NOTE: An erratum is posted on pages 301-302 that updates information on pages 7, 15-16, and 67.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF 2-METHYLIMIDAZOLE

(CAS NO. 693-98-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

December 2004

NTP TR 516

NIH Publication No. 05-4456

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

FOREWORD

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

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

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

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

Details about ongoing and completed NTP studies, abstracts of all NTP Technical Reports, and full versions of the completed reports are available at the NTP's World Wide Web site: http://ntp.niehs.nih.gov. In addition, printed copies of these reports are available from the NTP as supplies last (919-541-1371).

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CONTENTS

SUMMARY

Background: 2-methylimidazole is used to make many other chemicals for drugs, photography, dyes, rubber, and agriculture. We studied the effects of 2-methylimidazole on male and female rats and mice to identify potential toxic or cancer-related hazards to humans.

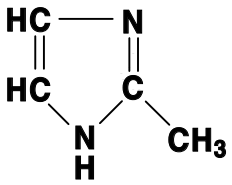
Methods: We studied the effects of 2-methylimidazole by mixing it in the feed of rats and mice for 2 years. The doses given were 300, 1,000, or 3,000 parts per million (ppm) 2-methylimidazole (equivalent to 0.03%, 0.1%, or 0.3%) for male rats; 1,000, 2,500, or 5,000 ppm for female rats; and 625, 1,250, or 2,500 ppm for male and female mice. There were 50 animals in each exposure group. Control animals received the same feed with no chemical added. Tissues from more than 40 sites were examined for every animal.

Results: For both male and female rats and mice, the groups receiving the highest amounts of 2-methylimidazole weighed less on average than the control animals. Male and female rats and male mice receiving 2-methylimidazole had higher rates of thyroid gland cancers than did the untreated control animals. The rates of liver tumors were greater in male and female mice receiving 2-methylimidazole and also slightly increased in male and female rats receiving 2-methylimidazole.

Conclusions: We concluded that 2-methylimidazole caused increased rates of cancer of the thyroid gland and liver in rats and mice.

NOTE: An erratum is posted on pages 301-302 that updates information on pages 7, 15-16, and 67.

ABSTRACT



2-METHYLIMIDAZOLE

CAS No. 693-98-1

Chemical Formula: $C_4H_6N_2$ Molecular Weight: 82.11

Synonyms: Imidazole,2-methyl; 2-MeI; 2-methylglyoxaline; 2-MI; 2-MZ

2-Methylimidazole is used in the manufacture of pharmaceuticals, photographic chemicals, dyes and pigments, agricultural chemicals, and rubber. It has been identified as a by-product in foods and has been detected in mainstream and sidestream tobacco smoke. 2-Methylimidazole was nominated by the National Cancer Institute for a long-term study because of the high potential for human exposure and a lack of carcinogenicity studies in rodents. Male and female F344/N rats and B6C3F$_1$ mice were exposed to 2-methylimidazole (99.5% pure) in feed for 2 years. Fifteen-day and 14-week toxicity studies of 2-methylimidazole in F344/N rats and B6C3F$_1$ mice are reported in NTP Toxicity Report No. 67 (NTP, 2004). Genetic toxicity studies were conducted in *Salmonella typhimurium*, rat and mouse bone marrow cells, and mouse peripheral blood.

2-Year Study in Rats

Groups of 60 male and 60 female rats were fed diets containing 0, 300, 1,000, or 3,000 ppm 2-methylimidazole (males) or 0, 1,000, 2,500, or 5,000 ppm 2-methylimidazole (females) (equivalent to average daily doses of approximately 13, 40, or 130 mg 2-methylimidazole/kg body weight to males and 50, 120, or 230 mg/kg to females) for 106 weeks. Ten male and 10 female rats were necropsied at 6 months. Additional groups of 20 male and 20 female special study rats were exposed to the same concentrations for 8 days or 14 weeks and were evaluated for clinical chemistry, liver enzyme activity, and organ weights. Survival of 2,500 ppm females was significantly less than that of the controls. The mean body weights of 3,000 ppm males and 2,500 and 5,000 ppm females were generally less than those of the controls during most of the study. Feed consumption by 5,000 ppm females was less than that by the control group.

The hematology results at 6 months indicated that exposure of rats to 2-methylimidazole induced a decreased erythron that was characterized as microcytic, normochromic, and nonresponsive. The thyroid hormone data indicated that rats administered 2-methylimidazole developed alterations in thyroid hormone concentrations; serum thyroxine and triiodothyronine concentrations were decreased, and thyroid stimulating hormone levels were increased. In general, the thyroid hormone

effects were most pronounced early in the study and ameliorated with time. The results for the tissue enzyme content analyses of these 2-year feed studies indicated that exposure of rats to 2-methylimidazole induced an increase in total hepatic UDP-glucuronosyltransferase at all time points evaluated through 6 months. The thyroid gland weights of 3,000 ppm males and 2,500 and 5,000 ppm females were significantly increased at 6 months.

At 6 months, two 5,000 ppm female rats had a thyroid gland follicular cell adenoma. The incidences of follicular cell adenoma, follicular cell carcinoma, and adenoma or carcinoma (combined) in the thyroid gland of 5,000 ppm females were significantly greater that those in the controls at 2 years. The incidence of follicular cell adenoma or carcinoma (combined) in the thyroid gland occurred with a positive trend in males. The incidences of follicular cell hyperplasia in all exposed groups of rats were significantly increased at 6 months and 2 years. The incidences of follicle mineralization of the thyroid gland in all exposed groups, except 300 ppm males at 6 months and in 1,000 and 5,000 ppm females at 2 years, were significantly greater than those of the controls.

In the liver, the incidences of hepatocellular adenoma or carcinoma (combined) in the two highest exposure groups of males and females exceeded the historical ranges for controls, and the incidences of hepatocellular adenoma in females occurred with a positive trend. The incidences of bile duct hyperplasia and granulomatous inflammation were increased in females, as were those of mixed cell focus in males and females. The incidence of granulomatous inflammation of the spleen in 5,000 ppm females was significantly increased. Lower body weights of female rats exposed to 5,000 ppm likely contributed to the decreased incidences of mammary gland fibroadenoma, pituitary gland adenoma, and clitoral gland adenoma in this group.

2-Year Study in Mice

Groups of 60 male and 60 female mice were fed diets containing 0, 625, 1,250, or 2,500 ppm 2-methylimidazole (equivalent to average daily doses of approximately 75, 150, or 315 mg/kg to males and 80, 150, or 325 mg/kg to females) for 105 weeks. Ten male and 10 female mice were necropsied at 6 months. Additional groups of 20 male and 20 female special study mice were exposed to the same concentrations for 8 days or 14 weeks and were evaluated for clinical chemistry, liver enzyme activity, and organ weights.

Survival of all exposed groups of mice was similar to that of the control groups. The mean body weights of 1,250 and 2,500 ppm males and 2,500 ppm females were less than those of the controls during most of the study. Feed consumption by all exposed groups of mice was similar to that by the control groups.

The hematology results at 6 months indicated that exposure of mice to 2-methylimidazole induced a decreased erythron that was characterized as macrocytic, normochromic to hypochromic, and responsive. The thyroid gland weights of 2,500 ppm male and female mice and 1,250 ppm females were increased at 6 months.

The incidence of follicular cell adenoma in the thyroid gland of 2,500 ppm males was significantly greater than that in the control group at 2 years. Follicular cell hypertrophy of the thyroid gland occurred in most exposed mice at 6 months, and the incidences of this lesion were significantly increased in the 1,250 and 2,500 ppm groups at 2 years; the incidences of follicular cell hyperplasia were significantly increased in 2,500 ppm males and females at 2 years.

The liver weights of 2,500 ppm female mice were significantly increased at 6 months. The incidences of hepatocellular adenoma occurred with positive trends in males and females and the incidences were significantly increased in the 2,500 ppm groups. The incidence of hepatocellular carcinoma was significantly increased in 1,250 ppm males and exceeded the historical control range in 2,500 ppm males. The incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in all exposed groups of males. The incidences of hepatocellular karyomegaly in 2,500 ppm males at 6 months and in 1,250 and 2,500 ppm males at 2 years, of hepatocellular cytoplasmic alteration in 1,250 and 2,500 ppm males at 2 years, and Kupffer cell pigmentation in 2,500 ppm males at 2 years were significantly increased.

In the spleen, the incidences of hematopoietic cell proliferation in all exposed groups of males and in 2,500 ppm females were significantly increased at 6 months and 2 years. Pigmentation was present in most 1,250 and 2,500 ppm mice at 6 months, and the incidences of this lesion were significantly increased in all exposed groups of males and in 1,250 and 2,500 ppm females at 2 years. The incidences of bone marrow hyperplasia were significantly increased in 1,250 and 2,500 ppm male mice at 2 years. Renal proximal tubule pigmentation was present in most 2,500 ppm male mice

at 6 months and 2 years. The responses in the spleen, bone marrow, and kidney were considered to be related to the responsive anemia. In males, the incidences of chronic active inflammation of the epididymis at 1,250 and 2,500 ppm, sperm granuloma at 2,500 ppm, and of germinal epithelial atrophy of the testis at 1,250 and 2,500 ppm were significantly increased at 2 years.

Genetic Toxicology

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

Conclusions

Under the conditions of this 2-year feed study, there was *some evidence of carcinogenic activity** of 2-methylimidazole in male F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. The increased incidences of hepatocellular neoplasms in males may have been related to exposure. There was *clear evidence of carcinogenic activity* of 2-methylimidazole in female F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. The increased incidences of hepatocellular adenoma in females may have been related to exposure. There was *some evidence of carcinogenic activity* in male $B6C3F_1$ mice based on increased incidences of thyroid gland follicular cell adenoma and hepatocellular neoplasms. There was *some evidence of carcinogenic activity* in female $B6C3F_1$ mice based on increased incidences of hepatocellular adenoma.

