734	734	734	734	734	734	735	735	735	735	735	735	735	735
Carcass ID Number	32 4	33 3	33 2	34 7	44 1	11 8	02 2	10 0	10 6	00 7	00 8	00 9	11 6	11 7	11 9	12 0	24 0	11 1	11 2	11 3	11 4	11 5	12 1	22 2	22 3
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																					X				
Histiocytic sarcoma																						X			
Luteoma																					X				
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																		X				X			
Leiomyoma																		X							
Sarcoma stromal																								X	
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+		+	+		+											+		+	+			
Pancreatic, squamous cell carcinoma, metastatic, stomach, forestomach								X																	
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma	X																								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle								+									+								
Squamous cell carcinoma, metastatic, stomach, forestomach								X									X								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X																			X	X	X
Alveolar/bronchiolar adenoma, multiple									X	X			X		X										
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 2 5	3 2 6	3 2 7	3 2 8	3 2 9	3 3 0	3 3 6	3 3 7	3 3 8	3 3 9	3 4 2	3 4 3	3 4 4	3 4 5	3 4 6	3 4 8	3 4 9	3 5 0	3 3 1	3 3 4	3 3 5	3 0 1	3 0 3	3 0 4	3 0 5	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cystadenoma																										1
Histiocytic sarcoma																										1
Luteoma	X																									2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Leiomyoma																										1
Sarcoma stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+				+								+				+	+								12
Pancreatic, squamous cell carcinoma, metastatic, stomach, forestomach																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Squamous cell carcinoma, metastatic, stomach, forestomach																										2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X						X	X	X					X			X	X			X		12
Alveolar/bronchiolar adenoma, multiple																										4
Alveolar/bronchiolar carcinoma				X												X										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	5 3 9	5 4 1	6 3 5	6 8 4	6 8 5	7 0 7	7 2 0	7 3 2	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 2 4	3 3 3	3 3 2	3 4 7	3 4 1	3 1 8	3 0 2	3 1 0	3 0 0	3 0 7	3 0 8	3 0 9	3 1 6	3 1 7	3 1 9	3 2 0	3 4 0	3 1 1	3 1 2	3 1 3	3 1 4	3 1 5	3 2 1	3 2 2	3 2 3
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X								X			X					X	X
Zymbal's gland																						+			
Carcinoma																						X			
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																						X			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X										X				X			
Lymphoma malignant		X	X		X	X	X					X							X						

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 625 ppm

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 2 5	3 2 6	3 2 7	3 2 8	3 2 9	3 3 0	3 3 6	3 3 7	3 3 8	3 3 9	3 4 2	3 4 3	3 4 4	3 4 5	3 4 6	3 4 8	3 4 9	3 5 0	3 3 1	3 3 4	3 3 5	3 0 1	3 0 3	3 0 4	3 0 5	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										5
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										3
Lymphoma malignant	X				X								X				X	X								12

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	4 0 9	4 4 7	5 5 2	5 6 9	6 4 0	6 4 7	6 6 5	6 8 5	6 8 7	7 1 1	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	3 9 4	3 8 0	3 6 1	3 9 8	3 5 7	3 7 0	3 7 6	3 8 9	3 6 0	3 8 7	3 5 6	3 5 8	3 5 9	3 9 1	3 9 2	3 9 3	3 9 5	3 9 6	3 5 1	3 5 2	3 5 3	3 5 4	3 5 5	3 8 1	3 8 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine large, rectum	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Intestine small, duodenum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Intestine small, jejunum	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular adenoma															X										
Histiocytic sarcoma						X										X									
Mast cell tumor malignant										X															
Mesentery															+										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																				X					
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Adenoma																									
Tongue																			+						
Tooth																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant				X																					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma											X			X											
Thyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma												X													
General Body System																									
Tissue NOS																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 8 3	3 8 4	3 8 5	3 6 2	3 6 3	3 6 4	3 6 5	3 7 1	3 7 2	3 7 3	3 7 4	3 7 5	3 7 7	3 7 8	3 7 9	3 9 7	3 9 9	4 0 0	3 8 6	3 8 8	3 9 0	3 6 6	3 6 7	3 6 8	3 6 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Histiocytic sarcoma																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																					X					1
Hepatocellular adenoma																										1
Histiocytic sarcoma																										2
Mast cell tumor malignant																										1
Mesentery		+														+				+						4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma								X																		2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																				X						1
Tongue														+		+										3
Tooth														+												1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma																										2
Thyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell, adenoma																										1
General Body System																										
Tissue NOS														+												1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	4 0 9	4 4 7	5 5 2	5 6 9	6 4 0	6 4 7	6 6 5	6 8 5	6 8 7	7 1 1	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	3 9 4	3 8 0	3 6 1	3 9 8	3 5 7	3 7 0	3 7 6	3 8 9	3 6 0	3 8 7	3 5 6	3 5 8	3 5 9	3 9 1	3 9 2	3 9 3	3 9 5	3 9 6	3 5 1	3 5 2	3 5 3	3 5 4	3 5 5	3 8 1	3 8 2
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									
Luteoma									X																
Tubulostromal adenoma						X																			
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Histiocytic sarcoma						X										X									
Polyp stromal																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma							X																		
Histiocytic sarcoma						X																			
Mast cell tumor malignant										X															
Lymph node	+	+			+	+					+											+			+
Mediastinal, histiocytic sarcoma						X																			
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Mast cell tumor malignant										X															
Spleen	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant										X															
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma								X																	
Subcutaneous tissue, hemangiosarcoma							X																		
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma			X																						
Skeletal muscle			+			+																			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant										X															
Peripheral nerve			+			+																			
Spinal cord			+																						
Osteosarcoma, metastatic, bone			X																						

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 8 3	3 8 4	3 8 5	3 6 2	3 6 3	3 6 4	3 6 5	3 7 1	3 7 2	3 7 3	3 7 4	3 7 5	3 7 7	3 7 8	3 7 9	3 9 7	3 9 9	4 0 0	3 8 6	3 8 8	3 9 0	3 6 6	3 6 7	3 6 8	3 6 9	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma								X																		1
Luteoma																			X							2
Tubulostromal adenoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma													X													1
Histiocytic sarcoma																										2
Polyp stromal					X				X																	2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Histiocytic sarcoma																										1
Mast cell tumor malignant																										1
Lymph node																										7
Mediastinal, histiocytic sarcoma																										1
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Mast cell tumor malignant																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Mast cell tumor malignant																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma													X													1
Carcinoma						X																				1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Skeletal muscle																										2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mast cell tumor malignant																										1
Peripheral nerve																										2
Spinal cord																										1
Osteosarcoma, metastatic, bone																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	4 0 9	4 4 7	5 5 2	5 6 9	6 4 0	6 4 7	6 6 5	6 8 5	6 8 7	7 1 1	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	3 9 4	3 8 0	3 6 1	3 9 8	3 5 7	3 7 0	3 7 6	3 8 9	3 6 0	3 8 7	3 5 6	3 5 8	3 5 9	3 9 1	3 9 2	3 9 3	3 9 5	3 9 6	3 5 1	3 5 2	3 5 3	3 5 4	3 5 5	3 5 8	3 8 1	3 8 2
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma															X		X					X			
Alveolar/bronchiolar adenoma, multiple											X							X							
Alveolar/bronchiolar carcinoma									X																
Alveolar/bronchiolar carcinoma, multiple																									
Histiocytic sarcoma						X										X									
Mast cell tumor malignant										X															
Osteosarcoma, metastatic, bone			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X															X	X	
Carcinoma										X															
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X										X									
Lymphoma malignant	X	X			X				X		X				X			X			X			X	X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4-Methylimidazole: 1,250 ppm

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 7	7 3 7	7 3 7	7 3 7	
Carcass ID Number	3 8 3	3 8 4	3 8 5	3 6 2	3 6 3	3 6 4	3 6 5	3 7 1	3 7 2	3 7 3	3 7 4	3 7 5	3 7 7	3 7 8	3 7 9	3 9 7	3 9 9	4 0 0	3 8 6	3 8 8	3 9 0	3 6 6	3 6 7	3 6 8	3 6 9	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X			X				X																	6
Alveolar/bronchiolar adenoma, multiple																										2
Alveolar/bronchiolar carcinoma									X			X		X				X						X		6
Alveolar/bronchiolar carcinoma, multiple																							X			1
Histiocytic sarcoma																										2
Mast cell tumor malignant																										1
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma	X				X															X						6
Carcinoma														X												2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymphoma malignant	X																					X			X	13

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Harderian Gland: Adenoma				
Overall rate[a]	6/50 (12%)	1/50 (2%)	5/50 (10%)	6/50 (12%)
Adjusted rate[b]	12.8%	2.1%	10.4%	13.0%
Terminal rate[c]	5/43 (12%)	1/40 (3%)	4/43 (9%)	5/40 (13%)
First incidence (days)	593	729 (T)	720	685
Poly-3 test[d]	P=0.323	P=0.054N	P=0.486N	P=0.608
Harderian Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	2/50 (4%)	5/50 (10%)	8/50 (16%)
Adjusted rate	14.9%	4.2%	10.4%	17.3%
Terminal rate	6/43 (14%)	2/40 (5%)	4/43 (9%)	6/40 (15%)
First incidence (days)	593	729 (T)	720	685
Poly-3 test	P=0.233	P=0.076N	P=0.365N	P=0.486
Liver: Hepatocellular Adenoma				
Overall rate	4/50 (8%)	5/50 (10%)	0/50 (0%)	1/50 (2%)
Adjusted rate	8.6%	10.5%	0.0%	2.2%
Terminal rate	4/43 (9%)	5/40 (13%)	0/43 (0%)	1/40 (3%)
First incidence (days)	729 (T)	729 (T)	—[e]	729 (T)
Poly-3 test	P=0.048N	P=0.516	P=0.057N	P=0.183N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	5/50 (10%)	6/50 (12%)	0/50 (0%)	2/50 (4%)
Adjusted rate	10.7%	12.5%	0.0%	4.3%
Terminal rate	5/43 (12%)	5/40 (13%)	0/43 (0%)	2/40 (5%)
First incidence (days)	729 (T)	656	—	729 (T)
Poly-3 test	P=0.065N	P=0.521	P=0.028N	P=0.221N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	0/50 (0%)	8/50 (16%)	16/50 (32%)	8/50 (16%)
Adjusted rate	0.0%	16.6%	33.2%	17.4%
Terminal rate	0/43 (0%)	7/40 (18%)	15/43 (35%)	8/40 (20%)
First incidence (days)	—	632	684	729 (T)
Poly-3 test	P=0.017	P=0.004	P<0.001	P=0.003
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)	7/50 (14%)
Adjusted rate	6.4%	0.0%	4.2%	15.2%
Terminal rate	3/43 (7%)	0/40 (0%)	2/43 (5%)	6/40 (15%)
First incidence (days)	729 (T)	—	729 (T)	687
Poly-3 test	P=0.019	P=0.114N	P=0.487N	P=0.154
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	3/50 (6%)	8/50 (16%)	17/50 (34%)	14/50 (28%)
Adjusted rate	6.4%	16.6%	35.3%	30.3%
Terminal rate	3/43 (7%)	7/40 (18%)	16/43 (37%)	13/40 (33%)
First incidence (days)	729 (T)	632	684	687
Poly-3 test	P=0.002	P=0.109	P<0.001	P=0.002
Pancreatic Islets: Adenoma				
Overall rate	0/49 (0%)	3/48 (6%)	0/47 (0%)	0/49 (0%)
Adjusted rate	0.0%	6.5%	0.0%	0.0%
Terminal rate	0/43 (0%)	2/40 (5%)	0/42 (0%)	0/39 (0%)
First incidence (days)	—	668	—[f]	—
Poly-3 test	P=0.314N	P=0.118	—	—

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	0/49 (0%)	4/48 (8%)	1/47 (2%)	0/49 (0%)
Adjusted rate	0.0%	8.6%	2.2%	0.0%
Terminal rate	0/43 (0%)	3/40 (8%)	1/42 (2%)	0/39 (0%)
First incidence (days)	—	668	729 (T)	—
Poly-3 test	P=0.304N	P=0.060	P=0.494	—
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	5/49 (10%)	4/46 (9%)	3/49 (6%)	2/50 (4%)
Adjusted rate	11.0%	9.0%	6.4%	4.3%
Terminal rate	5/42 (12%)	4/38 (11%)	3/43 (7%)	2/40 (5%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.145N	P=0.519N	P=0.340N	P=0.212N
Stomach (Forestomach): Squamous Cell Papilloma or Carcinoma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.3%	0.0%	6.3%	4.3%
Terminal rate	2/43 (5%)	0/40 (0%)	3/43 (7%)	2/40 (5%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P=0.410	P=0.232N	P=0.513	P=0.689
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	4/50 (8%)	0/50 (0%)	2/50 (4%)
Adjusted rate	6.4%	8.4%	0.0%	4.3%
Terminal rate	2/43 (5%)	3/40 (8%)	0/43 (0%)	1/40 (3%)
First incidence (days)	706	721	—	665
Poly-3 test	P=0.263N	P=0.513	P=0.114N	P=0.504N
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.2%	2.1%	6.3%	4.3%
Terminal rate	1/43 (2%)	0/40 (0%)	3/43 (7%)	1/40 (3%)
First incidence (days)	729 (T)	525	729 (T)	647
Poly-3 test	P=0.316	P=0.752N	P=0.316	P=0.498
All Organs: Malignant Lymphoma				
Overall rate	7/50 (14%)	10/50 (20%)	12/50 (24%)	13/50 (26%)
Adjusted rate	15.0%	20.6%	24.4%	27.0%
Terminal rate	7/43 (16%)	6/40 (15%)	7/43 (16%)	9/40 (23%)
First incidence (days)	729 (T)	629	541	409
Poly-3 test	P=0.100	P=0.329	P=0.187	P=0.119
All Organs: Benign Neoplasms				
Overall rate	18/50 (36%)	23/50 (46%)	23/50 (46%)	21/50 (42%)
Adjusted rate	38.2%	47.5%	47.7%	45.0%
Terminal rate	17/43 (40%)	20/40 (50%)	21/43 (49%)	18/40 (45%)
First incidence (days)	593	632	684	647
Poly-3 test	P=0.347	P=0.241	P=0.234	P=0.325
All Organs: Malignant Neoplasms				
Overall rate	20/50 (40%)	20/50 (40%)	23/50 (46%)	29/50 (58%)
Adjusted rate	41.7%	40.3%	46.2%	58.0%
Terminal rate	16/43 (37%)	12/40 (30%)	17/43 (40%)	19/40 (48%)
First incidence (days)	539	525	539	409
Poly-3 test	P=0.038	P=0.525N	P=0.406	P=0.078

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	29/50 (58%)	36/50 (72%)	37/50 (74%)	40/50 (80%)
Adjusted rate	59.9%	72.0%	74.0%	80.0%
Terminal rate	24/43 (56%)	27/40 (68%)	30/43 (70%)	30/40 (75%)
First incidence (days)	539	525	539	409
Poly-3 test	P=0.025	P=0.146	P=0.101	P=0.023

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, pancreatic islets, and pituitary gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE D4
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F$_1$ Mice

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Feed Studies			
Benzophenone	0/50	1/50	1/50
trans-Cinnamaldehyde	3/100	2/100	5/100
Citral	5/99	6/99	11/99
p,p′-Dichlorodiphenyl sulfone	0/50	0/50	0/50
2-Methylimidazole	4/50	0/50	4/50
4-Methylimidazole	0/50	3/50	3/50
o-Nitrotoluene	2/60	3/60	5/60
p-Nitrotoluene	5/50	1/50	6/50
Overall Historical Incidence: Feed Studies			
Total (%)	19/509 (3.7%)	16/509 (3.1%)	35/509 (6.9%)
Mean ± standard deviation	3.7% ± 3.8%	2.9% ± 2.5%	6.6% ± 4.2%
Range	0%-10%	0%-6%	0%-12%
Overall Historical Incidence			
Total (%)	80/1,552 (5.2%)	40/1,552 (2.6%)	117/1,552 (7.5%)
Mean ± standard deviation	5.1% ± 3.5%	2.5% ± 2.6%	7.4% ± 3.8%
Range	0%-12%	0%-12%	0%-14%

[a] Data as of January 28, 2005

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 4-Methylimidazole[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	3	4	3	2
Natural death	4	6	4	8
Survivors				
Died last week of study	1		2	1
Terminal sacrifice	42	40	41	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(49)	(48)	(46)	(46)
Edema	3 (6%)	4 (8%)	2 (4%)	2 (4%)
Intestine small, jejunum	(50)	(48)	(47)	(47)
Hyperplasia, lymphoid	4 (8%)	2 (4%)	2 (4%)	
Intestine small, ileum	(48)	(45)	(48)	(48)
Hyperplasia, lymphoid	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis			2 (4%)	
Basophilic focus	1 (2%)		1 (2%)	1 (2%)
Cyst	3 (6%)			1 (2%)
Eosinophilic focus	1 (2%)	2 (4%)	1 (2%)	
Hematopoietic cell proliferation	6 (12%)		5 (10%)	3 (6%)
Hemorrhage				1 (2%)
Hyperplasia, lymphoid	2 (4%)	3 (6%)	1 (2%)	
Infiltration cellular, mixed cell	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Mixed cell focus	1 (2%)		1 (2%)	
Necrosis, focal	4 (8%)	3 (6%)	2 (4%)	6 (12%)
Centrilobular, necrosis	1 (2%)		1 (2%)	1 (2%)
Hepatocyte, hypertrophy	1 (2%)			
Hepatocyte, vacuolization cytoplasmic	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Kupffer cell, pigmentation	1 (2%)	1 (2%)	2 (4%)	
Mesentery	(3)	(7)	(7)	(4)
Hemorrhage				1 (25%)
Fat, necrosis	3 (100%)	3 (43%)	3 (43%)	3 (75%)
Pancreas	(49)	(48)	(48)	(49)
Atrophy		1 (2%)		
Cyst	3 (6%)	1 (2%)		
Hyperplasia, lymphoid		1 (2%)		1 (2%)
Necrosis	1 (2%)			
Acinus, cytoplasmic alteration	1 (2%)	1 (2%)		
Salivary glands	(50)	(48)	(50)	(49)
Atrophy				2 (4%)
Hyperplasia, lymphoid	3 (6%)	4 (8%)	3 (6%)	3 (6%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum	2 (4%)			
Hyperplasia, squamous			1 (2%)	
Inflammation, chronic			1 (2%)	1 (2%)
Ulcer	1 (2%)	1 (2%)		
Epithelium, hyperplasia	3 (6%)	5 (10%)	2 (4%)	5 (10%)
Stomach, glandular	(49)	(49)	(50)	(49)
Erosion			2 (4%)	1 (2%)

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

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		1 (2%)	
Mineralization				4 (8%)
Thrombosis			1 (2%)	
Myocardium, necrosis		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	9 (18%)	9 (18%)	9 (18%)	5 (10%)
Hyperplasia, diffuse	1 (2%)			
Hyperplasia, focal			1 (2%)	2 (4%)
Capsule, hyperplasia		2 (4%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia			1 (2%)	2 (4%)
Islets, pancreatic	(49)	(48)	(47)	(49)
Hyperplasia	1 (2%)			2 (4%)
Parathyroid gland	(49)	(49)	(48)	(47)
Cyst		1 (2%)	5 (10%)	2 (4%)
Pituitary gland	(49)	(46)	(49)	(50)
Pars distalis, cyst	1 (2%)	3 (7%)	4 (8%)	
Pars distalis, hyperplasia, focal	2 (4%)	4 (9%)	1 (2%)	
Thyroid gland	(50)	(49)	(50)	(48)
Follicle, cyst	20 (40%)	22 (45%)	29 (58%)	30 (63%)
Follicular cell, hyperplasia	1 (2%)		1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Inflammation, chronic	1 (2%)	3 (6%)	5 (10%)	3 (6%)
Ovary	(48)	(49)	(49)	(50)
Angiectasis	9 (19%)	9 (18%)	7 (14%)	6 (12%)
Cyst	16 (33%)	20 (41%)	14 (29%)	19 (38%)
Inflammation, granulomatous		1 (2%)		
Corpus luteum, hyperplasia				1 (2%)
Granulosa cell, hyperplasia	1 (2%)	1 (2%)		
Thecal cell, hyperplasia			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Angiectasis		3 (6%)		3 (6%)
Hyperplasia, cystic	45 (90%)	47 (94%)	46 (92%)	47 (94%)
Inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Metaplasia, squamous	2 (4%)	3 (6%)	1 (2%)	5 (10%)
Thrombosis			1 (2%)	

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm		312 ppm		625 ppm		1,250 ppm	
Hematopoietic System								
Bone marrow	(50)		(50)		(50)		(50)	
Hyperplasia	13	(26%)	13	(26%)	10	(20%)	17	(34%)
Infiltration cellular, mast cell			1	(2%)				
Lymph node	(3)		(9)		(12)		(7)	
Bronchial, hyperplasia, lymphoid					1	(8%)		
Iliac, hemorrhage			1	(11%)			1	(14%)
Iliac, hyperplasia, lymphoid			2	(22%)	1	(8%)	1	(14%)
Iliac, pigmentation							1	(14%)
Inguinal, hyperplasia, lymphoid			2	(22%)	1	(8%)		
Mediastinal, hyperplasia, lymphoid			2	(22%)				
Renal, hematopoietic cell proliferation					1	(8%)		
Renal, hyperplasia, lymphoid					1	(8%)	1	(14%)
Renal, pigmentation							1	(14%)
Lymph node, mandibular	(47)		(47)		(48)		(48)	
Hematopoietic cell proliferation	1	(2%)			2	(4%)		
Hemorrhage					1	(2%)	3	(6%)
Hyperplasia, lymphoid	6	(13%)	8	(17%)	7	(15%)	13	(27%)
Pigmentation	12	(26%)	19	(40%)	18	(38%)	19	(40%)
Lymph node, mesenteric	(49)		(47)		(49)		(50)	
Atrophy			2	(4%)				
Ectasia	1	(2%)			1	(2%)		
Hematopoietic cell proliferation	2	(4%)	1	(2%)	2	(4%)		
Hyperplasia, lymphoid	3	(6%)	8	(17%)	9	(18%)	10	(20%)
Spleen	(49)		(49)		(49)		(49)	
Hematopoietic cell proliferation	15	(31%)	16	(33%)	13	(27%)	11	(22%)
Hyperplasia, lymphoid	8	(16%)	11	(22%)	8	(16%)	11	(22%)
Pigmentation			1	(2%)	1	(2%)		
Lymphoid follicle, atrophy	1	(2%)	1	(2%)			1	(2%)
Lymphoid follicle, hyperplasia	14	(29%)	12	(24%)	12	(24%)	13	(27%)
Red pulp, congestion							1	(2%)
Thymus	(49)		(49)		(50)		(48)	
Atrophy	3	(6%)	6	(12%)	2	(4%)	5	(10%)
Hyperplasia, lymphoid	3	(6%)	1	(2%)	2	(4%)		
Integumentary System								
Mammary gland	(50)		(50)		(49)		(49)	
Galactocele							1	(2%)
Hyperplasia	16	(32%)	10	(20%)	14	(29%)	24	(49%)
Skin	(50)		(50)		(50)		(50)	
Edema					1	(2%)	1	(2%)
Epidermis, hyperplasia			1	(2%)				
Subcutaneous tissue, inflammation, granulomatous	1	(2%)						
Musculoskeletal System								
Bone	(50)		(50)		(50)		(50)	
Hyperostosis	10	(20%)	12	(24%)	10	(20%)	6	(12%)
Skeletal muscle	(1)		(1)		(2)		(2)	
Mineralization							1	(50%)

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 4-Methylimidazole

	0 ppm	312 ppm	625 ppm	1,250 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression				1 (2%)
Hemorrhage		2 (4%)	1 (2%)	2 (4%)
Peripheral nerve		(2)		(2)
Atrophy				1 (50%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)	2 (4%)	3 (6%)	4 (8%)
Hyperplasia, lymphoid	6 (12%)	6 (12%)	8 (16%)	8 (16%)
Infiltration cellular, histiocyte	1 (2%)	5 (10%)	1 (2%)	8 (16%)
Metaplasia, osseous	1 (2%)			
Alveolar epithelium, hyperplasia	3 (6%)	2 (4%)	3 (6%)	11 (22%)
Nose	(50)	(49)	(50)	(50)
Inflammation, chronic	1 (2%)			
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract			2 (4%)	1 (2%)
Inflammation, chronic			1 (2%)	1 (2%)
Cornea, hyperplasia			1 (2%)	
Cornea, mineralization			1 (2%)	
Retina, degeneration			1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Hyperplasia, focal	2 (4%)	3 (6%)	2 (4%)	
Inflammation, chronic	1 (2%)			
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Cyst	1 (2%)	1 (2%)		
Hydronephrosis		1 (2%)		
Hyperplasia, lymphoid	1 (2%)	2 (4%)	6 (12%)	3 (6%)
Metaplasia, osseous	1 (2%)	1 (2%)		1 (2%)
Nephropathy	21 (43%)	20 (40%)	17 (34%)	25 (50%)
Renal tubule, accumulation, hyaline droplet	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Renal tubule, hyperplasia	1 (2%)			
Renal tubule, necrosis		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Edema			1 (2%)	1 (2%)
Hemorrhage		1 (2%)		
Hyperplasia, lymphoid	1 (2%)	3 (6%)	4 (8%)	3 (6%)
Inflammation, chronic	1 (2%)	1 (2%)		

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1988). 4-Methylimidazole was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella 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 4-methylimidazole. For the study conducted at SRI International, the high dose was limited by experimental design to 10,000 µg/plate. For the study conducted at Environmental Health Research and Testing, Inc., the highest concentration tested was limited to 33 µg/plate by toxicity. All trials in both laboratories were repeated at the same or a higher S9 fraction.

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. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by 4-methylimidazole exposure. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Groups of male F344/N rats and B6C3F$_1$ mice were injected intraperitoneally (three times at 24-hour intervals) with 4-methylimidazole dissolved in phosphate-buffered saline. Solvent control animals were injected with solvent only. The positive control animals 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 for the frequency of micronucleated cells in each of up to five animals per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the bone marrow was scored for each dose group as a measure of cytotoxicity.

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 dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed 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 dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted previously). 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 study, in which 4-methylimidazole was administered via dosed feed, peripheral blood samples were obtained from male and female mice (NTP, 2004a). Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were 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 dose group. In addition, the percentage of PCEs among the total erythrocyte population in peripheral blood was scored for each group as a measure of bone marrow toxicity.

The results for NCEs were tabulated as described for PCEs in the bone marrow micronucleus test. Results of the 14-week studies were accepted without repeat tests because additional data could not be obtained.

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 summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

4-Methylimidazole (1 to 10,000 µg/plate) was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535, when tested with and without 10% or 30% hamster or rat liver S9 activation enzymes (Table E1). In addition, no increases in the frequencies of micronucleated erythrocytes were seen in bone marrow of male rats (Table E2) or male mice (Table E3) administered 4-methylimidazole by intraperitoneal injection three times at 24-hour intervals or in peripheral blood samples from male or female mice administered the compound in dosed feed for 14 weeks (Table E4). In the mouse bone marrow micronucleus test, two trials were conducted; a significant increase in micronucleated PCEs was seen in the first trial, but the response was not replicated in a repeat trial (Trial 2), and the test was judged to be negative overall. No significant alterations in percent PCEs, a rough indicator of bone marrow toxicity, were seen in the mouse bone marrow or peripheral blood tests, but in bone marrow of male rats, percent PCEs declined with increasing dose of 4-methylimidazole and was significantly depressed at the highest dose.

TABLE E1
Mutagenicity of 4-Methylimidazole in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 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 E1
Mutagenicity of 4-Methylimidazole in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 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 E2
Induction of Micronuclei in Bone Marrow Erythrocytes of Male Rats Treated with 4-Methylimidazole by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Male Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	Pairwise P Value[c]	PCEs[b] (%)
Phosphate-buffered saline[d]					
	0	5	1.70 ± 0.25		47.80 ± 3.04
4-Methylimidazole	25	5	1.60 ± 0.19	0.5692	42.6 ± 3.5
	50	5	1.40 ± 0.29	0.7051	40.5 ± 3.6
	100	4	0.88 ± 0.24	0.9341	30.8 ± 2.5
			P = 0.939[e]		
Cyclophosphamide[f]	7.5	5	22.30 ± 1.62	0.0000	33.0 ± 3.5

[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; dosed groups significant at P≤0.008; positive control significant at P≤0.05 (ILS, 1990)
[d] Solvent control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)
[f] Positive control

TABLE E3
Induction of Micronuclei in Bone Marrow Erythrocytes of Male Mice Treated with 4-Methylimidazole by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Male Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	Pairwise P Value[c]	PCE[b] (%)
Trial 1					
Phosphate-buffered saline[d]	0	5	2.20 ± 0.44		54.4 ± 0.8
4-Methylimidazole	25	5	2.50 ± 0.22	0.3307	51.4 ± 2.3
	50	5	4.30 ± 1.08	0.0045	53.8 ± 2.9
	100	5	4.10 ± 0.58	0.0083	48.7 ± 2.3
			P = 0.003[e]		
Cyclophosphamide[f]	25	5	31.30 ± 1.81	0.0000	44.0 ± 1.5
Trial 2					
Phosphate-buffered saline	0	5	2.50 ± 0.22		48.1 ± 3.6
4-Methylimidazole	25	5	3.00 ± 0.27	0.2498	51.8 ± 5.7
	50	5	3.10 ± 0.66	0.2110	46.8 ± 3.3
	100	5	2.40 ± 0.56	0.5569	53.4 ± 2.3
			P = 0.614		
Cyclophosphamide	10	5	12.90 ± 1.26	0.0000	49.0 ± 1.9

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al.* (1993).
[b] PCE=polychromatic erythrocyte
[c] Mean ± standard error
[d] Pairwise comparison with the solvent control; dosed groups significant at $P \leq 0.008$; positive control significant at $P \leq 0.05$ (ILS, 1990)
[e] Solvent control
[f] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at $P \leq 0.025$ (ILS, 1990)
Positive control

TABLE E4
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice 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]	Pairwise P Value[c]	PCE[b] (%)
Male					
NIH-07 feed[d]	0	5	1.90 ± 0.56		7.3 ± 0.7
4-Methylimidazole	625	5	1.70 ± 0.20	0.6307	9.0 ± 0.8
	1,250	5	1.90 ± 0.33	0.5000	8.2 ± 0.7
	2,500	5	2.10 ± 0.24	0.3758	7.2 ± 1.0
	5,000	5	2.50 ± 0.59	0.1826	6.7 ± 1.2
	10,000	3	1.83 ± 0.33	0.5376	8.0 ± 1.3
			P = 0.326[e]		
Female					
NIH-07 feed	0	5	2.30 ± 0.25		8.1 ± 1.6
4-Methylimidazole	625	5	2.40 ± 0.43	0.4419	5.8 ± 0.8
	1,250	5	2.50 ± 0.35	0.3863	7.3 ± 0.5
	2,500	5	1.70 ± 0.44	0.8289	7.5 ± 1.1
	5,000	5	2.50 ± 0.32	0.3863	6.7 ± 0.4
	10,000	5	2.90 ± 0.70	0.2024	6.6 ± 0.4
			P = 0.153		

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

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF 4-METHYLIMIDAZOLE

4-Methylimidazole was obtained from Sigma Chemical Company (St. Louis, MO) in one lot (116H0901), which was used during the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Battelle Columbus Operations (Columbus, OH), Galbraith Laboratories, Inc. (Knoxville, TN), and the study laboratory, Southern Research Institute (Birmingham, AL). Stability analyses were performed by the analytical chemistry laboratory. Reports on analyses performed in support of the 4-methylimidazole studies are on file at the National Institute of Environmental Health Sciences.

Lot 116H0901 of the chemical, a white powder, was identified as 4-methylimidazole by the analytical chemistry laboratory using infrared (IR), ultraviolet/visible, and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using IR and proton NMR spectroscopy. All spectra were consistent with literature spectra (Aldrich, 1985, 1992) and the structure of 4-methylimidazole. The IR, proton NMR, and carbon-13 NMR spectra are presented in Figures F1 through F3, respectively. The melting point range (51° to 53° C) was consistent with that given on the manufacturer's Certificate of Analysis for a different lot obtained from TCI America (Portland, OR).

The moisture content of lot 116H0901 was determined by Galbraith Laboratories, Inc., using Karl Fischer titration; this laboratory also performed elemental analyses of lot 116H0901. The purity of lot 116H0901 was determined by the analytical chemistry laboratory by titration of the amine functional group of 4-methylimidazole with certified 0.0998 N HCl to neutrality. The test chemical was dissolved in water, and the titration was monitored with a combined pH glass electrode. Additional purity determinations conducted by the analytical chemistry laboratory included gas chromatography (GC) and high-performance liquid chromatography (HPLC) by system A (Table F1). GC was performed with a gas chromatograph (Hewlett-Packard, Palo Alto, CA) using a flame ionization detector with a helium carrier gas flow rate of 9 mL/minute. A Carbowax™-amine column (30 m × 0.53 mm ID, 1.0 µm film thickness; Supelco, Inc., Bellefonte, PA) was used with an oven temperature program of 100° C for 3 minutes, then 10° C per minute to 200° C, followed by a 15-minute hold. GC by a similar system was used by the study laboratory to assess the purity of lot 116H0901.

For lot 116H0901, Karl Fischer titration indicated 0.12% water. Elemental analyses for carbon, hydrogen, and nitrogen were consistent with the theoretical values for 4-methylimidazole. Amine functional group titration showed a purity of approximately 100%. The GC purity profile determined by the analytical chemistry laboratory indicated a major peak and two unidentified volatile impurities of 0.1% and 0.6% relative to the major peak area; sample purity was estimated to be 99.3%. HPLC analysis by system A detected two volatile impurities with a combined relative area of 0.5% of the major peak. GC conducted by the study laboratory indicated an area percent purity of 99.3% for lot 116H0901 and a relative purity of 98% when compared to a frozen reference sample of the same lot obtained from the analytical chemistry laboratory. The overall purity of lot 116H0901 was determined to be greater than 99%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC as described for the purity analyses. These studies indicated that 4-methylimidazole was stable as a bulk chemical for at least 14 days when stored in Teflon®-sealed amber glass vials at temperatures up to 60° C. However, refrigeration was recommended to maintain the powder form of the chemical to facilitate mixing. To ensure stability, the bulk chemical was stored at 5° C in Teflon®-sealed containers, protected from light and moisture. Stability was monitored by the study laboratory during the 2-year studies using the GC system described for the purity analyses. No degradation of the bulk chemical relative to a frozen reference sample of the same lot was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared every 2 weeks by mixing 4-methylimidazole with feed (Table F2). A premix was prepared by hand and then blended with additional feed in a Patterson-Kelly V-shell blender for 30 minutes using an intensifier bar. Dose formulations in double-thickness plastic bags were placed into heavy-duty opaque plastic bags, sealed in plastic containers, and stored at approximately 5° C for up to 36 days.

Homogeneity studies of 100 and 2,400 ppm dose formulations or 300 and 5,000 ppm dose formulations were performed by the analytical chemistry laboratory and the study laboratory with HPLC by systems B and C, respectively. Stability studies of a 100 ppm dose formulation were performed by the analytical chemistry laboratory using HPLC by system B. Homogeneity was confirmed, and stability was confirmed for at least 36 days for dose formulations stored in refrigerated, sealed, opaque plastic containers and for at least 7 days under simulated animal room conditions.

Periodic analyses of the dose formulations of 4-methylimidazole were conducted by the study laboratory using HPLC by system C. During the 2-year studies, the dose formulations were analyzed at least every 12 weeks; animal room samples were also analyzed (Table F3). Of the dose formulations analyzed and used, 140 of 141 for rats and all 74 for mice were within 10% of the target concentrations. Of the animal room samples analyzed, all 16 samples for rats and 9 of 12 samples for mice were within 10% of the target concentrations. All animal room samples were within 15% of target concentrations.