Exposure to 2-methylimidazole resulted in nonneoplastic lesions in the thyroid gland and liver of male rats; the thyroid gland, liver, and spleen of female rats; the thyroid gland, liver, spleen, bone marrow, kidney, epididymis and testes of male mice; and the thyroid gland and spleen of female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

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

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in feed	0, 300, 1,000, or 3,000 ppm	0, 1,000, 2,500, or 5,000 ppm	0, 625, 1,250, or 2,500 ppm	0, 625, 1,250, or 2,500 ppm
Body weights	3,000 ppm group less than the control group	2,500 and 5,000 ppm groups less than the control group	1,250 and 2,500 ppm groups less than the control group	2,500 ppm group less than the control group
Survival rates	35/50, 40/50, 36/50, 35/50	40/50, 39/50, 28/50, 42/50	43/50, 46/50, 36/50, 40/50	46/50, 43/49, 43/49, 45/50
Nonneoplastic effects	Thyroid Gland: follicular cell hyperplasia (0/48, 17/46, 37/43, 43/50) Liver: mixed cell focus (14/50, 16/50, 23/50, 26/50)	Thyroid Gland: follicular cell hyperplasia (0/49, 41/48, 34/42, 46/48); mineralization (42/49, 47/48, 41/42, 48/48) Liver: bile duct hyperplasia (20/50, 29/49, 20/50, 40/50); inflammation granulomatous (18/50, 23/49, 22/50, 42/50); mixed cell focus (15/50, 14/49, 11/50, 26/50) Spleen: granulamatous inflammation (3/50, 2/49, 4/48, 27/50)	Thyroid Gland: follicular cell hyperplasia (0/50, 2/50, 3/50, 33/50); follicular cell hypertrophy (1/50, 0/50, 6/50, 25/50) Liver: hepatocyte cytoplasmic alteration (0/50, 0/50, 11/50, 37/50); hepatocyte karyomegaly (0/50, 0/50, 10/50, 29/50); Kupffer cell pigmentation (0/50, 1/50, 1/50, 19/50) Spleen: hematopoietic cell proliferation (10/50, 21/50, 38/49, 45/50); pigmentation (1/50, 16/50, 33/49, 43/50); lymphoid follicle atrophy (0/50, 4/50, 14/49, 30/50) Bone Marrow: hyperplasia (4/50, 10/50, 20/50, 42/50) Kidney: renal tubule pigmentation (1/50, 0/50, 2/50, 45/50) Epididymis: inflammation chronic active (1/50, 3/50, 7/50, 8/50); sperm granuloma (0/50, 0/50, 2/50, 5/50) Testes: germinal epithelium atrophy (1/50, 4/50, 8/50, 14/50)	Thyroid Gland: follicular cell hyperplasia (1/49, 1/48, 1/48, 9/50); follicular cell hypertrophy (6/49, 3/48, 23/48, 46/50) Spleen: hematopoietic cell proliferation (15/50, 20/49, 24/49, 39/50); pigmentation (0/50, 4/49, 11/49, 34/50)

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

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects	Thyroid Gland: follicular cell adenoma or carcinoma (1/48, 2/46, 1/43, 5/50)	Thyroid Gland: follicular cell adenoma (0/49, 0/48, 0/42, 5/48); follicular cell carcinoma (1/49, 1/48, 1/42, 7/48); follicular cell adenoma or carcinoma (1/49, 1/48, 1/42, 11/48)	Thyroid Gland: follicular cell adenoma (0/50, 1/50, 0/50, 7/50) Liver: hepatocellular adenoma (7/50, 14/50, 13/50, 18/50); hepatocellular carcinoma (4/50, 8/50, 14/50, 6/50); hepatocellular adenoma or carcinoma (10/50, 22/50, 22/50, 22/50)	Liver: hepatocellular adenoma (3/50, 4/49, 6/49, 10/50)
Equivocal findings	Liver: hepatocellular adenoma or carcinoma (0/50, 1/50, 3/50, 3/50)	Liver: hepatocellular adenoma (1/50, 0/49, 2/50, 4/50)	None	None
Level of evidence of carcinogenic activity	Some evidence	Clear evidence	Some evidence	Some evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative in strains TA97, TA98, TA100, and TA1537 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:*	Positive			

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 2-methylimidazole on May 22, 2003, 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 May 22, 2003, the draft Technical Report on the toxicology and carcinogenesis studies of 2-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, Research Triangle Park, NC.

Dr. P.C. Chan, NIEHS, introduced the toxicology and carcinogenicity studies of 2-methylimidazole by describing the uses of the chemical, the design of the previous short-term studies, and the results of the current 2-year feed studies. The proposed conclusions were *some evidence of carcinogenic activity* of 2-methylimidazole in male F344/N rats, *clear evidence of carcinogenic activity* of 2-methylimidazole in female F344/N rats, and *some evidence of carcinogenic activity* in male and female B6C3F_1 mice.

Dr. Piegorsch, the first principal reviewer, agreed with the proposed conclusions for rats and female mice but suggested the weight of evidence for male mice might be considered *clear evidence*.

Dr. Elwell, the second principal reviewer, agreed with the overall proposed conclusions and suggested some enhancements to the discussion about the possible relation between various proliferative lesions of the thyroid gland and about the granulomatous changes in the liver.

Dr. Vore, the third principal reviewer, also concurred with the proposed conclusions but questioned directly linking the liver neoplasms to the induction of UDP-glucoronosyl transferase.

Dr. Chan described the reasoning that led to the conclusion for the male mouse study, noting that there was not an exposure concentration-related increase in neoplasms, and that the neoplasm incidences in all groups were within the historical control range. Responding to Dr. Vore's question, he said that the T_4 related mechanism was mentioned because data on those measures were gathered in these studies, but the entire spectrum of the thyroid synthesis pathways and secretion were mentioned in the discussion as possible causes of the liver carcinogenesis.

Dr. R.C. Sills, NIEHS, addressed the pathogenesis of the thyroid gland lesions and, following a review of other NTP studies, concluded that there was no relationship between hypertrophy and subsequent development of thyroid gland hyperplasia or neoplasms.

Dr. Sills also indicated that the granulomatous inflammation of the liver and spleen were considered treatment related, and that a similar effect was seen in a preliminary review of tissues from studies on the related chemical 4-methylimidazole.

Dr. Storer suggested that the possible confounding effects of anemia and hematopoietic cell proliferation be added to the discussion of increases in micronuclei.

Dr. Piegorsch asked if the liver and thyroid gland neoplasms in male mice might warrant a conclusion of *clear evidence*. He also suggested that formal statistical analyses incorporating historical incidences, rather than just noting whether incidences occurred within the historical range, might be another useful metric for study evaluation.

Dr. Carpenter inquired if relatively more importance was attached to carcinomas than to adenomas in evaluating the response.

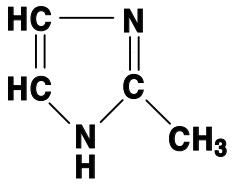
Dr. J.R. Bucher, NIEHS, noted that the studies are truncated at 2 years, and that progression and earlier onset of neoplasms are contributors to evidence of carcinogenic activity.

Dr. Boekelheide questioned the linking of testicular atrophy to epididymal alterations and suggested they could be independent responses to the chemical.

Dr. Piegorsch moved that the conclusions be accepted as drafted, with the exception that the conclusion for male mice be *clear evidence*. The motion failed for lack of a second. Dr. Piegorsch then moved that the conclusions be accepted as originally written. Dr. Boekelheide seconded the motion, which was approved unanimously with eight votes.

NOTE: An erratum is posted on pages 301-302 that updates information on pages 7, 15-16, and 67.

INTRODUCTION



2-METHYLIMIDAZOLE

CAS No. 693-98-1

Chemical Formula: $C_4H_6N_2$ Molecular Weight: 82.11

Synonyms: Imidazole,2-methyl; 2-MeI; 2-methylglyoxaline; 2-MI; 2-MZ

Chemical and Physical Properties

2-Methylimidazole is a colorless, crystalline solid with a melting point of 142° C and a boiling point of 267° C (MSDS, 2003); it is soluble in water and ethanol and sparingly soluble in cold benzene (Lewis, 1996).

Production, Use, and Human Exposure

2-Methylimidazole is produced by cyclocondensation of aldehyde and ammonia with methylglyoxal or by platinum/alumina cyclization of ethylenediamine with acetic acid (Lewis, 1996). Current production figures for 2-methylimidazole are not available. The public file of the Toxic Substances Control Act Chemical Substance Inventory (USEPA, 2000) reported that between 100,000 and 500,000 pounds of 2-methylimidazole were produced or imported in 1998.

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

Ammoniation of carbohydrate-containing material, including hay, to increase nonprotein nitrogen content is a common practice on farms (Ray *et al.*, 1984). 2-Methylimidazole, formed by interaction of ammonia with reducing sugars, has been identified as a toxic by-product in ammoniated hay forage for livestock animals (Ray *et al.*, 1984), and it has been identified in milk, plasma, and urine from cows and sheep given ammoniated feed (Morgan and Edwards, 1986; Perdok and Leng, 1987; Sivertsen *et al.*, 1997; Muller *et al.*, 1998). 2-Methylimidazole has been identified as an undesirable by-product in fermented foods and is found 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). During cooking, 2-methylimidazole may be formed when ammonium hydroxide, glycine, and monosodium glutamate are present (Wong and Bernhard, 1988). Humans may also be exposed to low concentrations of 2-methylimidazole in mainstream and sidestream cigarette smoke (Moree-Testa *et al.*, 1984; Sakuma *et al.*, 1984).

A joint committee of the Food and Agriculture Organization of the United Nations recommended in 1971 that daily intake of caramel should be restricted to no more than 100 mg caramel/kg of body weight (Chappel and Howell, 1992). Citing a cancer risk, legislation enacted in August of 1976 restricted the use of caramel coloring in food and beverages in Denmark (Chappel and Howell, 1992). The National Occupational Exposure Survey conducted from 1980 to 1983 estimated that 7,000 workers in 22 occupations at 318 facilities in 11 industries were potentially exposed to 2-methylimidazole annually in the United States (NIOSH, 1990). No standard or guideline has been set in the United States for allowable occupational exposure or environmental concentration of 2-methylimidazole (Chappel and Howell, 1992).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

[^{14}C]-2-Methylimidazole administered orally at up to 150 mg/kg was well absorbed in male and female Fischer rats (Sanders *et al.*, 1998). Urinary excretion of radioactivity was essentially complete by 12 hours and was similar following intravenous and oral administration implying near 100% absorption. About 90% of the administered radioactivity was excreted in urine; parent chemical contributed 64% to 75% of that radioactivity. In a time course study, tissue concentrations of 2-methylimidazole-derived radioactivity were highest in kidney, liver, muscle, and the thyroid gland 15 minutes after intravenous administration of a 5 mg/kg dose. Absolute tissue concentrations declined rapidly with time, but remained high relative to blood in kidney, liver, and thyroid gland throughout the 24-hour study.

Ohta *et al.* (1996) reported that within 24 hours of intravenous administration of 250 µg/kg of [^{14}C]-2-methylimidazole to male Wistar rats 76% to 88% of radioactivity was excreted in urine. Parent chemical accounted for 65% the radioactivity. Ohta *et al.* (1998) identified a mono-oxygenated urinary metabolite, 2-methylimidazolone.

Miyachi and Nagatsu (2002) demonstrated that the metabolite 2-methylimidazolone could be produced by oxidation of 2-methylimidazole using an iodosylbenzene/manganese porphyrin cytochrome P450 model system.

Imidazoles bind to the sixth ligand position of the cytochrome P450 heme, resulting in inhibition of catalysis. However, 2-substituted imidazoles are considered to be poor inhibitors (Murray, 1987). 2-Methylimidazole was shown to have a sevenfold higher K_i for inhibition of *p*-nitrophenol hydroxylase (a cytochrome P4502E1 substrate) than imidazole (Hargreaves *et al.*, 1994). The inhibition by both imidazole and 2-methylimidazole was characterized as noncompetitive. The interpretation the authors offer for the kinetic behavior is that the inhibitor may be complexed to the active site at the same time as the substrate. The decrease in inhibition between 2-methylimidazole and imidazole was attributed to the steric effect of the methyl group.

Imidazole is considered to be an inducer of cytochrome P4502E1. In general, inducers of this isozyme stabilize the enzyme by preventing phosphorylation of a serine which leads to heme loss (Lewis, 1996). Thus, "induction" does not necessarily mean more enzyme is synthesized.