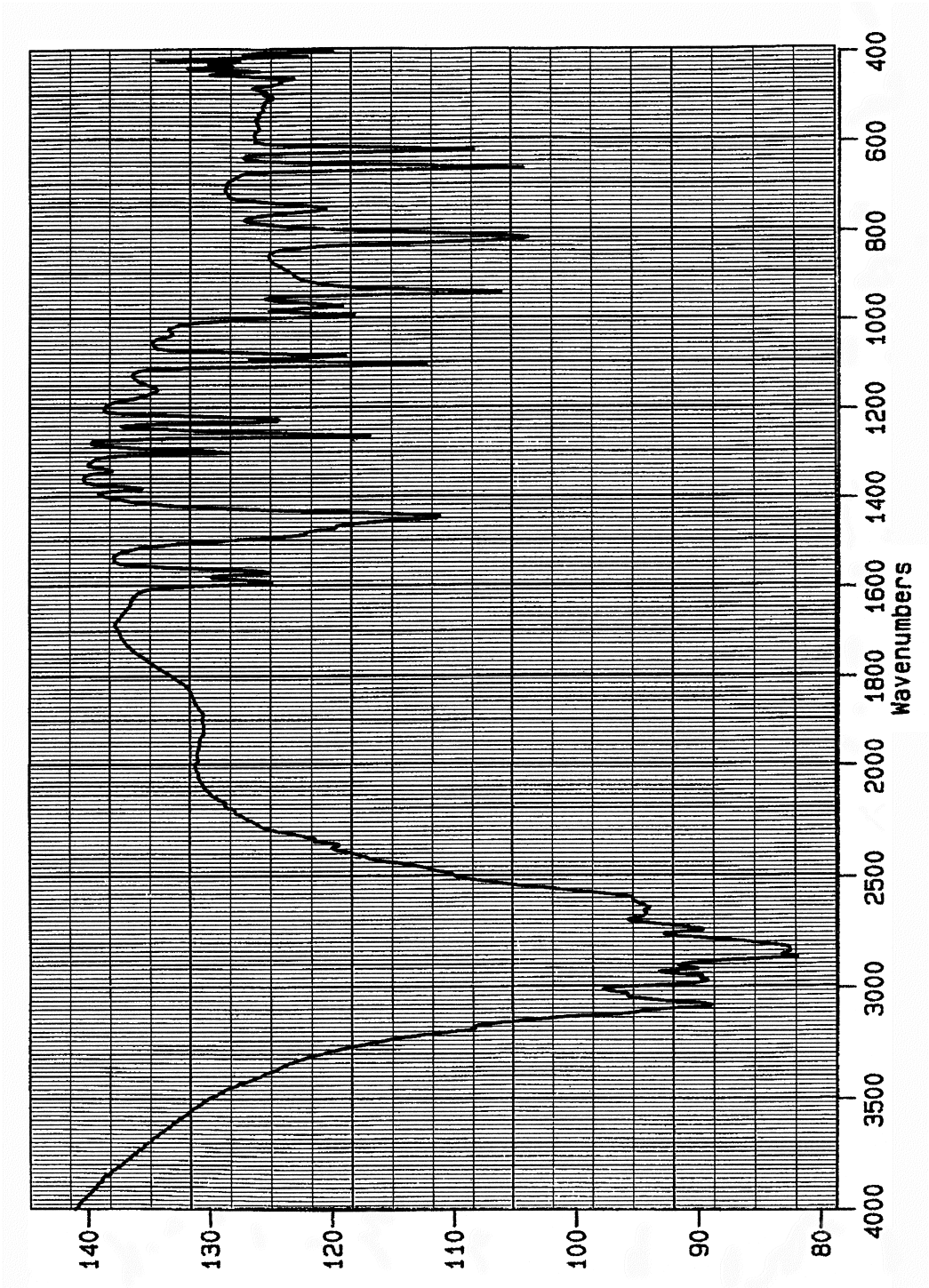

FIGURE F1
Infrared Absorption Spectrum of 4-Methylimidazole

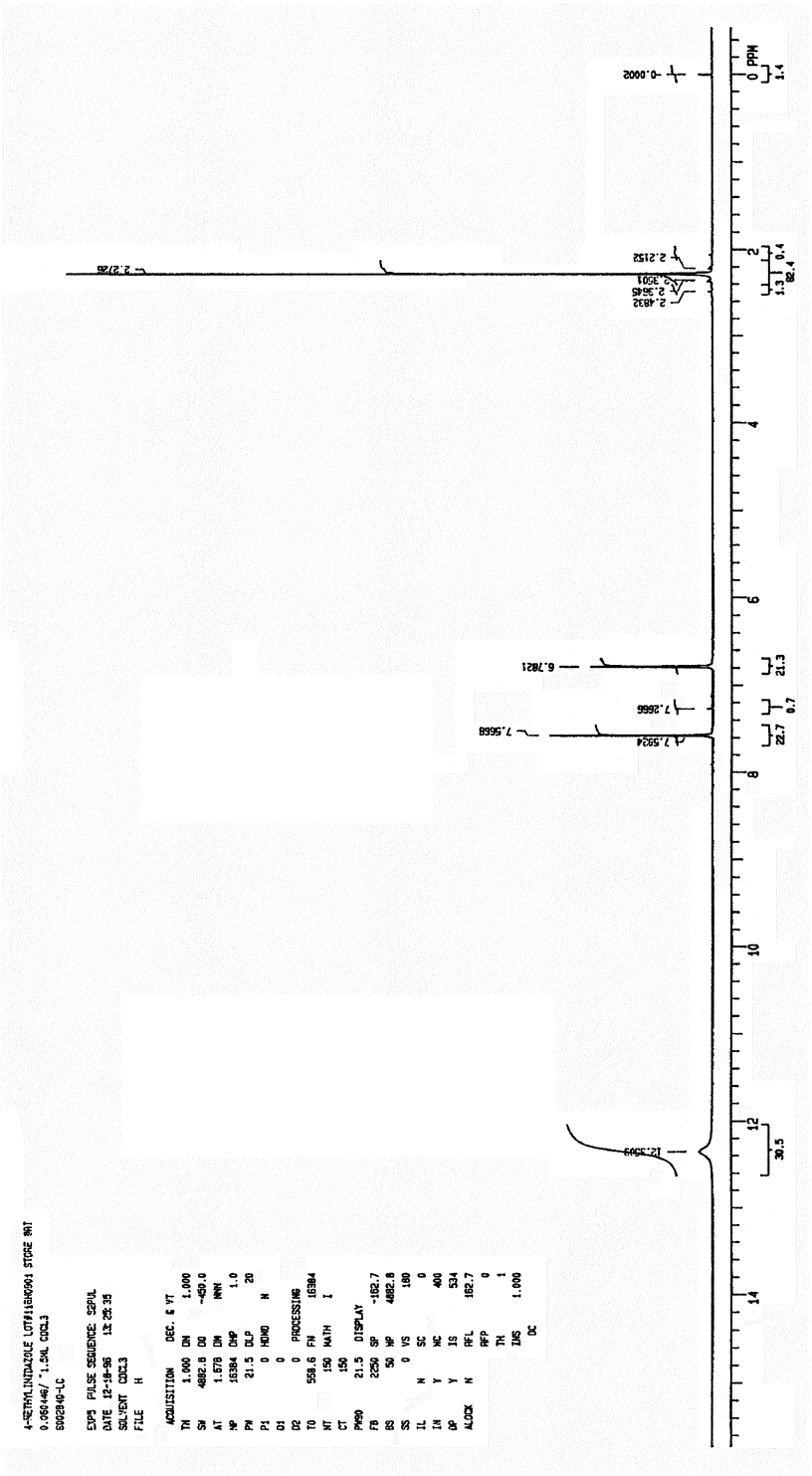

FIGURE F2
Proton Nuclear Magnetic Resonance Spectrum of 4-Methylimidazole

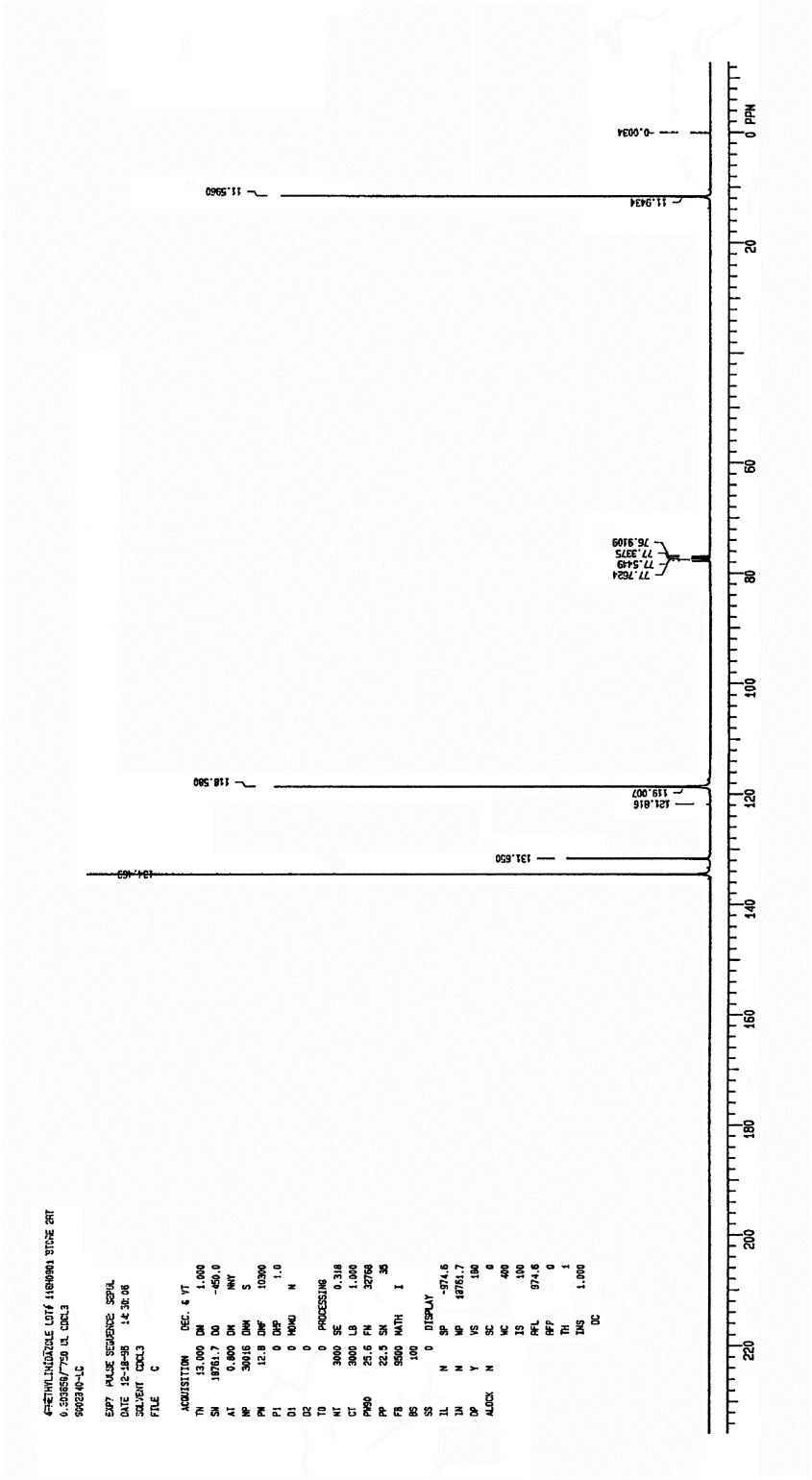

FIGURE F3
Carbon-13 Nuclear Magnetic Resonance Spectrum of 4-Methylimidazole

TABLE F1
High-Performance Liquid Chromatography Systems Used in the 2-Year Feed Studies of 4-Methylimidazole[a]

Detection System	Column	Solvent System
System A Ultraviolet (221 nm) light	Prodigy™ C8, 250 mm × 4.6 mm, 5µ (Phenomenex, Inc., Torrance, CA)	A) 0.0375 M sodium dihydrogen phosphate monohydrate and 0.00375 M sodium dodecyl sulfate in solvent A and B) methanol (50% A; 50% B), isocratic; flow rate = 0.8 mL/minute
System B Ultraviolet (215 nm) light	Prodigy™ C8, 250 mm × 4.6 mm, 5µ (Phenomenex, Inc.)	A) 0.075 M sodium dihydrogen phosphate monohydrate and 0.0075 M sodium dodecyl sulfate in water and B) methanol (50% A; 50% B), isocratic; flow rate = 0.8 mL/minute
System C Ultraviolet (215 nm) light	Primesphere™ C18-HC, 250 mm × 4.6 mm, 5µ (Phenomenex, Inc.)	A) 0.075 M sodium dihydrogen phosphate monohydrate and 0.0075 M sodium dodecyl sulfate in water and B) methanol (60% A; 40% B), isocratic; flow rate = 1.0 mL/minute

[a] The high-performance liquid chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA).

TABLE F2
Preparation and Storage of Dose Formulations in the 2-Year Feed Studies of 4-Methylimidazole

Preparation
Dose formulations were prepared every 2 weeks. A premix was prepared by grinding the required weight of 4-methylimidazole and a portion of preweighed feed with a glass mortar and pestle. The thoroughly ground and mixed contents of the mortar were combined with an additional portion of preweighed feed in a stainless steel mixing bowl, and the contents were stirred with a stainless steel spatula until visibly homogenous. Additional plain feed was used to rinse the mortar and approximately double the premix volume in several steps until a total of 500 to 1,500 grams of premix was prepared in the mixing bowl. Layers of the premix and plain feed were interleaved in the Patterson Kelly blender until the mixing bowl was empty. The mixing bowl was rinsed with a portion of the remaining plain feed, and the rinsate was added to the blender. All remaining feed in the preweighed bag was added directly into the blender, and blending was conducted for 30 minutes with the intensifier bar turned on.

Chemical Lot Number
116H0901

Maximum Storage Time
36 days

Storage Conditions
Dose formulations in double-thickness plastic bags were placed into heavy-duty opaque plastic bags, sealed in plastic containers, and stored at approximately 5° C.

Study Laboratory
Southern Research Institute (Birmingham, AL)

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration[a] (mg/g)	Difference from Target (%)
Rats				
January 12-14, 2000	January 13-15, 2000	0.625	0.617	–1
		0.625	0.610	–2
		0.625	0.638	+2
		0.625	0.623	0
		0.625	0.624	0
		1.25	1.26	+1
		1.25	1.28	+2
		1.25	1.28	+2
		1.25	1.27	+2
		1.25	1.24	–1
		1.25	1.25	0
		2.50	2.54	+2
		2.50	2.38	–5
		2.50	2.49	0
		2.50	2.44	–2
		2.50	2.55	+2
		2.50	2.56	+2
		2.50	2.55	+2
		5.00	5.00	0
		5.00	5.15	+3
		5.00	5.07	+1
	February 10-11, 2000[b]	0.625	0.581	–7
		1.25	1.17	–6
		2.50	2.38	–5
		5.00	4.82	–4
March 22 and 24, 2000	March 27-29, 2000	0.625	0.579	–7
		0.625	0.594	–5
		0.625	0.661	+6
		1.25	1.14	–9
		1.25	1.17	–6
		1.25	1.23	–2
		1.25	1.23	–2
		2.50	2.43	–3
		2.50	2.17[c]	–13
		2.50	2.40	–4
		5.00	4.79	–4
		5.00	4.81	–4
April 5, 2000	April 5-6, 2000	2.50	2.50[d]	0

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
June 14 and 16, 2000	June 19-20, 2000	0.625	0.611	–2
		0.625	0.597	–4
		0.625	0.631	+1
		0.625	0.611	–2
		1.25	1.20	–4
		1.25	1.18	–6
		1.25	1.21	–3
		1.25	1.24	–1
		1.25	1.23	–2
		2.50	2.51	0
		2.50	2.50	0
		2.50	2.49	0
		2.50	2.36	–6
		5.00	4.87	–3
		5.00	4.81	–4
		5.00	4.90	–2
August 23, 2000	August 28-29, 2000	0.625	0.629	+1
		0.625	0.626	0
		0.625	0.607	–3
		1.25	1.25	0
		1.25	1.25	0
		1.25	1.24	–1
		1.25	1.24	–1
		2.50	2.44	–2
		2.50	2.48	–1
		2.50	2.48	–1
		2.50	2.46	–2
		5.00	4.92	–2
		5.00	5.00	0
	September 22-23, 2000[b]	0.625	0.601	–4
		1.25	1.16	–7
		2.50	2.41	–4
		5.00	4.84	–3
November 15-16, 2000	November 16-18, 2000	0.625	0.629	+1
		0.625	0.606	–3
		0.625	0.606	–3
		0.625	0.614	–2
		1.25	1.25	0
		1.25	1.25	0
		1.25	1.27	+2
		1.25	1.22	–2
		2.50	2.57	+3
		2.50	2.57	+3
		2.50	2.53	+1
		2.50	2.47	–1
		5.00	5.06	+1
		5.00	5.03	+1

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
February 7, 2001	February 8-9, 2001	0.625	0.616	–1
		0.625	0.617	–1
		0.625	0.612	–2
		1.25	1.15	–8
		1.25	1.21	–3
		1.25	1.21	–3
		1.25	1.24	–1
		2.50	2.47	–1
		2.50	2.45	–2
		2.50	2.42	–3
		2.50	2.52	+1
		5.00	4.52	–10
		5.00	4.87	–3
April 18-19, 2001	April 19-20, 2001	0.625	0.608	–3
		0.625	0.613	–2
		0.625	0.633	+1
		1.25	1.23	–2
		1.25	1.23	–2
		1.25	1.22	–2
		1.25	1.23	–2
		2.50	2.46	–2
		2.50	2.47	–1
		2.50	2.45	–2
		2.50	2.46	–2
		5.00	5.05	+1
		5.00	4.97	–1
	May 17-19, 2001[b]	0.625	0.579	–7
		1.25	1.19	–5
		2.50	2.34	–6
		5.00	4.69	–6
June 27-28, 2001	June 28-29, 2001	0.625	0.588	–6
		0.625	0.610	–2
		0.625	0.613	–2
		1.25	1.25	0
		1.25	1.24	–1
		1.25	1.23	–2
		1.25	1.23	–2
		2.50	2.49	0
		2.50	2.55	+2
		2.50	2.52	+1
		2.50	2.52	+1
		5.00	5.05	+1
		5.00	4.99	0

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
September 5, 2001	September 6-7, 2001	0.625	0.608	–3
		0.625	0.596	–5
		0.625	0.650	+4
		1.25	1.19	–5
		1.25	1.23	–2
		1.25	1.21	–3
		1.25	1.23	–2
		2.50	2.43	–3
		2.50	2.47	–1
		2.50	2.45	–2
		2.50	2.47	–1
		5.00	4.97	–1
		5.00	4.83	–3
November 14, 2001	November 15-16, 2001	0.625	0.616	–1
		0.625	0.629	+1
		1.25	1.27	+2
		1.25	1.23	–2
		1.25	1.23	–2
		1.25	1.22	–2
		2.50	2.52	+1
		2.50	2.53	+1
		2.50	2.47	–1
		2.50	2.48	–1
		5.00	5.02	0
		5.00	4.88	–2
	December 13-14, 2001[b]	0.625	0.603	–4
		1.25	1.16	–7
		2.50	2.47	–1
		5.00	4.63	–7

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice				
January 12-13, 2000	January 13-15, 2000	0.312	0.298	–4
		0.312	0.305	–2
		0.625	0.617	–1
		0.625	0.638	+2
		1.25	1.25	0
	February 10-11, 2000[b]	0.312	0.268	–14
		0.625	0.566	–9
		1.25	1.17	–6
March 22, 2000	March 27-29, 2000	0.312	0.306	–2
		0.312	0.315	+1
		0.312	0.292	–6
		0.625	0.592	–5
		0.625	0.612	–2
		0.625	0.661	+6
		1.25	1.14	–9
		1.25	1.23	–2
		1.25	1.23	–2
June 14 and 16, 2000	June 19-20, 2000	0.312	0.299	–4
		0.312	0.298	–4
		0.312	0.313	0
		0.625	0.618	–1
		0.625	0.611	–2
		0.625	0.611	–2
		1.25	1.20	–4
		1.25	1.20	–4
		1.25	1.23	–2
August 23, 2000	August 28-29, 2000	0.312	0.313	0
		0.312	0.311	0
		0.625	0.625	0
		0.625	0.607	–3
		1.25	1.25	0
		1.25	1.25	0
		1.25	1.24	–1
	September 22-23, 2000[b]	0.312	0.298	–4
		0.625	0.547	–12
		1.25	1.11	–11
November 15, 2000	November 16-18, 2000	0.312	0.298	–4
		0.312	0.306	–2
		0.625	0.606	–3
		0.625	0.606	–3
		0.625	0.614	–2
		1.25	1.26	+1
		1.25	1.27	+2
		1.25	1.26	+1

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice (continued)				
February 7, 2001	February 8-9, 2001	0.312	0.307	–2
		0.312	0.305	–2
		0.625	0.616	–1
		0.625	0.610	–2
		0.625	0.617	–1
		0.625	0.612	–2
		1.25	1.23	–2
		1.25	1.20	–4
April 18, 2001	April 19-20, 2001	0.312	0.306	–2
		0.312	0.304	–3
		0.625	0.608	–3
		0.625	0.613	–2
		0.625	0.633	+1
		0.625	0.620	–1
		1.25	1.24	–1
		1.25	1.26	+1
	May 17-19, 2001[b]	0.312	0.290	–7
		0.625	0.574	–8
		1.25	1.19	–5
June 27-28, 2001	June 28-29, 2001	0.312	0.304	–3
		0.312	0.305	–2
		0.625	0.621	–1
		0.625	0.610	–2
		1.25	1.25	0
		1.25	1.26	+1
September 5, 2001	September 6-7, 2001	0.312	0.314	+1
		0.312	0.316	+1
		0.625	0.608	–3
		0.625	0.630	+1
		1.25	1.22	–2
		1.25	1.22	–2

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 4-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice (continued)				
November 14, 2001	November 15-16, 2001	0.312	0.315	+1
		0.312	0.309	–1
		0.625	0.615	–2
		0.625	0.616	–1
		0.625	0.616	–1
		1.25	1.22	–2
		1.25	1.21	–3
		1.25	1.22	–2
	December 13-14, 2001[b]	0.312	0.308	–1
		0.625	0.581	–7
		1.25	1.17	–6

[a] Results of duplicate or triplicate (0.312 mg/g formulations only) analyses: 0.312 mg/g = 312 ppm; 0.625 mg/g = 625 ppm; 1.25 mg/g = 1,250 ppm; 2.50 mg/g = 2,500 ppm; 5.00 mg/g = 5,000 ppm.
[b] Animal room samples
[c] Remixed; used in error for approximately 5 hours
[d] Results of remix

APPENDIX G
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES OF 4-METHYLIMIDAZOLE

TABLE G1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of 4-Methylimidazole

Week	0 ppm		625 ppm			1,250 ppm			2,500 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	17.5	124	16.3	124	82	14.5	123	147	11.7	124	235
2	17.2	179	17.1	173	62	16.3	165	124	15.6	151	259
3	19.2	210	18.6	205	57	17.3	193	112	15.8	180	219
4	19.4	240	18.5	235	49	17.3	221	97	16.2	205	198
5	19.4	255	19.4	250	49	18.3	237	96	16.6	223	186
6	18.5	270	18.0	266	42	17.3	252	86	16.1	239	168
7	18.8	286	17.6	280	39	16.9	265	80	15.8	250	158
8	17.8	304	17.3	297	36	16.4	280	73	15.2	262	145
9	17.6	313	16.9	307	34	15.6	288	68	15.0	270	139
10	17.9	324	17.3	317	34	15.7	295	66	14.9	278	134
11	17.8	330	16.4	321	32	15.2	297	64	14.4	280	129
12	17.7	342	16.2	331	31	15.0	304	62	14.4	285	127
13	17.4	345	16.6	332	31	15.0	303	62	14.2	284	125
17	17.8	365	16.9	352	30	15.6	322	60	14.1	292	121
21	18.9	382	17.2	368	29	16.9	340	62	16.1	313	129
25	18.0	395	16.4	383	27	15.7	351	56	14.6	323	113
29	18.4	410	17.7	396	28	16.3	363	56	15.7	336	117
33	17.5	418	17.2	402	27	15.6	370	53	14.7	340	108
37	17.7	426	16.7	408	26	15.9	379	52	15.8	343	115
41	17.0	429	16.9	415	25	16.9	380	55	15.3	349	110
45	17.1	433	16.7	419	25	15.4	384	50	14.8	353	105
49	17.1	437	16.9	421	25	15.5	385	50	15.4	357	108
53	16.5	440	16.2	429	24	15.1	386	49	14.4	359	100
57	17.1	438	16.6	425	24	15.5	390	50	15.3	359	107
61	17.9	445	16.9	428	25	15.8	396	50	15.2	364	105
65	16.6	448	16.1	435	23	15.9	396	50	15.3	366	104
69	16.5	445	15.3	430	22	15.1	402	47	16.3	367	111
73	16.6	444	16.0	424	24	15.3	395	48	14.4	366	98
77	15.8	438	16.1	425	24	14.8	396	47	15.9	365	109
81	15.5	438	14.9	419	22	14.4	386	46	14.7	364	101
85	15.6	433	15.2	416	23	14.6	388	47	14.4	362	99
89	14.9	421	14.1	408	22	14.2	387	46	13.9	354	98
93	15.9	426	16.9	414	25	13.9	383	45	12.3	349	88
97	16.4	422	15.8	408	24	15.3	381	50	14.7	350	105
101	15.3	400	14.1	405	22	14.4	378	48	15.0	350	107
Mean for weeks											
1-13	18.2	271	17.4	264	45	16.2	248	87	15.1	233	171
14-52	17.7	411	16.9	396	27	16.0	364	55	15.2	334	114
53-101	16.2	434	15.7	421	23	14.9	390	48	14.8	360	103

[a] Grams of feed consumed per animal per day
[b] Milligrams of 4-methylimidazole consumed per kilogram body weight per day

TABLE G2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of 4-Methylimidazole