It has been reported that several drugs containing an imidazole moiety were retained in connective tissue when they were administered to laboratory animals (Ohta *et al*., 1996). Ohta *et al.* (1996) studied the retention of [^{14}C]-2-methylimidazole-derived radioactivity after intravenous administration to male Wistar rats. Ohta *et al.* (1996) found the highest concentration of radioactivity in the aorta 24 hours after dosing, followed by skin and cartilage. The radioactivity could not be extracted by organic solvents from trichloroacetic acid-treated tissue homogenates. The bound material was primarily recovered in the elastolytic fraction (70% of the total), and secondly in the collagenolytic fraction. The reactive species is postulated to be 2-methylimidazolone which reacts with aldehydes to give aldol condensation products (Ohta *et al.*, 1998). The metabolic activation of 2-methylimidazole appears to involve metabolism by other enzymes in addition to cytochrome P450. Pretreatment with SKF525-A, cimetidine, or piperonyl

butoxide, all inhibitors of cytochrome P450, increased the amount of radioactivity bound to aortic tissue. Also, induction with phenobarbital or 3-methylcholanthrene did not change the amount of irreversible binding. In a subsequent paper, Ohta *et al.* (1998) reported SKF525-A pretreatment had little effect on the urinary excretion of 2-methylimidazole.

Humans

No information on the absorption, distribution, metabolism, or excretion of 2-methylimidazole in humans was found in a review of the literature.

TOXICOKINETIC MODEL

Experimental Animals

After a single gavage dose of 2-methylimidazole (25, 50, or 100 mg/kg) to male and female Fischer rats, the plasma concentration versus time data could be described by a one-compartment model with no lag phase and first-order absorption and elimination for both males and females (Johnson *et al.*, 2002). The absorption and elimination half-lives were approximately 10 to 15 minutes and 1 hour, respectively. The plasma concentration versus time data following an intravenous administration of 10 mg/kg were described as a two-compartment model with first-order elimination. The distribution and elimination half-lives were approximately 5 and 45 minutes, respectively. Bioavailability was approximately 97%, based on comparison of the area under the concentration versus time curves for the two modes of administration. Johnson *et al*. (2002) concluded that absorption was not saturated over the dose range used. However, a clear sex difference in clearance was observed with a dose-dependent response in females. A similar study was conducted in male and female B6C3F$_1$ mice at the same doses (Appendix M). The single gavage doses gave results that were described by the same model as the Johnson *et al.* (2002) study, with absorption and elimination half-lives of approximately 2 and 20 minutes for males and females (Appendix M). The plasma concentration versus time data from the intravenous portion of the study was best fit with a one-compartment model with first order elimination. The half-life for the terminal phase was approximately 16 minutes. Bioavailability was approximately 90%. Clearance rates decreased with increasing dose, but were comparable between male and female mice.

TOXICITY

Experimental Animals

The LD_{50} values of 2-methylimidazole for mice were 1,400 mg/kg orally and 480 mg/kg intraperitoneally (Nishie *et al.*, 1969). Acute toxicity information for 2-methylimidazole in rats is not available.

2-Methylimidazole has been associated with acute toxicity including convulsant activity in foraging animals fed commercially ammoniated grasses or grains (Nishie *et al.*, 1969). 2-Methylimidazole has produced similar neurologic effects (convulsive activity) in mice (Nishie *et al.*, 1969, 1970). Ewes fed ammoniated hay showed facial twitching and general body tremors followed by opisthotonus and convulsions; two of three convulsing animals died (Weiss *et al.*, 1986). Although the causative chemical was not identified, calves nursing from cows fed ammoniated hay would run in circles and into walls and were easily excited by noise and touch (Morgan and Edwards, 1986; Weiss *et al.*, 1986; Motoi *et al.*, 1997).

Neurologic signs and convulsant activity have been observed in cattle fed ammoniated molasses (Nishie *et al.*, 1970). The signs included restlessness, bellowing, frothing at the mouth, and paralysis (Wiggins, 1956). In mice, 2-methylimidazole induced similar toxic neurologic effects; at high doses, 2-methylimidazole induced tremor, restlessness, running, sialorrhea, opisthotonus, Straub tail, and tonic extensor seizure terminating in death; at lower doses, loss of balance was the common finding. The convulsant doses (CD_{50}) were 1,300 mg/kg orally and 500 mg/kg intraperitoneally (Nishie *et al.*, 1969, 1970). Ferrari *et al.* (1987) reported that intraperitoneal injection of 2-methylimidazole induced aggressive behavior in male Wistar rats treated with lisuride.

The NTP (2004) also conducted 15-day and 14-week studies in F344/N rats and B6C3F$_1$ mice in which 2-methylimidazole was administered in feed to groups of five male and five female rats and mice at 0, 1,200, 3,300, or 10,000 ppm, and to groups of 10 male and 10 female rats and mice at 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm, respectively. In the 15-day study in male and female rats, 2-methylimidazole exposure increased the incidences of follicular cell hyperplasia in the thyroid gland and pars distalis hypertrophy in the pituitary gland. In the 15-day study in male and female mice,

2-methylimidazole exposure increased the incidences of follicular cell hypertrophy in the thyroid gland; the severity of hematopoietic cell proliferation in the spleen was generally increased with exposure concentration. In the 14-week study in rats, exposure concentration-related decreases in mean body weights and serum thyroid hormone (T_3 and T_4) concentrations were observed. In addition, increases in serum thyroid stimulating hormone (TSH) concentrations, anemia, and the incidences of thyroid gland follicular cell hyperplasia were observed in exposed groups of rats compared to the controls. Table 1 summarizes the 14-week study findings in F344/N rats and B6C3F_1 mice. In this study, follicular cell adenoma of the thyroid gland was diagnosed in two 10,000 ppm male rats. Degeneration of the testes was also observed in 10,000 ppm males. In the 14-week study in mice, exposure concentration-related decreases in mean body weights and in serum T_4 concentrations (females only) and increases in serum T_3 concentrations and anemia were observed. In addition, increased incidences of thyroid gland follicular cell hypertrophy, splenic hematopoietic cell proliferation, and hemosiderin in kidney proximal tubules were observed in exposed groups of mice. No tremors or convulsions were observed in rats or mice in these studies.

Humans

No data on adverse health effects of 2-methylimidazole in humans were found in the literature.

Reproductive and Developmental Toxicity

Imidazoles have been reported to disrupt male fertility through suppression of testicular function. Adams *et al.* (1998) reported that 2-methylimidazole decreased luteinizing hormone secretion and tissue interstitial fluid testosterone concentration 2 hours after injection to Sprague-Dawley rats.

No data on the reproductive or teratogenic effects of 2-methylimidazole in animals or humans were found in the literature.

Carcinogenicity

No data on the carcinogenicity of 2-methylimidazole in animals or epidemiology studies or case reports associating 2-methylimidazole exposure with cancer risk in humans were found in the literature.

Genetic Toxicity

2-Methylimidazole exhibited no mutagenicity suppressing effects on 3-amino-1-methyl-5H-pyrido[2,3-b]indol, 2-acetylaminofluorene, or benzo[a]pyrene in *Salmonella typhimurium* strains TA98 and TA100, as reported by Yamaguchi and Nakagawa (1983). A broad screening study of 51 imidazole compounds in two assays, the fluctuation test with *Klebsiella pneumoniae* and the *S. typhimurium* plate incorporation mutagenicity test using strain TA100 only and no metabolic activation enzymes was reported by Voogd *et al*. (1979). No mutagenic activity for 2-methylimidazole (sample obtained from BASF) was detected in either assay at dose levels up to 12 mmol/L.

Study Rationale

2-Methylimidazole was among the imidazole class study of chemicals used in the electronics industry. Both 2-methylimidazole and 4-methylimidazole were nominated by the National Cancer Institute Chemical Selection Working Group to the NTP for carcinogenicity testing with high priority because of widespread use, the potential for exposure by several possible routes, and a lack of specific chronic toxicity and carcinogenicity test data in the literature.

TABLE 1
Incidences of Neoplasms and Selected Nonneoplastic Lesions in Rats and Mice in the 14-Week Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Rats						
Male						
Thyroid Gland[b]	10	10	10	10	10	10
Follicular Cell, Hyperplasia, Diffuse[c]	2 (1.5)[d]	0	8* (1.1)	10** (1.1)	10** (1.9)	10** (2.9)
Follicular Cell Adenoma	0	0	0	0	0	2
Testes	10	10	10	10	10	10
Degeneration	2 (2.5)	2 (1.0)	1 (1.0)	2 (1.0)	2 (1.0)	9** (1.2)
Female						
Thyroid Gland	10	9	10	10	10	10
Follicular Cell, Hyperplasia, Diffuse	0	0	0	10** (1.0)	10** (2.0)	10** (3.0)
Mice						
Male						
Thyroid Gland	10	9	10	9	10	10
Follicular Cell, Hypertrophy	0	0	0	9** (1.0)	10** (1.9)	10** (2.8)
Spleen	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	1 (1.0)	10** (1.3)	10** (2.3)	10** (2.6)	10** (3.6)
Kidney	10	10	10	10	10	10
Renal Tubule, Pigmentation, Hemosiderin	0	0	10** (1.0)	10** (2.3)	10** (2.7)	10** (3.0)
Female						
Thyroid Gland	10	9	8	9	10	10
Follicular Cell, Hypertrophy	0	0	0	7** (1.0)	10** (1.7)	10** (2.4)
Spleen	10	10	10	10	10	10
Hematopoietic Cell Proliferation	2 (1.0)	1 (1.0)	1 (2.0)	10** (1.3)	10** (2.4)	10** (2.8)
Kidney	10	10	10	10	10	10
Renal Tubule, Pigmentation, Hemosiderin	0	0	0	3 (1.0)	9** (1.4)	10** (1.5)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] NTP (2004)
[b] Number of animals with organ examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 2-METHYLIMIDAZOLE

2-Methylimidazole was obtained from Aldrich Chemical Company (Milwaukee, WI) in two lots (08222CN and 04209TQ). The two lots were combined at the analytical chemistry laboratory, Battelle Columbus (Columbus, OH), and the study laboratory, Southern Reserch Institute (Birmingham, IL), assigned a new lot number (081497) to the combined lot. Lot 081497 was used during the 2-year studies. Identity and purity analyses on lot 081497 were conducted by the analytical chemistry laboratory and the study laboratory; stability analyses were performed by the study laboratory (Appendix I). Reports on analyses performed in support of the 2-methylimidazole studies are on file at the National Institute of Environmental Health Sciences.

Lot 081497, a dry white powder, was identified as 2-methylimidazole by the analytical chemistry laboratory using infrared, proton nuclear magnetic resonance (NMR), carbon-13 NMR, and elemental analyses and melting point determination.

The purity of lot 081497 was determined using capillary gas chromatography (GC) and high-performance liquid chromatography (HPLC). The moisture content of lot 081497 was determined using Karl Fischer titration.

For lot 081497, GC at the analytical chemistry laboratory and the study laboratory indicated a purity of 100% with one major peak and no impurities. HPLC at the analytical chemistry laboratory indicated a purity of 100% with no UV absorbing impurities. Karl Fischer titration indicated a moisture content of less than 0.16%. The overall purity of lot 081497 was determined to be greater than 99.5%.

Stability studies of the bulk chemical were performed using GC. Samples stored under minimal headspace in sealed glass containers, protected from light, were stable for at least 2 weeks at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in glass bottles. Stability was monitored during the 2-year studies using capillary 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 2-methylimidazole with feed (Table I1). Formulations were stored in double thickness plastic bags placed inside heavy-duty opaque plastic bags and sealed inside plastic containers, protected from light at approximately 5° C for up to 35 days.

Homogeneity studies of the 300 and 5,000 ppm dose formulations were performed by the study laboratory by HPLC. Stability studies of the 100 ppm dose formulation were performed by the analytical chemistry laboratory by HPLC. Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored in sealed plastic containers, protected from light at approximately 5° C and 25° C, and for at least 7 days when exposed to light and air.

Analyses of the dose formulations of 2-methylimidazole were conducted by the study laboratory every 8 to 12 weeks by HPLC. In the rat study, 208 of 223 (93%) of the dose formulations analyzed were within specifications. Of the 15 formulations that did not meet specifications, three were used and not remixed. Of those three, none were more than 13% from the target concentration. The concentrations of animal room samples for rats ranged from 89% to 104% of the target concentrations. In the mouse study, 89 of 95 (94%) of the dose formulations analyzed were within specifications. Of the four formulations not within specifications, one was used and not remixed. Animal room samples ranged from 90% to 101% of the target concentrations.

2-YEAR STUDIES

Study Design

Groups of 60 male and 60 female rats were fed diets containing 0, 300, 1,000, or 3,000 ppm 2-methylimidazole (males) or 0, 1,000, 2,500, or 5,000 ppm 2-methylimidazole (females) for 106 weeks. Groups of 60 male and 60 female mice were fed diets containing 0, 625, 1,250, or 2,500 ppm 2-methylimidazole for 105 weeks. Ten animals from each group were designated for interim evaluation and were sacrificed at 6 months. Additional groups of 20 male and 20 female special study rats and mice were exposed to the same concentrations for 8 days or 14 weeks.

The exposure concentrations selected for the 2-year study in rats were 0, 300, 1,000, and 3,000 ppm for males and 0, 1,000, 2,500, and 5,000 ppm for females. Lower concentrations were selected for male rats because they appeared to be more sensitive to the effects of 2-methylimidazole than were the female rats in the 14-week study (NTP, 2004). In that study, the no-observed-adverse-effect level (NOAEL) for thyroid gland follicular cell hyperplasia was 625 ppm in males and 1,250 ppm in females, body weights were significantly decreased in 5,000 ppm males but not in 5,000 ppm females, two thyroid gland follicular cell adenomas were found in 10,000 ppm males but not in 10,000 ppm females.

The exposure concentrations selected for the 2-year study in mice were 0, 625, 1,250, and 2,500 ppm based on the findings in the 14-week study that there were decreased body weights in 5,000 and 10,000 ppm males and females compared to the controls, a NOAEL of 1,250 ppm for thyroid gland follicular cell hypertrophy in males and females, and NOAELs of 625 and 1,250 ppm for renal tubule pigmentation in males and females, respectively. The highest exposure concentrations selected for the 2-year studies provided internal doses lower than the 100 mg/kg bolus dose used in the toxicokinetic studies (Appendix M), which demonstrated evidence of saturation. When translated to concentrations in dosed feed, the highest exposure concentrations selected for each species were insufficient to reach this level.

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 7 (31 female rats received on July 22, 1998) or 13 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 L).

Animal Maintenance

Male rats were housed two or three per cage, female rats and mice were housed five per cage, and male mice were housed individually. Feed and water were available *ad libitum*. Feed consumption was measured for one week at 4-week intervals throughout the study for core study rats and mice. Cages were changed once (males) or twice (females) weekly, and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks and body weights were recorded initially after 3 weeks, then every 4 weeks, and at study termination for core study animals.

At each interim sacrifice day, the animals were anesthetized with a CO_2/O_2 mixture, and blood was collected from 10 male and 10 female rats and mice for hormone (8 days, 14 weeks, and 6 months) and hematology (6 months) analyses. All blood samples were drawn from the retroorbital sinus. The animals were sacrificed after blood was drawn and tissues from liver and pituitary gland, spleen, and thyroid gland were removed, weighed, and prepared for enzyme analyses and pathology examination.

Blood for hematology determinations was placed in tubes containing EDTA as an anticoagulant. Erythrocyte and leukocyte counts, hemoglobin concentration, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet counts, and differential counts were performed on a Technicon H-1 hematology analyzer (Technicon Corporation, Tarrytown, NY). Reagents were supplied by Bayer, Inc. (Tustin, CA), R&D Systems (Minneapolis, MN), or Fisher Scientific, Inc. (Norcross, GA). Reticulocyte counts were performed on a Coulter Elite™ Flow

Cytometer (Coulter Corporation, Miami, FL); reagents were supplied by Coulter Corporation or Molecular Probes (Eugene, OR).

Blood for thyroid hormone analysis was collected into tubes containing no anticoagulant and processed for serum. Serum was prepared, aliquoted into two tubes, and frozen at approximately –70° C until analyzed for triiodothyronine, thyroxine, and thyroid stimulating hormone concentrations.

For liver enzyme analysis, the entire liver was collected from 10 male and 10 female special study rats and mice on day 8 and at 14 weeks and the left and right lateral liver lobes were collected from 10 male and 10 female core study rats and mice at 6 months. The samples were frozen on dry ice for determination of total cytochrome P450 and UDP-glucuronosyltransferase (UDPGT) activity (Temple *et al.*, 1971). Liver samples were homogenized and centrifuged; calcium chloride was mixed with the supernatant and centrifuged to form microsome pellets, which were resuspended in Tris-chloride (pH 7.4) containing potassium chloride (KCl), and centrifuged again. The final suspensions of microsome pellets in Tris-chloride (pH 7.4) with KCl at approximately 10 mg protein/mL were stored at –80° C. For total cytochrome P450 analysis, suspensions were thawed, diluted to 1.5 mg/mL protein with potassium phosphate buffer (pH 7.5), and analyzed for carbon monoxide-difference spectra at 450 and 490 nm using a Cary 3E spectrophotometer. For UDPGT, microsomal suspensions were thawed and diluted with potassium sulfate buffer (pH 7.4) or Triton X-100 (final concentration of detergent in the suspension 0.1%). Buffer [Tris-maleate (pH 7.4) containing magnesium chloride and 4-nitrophenol] and UPD-glucuronic acid were added to aliquots of the microsomal suspension and the samples were incubated at 37° C. Ice-cold trichloroacetic acid was added and the samples were vortexed, chilled on ice, and centrifuged. The supernatant was removed, mixed with an equal volume of 2 M sodium hydroxide, diluted with distilled water, and analyzed at 405 nm using a Cary 3E spectrophotometer. The biuret and Lowry procedures for protein determination used a Sigma Diagnostics® Micro Protein Determination kit. Complete necropsies and microscopic examinations were performed on all core study rats and mice. The liver, pituitary gland, spleen, and thyroid gland were weighed from 10 male and 10 female rats and mice at the 6-month interim evaluation in the core study and from 10 male and 10 female special study rats and mice on day 8 and at week 14. 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, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 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 2.

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 including the thyroid gland of rats and mice, the liver and spleen of mice and female rats, the bone marrow of mice, and the epididymis, testis, and kidney of male mice.

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 2
Experimental Design and Materials and Methods in the 2-Year Feed Studies of 2-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
13 days (except 31 female rats held 7 days)

Average Age When Studies Began
6 weeks

Date of First Exposure
Rats: July 15, 1998 (core study) or
July 29, 1998 (special studies)
Mice: July 29, 1998 (core study) or
August 12, 1998 (special studies)

Duration of Exposure
Core studies, 105 (mice) or 106 (rats) weeks; special studies, 8 days or 14 weeks

Date of Last Exposure
Rats: core groups - July 12 - 20, 2000;
special study - October 28, 1998;
Mice: core groups - July 26 - 31, 2000;
special study - November 11, 1998;

Necropsy Dates
Rats: July 12 - 20, 2000
Mice: July 26 - 31, 2000

Average Age at Necropsy
110-111 weeks

Size of Study Groups
60 males and 60 females (core studies), 20 males and 20 females (special studies).

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

Animals per Cage
Rats: 2-3 (males) or 5 (females)
Mice: 1 (male) or 5 (females)

Method of Animal Identification
Tail tattoo

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

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

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

Cages
Solid-bottom polycarbonate (Lab Products, Inc., Maywood, NJ) changed weekly (males) or twice weekly (females)

Bedding
Irradiated, heat-treated hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changes weekly (males) or twice weekly (females)

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

Animal Room Environment
Temperature: 72° ± 3° F
Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: 10/hour

Exposure Concentrations
0, 300, 1,000, or 3,000 ppm (male rats), 0, 1,000, 2,500, or 5,000 ppm (female rats), or 0, 625, 1,250, or 2,500 ppm (mice) in feed

Type and Frequency of Observation
Observed twice daily; core study animals were weighed initially, after 3 weeks, then every 4 weeks, and at the end of the studies; clinical findings and feed consumption were recorded every 4 weeks.

Method of Sacrifice
CO_2 asphyxiation

Necropsy
Necropsies were performed on core study animals. Organs weighed were liver, pituitary gland, spleen, and thyroid gland from core study animals at the 6-month interim evaluation and special study animals on day 8 and at week 14.

Clinical Pathology
Blood was collected from the retroorbital sinus of 10 male and 10 female special study animals on day 8 and at 14 weeks for thyroid hormone analyses and from 10 male and 10 female core study animals at 6 months for hematology analysis and thyroid hormone analyses.
Hematology: automated and manual hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials
Thyroid Hormone: triiodothyronine (T_3), thyroxine (T_4), and thyroid stimulating hormone (TSH).

Liver Enzyme Analysis
Liver samples were collected from 10 male and 10 female special study rats and mice on day 8 and at week 14 and 10 male and 10 female core study rats and mice at 6 months. The livers were frozen on dry ice for determination of total cytochrome P450 and UDP-glucuronosyltransferase activity.

Histopathology
Complete histopathology was performed on all animals. In addition to gross lesions and tissues masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, 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 epididymus and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, uterus, and Zymbal's gland (mice).

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, A5, 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, to 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 upon 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

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, thyroid hormone, and liver enzyme data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive

test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

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 NTP historical database for this Technical Report contains all 21 studies that use the NTP-2000 diet with histopathology findings completed up to the time of this study. 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.

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 2-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. 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, 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

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 3 and in the Kaplan-Meier survival curves (Figure 1). Survival of 2,500 ppm females was significantly less than that of the controls.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of 3,000 ppm males and 2,500 and 5,000 ppm females were generally less than those of the controls during most of the study (Figure 2 and Tables 4 and 5). Feed consumption by 3,000 ppm males was less

TABLE 3
Survival of Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Male				
Animals initially in study	60	60	60	60
6-Month interim evaluation[a]	10	10	10	10
Moribund	13	6	7	13
Natural deaths	2	4	7	2
Animals surviving to study termination	35[e]	40[e]	36[e]	35
Percent probability of survival at end of study[b]	70	80	72	70
Mean survival (days)[c]	704	704	693	687
Survival analysis[d]	P=0.606	P=0.438N	P=1.000N	P=1.000
	0 ppm	**1,000 ppm**	**2,500 ppm**	**5,000 ppm**
Female				
Animals initially in study	60	60	60	60
6-Month interim evaluation	10	10	10	10
Moribund	6	8	12	7
Natural deaths	4	3	10	1
Animals surviving to study termination	40	39	28	42[e]
Percent probability of survival at end of study	80	78	56	84
Mean survival (days)	703	697	667	717
Survival analysis	P=0.821N	P=0.958	P=0.016	P=0.697N

[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 lower mortality in an exposed group is indicated by **N**.
[e] Includes one animal that died during the last week of study