Week	0 ppm		1,250 ppm			2,500 ppm			5,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	12.7	100	11.1	98	142	8.8	100	221	5.5	99	278
2	12.1	127	12.1	124	122	11.7	118	247	9.6	96	499
3	12.4	138	12.4	137	113	11.4	133	216	10.1	109	461
4	12.2	149	11.5	148	97	11.3	144	197	9.0	118	381
5	11.9	156	11.6	155	94	11.0	152	181	9.4	126	374
6	11.6	163	11.2	162	87	10.9	161	169	9.4	132	357
7	11.8	169	10.7	166	80	11.3	166	170	9.6	137	349
8	11.3	176	10.4	172	75	10.3	172	149	9.3	142	329
9	11.0	177	10.2	173	73	10.2	175	145	8.7	143	305
10	11.1	182	9.8	176	70	9.9	178	139	8.6	145	296
11	10.9	186	9.8	178	69	9.6	180	134	8.3	146	284
12	10.7	187	9.6	180	67	9.1	181	126	8.3	149	279
13	10.8	190	9.9	182	68	9.0	181	124	9.4	152	311
17	11.0	194	10.1	189	67	9.5	189	126	8.7	154	283
21	11.8	204	10.2	194	66	9.9	193	128	8.3	158	265
25	11.2	212	9.7	202	60	9.5	202	118	8.3	164	254
29	11.4	217	10.4	209	62	9.3	203	115	8.7	167	261
33	11.8	223	10.8	215	63	9.0	209	108	8.6	172	252
37	12.3	229	9.8	218	56	10.0	215	116	8.7	174	252
41	9.6	228	10.2	220	58	9.7	216	112	9.1	178	256
45	11.1	238	9.8	223	55	9.3	217	107	8.8	179	246
49	11.7	242	10.2	227	56	9.7	221	109	9.1	183	248
53	11.3	251	9.1	232	49	9.1	224	101	8.6	187	231
57	12.5	255	9.8	234	52	9.7	224	108	9.3	187	248
61	12.3	270	10.2	239	53	10.0	227	110	10.0	193	260
65	12.6	275	9.6	244	49	9.6	231	104	9.3	195	239
69	12.1	278	9.9	248	50	9.8	232	106	8.2	192	214
73	12.5	284	10.5	248	53	9.7	233	104	10.0	195	256
77	12.8	291	10.4	254	51	10.1	237	107	9.9	197	250
81	12.8	296	9.9	256	49	9.9	237	105	10.1	200	252
85	12.2	297	10.3	258	50	10.4	241	108	9.7	197	245
89	12.3	301	10.3	258	50	10.1	244	104	9.7	200	242
93	12.3	305	9.8	259	47	10.0	247	101	9.5	202	235
97	12.9	300	11.0	265	52	10.7	250	107	10.2	203	252
101	13.0	311	10.6	264	51	11.2	253	110	10.4	202	257
Mean for weeks											
1-13	11.6	161	10.8	158	89	10.3	157	171	8.9	130	346
14-52	11.3	221	10.1	211	60	9.5	207	115	8.7	170	257
53-101	12.4	286	10.1	251	50	10.0	237	106	9.6	196	245

[a] Grams of feed consumed per animal per day
[b] Milligrams of 4-methylimidazole consumed per kilogram body weight per day

TABLE G3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of 4-Methylimidazole

Week	0 ppm		312 ppm			625 ppm			1,250 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	5.1	21.2	4.8	21.3	71	5.1	21.1	150	4.9	21.2	291
2	5.5	22.1	5.4	22.3	75	5.4	21.9	154	5.6	22.0	320
3	5.3	23.2	5.3	22.8	73	5.4	23.1	145	5.4	22.8	294
4	5.4	24.4	5.4	24.7	69	5.4	24.3	138	5.3	24.0	275
5	6.0	25.5	5.5	25.7	67	5.5	25.3	134	5.4	25.0	271
6	6.2	26.8	5.6	26.7	65	6.1	26.6	145	5.9	26.2	279
7	5.6	27.4	5.7	27.4	65	5.6	27.1	129	5.5	26.8	257
8	5.6	28.3	5.2	28.3	58	5.1	27.8	115	5.2	27.5	237
9	5.2	28.8	4.9	28.9	53	5.0	28.4	109	4.9	27.9	219
10	5.4	29.1	5.1	29.1	55	5.1	28.8	110	5.0	28.4	219
11	5.1	29.6	5.1	29.7	53	5.1	29.1	109	5.0	28.8	217
12	5.1	30.8	5.5	30.6	56	5.3	30.3	108	5.4	29.4	231
13	5.1	31.3	5.3	31.2	53	5.2	31.0	104	5.3	30.3	217
17	5.3	34.4	5.4	34.0	49	5.3	33.6	99	5.2	32.6	201
21	5.1	36.1	4.8	36.1	42	4.9	35.3	87	4.9	33.5	183
25	5.2	37.6	5.2	37.6	43	5.1	36.7	87	5.1	34.7	184
29	4.8	40.1	5.0	40.3	39	4.9	39.3	78	4.9	37.1	164
33	4.9	41.4	4.9	41.7	37	4.5	40.2	71	4.4	37.5	148
37	5.2	42.3	5.2	42.4	38	5.2	41.0	80	5.1	38.3	166
41	4.3	44.1	4.6	43.9	33	4.6	42.8	67	4.5	39.8	141
45	4.6	43.5	4.5	44.2	32	4.6	43.0	66	4.4	40.0	138
49	4.7	43.9	4.9	44.1	35	4.8	42.7	70	4.6	39.5	146
53	4.7	45.3	4.5	45.7	31	4.6	44.2	65	4.4	41.1	135
57	5.0	46.1	5.0	46.5	34	5.0	45.1	69	4.9	41.1	150
61	5.1	45.9	5.0	46.1	33	5.1	45.2	70	4.7	40.7	143
65	4.7	44.3	4.7	44.9	33	4.7	43.6	68	4.6	39.5	147
69	4.8	44.8	5.1	43.7	36	5.0	42.5	73	4.9	38.8	156
73	5.0	44.9	5.2	44.1	37	5.3	42.9	77	5.1	39.0	162
77	5.6	45.7	5.8	44.2	41	5.4	42.3	80	5.4	38.4	176
81	5.3	45.2	5.6	43.1	40	5.4	42.0	80	5.2	38.0	170
85	4.8	45.7	5.0	43.9	36	5.1	42.9	75	5.0	38.4	163
89	4.8	45.0	4.9	43.7	35	4.8	42.7	70	4.6	38.0	152
93	5.3	44.9	5.3	43.7	38	5.3	43.0	78	5.0	38.1	164
97	5.5	44.7	5.1	43.7	36	4.9	42.8	72	4.6	38.4	148
101	4.9	43.7	5.4	42.6	40	5.2	41.9	78	5.1	37.4	171
Mean for weeks											
1-13	5.4	26.8	5.3	26.8	62	5.3	26.5	127	5.3	26.2	256
14-52	4.9	40.4	4.9	40.5	39	4.9	39.4	78	4.8	37.0	163
53-101	5.0	45.1	5.1	44.3	36	5.1	43.2	73	4.9	39.0	157

[a] Grams of feed consumed per animal per day
[b] Milligrams of 4-methylimidazole consumed per kilogram body weight per day

TABLE G4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of 4-Methylimidazole

Week	0 ppm		312 ppm			625 ppm			1,250 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	3.7	17.4	3.5	17.3	63	3.8	17.4	135	3.3	17.4	239
2	4.0	18.3	3.8	18.0	65	3.8	18.6	129	3.8	17.7	265
3	3.6	19.0	3.7	18.8	62	3.8	19.1	125	3.7	18.9	245
4	4.3	20.5	4.2	20.3	64	4.1	20.1	126	3.9	19.6	251
5	4.3	21.3	4.0	20.4	60	4.1	21.2	120	3.8	20.0	239
6	4.5	22.3	4.6	22.0	66	4.6	22.2	130	4.4	21.2	262
7	4.5	23.1	4.5	22.5	63	4.6	22.5	127	4.4	21.6	254
8	4.5	23.1	4.4	23.3	59	4.6	23.2	123	4.5	22.2	255
9	4.1	23.9	4.0	23.7	52	4.0	23.6	106	3.9	22.9	216
10	4.2	24.6	4.2	24.4	54	4.5	24.3	114	4.2	23.5	224
11	4.1	24.9	4.0	24.7	51	4.4	24.7	111	4.0	23.7	212
12	4.6	25.0	4.6	25.3	57	4.5	25.1	112	4.5	23.8	235
13	4.4	25.7	4.5	25.5	55	4.3	25.1	108	4.4	24.1	229
17	4.6	28.7	4.6	28.5	50	4.5	27.6	101	4.4	25.9	211
21	4.5	30.4	4.5	30.1	46	4.4	30.0	93	4.3	27.5	195
25	4.8	32.5	4.8	32.1	46	4.7	31.2	93	4.7	28.3	206
29	4.7	35.9	4.7	34.5	43	4.7	33.9	86	4.6	30.1	191
33	4.8	36.0	4.6	35.1	41	4.6	33.1	87	4.2	29.3	180
37	4.5	37.1	4.6	35.8	40	4.8	35.5	84	4.4	31.1	179
41	3.7	37.7	4.0	37.6	33	3.8	37.4	63	3.7	31.8	145
45	4.3	39.0	4.1	39.0	32	4.3	38.1	70	4.3	33.4	159
49	4.3	40.0	4.5	39.5	35	4.4	38.9	71	4.1	33.1	156
53	4.7	41.6	4.3	40.6	33	4.6	39.9	72	4.5	34.5	163
57	4.2	43.3	4.4	41.8	33	4.4	41.2	68	4.1	35.0	145
61	4.9	43.0	4.9	41.1	37	4.6	41.2	70	4.3	34.5	156
65	4.4	42.6	4.5	40.7	35	4.4	40.3	68	4.1	34.5	150
69	4.9	43.4	5.0	41.1	38	4.8	39.6	76	4.5	35.1	159
73	4.9	42.9	5.1	41.0	39	5.2	39.8	81	4.6	35.4	161
77	4.6	42.9	4.9	41.5	37	4.7	39.3	75	4.5	35.4	158
81	4.7	42.5	4.8	40.5	37	4.7	39.1	74	4.7	34.7	171
85	4.9	43.1	5.0	41.4	38	4.5	40.0	70	3.8	35.5	134
89	5.0	44.2	5.1	41.0	39	4.7	40.2	73	4.2	35.6	148
93	4.9	45.3	5.1	42.4	37	4.8	39.9	75	4.3	35.4	153
97	4.8	45.6	4.9	43.2	35	4.7	40.3	72	4.1	36.3	141
101	5.0	44.6	5.3	41.8	39	4.9	41.5	74	4.9	36.2	169
Mean for weeks											
1-13	4.2	22.2	4.2	22.0	59	4.2	22.1	120	4.1	21.3	240
14-52	4.5	35.3	4.5	34.7	41	4.5	34.0	83	4.3	30.0	180
53-101	4.8	43.5	4.9	41.4	37	4.7	40.2	73	4.4	35.2	154

[a] Grams of feed consumed per animal per day
[b] Milligrams of 4-methylimidazole consumed per kilogram body weight per day

APPENDIX H
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE H1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE H2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE H3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.1 ± 0.67	13.2 – 15.7	24
Crude fat (% by weight)	8.1 ± 0.27	7.6 – 8.5	24
Crude fiber (% by weight)	9.1 ± 0.56	8.0 – 10.5	24
Ash (% by weight)	5.2 ± 0.26	4.8 – 5.8	24
Amino Acids (% of total diet)			
Arginine	0.748 ± 0.053	0.670 – 0.850	12
Cystine	0.223 ± 0.027	0.150 – 0.250	12
Glycine	0.702 ± 0.043	0.620 – 0.750	12
Histidine	0.343 ± 0.023	0.310 – 0.390	12
Isoleucine	0.534 ± 0.041	0.430 – 0.590	12
Leucine	1.078 ± 0.059	0.960 – 1.140	12
Lysine	0.729 ± 0.065	0.620 – 0.830	12
Methionine	0.396 ± 0.053	0.260 – 0.460	12
Phenylalanine	0.611 ± 0.038	0.540 – 0.660	12
Threonine	0.492 ± 0.045	0.430 – 0.590	12
Tryptophan	0.129 ± 0.016	0.110 – 0.160	12
Tyrosine	0.378 ± 0.054	0.280 – 0.460	12
Valine	0.658 ± 0.049	0.550 – 0.710	12
Essential Fatty Acids (% of total diet)			
Linoleic	3.89 ± 0.278	3.49 – 4.54	12
Linolenic	0.30 ± 0.038	0.21 – 0.35	12
Vitamins			
Vitamin A (IU/kg)	4,874 ± 910	3,060 – 6,810	24
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.3 ± 17.06	52.0 – 110.0	12
Thiamine (ppm)[b]	7.3 ± 0.82	6.0 – 8.8	24
Riboflavin (ppm)	6.4 ± 2.11	4.20 – 11.20	12
Niacin (ppm)	78.6 ± 10.86	66.4 – 98.2	12
Pantothenic acid (ppm)	23.1 ± 3.61	17.4 – 29.1	12
Pyridoxine (ppm)[b]	8.88 ± 2.05	6.4 – 12.4	12
Folic acid (ppm)	1.84 ± 0.56	1.26 – 3.27	12
Biotin (ppm)	0.337 ± 0.13	0.225 – 0.704	12
Vitamin B_{12} (ppb)	64.8 ± 50.9	18.3 – 174.0	12
Choline (ppm)[b]	3,094 ± 292	2,700 – 3,790	12
Minerals			
Calcium (%)	1.038 ± 0.046	0.964 – 1.140	24
Phosphorus (%)	0.601 ± 0.039	0.552 – 0.701	24
Potassium (%)	0.668 ± 0.023	0.627 – 0.694	12
Chloride (%)	0.368 ± 0.033	0.300 – 0.423	12
Sodium (%)	0.189 ± 0.016	0.160 – 0.212	12
Magnesium (%)	0.200 ± 0.009	0.185 – 0.217	12
Sulfur (%)	0.176 ± 0.026	0.116 – 0.209	12
Iron (ppm)	177 ± 46.2	135 – 311	12
Manganese (ppm)	53.4 ± 6.42	42.1 – 63.1	12
Zinc (ppm)	52.5 ± 6.95	43.3 – 66.0	12
Copper (ppm)	6.64 ± 1.283	5.08 – 9.92	12
Iodine (ppm)	0.535 ± 0.242	0.233 – 0.972	12
Chromium (ppm)	0.545 ± 0.125	0.330 – 0.751	12
Cobalt (ppm)	0.23 ± 0.041	0.20 – 0.30	12

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE H4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.21 ± 0.042	0.16 – 0.37	24
Cadmium (ppm)	0.04 ± 0.004	0.04 – 0.06	24
Lead (ppm)	0.09 ± 0.097	0.05 – 0.54	24
Mercury (ppm)	<0.02		24
Selenium (ppm)	0.22 ± 0.055	0.14 – 0.36	24
Aflatoxins (ppb)	<5.00		24
Nitrate nitrogen (ppm)[c]	11.3 ± 3.41	6.85 – 21.1	24
Nitrite nitrogen (ppm)[c]	<0.61		24
BHA (ppm)[d]	<1.0		24
BHT (ppm)[d]	<1.0		24
Aerobic plate count (CFU/g)	14 ± 13	10 – 70	24
Coliform (MPN/g)	2.4 ± 1.6	0.0 – 3.6	24
Escherichia coli (MPN/g)	<10		24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[e]	4.8 ± 1.11	3.1 – 7.5	24
N-Nitrosodimethylamine (ppb)[e]	2.1 ± 0.58	1.0 – 3.2	24
N-Nitrosopyrrolidine (ppb)[e]	2.7 ± 1.01	1.0 – 5.1	24
Pesticides (ppm)			
α-BHC	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.10		24
Estimated PCBs	<0.20		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.10		24
Methyl chlorpyrifos	0.173 ± 0.112	0.020 – 0.499	24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion	0.178 ± 0.141	0.020 – 0.557	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

APPENDIX I
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from male and female sentinel rats and mice at 6, 12, and 18 months and from randomly selected 2,500 ppm male rats, 5,000 ppm female rats, and 1,250 ppm male and female mice at the end of the studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	6, 12, and 18 months, study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	6, 12, and 18 months, study termination
RCV/SDA	18 months
Sendai	6 months

Method and Test	Time of Analysis
MICE	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM (epizootic diarrhea of infant mice)	6, 12, and 18 months, study termination
GDVII (mouse encephalomyelitis virus)	6, 12, and 18 months, study termination
LCM (lymphocytic choriomeningitis virus)	6, 12, and 18 months, study termination
Mouse adenoma virus-FL	6, 12, and 18 months, study termination
MHV (mouse hepatitis virus)	6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
EDIM	6 months and study termination
GDVII	6 months
LCM	6 and 12 months, study termination
Mouse adenoma virus-FL	6 months
MCMV (mouse cytomegalovirus)	Study termination
Parvovirus	6, 12, and 18 months, study termination
PVM	6 months and study termination
Reovirus 3	18 months
Sendai	12 months

RESULTS

All test results were negative.

APPENDIX J
SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F$_1$ MICE

SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F$_1$ MICE

INTRODUCTION

Single-dose intravenous injection and gavage toxicokinetic studies were designed to estimate toxicokinetic parameters for the elimination of 4-methylimidazole from the plasma of F344/N rats and B6C3F$_1$ mice. Male and female F344/N rats and B6C3F$_1$ mice received a single intravenous injection of 10 mg 4-methylimidazole/kg body weight or a single gavage dose of 10, 50, or 100 mg/kg. Post-dose plasma samples were analyzed for 4-methylimidazole, and the results were used to calculate toxicokinetic parameters.

MATERIALS AND METHODS

4-Methylimidazole (Lot 116H0901) was obtained from Sigma Chemical Company (St. Louis, MO), repackaged in 250 mL amber bottles, and stored refrigerated. The material was analyzed for identity and purity; the results and analytical systems are described in Appendix F.

On the day of dosing, male and female F344/N rats were 14 weeks old and ranged in weight from 231.2 to 322.4 and 166.6 to 197.1 grams, respectively; male and female B6C3F$_1$ mice were 12 or 13 weeks old and ranged in weight from 19.3 to 35.6 and 16.3 to 26.3 grams, respectively. The gavage doses were formulated in 0.05 M phosphate-buffered saline (pH 7.4 ± 0.1).

After dosing, animals were anesthetized with CO_2/O_2 prior to bleeding. Blood samples were collected using the retroorbital puncture method for rats and cardiac puncture for mice. Three rats and three mice were bled at each time point. Blood samples of approximately 1 mL (rats) and the maximum volume obtainable for mice (approximately 0.4 to 1 mL) were placed into individual tubes containing EDTA as an anticoagulant. Whole blood samples were gently rocked by hand to ensure adequate mixing with the anticoagulant and then placed on wet ice. Approximately 60 minutes after collection, the whole blood was centrifuged and the plasma transferred to a plastic storage vial. The plasma was stored at –70° C until analyzed. After blood collection, the animals were sacrificed and discarded.

For analysis, plasma samples were thawed to room temperature. Aliquots of 200 µL of plasma were combined with 50 µL of 0.2 M sodium hydroxide saturated with sodium chloride and 50 µL of 3-pyridinepropanol internal standard solution (40 µg/mL in methanol), and the mixture was vortexed for approximately 30 seconds.

Ethyl acetate (1.0 mL) was added, and the mixture was vortexed for an additional 30 seconds and then centrifuged for approximately 5 minutes at 1,700 rpm. The organic layer was transferred to automated liquid sampler vials for analysis. Chromatography was performed on a gas chromatography system equipped with a nitrogen phosphorous detector and a Carbowax™-amine column (30 m × 0.53 mm ID, 1.00 µm film or 30 m × 0.32 mm ID, 0.25 µm film; Supelco, Bellefonte, PA). The column oven was programmed at 110° C for 3 minutes, then to 210° C at 8° C per minute, held for 3 minutes, and then increased to 220° C at 10° C per minute followed by a 5-minute hold. Sample concentrations of 4-methylimidazole were calculated using 1/x-weighted quadratic regression analysis of instrument response to calibration standards prepared in blank F344/N rat plasma.

The analytical method for determining 4-methylimidazole in plasma samples was validated within a range of 50 to 20,000 ng/mL in F344/N rat plasma. The limit of quantitation ranged from 50 to 100 ng/mL. Precision, based on standard deviation of quality control samples, was less than or equal to 15%. Accuracy, based on percent relative errors in the determined versus the prepared concentration of calibration standards, was less than or equal to 15%.

Toxicokinetics

4-Methylimidazole plasma concentration versus time data was evaluated using WinNONLIN® (version 1.5A; Scientific Consulting, Inc., Freeman, SD). A one-compartment model with no lag time and first-order absorption and elimination was used to fit the data:

$$C(t)=D \bullet K_{01} / V / (K_{01} - K_{10}) \bullet [\exp(K_{10} \bullet t) - \exp(K_{01} \bullet t)]$$

where C(t) is the plasma concentration at time t, D is dose, and V is volume of distribution, K_{01} is the absorption rate constant, and K_{10} is the elimination rate constant. These parameters were estimated by nonlinear regression using a least-squares method and a weighting factor ($1/y^2$ predicted).

AUC (areas under the plasma concentration versus time curve) values were calculated using the trapezoidal rule:

$$AUC_t = \Sigma [(C_{n-1} + C_n) / 2 \times (t_n - t_{n-1})]$$

And the AUC extrapolated to infinity was calculated as:

$$AUC_{infinity} = AUC_t + (C_f / K_{10})$$

Clearance was calculated as $D/AUC_{infinity}$, and the half-lives for the absorption and elimination phases were calculated as $0.693/K_{01}$ and $0.693/K_{10}$, respectively.