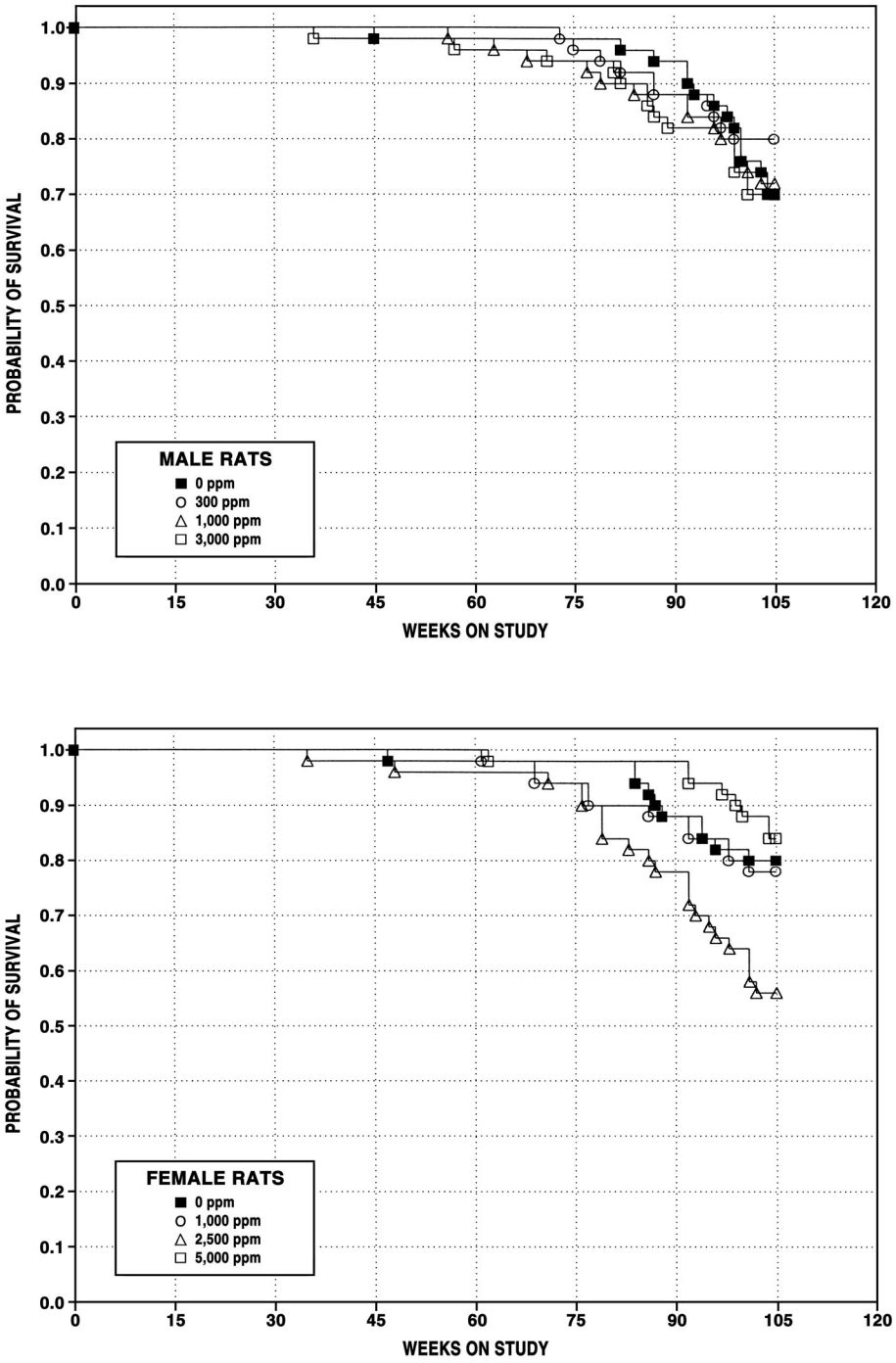


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to 2-Methylimidazole in Feed for 2 Years

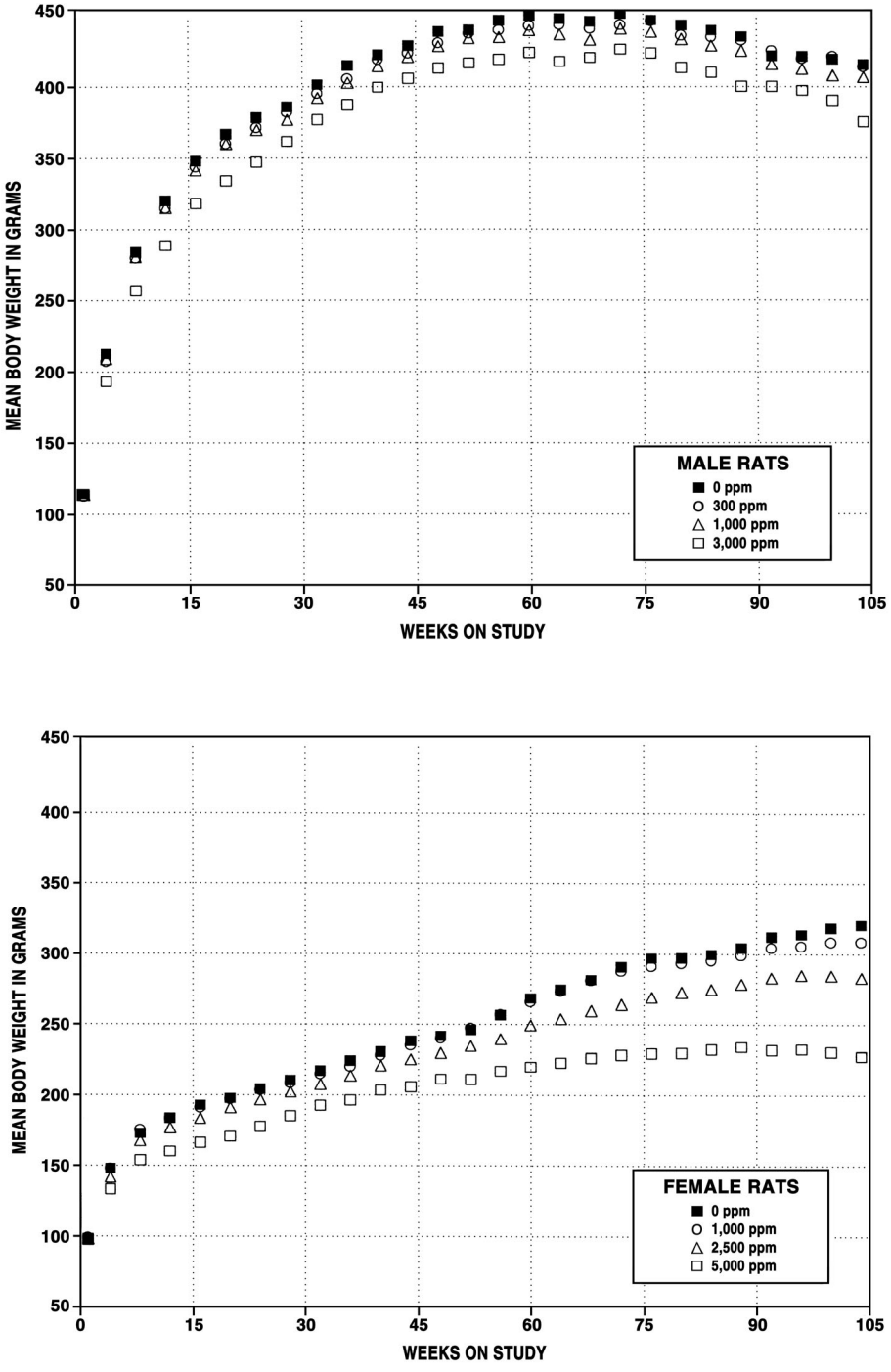


FIGURE 2
Growth Curves for Male and Female Rats
Exposed to 2-Methylimidazole in Feed for 2 Years

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

Weeks on Study	0 ppm		300 ppm			1,000 ppm			3,000 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	114	60	113	99	60	114	99	60	113	99	60
4	213	60	207	97	60	209	98	60	193	91	60
8	284	60	280	99	60	280	99	60	257	90	60
12	320	60	315	98	60	315	98	60	289	90	60
16	348	60	344	99	60	341	98	60	318	92	60
20	367	60	360	98	60	360	98	60	334	91	60
24	378	60	371	98	60	369	98	60	347	92	60
28[a]	386	50	382	99	50	377	98	50	362	94	50
32	401	50	395	98	50	392	98	50	377	94	50
36	415	50	405	98	50	403	97	50	388	94	50
40	422	50	419	99	50	414	98	50	400	95	49
44	429	50	423	99	50	421	98	50	406	95	49
48	436	49	428	98	50	426	98	50	411	94	49
52	437	49	435	99	50	432	99	50	414	95	49
56	444	49	437	98	50	432	97	50	417	94	49
60	446	49	438	98	50	435	98	49	420	94	48
64	444	49	440	99	50	433	98	48	413	93	48
68	443	49	438	99	50	429	97	48	417	94	48
72	447	49	439	98	50	437	98	47	422	95	47
76	443	49	443	100	48	435	98	47	420	95	47
80	440	49	433	98	47	430	98	45	410	93	47
84	436	48	431	99	46	425	98	44	407	93	45
88	431	47	429	99	44	422	98	44	400	93	42
92	422	46	425	101	44	416	99	44	400	95	41
96	421	44	420	100	43	412	98	42	397	94	41
100	419	39	421	100	40	408	97	39	390	93	37
104	415	36	414	100	40	407	98	36	375	90	35
Mean for weeks											
1-13	233		229	98		230	99		213	93	
14-52	402		396	99		394	98		376	94	
53-104	435		431	99		425	98		407	94	

[a] Interim evaluation occurred during week 27.

TABLE 5
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of 2-Methylimidazole

Weeks on Study	0 ppm		1,000 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	99	60	99	101	60	98	100	60	98	99	60
4	148	60	148	100	60	142	96	60	133	90	60
8	173	60	175	101	60	168	97	60	154	89	60
12	184	60	184	100	60	177	96	60	160	87	60
16	193	60	191	99	60	184	95	60	166	86	60
20	198	60	197	100	60	191	97	60	171	86	60
24	204	60	203	100	60	197	96	60	178	87	60
28[a]	210	50	208	99	50	202	96	50	185	88	50
32	217	50	215	99	50	208	96	50	193	89	50
36	224	50	220	98	50	214	95	49	197	88	50
40	231	50	228	99	50	221	96	49	204	88	50
44	238	50	235	99	50	225	95	49	206	87	50
48	242	49	240	99	50	230	95	49	211	87	50
52	246	49	247	101	50	235	95	48	211	86	50
56	257	49	257	100	50	240	93	48	217	85	50
60	268	49	266	99	50	249	93	48	220	82	50
64	275	49	273	100	49	254	93	48	223	81	49
68	282	49	281	100	49	260	92	48	226	80	49
72	291	49	288	99	47	264	91	47	228	79	49
76	297	49	291	98	47	269	91	46	230	77	49
80	297	49	293	99	45	273	92	42	230	77	49
84	300	48	295	99	45	275	92	41	232	78	49
88	304	45	299	98	44	279	92	39	234	77	49
92	312	44	304	98	42	283	91	37	232	74	48
96	314	42	306	97	42	285	91	33	233	74	47
100	319	41	308	97	40	285	89	32	231	72	45
104	321	40	309	96	39	283	88	28	228	71	42
Mean for weeks											
1-13	151		152	101		146	97		136	91	
14-52	220		218	99		211	96		192	87	
53-104	295		290	98		269	91		228	77	

[a] Interim evaluation occurred during week 27.

than that by the controls from week 4 through week 28, and that by 5,000 ppm females was less throughout the study (Tables J1 and J2). Dietary concentrations of 300, 1,000, or 3,000 ppm for males and 1,000, 2,500, or 5,000 ppm for females resulted in average daily doses of approximately 13, 40, or 130 mg 2-methylimidazole/kg body weight to males and 50, 120, or 230 mg/kg to females. Clinical findings for rats included a thin body condition in 3,000 ppm males and 5,000 ppm females. This was attributed to the poor palatability of the feed rather than a toxic effect of 2-methylimidazole.

Clinical Pathology and Organ Weights

The hematology and thyroid hormone data for rats are presented in Tables 6, 7, and F1. Rats that were exposed to 2-methylimidazole for up to 6 months had alterations in thyroid hormone concentrations. On day 8, the thyroxine and triiodothyronine concentrations were decreased and thyroid stimulating hormone increased in exposed groups of males and females. By week 14, the effects for the thyroid hormones had ameliorated and the only evidence of an effect was increased thyroid stimulating hormone levels in the 5,000 ppm females. At 6 months, the increase in thyroid stimulating hormone persisted in the females and was accompanied by a slight decrease in triiodothyronine concentrations. In general, exposure-related increases in absolute and relative thyroid gland weights were observed at 8 days, 14 weeks, and 6 months in male and female rats (Tables 6, 7, G1, G2, and G3). There are no differences in absolute and relative pituitary gland weights between exposed and control groups of males at these time points, but the weights of the pituitary gland were significantly decreased in 5,000 ppm females at all three time points. The thyroid hormone findings in this 2-year study were similar to those observed in an earlier 14-week study of 2-methylimidazole (NTP, 2004). In the 14-week study, exposure to 625 ppm or more 2-methylimidazole in the feed induced changes in thyroid hormone concentrations. Additionally, the thyroid hormone effects were most pronounced early in the study and ameliorated with time.

A minimal (less than 10%) decrease in the erythron characterized by a lack of a reticulocyte response and minimal (approximately 6%) decreases in mean cell volume and mean cell hemoglobin values was observed in male and female rats after 6 months of 2-methylimidazole exposure. No Heinz body was found. These findings were also similar to those observed in the earlier 14-week study (NTP, 2004) where exposure to 2,500 ppm induced a decreased erythron that was characterized as minimal, dose related, microcytic, normochromic, and nonresponsive. The lack of an erythropoietic response may indicate that either the decrease in the erythron was not sufficient to stimulate a demonstrably increased erythropoiesis or that there was a minimal hypoproliferative or suppressive erythropoietic effect. The mechanism for the erythron decrease was unknown. However, the decreases in the mean cell volume and mean cell hemoglobin values could have been consistent with an altered iron metabolism and/or hemoglobin production resulting in an iron deficiency-like syndrome. Thyroid hormones have a stimulatory effect on erythroid colony growth (Malgor *et al.*, 1995), and iron deficiency anemia has been associated with lower thyroid hormone concentrations in rats (Beard *et al.*, 1998). Therefore, there may be some relationship between the altered thyroid hormones and the decreased erythron in this study.

Liver Enzyme Results and Liver Weights

As shown in Tables 8, 9, and H1, in rats exposed to 2-methylimidazole for up to 6 months, hepatic UDP-glucuronosyltransferase activity (calculated as total activity per liver) was generally up twofold or more at the high dose (3,000 ppm for males and 5,000 ppm for females). In addition, UDP-glucuronosyltransferase activity was significantly increased for the mid doses (1,000 ppm for males and 2,500 ppm for females) and the low doses (300 ppm for males and 1,000 ppm for females) for all but one group. In contrast, the only significant effect on total hepatic cytochrome P450 was a decrease in a few groups late in the study.

Correlating to the increases in UDP-glucuronosyltransferase activity, relative liver weights were increased in 1,000 and 3,000 ppm males at 8 days and in 3,000 ppm males at 6 months (Tables 8, G1, and G3). In exposed groups of female rats, there were general trends of increased relative liver weights at 8 days, 14 weeks, and 6 months (Tables 9, G1, G2, and G3). At 6 months, significant increases in absolute liver weight were observed in the 1,000 ppm and 2,500 ppm groups of females. These findings suggested a relationship between UDP-glucuronosyltransferase activity and increased liver weight.

TABLE 6
Thyroid Hormone Concentrations and Selected Organ Weights in Male Rats in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
n	10	10	10	10
Thyroid Hormones				
Thyroid-stimulating hormone (TSH) (ng/mL)				
Day 8	3.01 ± 0.15	3.82 ± 0.32*	8.85 ± 0.89**	19.60 ± 0.61**
Week 14	2.84 ± 0.37[b]	2.95 ± 0.31	2.25 ± 0.32	3.22 ± 0.27
Month 6	2.27 ± 0.49	1.71 ± 0.18	1.85 ± 0.23	2.50 ± 0.36
Triiodothyronine (T_3) (ng/dL)				
Day 8	120.40 ± 7.03	130.30 ± 7.20	102.40 ± 5.10	53.90 ± 2.46**
Week 14	102.60 ± 5.93	105.90 ± 8.08	100.40 ± 4.91	88.50 ± 6.06
Month 6	94.70 ± 4.54	95.44 ± 6.73[b]	95.11 ± 2.09[b]	87.20 ± 4.79
Thyroxine (T_4) (µg/dL)				
Day 8	5.73 ± 0.33	5.98 ± 0.33	5.23 ± 0.21	1.07 ± 0.10**
Week 14	5.13 ± 0.19	4.55 ± 0.25	4.78 ± 0.15	4.89 ± 0.19
Month 6	3.78 ± 0.20	3.93 ± 0.30	3.96 ± 0.16	3.80 ± 0.27
Organ Weights				
Pituitary gland				
Day 8				
Absolute	0.0066 ± 0.0003	0.0067 ± 0.0004	0.0070 ± 0.0003	0.0076 ± 0.0005
Relative	0.046 ± 0.002	0.045 ± 0.002	0.048 ± 0.003	0.054 ± 0.004
Week 14				
Absolute	0.0094 ± 0.0006	0.0098 ± 0.0009[b]	0.0101 ± 0.0004	0.0095 ± 0.0004
Relative	0.027 ± 0.001	0.029 ± 0.003[b]	0.031 ± 0.001	0.032 ± 0.001
Month 6				
Absolute	0.0105 ± 0.0003	0.0100 ± 0.0004	0.0105 ± 0.0004	0.0094 ± 0.0003
Relative	0.026 ± 0.001	0.026 ± 0.001	0.027 ± 0.001	0.026 ± 0.001
Thyroid gland				
Day 8				
Absolute	0.011 ± 0.000	0.012 ± 0.001	0.021 ± 0.002▲▲	0.023 ± 0.002▲▲
Relative	0.075 ± 0.006	0.079 ± 0.008	0.146 ± 0.015▲▲	0.160 ± 0.012▲▲
Week 14				
Absolute	0.015 ± 0.001	0.015 ± 0.001	0.020 ± 0.001▲▲	0.025 ± 0.001▲▲
Relative	0.044 ± 0.002	0.046 ± 0.002	0.062 ± 0.003▲▲	0.083 ± 0.003▲▲
Month 6				
Absolute	0.021 ± 0.001	0.019 ± 0.001	0.024 ± 0.001	0.029 ± 0.001▲▲
Relative	0.052 ± 0.002	0.048 ± 0.003	0.060 ± 0.002	0.079 ± 0.004▲▲

* Significantly different ($P \le 0.05$) from the control group by Shirley's test
** $P \le 0.01$
▲▲ Significantly different ($P \le 0.01$) from the control group by Williams' test
[a] Mean ± standard error. Statistical tests were performed on unrounded data. Organ weights (absolute weights) are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight.
[b] n=9

TABLE 7
Thyroid Hormone Concentrations and Selected Organ Weights in Female Rats in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
n	10	10	10	10
Thyroid Hormones				
Thyroid-stimulating hormone (TSH) (ng/mL)				
Day 8	2.00 ± 0.08	4.00 ± 0.32**	16.73 ± 0.80**	17.36 ± 0.62**
Week 14	1.61 ± 0.18[b]	1.98 ± 0.23[c]	2.65 ± 0.44	7.42 ± 1.19**
Month 6	1.31 ± 0.19[c]	1.37 ± 0.11	1.92 ± 0.19*	3.75 ± 0.42**
Triiodothyronine (T_3) (ng/dL)				
Day 8	112.90 ± 4.08	98.30 ± 3.77*	60.20 ± 3.65**	41.40 ± 0.95**
Week 14	113.60 ± 3.68	113.80 ± 6.04	108.40 ± 5.20	97.10 ± 3.57
Month 6	108.50 ± 2.40	102.80 ± 4.54	95.10 ± 7.02*	80.90 ± 2.42**
Thyroxine (T_4) (µg/dL)				
Day 8	4.07 ± 0.24	3.32 ± 0.34	1.29 ± 0.14**	0.57 ± 0.05**
Week 14	3.98 ± 0.30	3.78 ± 0.30	3.97 ± 0.25	3.16 ± 0.14
Month 6	3.55 ± 0.24	3.25 ± 0.33	3.34 ± 0.28	2.71 ± 0.16
Organ Weights				
Pituitary gland				
Day 8				
Absolute	0.0084 ± 0.0003	0.0075 ± 0.0002▲	0.0080 ± 0.0002▲	0.0065 ± 0.0002▲▲
Relative	0.070 ± 0.003	0.067 ± 0.002	0.068 ± 0.003	0.060 ± 0.002▲
Week 14				
Absolute	0.0139 ± 0.0005	0.0139 ± 0.0007	0.0123 ± 0.0008	0.0084 ± 0.0003▲▲
Relative	0.075 ± 0.003	0.074 ± 0.004	0.069 ± 0.005	0.051 ± 0.002▲▲
Month 6				
Absolute	0.0156 ± 0.0008	0.0158 ± 0.0006	0.0162 ± 0.0005	0.0110 ± 0.0006▲▲
Relative	0.077 ± 0.004	0.077 ± 0.003	0.080 ± 0.003	0.060 ± 0.002▲▲
Thyroid gland				
Day 8				
Absolute	0.011 ± 0.001	0.014 ± 0.001▲	0.022 ± 0.001▲▲	0.019 ± 0.001▲▲
Relative	0.090 ± 0.008	0.129 ± 0.011▲▲	0.187 ± 0.010▲▲	0.174 ± 0.009▲▲
Week 14				
Absolute	0.014 ± 0.001	0.014 ± 0.000	0.019 ± 0.001▲▲	0.028 ± 0.001▲▲
Relative	0.075 ± 0.004	0.072 ± 0.003	0.107 ± 0.004▲▲	0.167 ± 0.006▲▲
Month 6				
Absolute	0.014 ± 0.001	0.016 ± 0.001	0.019 ± 0.001▲▲	0.027 ± 0.001▲▲
Relative	0.068 ± 0.006	0.077 ± 0.003	0.096 ± 0.005▲▲	0.150 ± 0.007▲▲

* Significantly different (P≤0.05) from the control group by Shirley's test
** P≤0.01
▲ Significantly different (P≤0.05) from the control group by Williams' test
▲▲ P≤0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data. Organ weights (absolute weights) are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight.
[b] n=8
[c] n=9