RESULTS AND DISCUSSION

4-Methylimidazole was rapidly absorbed when administered by gavage in an aqueous formulation to male and female F344/N rats and B6C3F$_1$ mice such that measurable concentrations of 4-methylimidazole were observed within 5 minutes of administration (Figures J1 and J2). The administration of 4-methylimidazole in an aqueous solution averted any dissolution phase, which is the rate-limiting step for solid dosage formulations such as dosed feed, and thereby precluded the presence of a lag time in the plasma concentration time profile. Absorption rate constants were larger than the elimination rate constants and were similar in males and females of each species. The absorption half-life values ranged from 5 to 23 minutes in rats and 2 to 5 minutes in mice and declined with dose (Tables J1 and J2). Elimination half-life values ranged from 65 to 499 minutes in rats and from 21 to 87 minutes in mice but increased with dose in both sexes of both species. Differences in clearance across the dosed groups were not all statistically significant, but clearance did show decreases with dose. These data indicate that the 100 mg/kg dose is approaching the upper limit of the linear dosing range and that higher doses would result in higher internal doses than expected based on the lower doses. Evaluation of the shape of the concentration versus time curves indicates that a flip-flop model may be important in explaining the kinetics of 4-methylimidazole. However, in a repeated dose scenario, the short absorption and elimination half-lives of the chemical would prevent accumulation from one dose to the next.

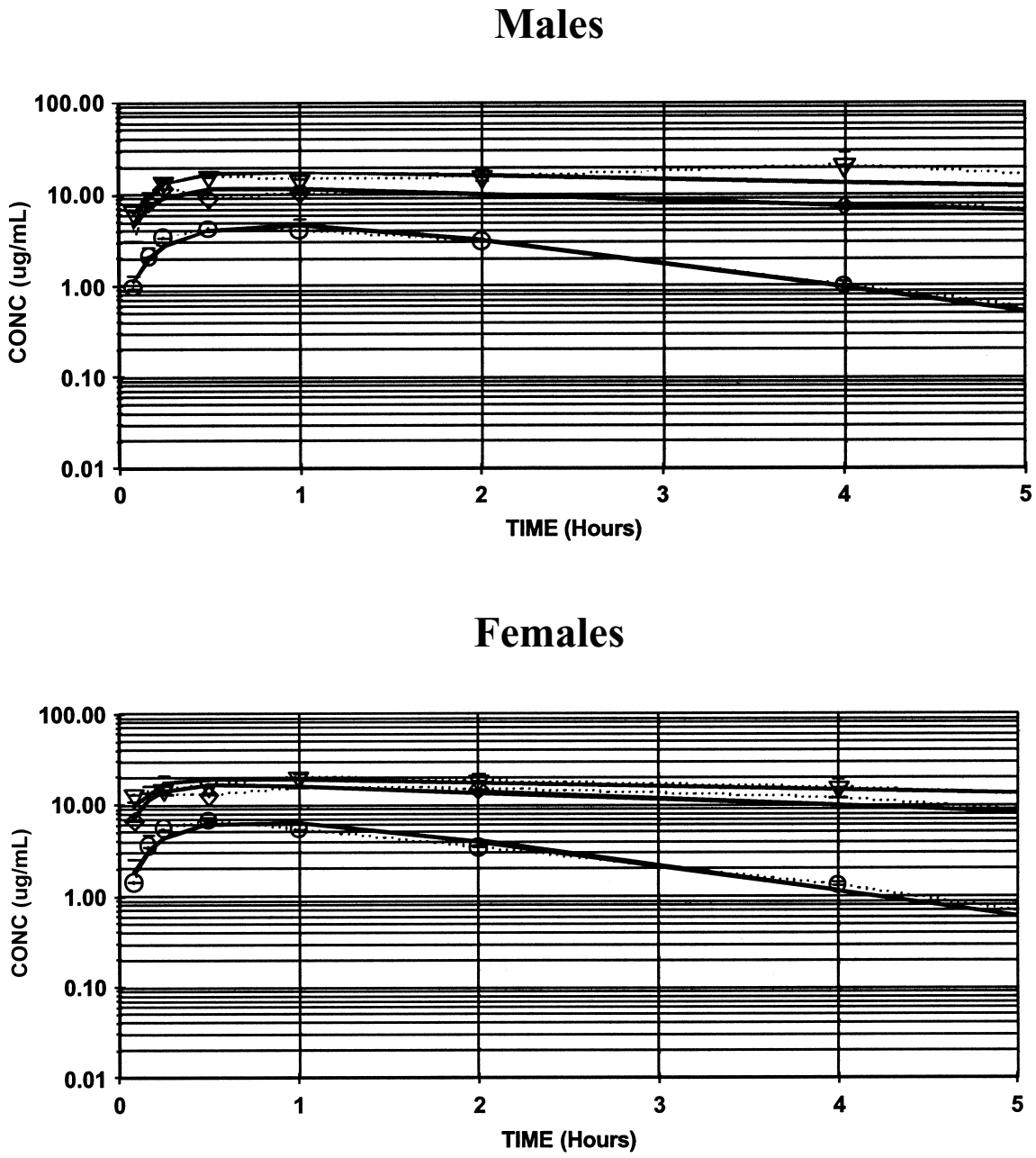

FIGURE J1
Plasma Concentrations of 4-Methylimidazole in Male and Female F344/N Rats after a Single Gavage Dose of 10, 50, or 100 mg/kg 4-Methylimidazole
Solid lines represent the best-fit curves (WinNONLIN®) plotted through the observed data points.
(Observed data: O – 10 mg/kg; ♦ – 50 mg/kg; ∇ – 100 mg/kg)

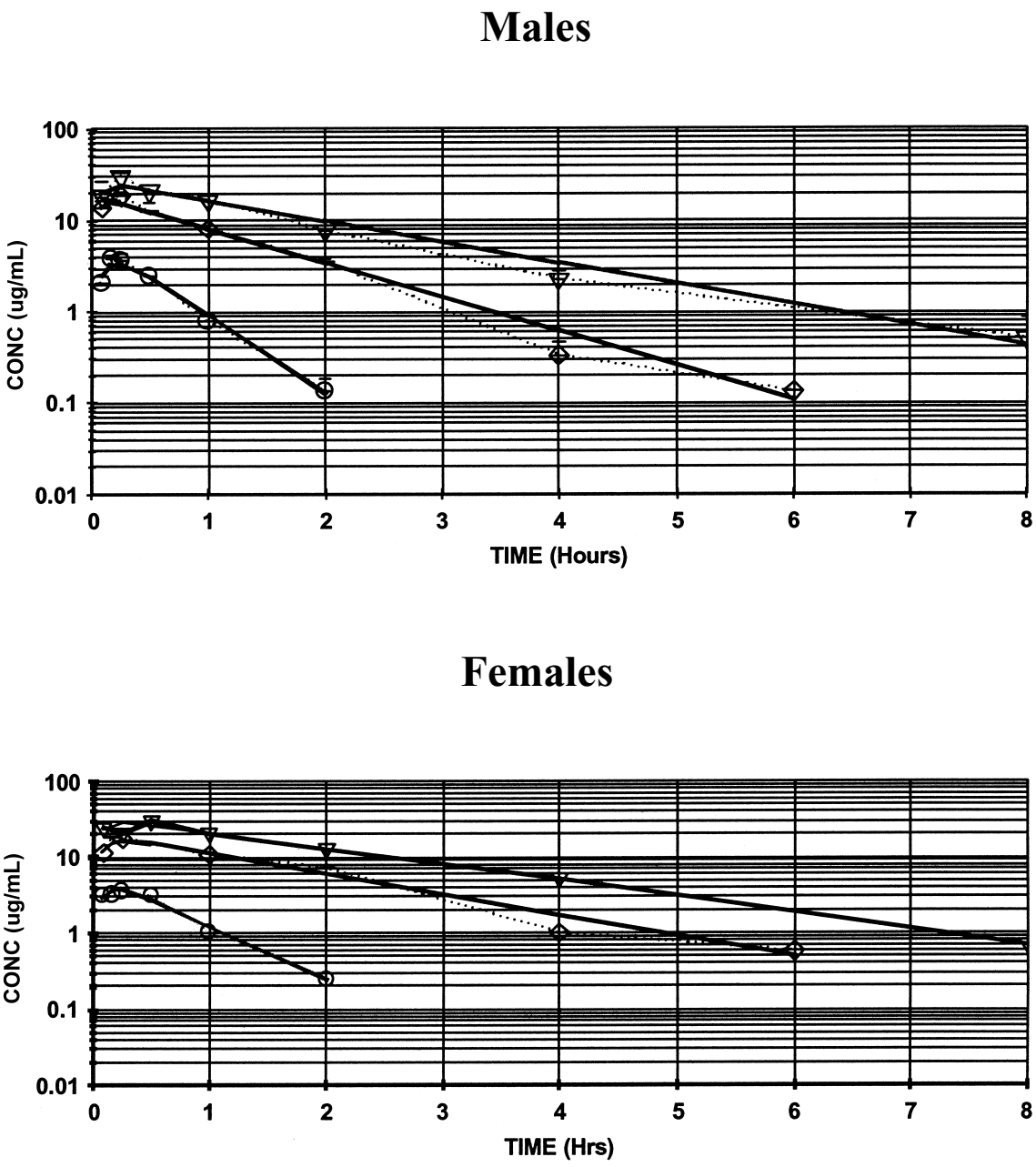

FIGURE J2
Plasma Concentrations of 4-Methylimidazole in Male and Female B6C3F$_1$ Mice after a Single Gavage Dose of 10, 50, or 100 mg/kg 4-Methylimidazole
Solid lines represent the best-fit curves (WinNONLIN®) plotted through the observed data points.
(Observed data: O – 10 mg/kg; ♦ – 50 mg/kg; ∇ – 100 mg/kg)

TABLE J1
Toxicokinetic Parameter Estimates for the Elimination of 4-Methylimidazole from the Plasma of F344/N Rats after a Single Gavage Dose of 4-Methylimidazole[a]

Parameter	10 mg/kg	50 mg/kg	100 mg/kg
Male			
C_{max} (µg/mL)	4.7 ± 0.3	11.8 ± 1.4	17.5 ± 3.6
C_{max}/dose	0.47 ± 0.03	0.236 ± 0.028	0.175 ± 0.036
AUC [(µg • hour)/mL]	12.9 ± 0.9	95.4 ± 15	225 ± 37
AUC/dose	1.29 ± 0.09	1.91 ± 0.3	2.25 ± 0.37
Absorption half-life (hours)	0.376 ± 0.066	0.129 ± 0.039	0.138 ± 0.081
Elimination half-life (hours)	1.09 ± 0.07	5.11 ± 1.29	8.31 ± 1.56
Clearance (L/hour per kg)	0.777 ± 0.070	0.524 ± 0.016	0.444 ± 0.16
Female			
C_{max} (µg/mL)	6.6 ± 0.6	16.8 ± 1.5	19.3 ± 3.3
C_{max}/dose	0.66 ± 0.06	0.336 ± 0.036	0.193 ± 0.033
AUC [(µg • hour)/mL]	16.8 ± 1.5	109 ± 10	235 ± 32
AUC/dose	1.68 ± 0.15	2.18 ± 0.2	2.35 ± 0.32
Absorption half-life (hours)	0.297 ± 0.064	0.117 ± 0.028	0.088 ± 0.053
Elimination half-life (hours)	1.08 ± 0.07	4.03 ± 0.62	8.01 ± 1.22
Clearance (L/hour per kg)	0.594 ± 0.089	0.459 ± 0.092	0.426 ± 0.14

[a] Data are reported as mean ± standard error. C_{max} = maximum plasma concentration; AUC = area under the plasma concentration versus time curve from 0 to infinity

TABLE J2
Toxicokinetic Parameter Estimates for the Elimination of 4-Methylimidazole from the Plasma of B6C3F$_1$ Mice after a Single Gavage Dose of 4-Methylimidazole[a]

Parameter	10 mg/kg	50 mg/kg	100 mg/kg
Male			
C_{max} (µg/mL)	3.42 ± 0.3	15.9 ± 1.8	24.3 ± 2.9
C_{max}/dose	0.342 ± 0.030	0.318 ± 0.036	0.243 ± 0.029
AUC [(µg • hour)/mL]	2.66 ± 0.21	21.3 ± 2.0	52.1 ± 5.3
AUC/dose	0.266 ± 0.021	0.426 ± 0.10	0.521 ± 0.053
Absorption half-life (hours)	0.080 ± 0.024	0.035 ± 0.024	0.041 ± 0.026
Elimination half-life (hours)	0.348 ± 0.030	0.805 ± 0.045	1.33 ± 0.10
Clearance (L/hour per kg)	3.76 ± 0.078	2.35 ± 0.094	1.92 ± 0.10
Female			
C_{max} (µg/mL)	3.73 ± 0.29	16.8 ± 1.8	29.3 ± 2.3
C_{max}/dose	0.373 ± 0.029	0.336 ± 0.046	0.293 ± 0.023
AUC [(µg • hour)/mL]	3.21 ± 0.22	31.9 ± 3.03	67.8 ± 4.4
AUC/dose	0.321 ± 0.22	0.638 ± 0.061	0.678 ± 0.044
Absorption half-life (hours)	0.062 ± 0.019	0.059 ± 0.026	0.041 ± 0.016
Elimination half-life (hours)	0.430 ± 0.036	1.11 ± 0.09	1.45 ± 0.08
Clearance (L/hour per kg)	3.12 ± 0.068	1.57 ± 0.095	1.47 ± 0.065

[a] Data are reported as mean ± standard error. C_{max} = maximum plasma concentration; AUC = area under the plasma concentration versus time curve from 0 to infinity

APPENDIX K
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION

A physiologically based pharmacokinetic (PBPK) model representing the uptake, distribution, and metabolism of 4-methylimidazole in rats and mice was developed to describe the processes involved in 4-methylimidazole toxicokinetics. Model development was based on published data for 4-methylimidazole disposition in male F344 rats (Yuan and Burka, 1995) and single-dose toxicokinetic data for male and female rats and mice presented in Appendix J. Most of the model parameters were obtained from estimates in the literature. However, the model has 10 parameters that did not have literature estimates. These parameters were estimated by least squares techniques. The model was fit to short timespan (less than 24 hours) intravenous injection and gavage data. The fitted model was used to make predictions of blood concentrations of 4-methylimidazole in the current dosed feed study using a time scale of months.

MODEL DEVELOPMENT

The PBPK model has separate compartments representing the amount of 4-methylimidazole in the liver, kidney, blood, adipose, stomach, and other aggregated tissues (Figure K1). For the metabolite, separate compartments are used to represent the amount of the metabolite in the blood and other aggregated tissues. All tissue compartments are modeled as flow limited. Uptake from the stomach and urinary excretion are modeled as saturable processes with Michaelis-Menten kinetics. Metabolism of 4-methylimidazole is assumed to follow Michaelis-Menten kinetics and takes place in the liver. A feed consumption rate function was derived for the current chronic feed study. Data from Yuan (1993) specifies the percent of total daily consumption by half-hour intervals. These values were used in an interpolation to define an instantaneous consumption rate as input to the stomach. A value of total feed consumed in a day coupled with this interpolation lets the model account for the eating pattern of the rats and mice. All model equations were encoded in MATLAB® (The Math Works, Inc., Natick, MA). Model equations are listed at the end of this section.

Physiological parameters for tissue volumes, tissue blood flows, and cardiac output were obtained from the literature (Table K1; Brown *et al.*, 1997). Cardiac output was derived from allometric relationships with body weight. Mean body weights from the single-dose toxicokinetic studies (Appendix J) were used in fitting the model. The 4-methylimidazole tissue:blood partition coefficients (Table K2) for adipose, liver, kidney, and muscle were derived from the octanol:water partition coefficient (Poulin and Krishnan, 1995). The muscle partition coefficient was used for the aggregated tissue partition coefficient. A search of the literature yielded no information that would help determine the values related to uptake from the stomach, metabolism, or elimination in the feces or urine; values for these parameters were determined by fitting model predictions to the toxicokinetic data from the current studies.

Short-term data from the literature and the NTP were used to estimate the unknown model parameters. Yuan and Burka (1995) published plasma concentrations of 4-methylimidazole and its metabolite in male F344 rats for up to 14 hours after oral doses of 5 or 50 mg 4-methylimidazole/kg body weight. Toxicokinetic data from the single-dose toxicokinetic studies (Appendix J) include blood concentrations of 4-methylimidazole for up to 30 hours following intravenous (10 mg/kg) or oral gavage (10, 50, 100 mg/kg) dosing of rats and mice of both sexes. These toxicokinetic data sets were used in the parameter fitting. The model with the fit parameters was evaluated against the blood data collected during the first 6 months of the 2-year bioassay. The 2-year feed study recorded the blood concentrations of 4-methylimidazole at 1, 14, and 26 weeks based on rats consuming feed containing 625 (males only), 1,250, 2,500, or 5,000 (females only) ppm 4-methylimidazole and mice of both sexes consuming feed containing 312, 625, or 1,250 ppm 4-methylimidazole. Body weights and doses of 4-methylimidazole consumed in the feed study (mg/kg per day) were determined weekly for the first 13 weeks and then monthly for the remainder of the 2 years.

Definitions of Abbreviations

A_i = Amount of 4-methylimidazole in compartment *i* (mg)
Q_i = Blood flow in compartment *I* (L/hour)
V_i = Volume of compartment *i* (L)
P_i = Tissue *i*:blood partition coefficient
C_i = Concentration of 4-methylimidazole in compartment *i* (mg/L)
CV_i = Concentration of 4-methylimidazole in the venous blood of compartment *i* (mg/L)
k_{Feces} = Fecal elimination rate constant ($hour^{-1}$)
k_1 = Rate constant for metabolite — blood to aggregate tissue compartment ($hour^{-1}$)
k_2 = Rate constant for metabolite — aggregate tissue to blood compartment ($hour^{-1}$)
k_{LS} = Rate constant for metabolite leaving the system ($hour^{-1}$)
V_{Max} = Maximum metabolism rate (mg/L per hour)
K_m = Michaelis-Menten constant associated with metabolism (mg/L)
OT = Other tissues
Ad = Adipose
L = Liver
K = Kidney
Bl = Blood
S = Stomach
M = Metabolite
Metab = Metabolism rate
MetBl = Metabolite in blood
MetRB = Metabolite in rest of body
MetLS = Metabolite leaving the system
Gav = Uptake from the stomach of a gavage dose

Model Equations

$$\frac{dA_{Ad}}{dt} = Q_{Ad}(C_{Bl} - CV_{Ad})$$

$$\frac{dA_{OT}}{dt} = Q_{OT}(C_{Bl} - CV_{OT})$$

$$\frac{dA_{k}}{dt} = Q_{K}(C_{Bl} - CV_{K}) - \frac{Vmax_{Urine}CV_{K}}{Km_{Urine} + CV_{K}}$$

$$\frac{dA_{L}}{dt} = Q_{L}(C_{Bl} - CV_{L}) - M + \frac{Vmax_{Gav}A_{S}}{Km_{Urine} + A_{S}}$$

$$\frac{dA_{S}}{dt} = - \frac{Vmax_{Gav}A_{S}}{Km_{Gav} + A_{S}} - k_{Feces}A_{S}$$

$$\frac{dA_{Urine}}{dt} = \frac{Vmax_{Urine}CV_{K}}{Km_{Urine} + CV_{K}}$$

$$\frac{dA_{Feces}}{dt} = k_{Feces}A_{S}$$

$$\frac{dA_{Bl}}{dt} = Q_{Ad}CV_{Ad} + Q_{OT}CV_{OT} + Q_{L}CV_{L} + Q_{K}CV_{K} - Q_{total}C_{Bl}$$

$$\frac{dA_{MetRB}}{dt} = k_{1}A_{MetBl} - K_{2}A_{MetRB}$$

$$\frac{dA_{MetBl}}{dt} = k_{2}A_{MetRB} - k_{1}A_{MetBl} - k_{LS}A_{MetBl}$$

$$\frac{dA_{MetLS}}{dt} = k_{LS}A_{MetBl}$$

$$M = \frac{Vmax_{Metab}CV_{L}}{Km_{Met} + CV_{L}}$$

$CV_i = \frac{A}{V_i P_i}$ $C_i = \frac{A}{V_i}$, where *i* denotes the tissue compartment

RESULTS

For rats, the estimates for the 10 fitted parameters are shown in Table K3. For mice, the values of three of the parameters (Table K4) were fixed based on estimates from male rats; the remaining seven parameters were fitted, and these estimates are shown in Table K5. Estimates were obtained by minimizing the sum of squares between model predictions and the blood concentration data.

The first test was for differences between sexes in rats. The full model had 20 parameters (10 for male rats and 10 for female rats), while the reduced model had 10 parameters. The value of the F-statistic was 1.2169, and the probability of a larger F was 0.27. Separate parameter values for male and female rats did not make a significant improvement to the fit of the data. The plots of the model predictions along with the data for the reduced model are shown in Figures K2 and K4.