TABLE 8
Liver Enzyme Activities and Liver Weights of Male Rats in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
n	10	10	10	10
Cytochrome P450 (nmol/mg protein)				
Day 8	0.828 ± 0.041	0.828 ± 0.031	0.855 ± 0.055	0.810 ± 0.031
Week 14	0.523 ± 0.045	0.290 ± 0.037**	0.400 ± 0.032	0.353 ± 0.044
Month 6	0.712 ± 0.012	0.665 ± 0.017*	0.642 ± 0.026*	0.628 ± 0.024**
Cytochrome P450 (nmol/g liver)				
Day 8	11.907 ± 0.686	12.038 ± 0.437	11.468 ± 1.125	9.672 ± 0.680
Week 14	5.656 ± 0.682	2.918 ± 0.510**	4.079 ± 0.524	3.745 ± 0.563
Month 6	7.512 ± 0.242	6.828 ± 0.247	7.027 ± 0.488	6.355 ± 0.361
Total Cytochrome P450 (nmol/liver)				
Day 8	76.2 ± 5.6	81.4 ± 4.4	78.2 ± 6.8	68.6 ± 5.4
Week 14	69.9 ± 7.9	37.6 ± 6.0**	49.3 ± 4.9	43.2 ± 6.2
Month 6	95.0 ± 2.8	85.1 ± 3.5*	86.3 ± 4.3*	74.7 ± 3.6**
UDP-Glucuronosyltransferase (nmol/mg protein per minute)				
Day 8	12.490 ± 0.835	19.310 ± 0.956**	25.320 ± 1.270**	18.870 ± 2.698**
Week 14	8.000 ± 0.533	10.040 ± 0.560*	12.550 ± 0.639**	17.700 ± 0.815**
Month 6	8.490 ± 0.224	10.290 ± 0.390**	10.910 ± 0.439**	18.290 ± 0.599**
UDP-Glucuronosyltransferase (nmol/g liver per minute)				
Day 8	182.120 ± 17.992	283.430 ± 19.239*	334.520 ± 25.076**	214.930 ± 25.316
Week 14	83.690 ± 7.235	96.320 ± 7.749	123.240 ± 7.508**	181.050 ± 5.794**
Month 6	89.490 ± 2.931	105.750 ± 5.109*	118.040 ± 5.348**	183.710 ± 7.333**
Total UDP-Glucuronosyltransferase (nmol/minute per liver)				
Day 8▼	1,120 ± 60	1,880 ± 70**	2,270 ± 80**	1,480 ± 130
Week 14▼	1,010 ± 70	1,260 ± 80*	1,520 ± 70**	2,120 ± 50**
Month 6▼	1,130 ± 30	1,310 ± 60*	1,460 ± 40**	2,170 ± 70**
Liver weight				
Day 8				
Absolute	6.427 ± 0.314	6.756 ± 0.230	6.947 ± 0.270	7.083 ± 0.226
Relative	44.110 ± 0.756	45.519 ± 0.403	47.278 ± 0.934▲▲	50.240 ± 0.815▲▲
Week 14				
Absolute	12.649 ± 0.598	13.223 ± 0.334	12.499 ± 0.450	11.778 ± 0.389
Relative	37.421 ± 1.072	39.557 ± 0.891	38.455 ± 1.020	39.584 ± 0.961
Month 6				
Absolute	12.676 ± 0.235	12.459 ± 0.214	12.453 ± 0.375	11.856 ± 0.262
Relative	31.270 ± 0.387	32.004 ± 0.496	31.490 ± 0.393	32.804 ± 0.331▲

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
▼ Significant trend (P≤0.01) by Jonckheere's test
▲ Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
▲▲ P≤0.01
[a] Mean ± standard error. Absolute liver weights are given in grams; relative liver weights are given as mg liver weight/g body weight.

TABLE 9
Liver Enzyme Activities and Liver Weights of Female Rats in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
n	10	10	10	10
Cytochrome P450 (nmol/mg protein)				
Day 8	0.687 ± 0.023	0.665 ± 0.010	0.768 ± 0.030	0.709 ± 0.025
Week 14	0.401 ± 0.021	0.409 ± 0.016	0.438 ± 0.019	0.408 ± 0.013
Month 6	0.584 ± 0.017	0.546 ± 0.017	0.522 ± 0.021	0.514 ± 0.016*
Cytochrome P450 (nmol/g liver)				
Day 8	11.583 ± 0.353	10.901 ± 0.325	10.525 ± 0.665	10.107 ± 0.472
Week 14	5.483 ± 0.402	5.578 ± 0.315	6.467 ± 0.430	5.865 ± 0.266
Month 6	8.705 ± 0.547	7.411 ± 0.288	6.733 ± 0.355*	7.227 ± 0.295
Total Cytochrome P450 (nmol/liver)				
Day 8	57.3 ± 2.2	52.2 ± 1.2	56.4 ± 2.7	49.8 ± 2.2
Week 14	32.1 ± 2.2	32.1 ± 2.0	38.2 ± 2.2	32.6 ± 1.3
Month 6	48.5 ± 2.8	44.9 ± 1.6	40.4 ± 2.0*	39.8 ± 2.0*
UDP-Glucuronosyltransferase (nmol/mg protein per minute)				
Day 8	10.670 ± 0.648	13.230 ± 1.043*	21.350 ± 1.318**	23.300 ± 1.745**
Week 14	7.030 ± 0.616	11.600 ± 0.614**	15.600 ± 0.543**	27.680 ± 1.496**
Month 6	9.450 ± 0.350	10.880 ± 0.412*	16.050 ± 0.573**	23.930 ± 0.646**
UDP-Glucuronosyltransferase (nmol/g liver per minute)				
Day 8	179.030 ± 9.764	217.150 ± 16.758*	290.540 ± 20.108**	330.270 ± 24.437**
Week 14	95.540 ± 8.841	158.160 ± 9.844**	230.870 ± 16.349**	393.410 ± 10.904**
Month 6	139.060 ± 5.403	147.740 ± 6.731	205.880 ± 7.911**	335.960 ± 11.896**
Total UDP-Glucuronosyltransferase (nmol/minute per liver)				
Day 8▼	881 ± 38	1,030 ± 70	1,550 ± 80**	1,620 ± 100**
Week 14▼	555 ± 49	907 ± 55**	1,370 ± 90**	2,200 ± 60**
Month 6▼	774 ± 21	891 ± 25**	1,240 ± 50**	1,840 ± 50**
Liver weight				
Day 8				
Absolute	4.952 ± 0.129	4.813 ± 0.133	5.421 ± 0.154	4.940 ± 0.127
Relative	40.753 ± 0.886	42.861 ± 0.875	45.696 ± 0.882▲▲	45.294 ± 0.906▲▲
Week 14				
Absolute	5.860 ± 0.147	5.745 ± 0.102	5.950 ± 0.100	5.584 ± 0.090
Relative	31.453 ± 0.733	30.321 ± 0.471	33.203 ± 0.821	33.758 ± 0.418▲
Month 6				
Absolute	5.591 ± 0.085	6.077 ± 0.132▲	6.015 ± 0.088▲	5.520 ± 0.200
Relative	27.401 ± 0.505	29.498 ± 0.536▲▲	29.775 ± 0.539▲▲	30.104 ± 0.518▲▲

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
▼ Significant trend (P≤0.01) by Jonckheere's test
▲ Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
▲▲ P≤0.01
[a] Mean ± standard error. Absolute liver weights are given in grams; relative liver weights are given as mg liver weight/g body weight.

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 thyroid gland, liver, spleen, preputial gland, mammary gland, clitoral gland, and pituitary 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 A for male rats and Appendix B for female rats.

Thyroid Gland: Two 5,000 ppm females had follicular cell adenomas at 6 months (Tables 10 and B1). The incidences of follicular cell adenoma, follicular cell carcinoma, and adenoma or carcinoma (combined) in 5,000 ppm females were significantly greater than those in the controls at 2 years, and the incidences exceeded the historical range in controls (Tables 10, B3, and B4a). The incidences of follicular cell adenoma or carcinoma (combined) occurred with a positive trend in males, and the incidence in 3,000 ppm males exceeded the historical control range (Tables 10, A3, and A4a).

Follicular cell adenomas were unilateral or bilateral masses that were well-circumscribed, expansile, compressive, and generally nonencapsulated (Plates 1 to 3). The neoplastic follicular epithelium was single layered and consisted of complex papillary or follicular structures (Plate 4). The neoplastic epithelial cells exhibited atypia with an increased nuclear to cytoplasmic ratio, hyperchromatic nuclei, and increased cytoplasmic basophilia. The adenomas in this study were commonly seen in association with diffuse follicular cell hyperplasia. Follicular cell carcinomas were poorly circumscribed masses in one or both thyroid glands and had a broad range of morphological patterns including papillary, follicular, and solid variants (Plate 5). Significant cellular pleomorphism, atypia, and invasion of the mass into the capsule or surrounding tissue distinguished carcinomas from adenomas (Plate 6).

Follicular cell hyperplasia is considered a precursor lesion to follicular cell neoplasms and was increased in males and females. Specifically, the incidences of follicular cell hyperplasia in all exposed groups of rats were significantly greater than those in the control groups at 6 months and at 2 years (Tables 10, A5, and B5). The findings of follicular cell hyperplasia were similar to those observed in males and females in the 14-week study (NTP, 2004); in addition, two 10,000 ppm males had follicular cell adenomas in the 14-week study.

Follicular cell hyperplasia was generally minimal to mild and diffuse and characterized by a gland having an overall increased cellularity with an increase in the ratio of small follicles to large follicles. The follicular epithelial cells were enlarged from low cuboidal to high cuboidal or tall columnar with increased amounts of vacuolated cytoplasm. The epithelial cells generally remained in a single layer. The colloid was usually rarified and less intensely stained. Follicles were occasionally distended and had irregular, angular contours with invaginations and piling up of follicular epithelial cells.

The incidences of follicle mineralization in 1,000 and 3,000 ppm males and all exposed groups of females were significantly greater than those in the controls at 6 months. The incidences of follicle mineralization in 1,000 and 5,000 ppm females were slightly increased at 2 years and the severity of these lesions generally increased with increasing exposure concentration (Tables 10, A5, and B5). Follicle mineralization was characterized by globular or ovoid shaped bodies within the colloid of some follicles. These bodies stained intensely basophilic and usually had a concentric laminated appearance. Follicle mineralization, a common background lesion, is probably degenerative remnants of follicular epithelial cells and may be referred to as corpora amylacea, calcospherules, or psammoma bodies. There was no mineralization of the intact follicular epithelial cells, the basement lamina, or any part of the parenchyma of the thyroid gland. The toxic effect of 2-methylimidazole exacerbated the incidence and severity of these mineralized bodies.

Liver: The incidences of hepatocellular adenoma or carcinoma (combined) in 1,000 and 3,000 ppm males exceeded the historical range for controls (Tables 11 and A4b). The incidences of hepatocellular adenoma in females occurred with a positive trend and the incidences in the 2,500 and 5,000 ppm groups exceeded the historical control range (Tables 11 and B4b). These neoplasms may have been related to 2-methylimidazole exposure because hepatocellular adenoma and carcinoma are relatively uncommon tumors in both males and females. Hepatocellular adenomas consisted of nodules of hepatocytes, which compressed adjacent hepatic parenchyma and lacked the normal lobular and sinusoidal pattern. Hepatocellular carcinomas consisted of

solid sheets of hepatocytes or trabeculae, three or more cells thick. Neoplastic hepatocytes were anaplastic, with prominent nuclei containing one or more nucleoli and variable amounts of cytoplasm.