The second test was for differences between sexes in mice. The full model had 14 parameters (seven for male mice and seven for female mice), while the reduced model had seven parameters. The value of the F-statistic was 3.43, and the probability of a larger F was 0.001. Therefore, a single set of parameters was not adequate for both male and female mice. The plots for the full model are given in Figures K5 and K6.

Unlike the reduced model fits for rats (Figures K7 to K12), the full model fits for mice (Figures K13 to K18) yielded accurate predictions of blood concentrations over 26 weeks in the dosed feed studies.

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Yuan, J.H., and Burka, L.T. (1995). Toxicokinetics of 4-methylimidazole in the male F344 rat. *Xenobiotica* **25**, 885-894.

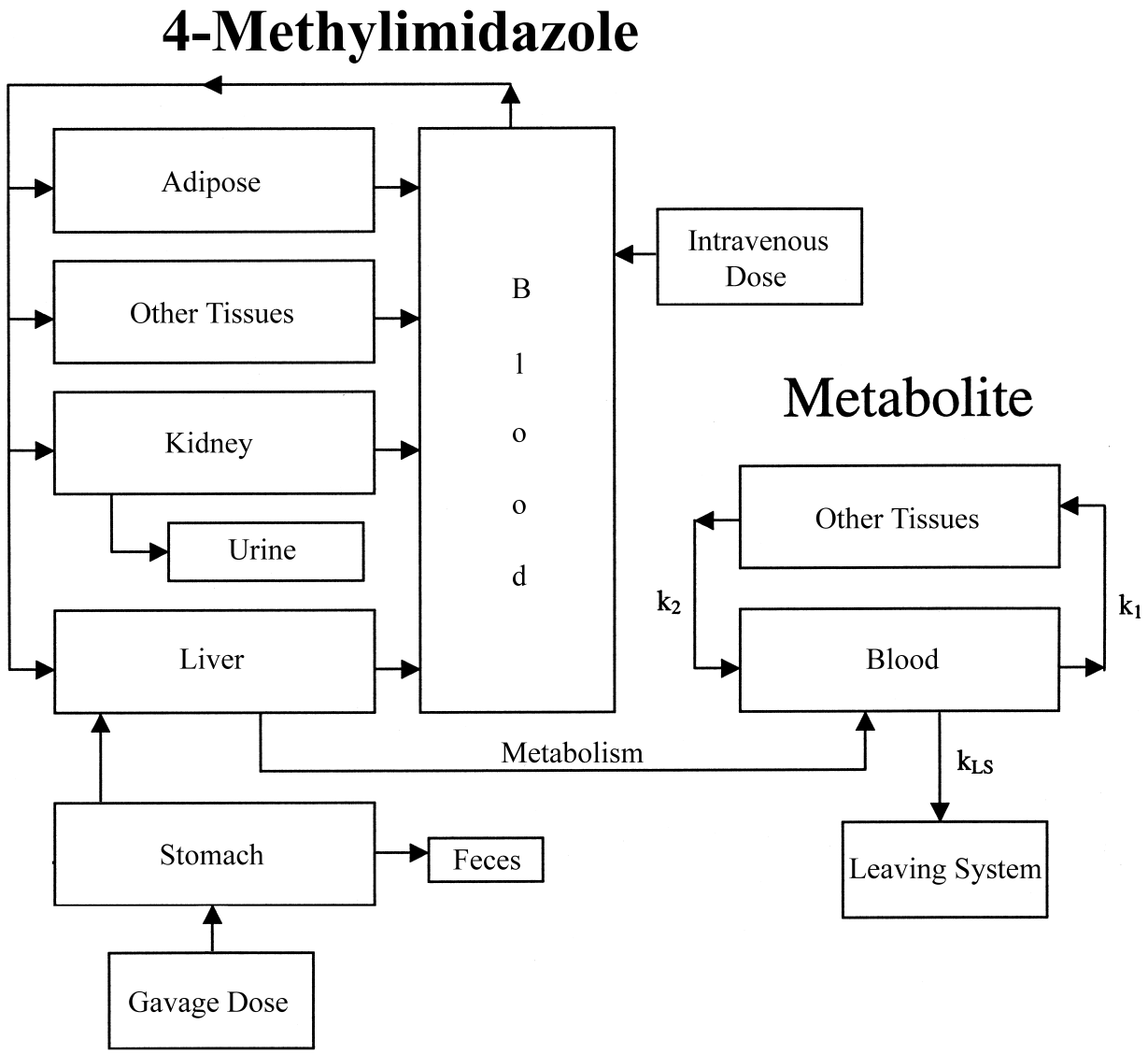

FIGURE K1
Physiologically Based Pharmacokinetic Model for Rats and Mice Exposed to 4-Methylimidazole by Single-Dose Intravenous Injection or Oral Gavage

TABLE K1
Physiological Parameters for Rats and Mice for the Physiologically Based Pharmacokinetics Model of 4-Methylimidazole[a]

Tissue	Tissue Space (%)	Blood Flow (%)
Rats		
Adipose	7.0	7.0
Liver	3.4	18.3
Kidney	0.7	14.1
Other	69.5	60.6
Blood	19.4	—
Mice		
Adipose	8.0	5.0
Liver	5.5	16.2
Kidney	1.67	9.1
Other	65.43	69.7
Blood	19.4	—

[a] Parameter estimates were derived from Brown *et al.* (1997) and allometric relationships with body weight.

TABLE K2
Partition Coefficients for 4-Methylimidazole for the Physiologically Based Pharmacokinetic Model of 4-Methylimidazole

Tissue	Partition Coefficient[a]
Adipose	4.238
Liver	1.048
Kidney	1.226
Other	0.9401[b]

[a] All coefficients are expressed as tissue:blood ratios. Coefficients for adipose, liver, kidney, and muscle were derived from the octanol:water partition coefficient for 4-methylimidazole (Poulin and Krishnan, 1995).
[b] Used the estimate for muscle (Poulin and Krishnan, 1995)

TABLE K3
Parameter Estimates for Rats from the Physiologically Based Pharmacokinetic Model of 4-Methylimidazole

Parameter	Reduced Model	Full Model	
	All Rats	Male Rats	Female Rats
V_{max} Gavage uptake (mg/L per hour)	15.8019	18.6080	14.7731
K_m Gavage uptake (mg/L)	1.6852	4.6096	1.1081
Fecal elimination rate ($hour^{-1}$)	5.5157	4.0735	6.1547
V_{max} Urinary excretion (mg/L per hour)	0.3068	0.4416	0.2642
K_m Urinary excretion (mg/L)	0.2640	0.6292	0.2610
V_{max} Metabolism (mg/L per hour)	0.2873	0.1984	0.2629
K_m Metabolism (mg/L)	2,393.1	1,895.8	3,004.8
k_1 ($hour^{-1}$)	0.0735	0.0312	0.075
k_2 ($hour^{-1}$)	6.8556	5.8479	6.85
k_{LS} ($hour^{-1}$)	0.00001	0.0005	0.001

TABLE K4
Fixed Parameter Values for Mice from the Physiologically Based Pharmacokinetic Model of 4-Methylimidazole[a]

k_1 ($hour^{-1}$)	0.03
k_2 ($hour^{-1}$)	5.85
k_{LS} ($hour^{-1}$)	0.0005

[a] Based on estimates from male rats

TABLE K5
Parameter Estimates for Mice from the Physiologically Based Pharmacokinetic Model of 4-Methylimidazole

Parameter	Reduced Model	Full Model	
	All Mice	Male Mice	Female Mice
V_{max} Gavage uptake (mg/L per hour)	7.9905	7.7701	12.2372
K_m Gavage uptake (mg/L)	0.6532	0.5402	1.5075
Fecal elimination rate ($hour^{-1}$)	8.1915	7.4807	8.4597
V_{max} Urinary excretion (mg/L per hour)	0.2181	0.2306	0.1486
K_m Urinary excretion (mg/L)	0.3235	0.2415	0.5801
V_{max} Metabolism (mg/L per hour)	0.3504	0.4252	0.3281
K_m Metabolism (mg/L)	81.0460	20.7664	106.9741

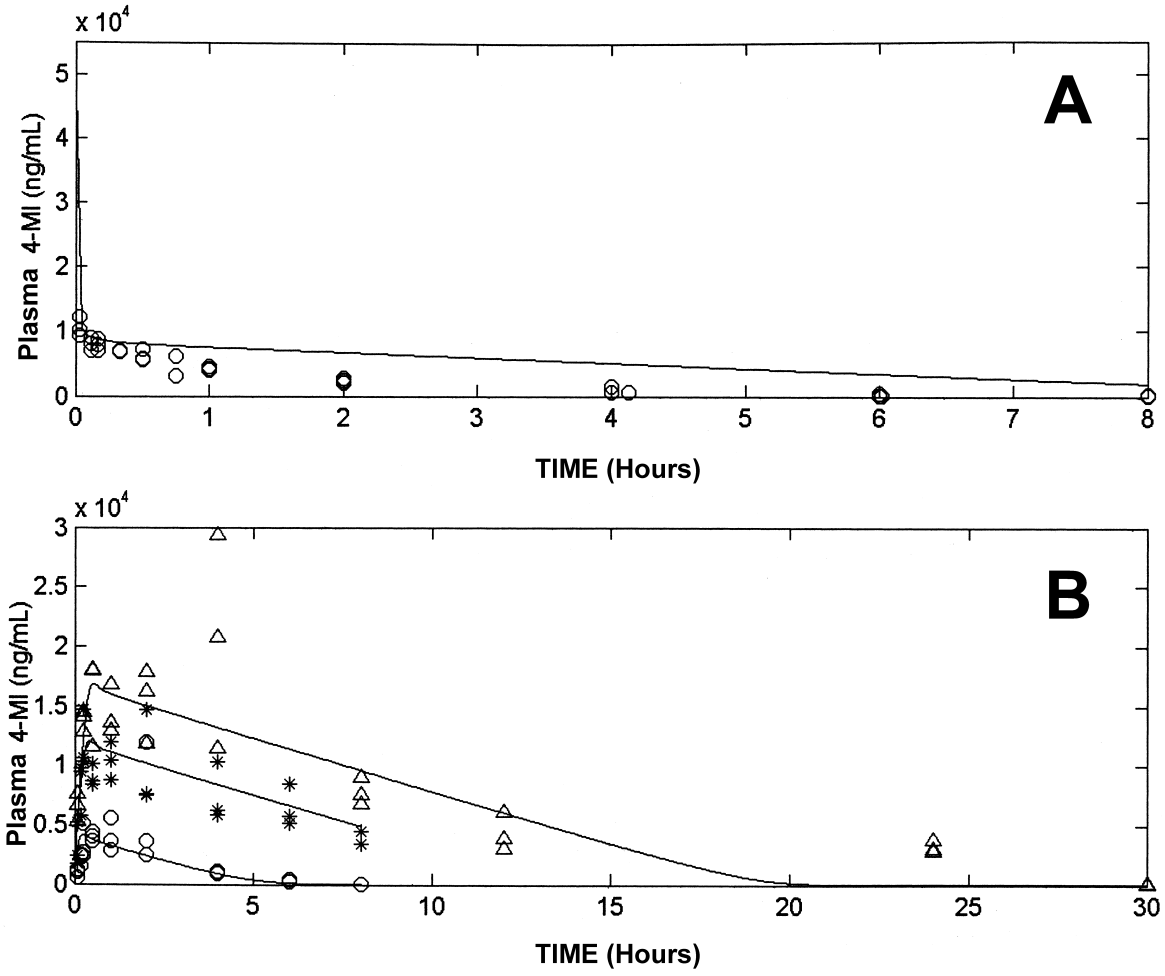

FIGURE K2
Plasma Concentrations of 4-Methylimidazole in Male Rats after a Single Intravenous Injection (Panel A) or a Single Gavage Dose (Panel B) of 4-Methylimidazole
Solid lines represent the predicted best-fit curves (from the reduced PBPK model) plotted through the observed data points. (Observed data: O – 10 mg/kg; ✻ – 50 mg/kg; Δ – 100 mg/kg)

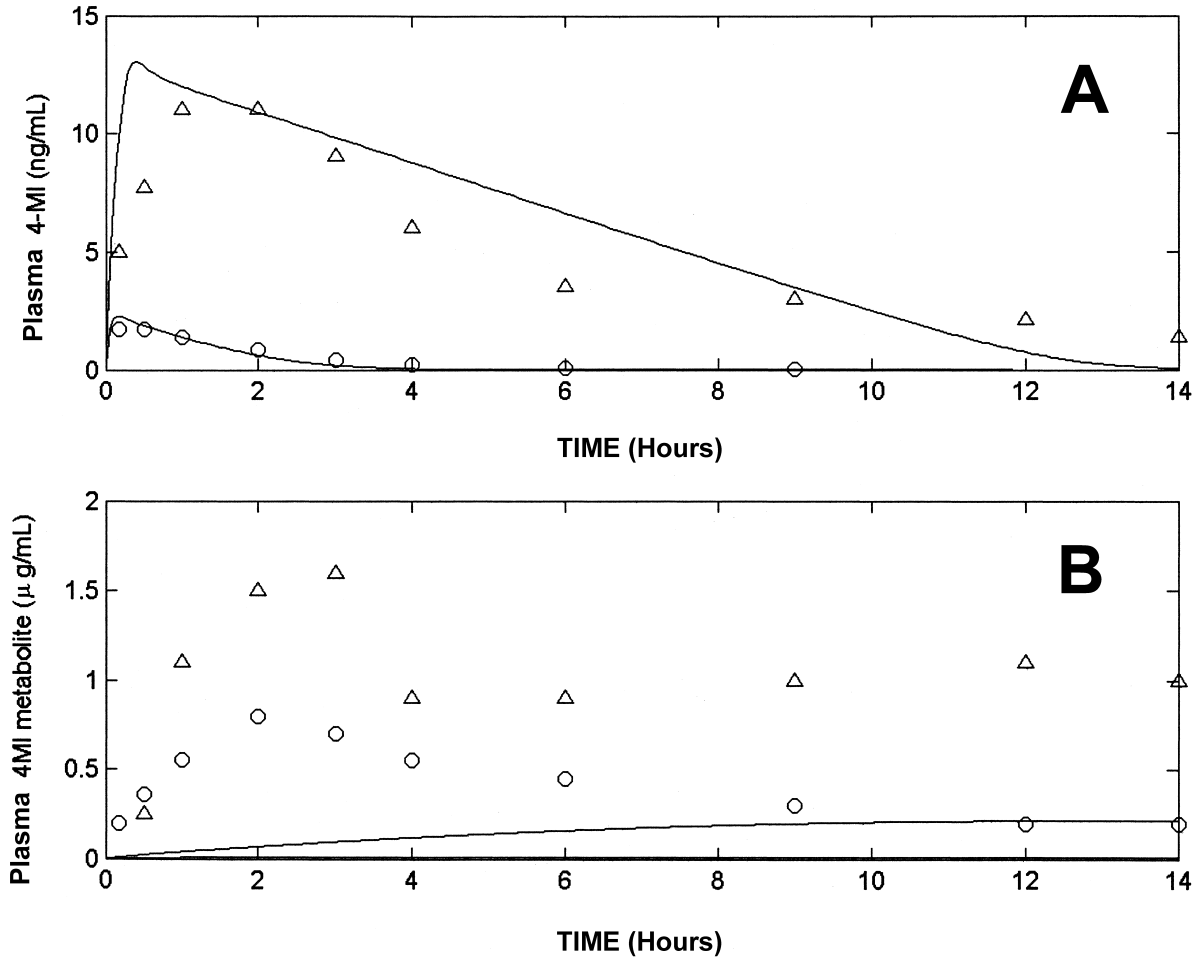

FIGURE K3
Yuan and Burka (1995) Data: Plasma Concentrations of 4-Methylimidazole (Panel A) or 4-Methylimidazole Metabolite (Panel B) in Male Rats after a Single Gavage Dose of 4-Methylimidazole
Solid lines represent the predicted best-fit curves (from the reduced PBPK model) plotted through the observed data points. (Observed data: O – 5 mg/kg; Δ – 50 mg/kg)

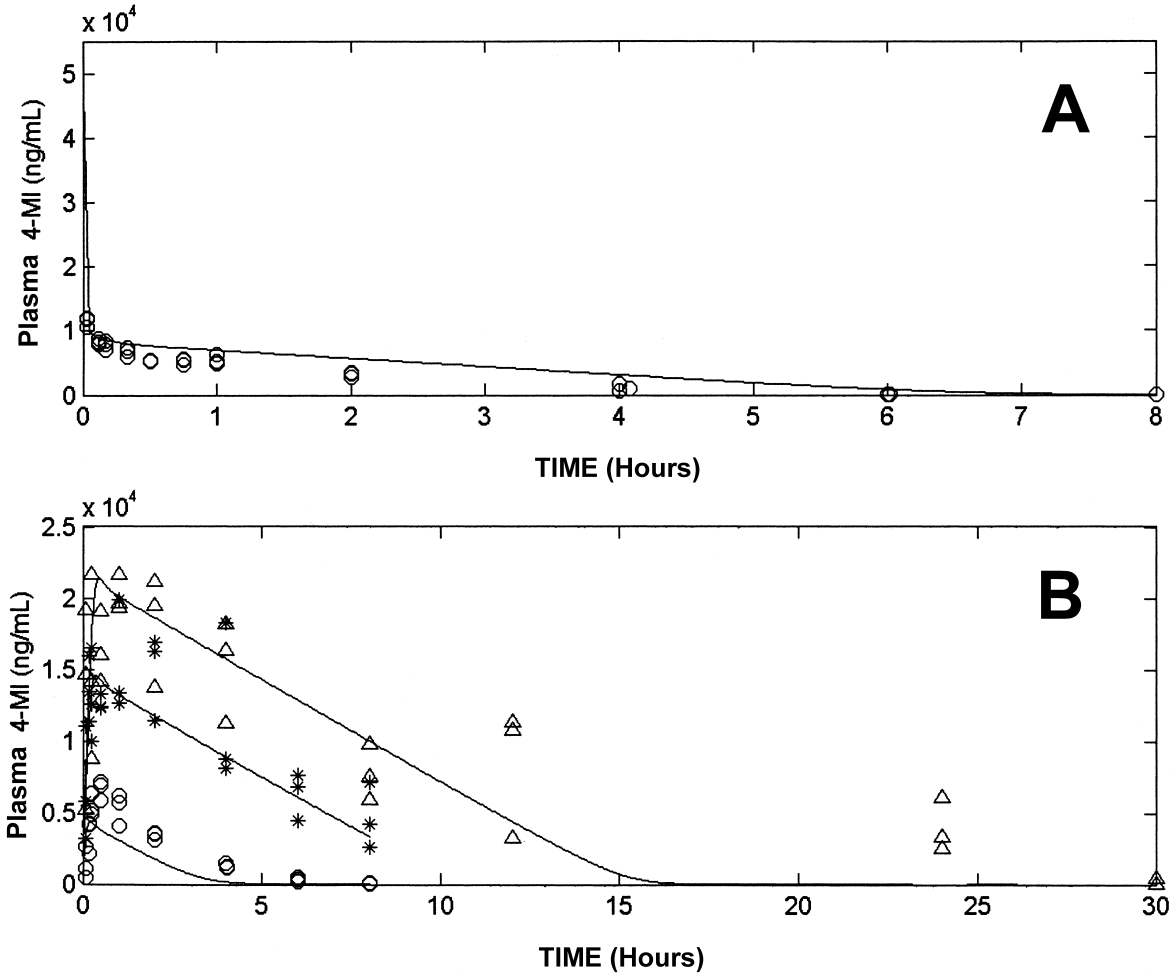

FIGURE K4
Plasma Concentrations of 4-Methylimidazole in Female Rats after a Single Intravenous Injection (Panel A) or a Single Gavage Dose (Panel B) of 4-Methylimidazole
Solid lines represent the predicted best-fit curves (from the reduced PBPK model) plotted through the observed data points. (Observed data: O – 10 mg/kg; ✻ – 50 mg/kg; Δ – 100 mg/kg)

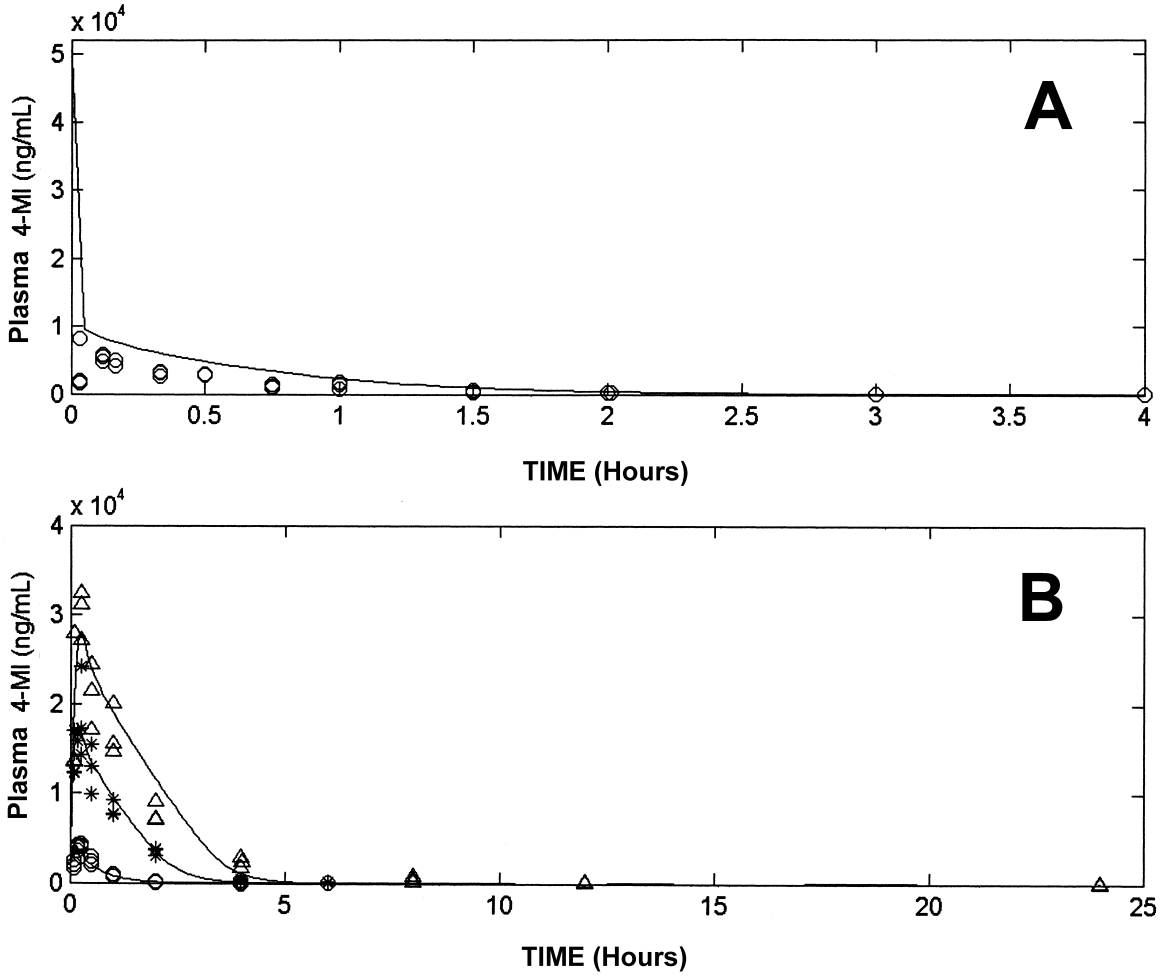

FIGURE K5
Plasma Concentrations of 4-Methylimidazole in Male Mice after a Single Intravenous Injection (Panel A) or a Single Gavage Dose (Panel B) of 4-Methylimidazole
Solid lines represent the predicted best-fit curves (from the full PBPK model) plotted through the observed data points. (Observed data: O – 10 mg/kg; ✻ – 50 mg/kg; Δ – 100 mg/kg)

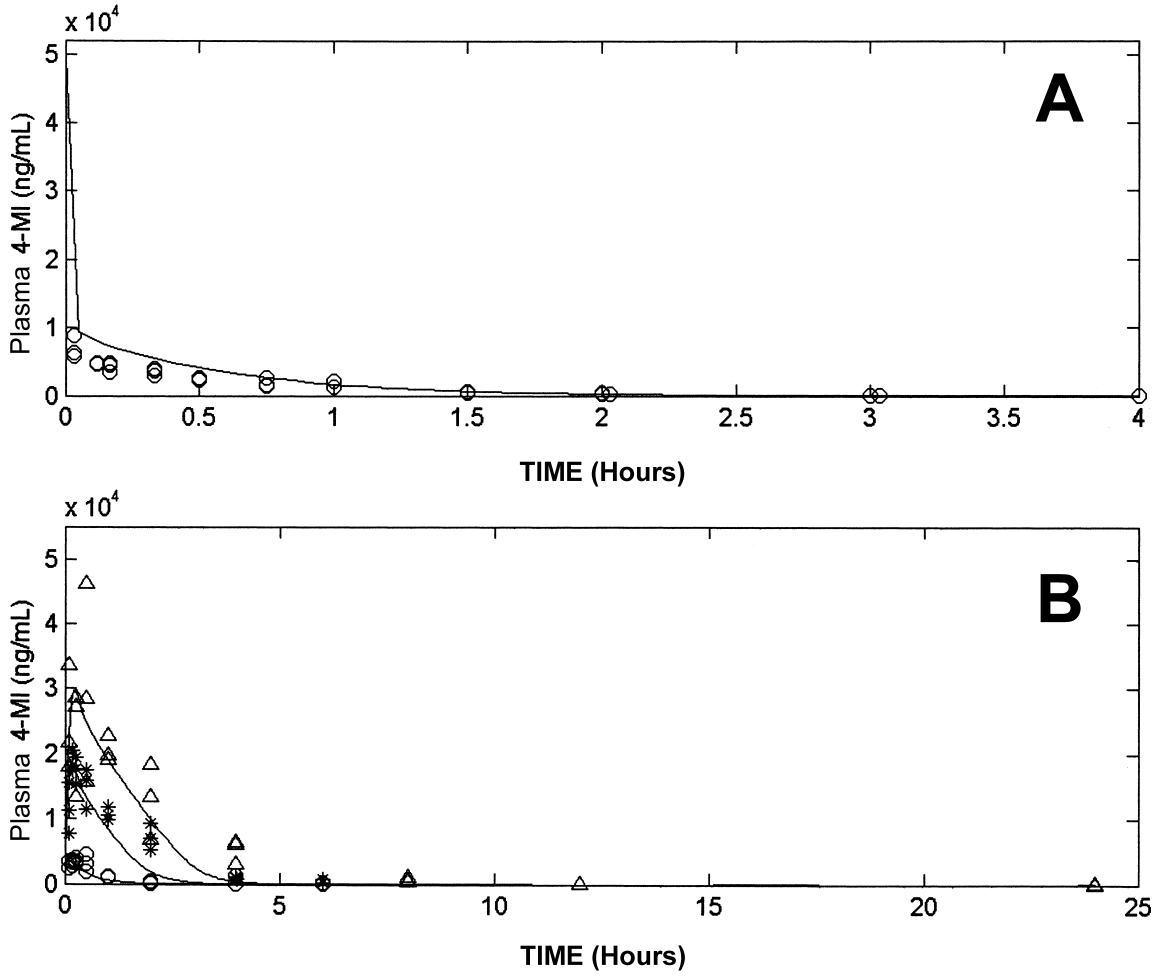

FIGURE K6
Plasma Concentrations of 4-Methylimidazole in Female Mice after a Single Intravenous Injection (Panel A) or a Single Gavage Dose (Panel B) of 4-Methylimidazole
Solid lines represent the predicted best-fit curves (from the full PBPK model) plotted through the observed data points. (Observed data: O – 10 mg/kg; ✻ – 50 mg/kg; Δ – 100 mg/kg)

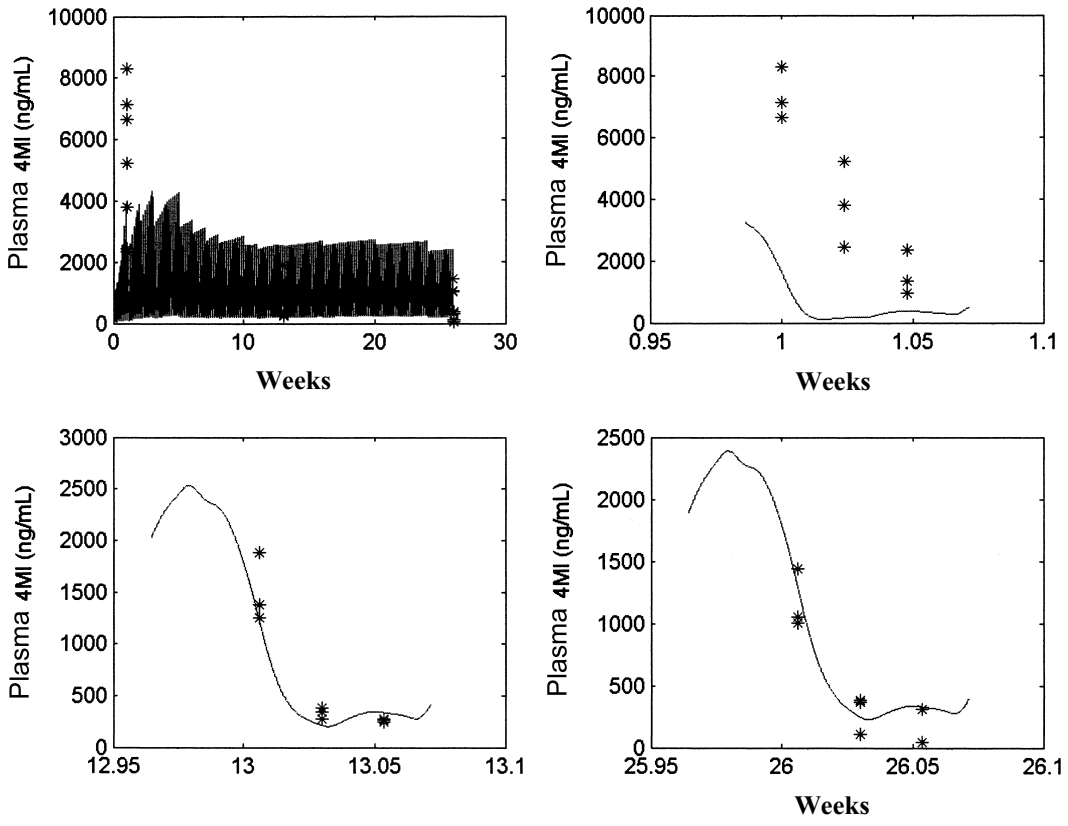

FIGURE K7
Reduced PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Male Rats Exposed to 625 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

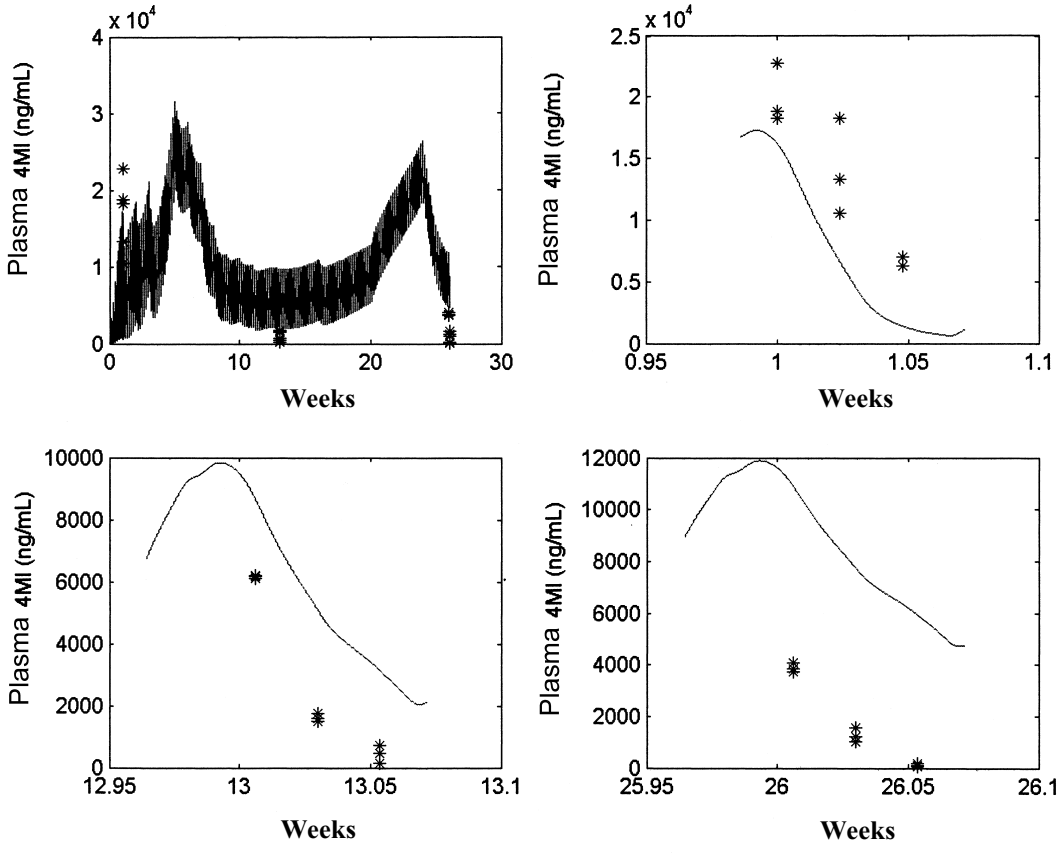

FIGURE K8
Reduced PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Male Rats Exposed to 1,250 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

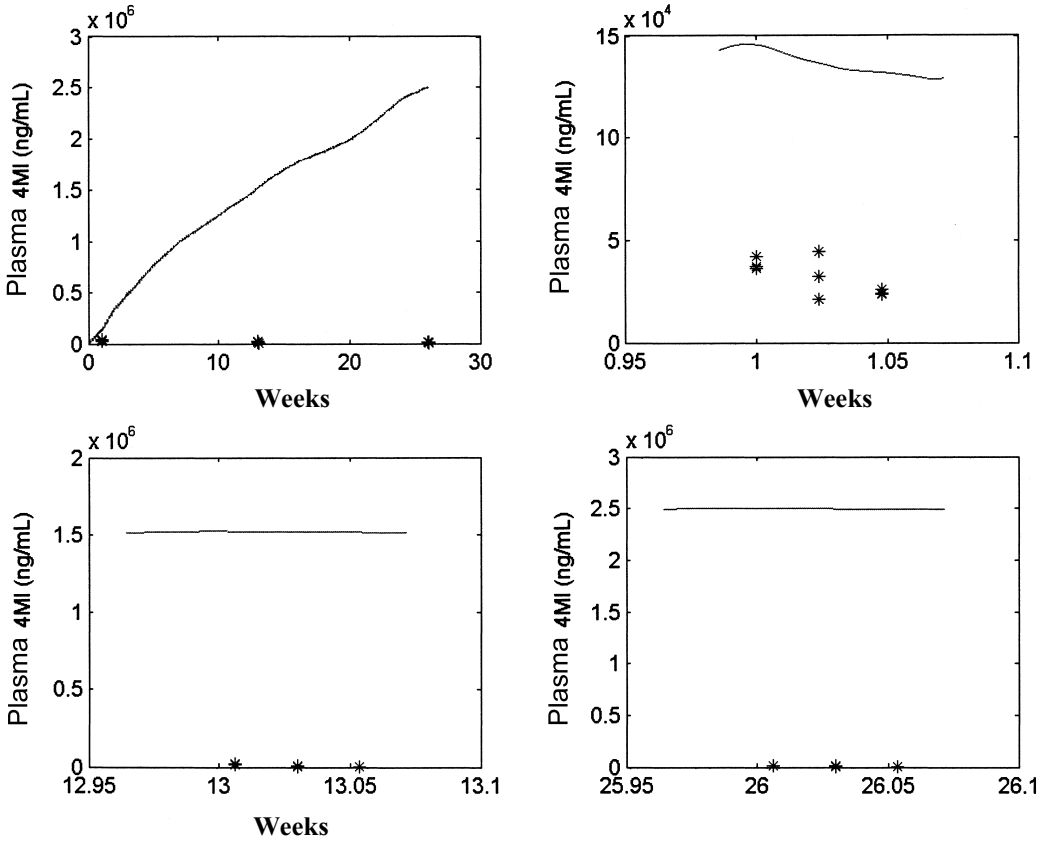

FIGURE K9
Reduced PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Male Rats Exposed to 2,500 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

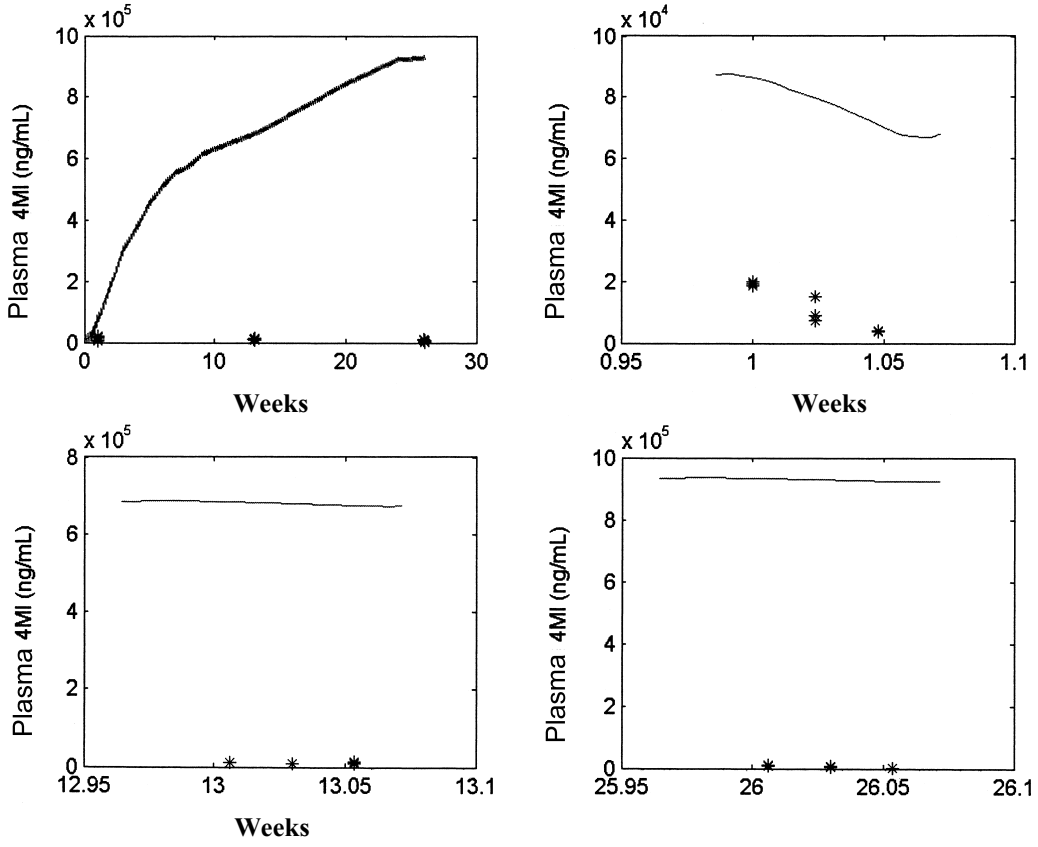

FIGURE K10
Reduced PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Female Rats Exposed to 1,250 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

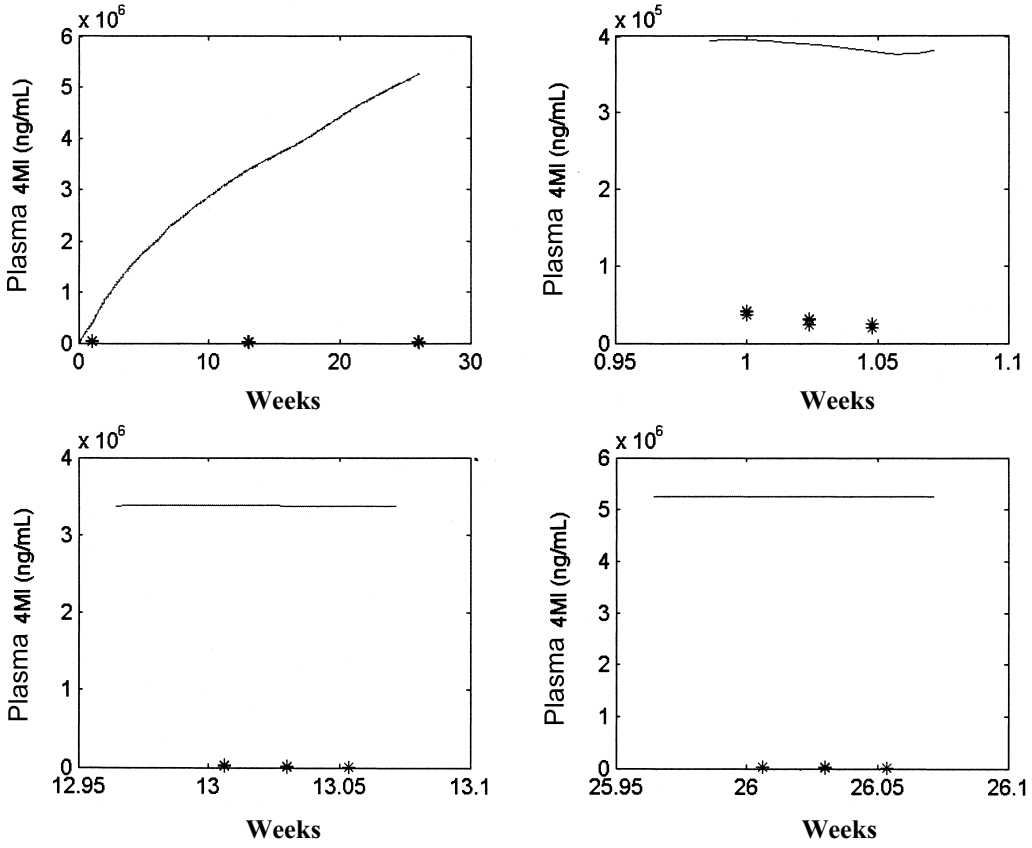

FIGURE K11
Reduced PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Female Rats Exposed to 2,500 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

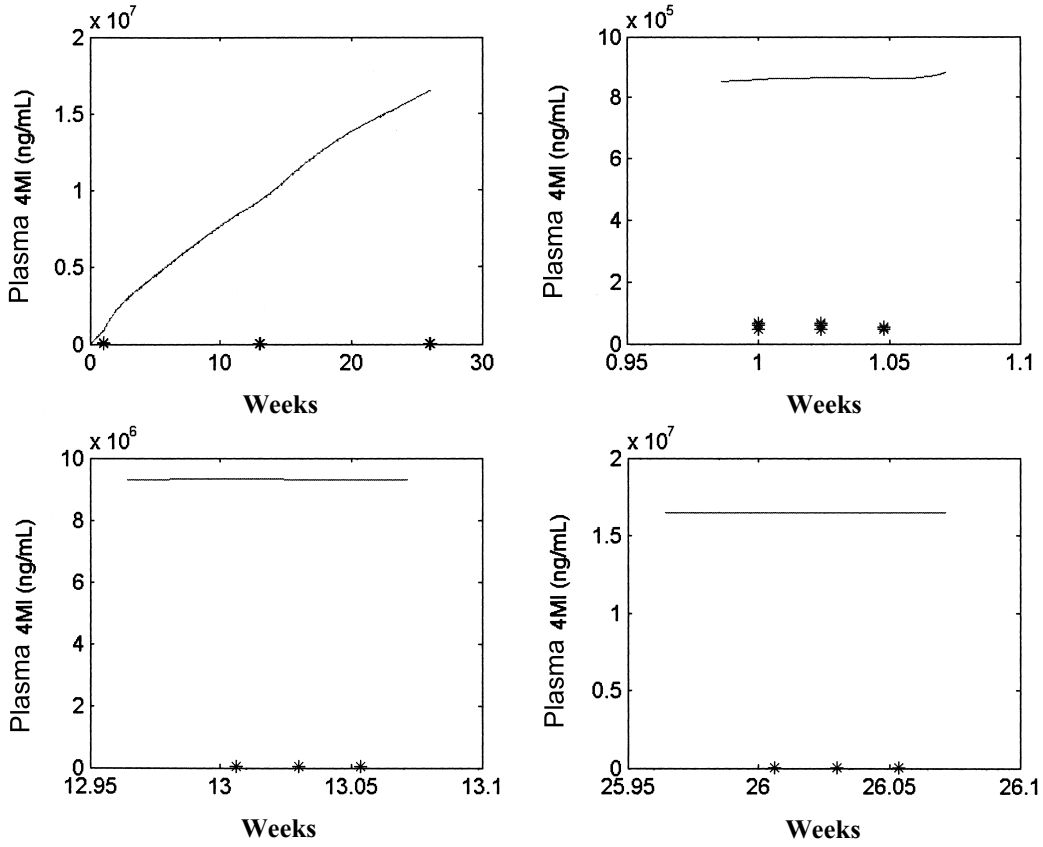

FIGURE K12
Reduced PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Female Rats Exposed to 5,000 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

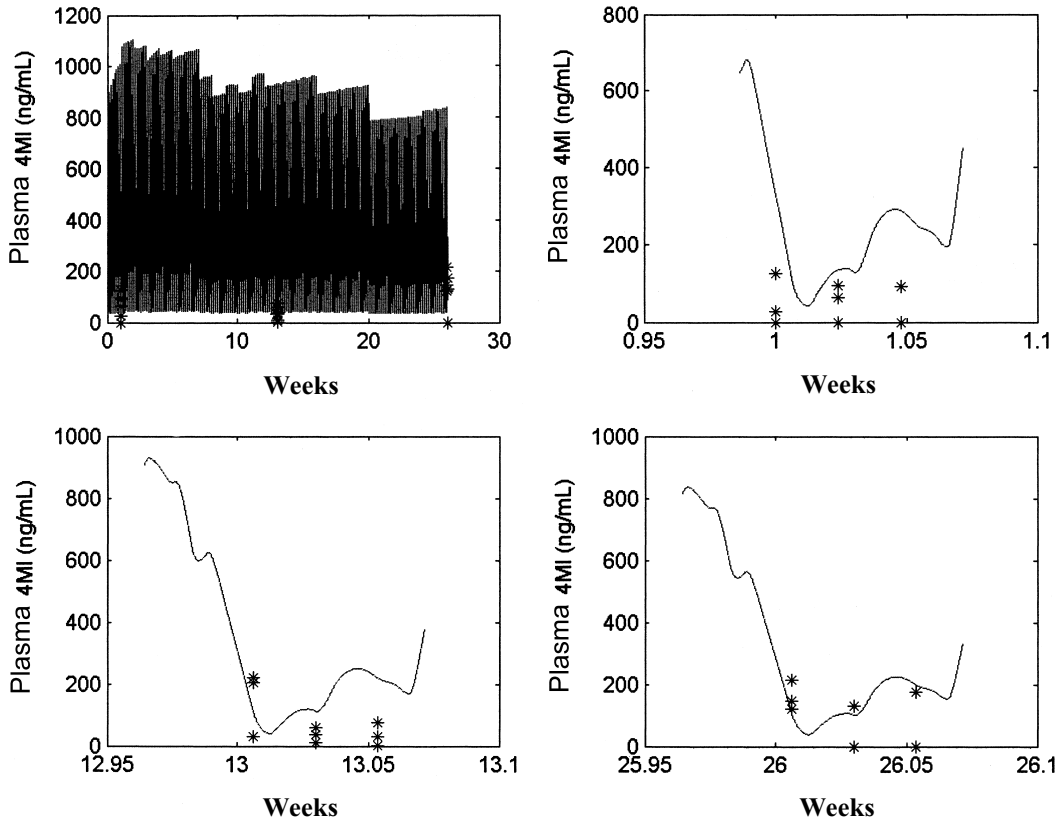

FIGURE K13
Full PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Male Mice Exposed to 312 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

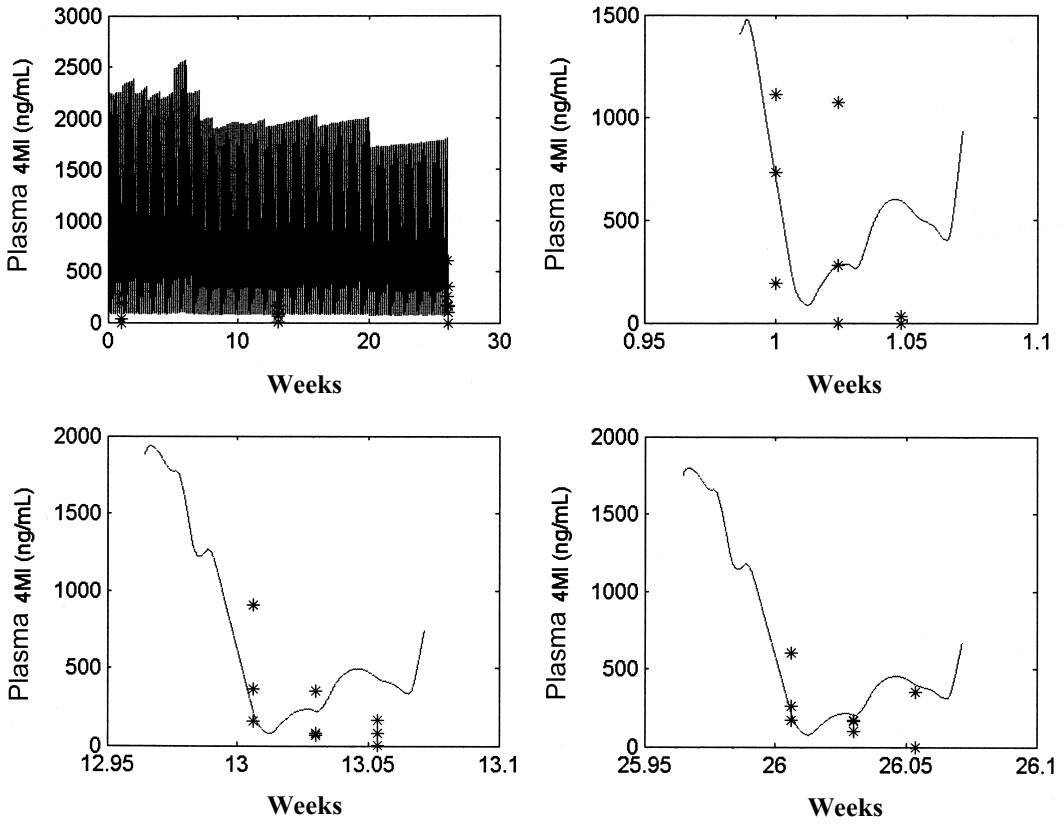

FIGURE K14
Full PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Male Mice Exposed to 625 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

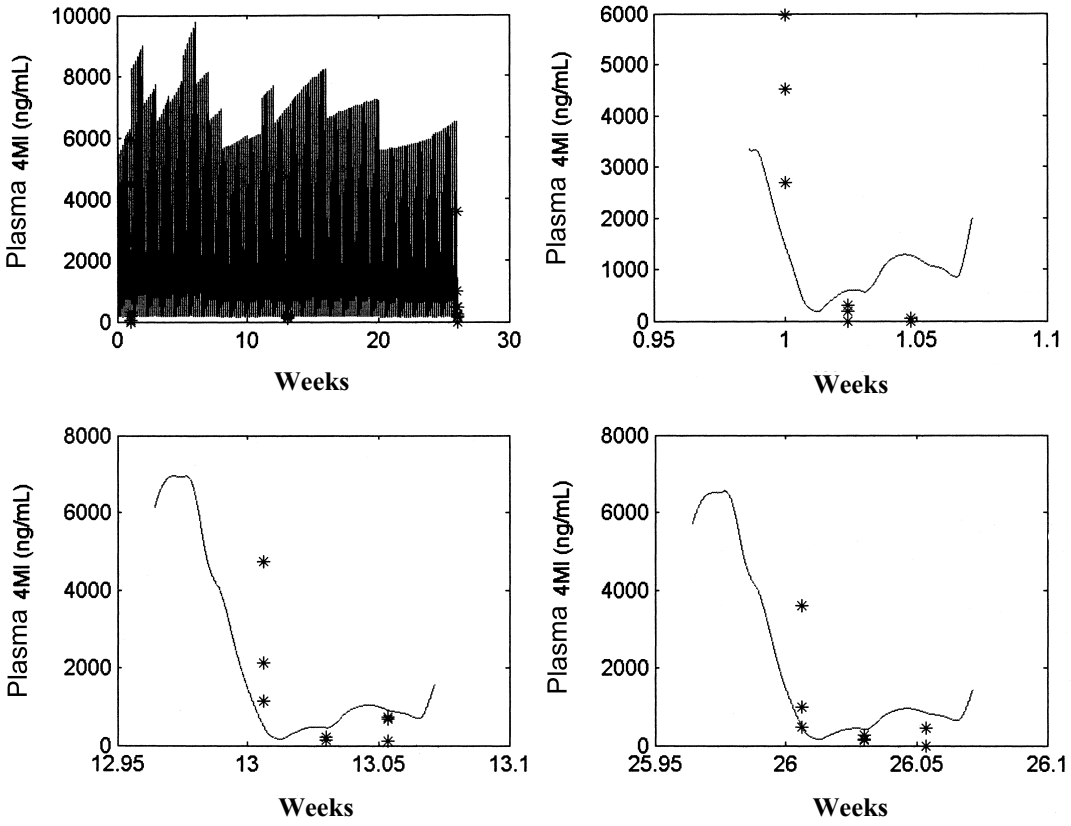

FIGURE K15
Full PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Male Mice Exposed to 1,250 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

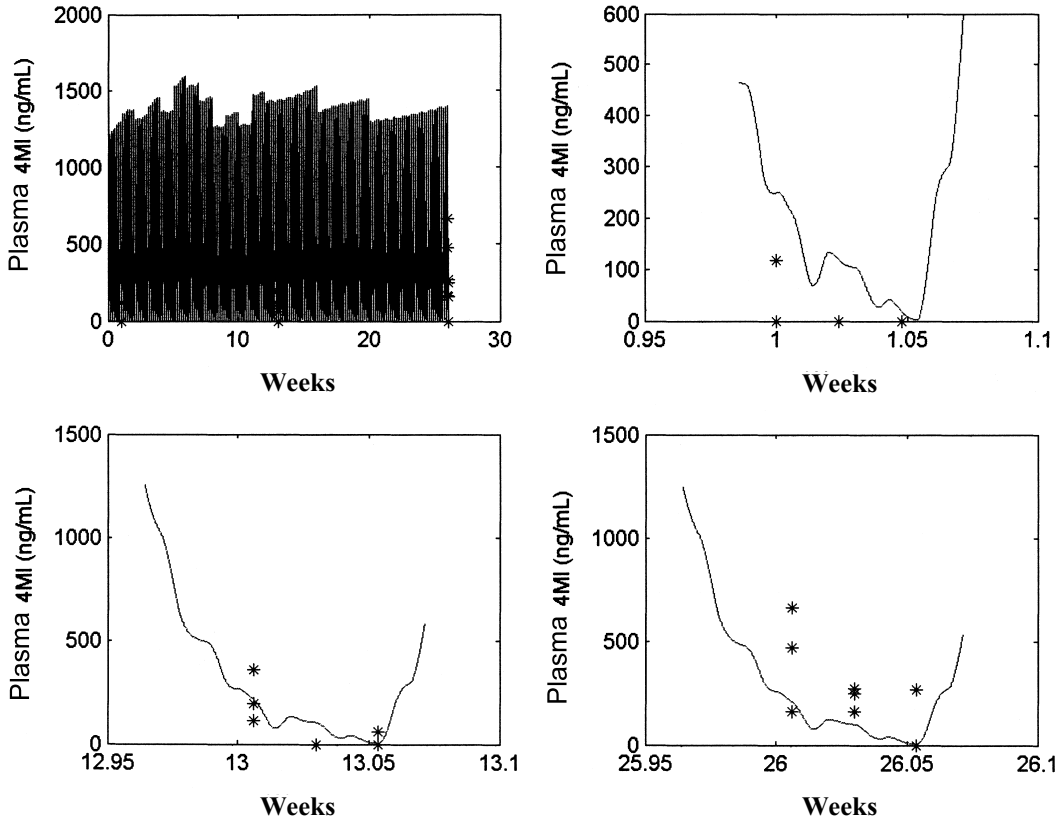

FIGURE K16
Full PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Female Mice Exposed to 312 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

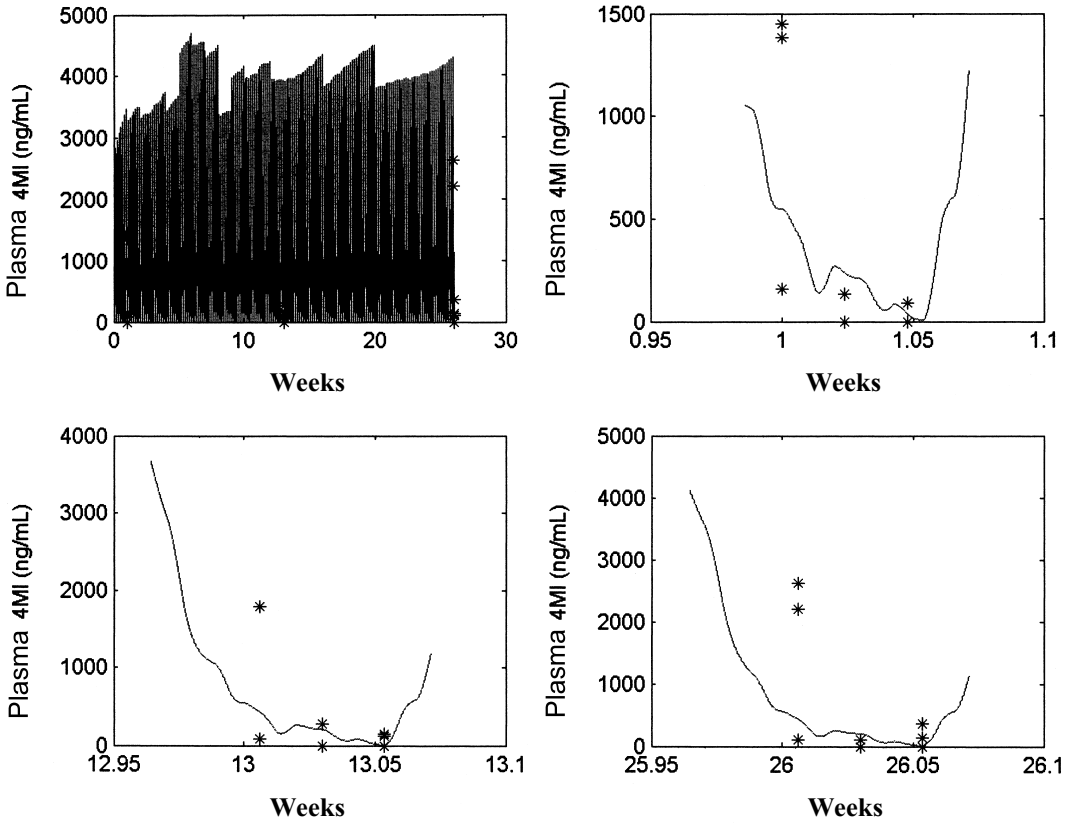

FIGURE K17
Full PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Female Mice Exposed to 625 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.

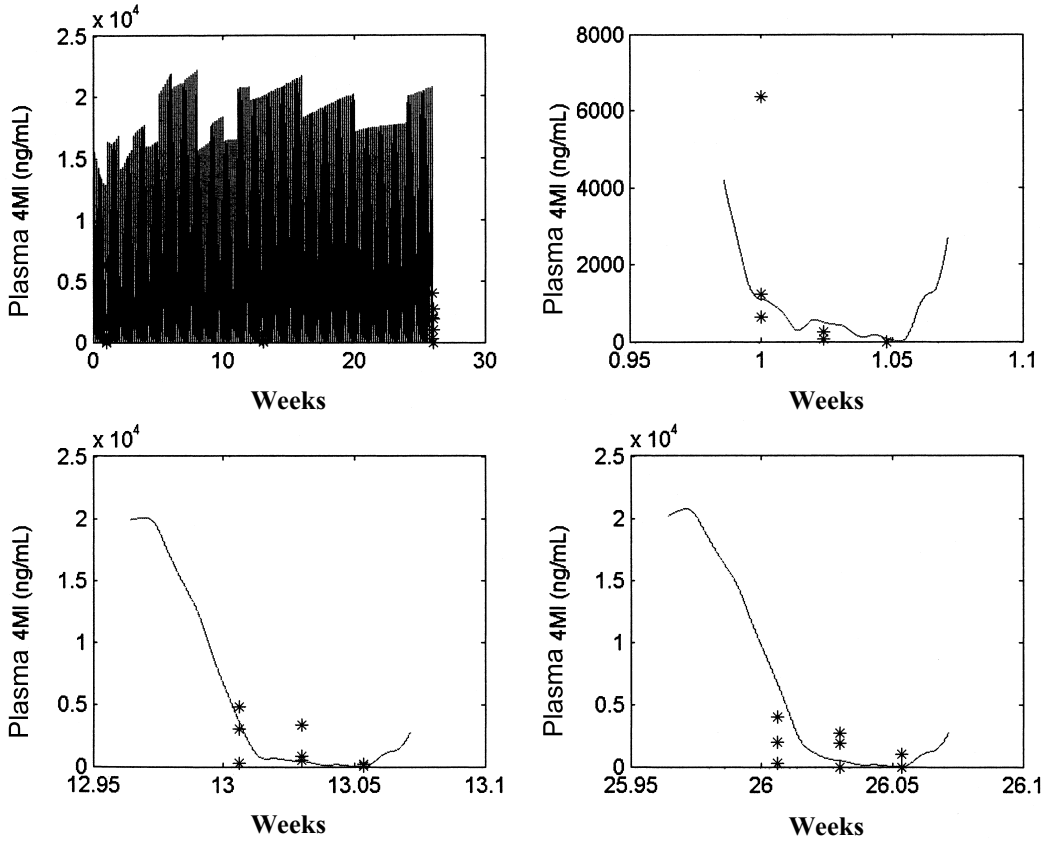

FIGURE K18
Full PBPK Model Predictions (lines) and Experimental Data (stars) for Plasma Concentrations of 4-Methylimidazole in Female Mice Exposed to 1,250 ppm in the 2-Year Feed Study of 4-Methylimidazole
The upper left panel plots the data and predictions for the first 26 weeks of the feed study; the other panels expand the plot at the times of data collection at 1, 13, and 26 weeks.