The incidences of mixed cell foci in 1,000 and 3,000 ppm males and 5,000 ppm females were significantly greater than those in the control groups at 2 years (Tables 11, A5, and B5). Mixed cell foci were well circumscribed and consisted of a mixture of vacuolated hepatocytes and hepatocytes with abundant eosinophilic cytoplasm. Hepatic cords within the foci merged with the surrounding hepatocytes. The incidences of basophilic foci were decreased in 1,000 and 3,000 ppm males when compared to controls. Experimental models suggests that some foci may progress to hepatocellular neoplasms. However, only a small number of foci may progress to neoplasia even with continued administration of the carcinogenic stimulus, and many foci regress when they are removed. Foci are usually observed in some exposed F344/N rats at 6 to 12 months of age and in almost all exposed rats at 2 years (Boorman *et al*., 1985). The increased incidence of cellular infiltration in 3,000 ppm males was considered to be due to biological variation and, therefore, unrelated to 2-methylimidazole exposure.

The incidences of bile duct hyperplasia in 1,000 ppm and 5,000 ppm females were significantly increased at 2 years (Tables 11 and B5). The low incidence of bile duct hyperplasia in 2,500 ppm females was due to decreased survival in this group. Generally, bile duct hyperplasia developed in rats that survived for more than 600 days. Bile duct hyperplasia consisted of increased numbers of small bile ducts in the portal areas with variable amounts of periductular fibrosis and mononuclear cell infiltrates. Bile duct hyperplasia was regarded as a normal aging change, which was accentuated by exposure to 2-methylimidazole. The severity of the bile duct hyperplasia increased with increasing exposure concentration. The incidence of granulomatous inflammation in 5,000 ppm females was significantly increased at 2 years (Tables 11 and B5). This lesion was seen near the portal areas, and consisted of focal aggregates of histiocytes and lymphocytes in varying proportions. The more advanced lesions consisted of clusters or whorls of histiocytes intermingled with or surrounded by a zone of lymphocytes. The histiocytes sometimes contained finely granular pale golden brown pigment. Focal granulomatous inflammation, a normal background lesion, was accentuated by 2-methylimidazole exposure. Whether female mice are more sensitive than male mice to granulomatous inflammation is not clear.

S*pleen:* The spleen weights of 3,000 ppm males and of 2,500 and 5,000 ppm females were significantly decreased on day 8 (Table G1). The incidence of granulomatous inflammation in the spleen of 5,000 ppm females was significantly increased at 2 years (0 ppm, 3/50; 1,000 ppm, 2/49; 2,500 ppm, 4/48; 5,000 ppm, 27/50; Table B5). The lesion was histologically similar to the granulomatous inflammation in the liver of 5,000 ppm females and was considered to be related to 2-methylimidazole exposure.

Preputial Gland: The incidence of preputial gland adenoma or carcinoma (combined) in 1,000 ppm males was significantly increased (0/50, 3/50, 5/50, 3/50; Table A3) but was not considered to be 2-methylimidazole exposure related since the incidence was within the historical range [21/310 (7% ± 4%), range 0%-10%]. Furthermore, there was no exposure concentration-response for these neoplasms and no supportive increases in the incidences of hyperplastic lesions. The significant finding of chronic inflammation of the preputial gland (4/50, 10/50, 10/50, 11/50; Table A5) was examined using an informal NTP database for non-neoplastic lesions to determine the relevance of this difference. Based on this review, it was determined that the significant difference was likely due to individual animal variation.

Other Organs: The incidence of fibroadenoma in the mammary gland of 5,000 ppm females was significantly lower than in controls at 2 years (26/50, 23/50, 24/50, 6/50; Table B3) and was below the historical range in controls [129/310 (42% ± 11%), range 28%-52%]. The incidence of clitoral gland adenoma in 5,000 ppm females was significantly lower than in controls at 2 years (5/50, 4/49, 6/50, 0/50; Table B3) and was below the historical range [38/304 (12% ± 7%), range 2%-20%]. The pituitary gland weights of 5,000 ppm females were significantly decreased on day 8 and at 14 weeks and 6 months (Tables 7, G1, G2, and G3). The incidence of pituitary gland pars distalis adenoma of 5,000 ppm females was significantly lower than in controls at 2 years (23/50, 24/48, 21/50, 13/50; Table B3); this incidence was at the lower end of the historical range [111/309 (36% ± 9%), range 24%-46%]. Lower body weight of 5,000 ppm female rats was likely a major contributing factor to the decreased incidences of mammary gland fibroadenoma, clitoral gland adenoma, and pituitary gland adenoma in this group.

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Male				
6-Month Interim Evaluation				
Number Examined Microscopically	10	10	10	10
Follicle, Mineralization, Focal[a]	1 (1.0)[b]	4 (1.0)	9**(1.0)	9**(2.8)
Follicular Cell Hyperplasia	0	7** (1.1)	10**(1.0)	10**(2.0)
2-Year Study				
Number Examined Microscopically	48	46	43	50
Follicle, Mineralization, Focal	48 (1.0)	45 (1.0)	43 (1.9)	49 (2.7)
Follicular Cell Hyperplasia	0	17** (1.1)	37**(1.1)	43**(1.8)
Follicular Cell Adenoma	1	0	1	3
Follicular Cell Carcinoma	0	2	0	2
Follicular Cell Adenoma or Carcinoma[c]				
Overall rate[d]	1/48 (2%)	2/46 (4%)	1/43 (2%)	5/50 (10%)
Adjusted rate[e]	2.2%	4.6%	2.5%	11.2%
Terminal rate[f]	1/35 (3%)	1/39 (3%)	1/35 (3%)	4/35 (11%)
First incidence (days)	729	693	729 (T)	395
Poly-3 test[g]	P=0.046	P=0.489	P=0.736	P=0.099

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
Female				
6-Month Interim Evaluation				
Number Examined Microscopically	10	10	10	10
Follicle, Mineralization, Focal	1 (1.0)	6* (1.0)	10**(1.0)	10**(1.0)
Follicular Cell Hyperplasia	0	5* (1.6)	10**(1.7)	10**(3.0)
Follicular Cell Adenoma	0	0	0	2
2-Year Study				
Number Examined Microscopically	49	48	42	48
Follicle, Mineralization, Focal	42 (1.0)	47* (1.7)	41 (2.4)	48* (2.7)
Follicular Cell Hyperplasia	0	41** (1.5)	34**(1.7)	46**(2.2)
Follicular Cell Adenoma, Bilateral	0	0	0	1
Follicular Cell Adenoma (includes bilateral)[h]				
Overall rate	0/49 (0%)	0/48 (0%)	0/42 (0%)	5/48 (10%)
Adjusted rate	0.0%	0.0%	0.0%	10.6%
Terminal rate	0/40 (0%)	0/39 (0%)	0/28 (0%)	4/41 (10%)
First incidence (days)	—[i]	—[j]	—	638
Poly-3 test	P<0.001		—	P=0.034
Follicular Cell Carcinoma, Bilateral	0	0	0	1
Follicular Cell Carcinoma (includes bilateral)[k]				
Overall rate	1/49 (2%)	1/48 (2%)	1/42 (2%)	7/48 (15%)
Adjusted rate	2.2%	2.3%	2.8%	14.9%
Terminal rate	0/40 (0%)	0/39 (0%)	1/28 (4%)	6/41 (15%)
First incidence (days)	583	538	729 (T)	722
Poly-3 test	P=0.003	P=0.754	P=0.703	P=0.033

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
Female (continued)				
2-Year Study (continued)				
Follicular Cell Adenoma or Carcinoma[l]				
Overall rate	1/49 (2%)	1/48 (2%)	1/42 (2%)	11/48 (23%)
Adjusted rate	2.2%	2.3%	2.8%	23.3%
Terminal rate	0/40 (0%)	0/39 (0%)	1/28 (4%)	9/41 (22%)
First incidence (days)	583	538	729 (T)	638
Poly-3 test	P<0.001	P=0.754	P=0.703	P=0.002

* Significantly different (P≤0.05) from the control group by the Fisher exact test (interim evaluation) or the Poly-3 test (2-year study)
** P≤0.01
(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 studies with controls given NTP-2000 diet (mean ± standard deviation): 8/307 (2.6% ± 3.0%), range 0%-8%
[d] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence: 1/309 (0.3% ± 0.8%), range 0%-2%
[i] Not applicable; no neoplasms in animal group
[j] Value of statistic cannot be computed.
[k] Historical incidence: 2/309 (0.7% ± 1.0%), range 0%-2%
[l] Historical incidence: 3/309 (1.0% ± 1.1%), range 0%-2%

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Basophilic Focus[a]	23	23	8**	2**
Mixed Cell Focus	14	16	23*	26**
Hepatocellular Adenoma	0	1	3	1
Hepatocellular Carcinoma	0	0	1	2
Hepatocellular Adenoma or Carcinoma[b]				
Overall rate[c]	0/50 (0%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate[d]	0.0%	2.2%	6.7%	6.8%
Terminal rate[e]	0/35 (0%)	1/40 (3%)	2/36 (6%)	2/35 (6%)
First incidence (days)	—[g]	729 (T)	696	702
Poly-3 test[f]	P=0.095	P=0.499	P=0.113	P=0.110

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
Female				
Number Examined Microscopically	50	49	50	50
Bile Duct, Hyperplasia	20 (1.3)[h]	29* (1.4)	20 (1.5)	40**(1.9)
Inflammation, Granulomatous	18 (1.6)	23 (2.0)	22 (1.5)	42**(2.1)
Mixed Cell Focus	15	14	11	26*
Hepatocellular Adenoma, Multiple	0	0	0	1
Hepatocellular Adenoma (includes multiple)[i]				
Overall rate	1/50 (2%)	0/49 (0%)	2/50 (4%)	4/50 (8%)
Adjusted rate	2.2%	0.0%	4.9%	8.3%
Terminal rate	1/40 (3%)	0/39 (0%)	2/28 (7%)	4/42 (10%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P=0.039	P=0.506N	P=0.458	P=0.192

* Significantly different (P≤0.05) from the control group by the Fisher exact test (interim evaluation) or the Poly-3 test (2- year study)
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Historical incidence for 2-year feed studies with controls given NTP-2000 diet (mean ± standard deviation): 7/310 (2.2% ± 2.0%), range 0%-5%
[c] Number of animals with neoplasm per number of animals wirh liver examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison 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 exposed group is indicated by **N**.
[g] Not applicable; no neoplasms in animal group
[h] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[i] Historical incidence: 2/310 (0.6% ± 1.0%), range 0%-2%

MICE
2-YEAR STUDY
Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 12 and in the Kaplan-Meier survival curves (Figure 3). Survival of all exposed groups of males and females was similar to that of the control groups.

TABLE 12
Survival of Mice in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
Animals initially in 2-year study	60	60	60	60
6-Month interim evaluation[a]	10	10	10	10
Moribund	4	1	5	5
Natural deaths	3	3	9	5
Animals surviving to study termination	43	46	36	40
Percent probability of survival at end of study[b]	86	92	72	80
Mean survival (days)[c]	706	713	634	663
Survival analysis[d]	P=0.197	P=0.497N	P=0.111	P=0.557
Female				
Animals initially in study	60	60	60	60
6-Month interim evaluation	10	10	10	10
Other[a]	0	1	1	0
Moribund	2	3	4	4
Natural deaths	2	3	2	1
Animals surviving to study termination	46	43[e]	43[e]	45
Percent probability of survival at end of study	92	88	88	90
Mean survival (days)	717	715	709	721
Survival analysis	P=0.989	P=0.703	P=0.708	P=1.000

[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 lower mortality in an exposed group is indicated by **N**.
[e] Includes one animal that died during the last week of study

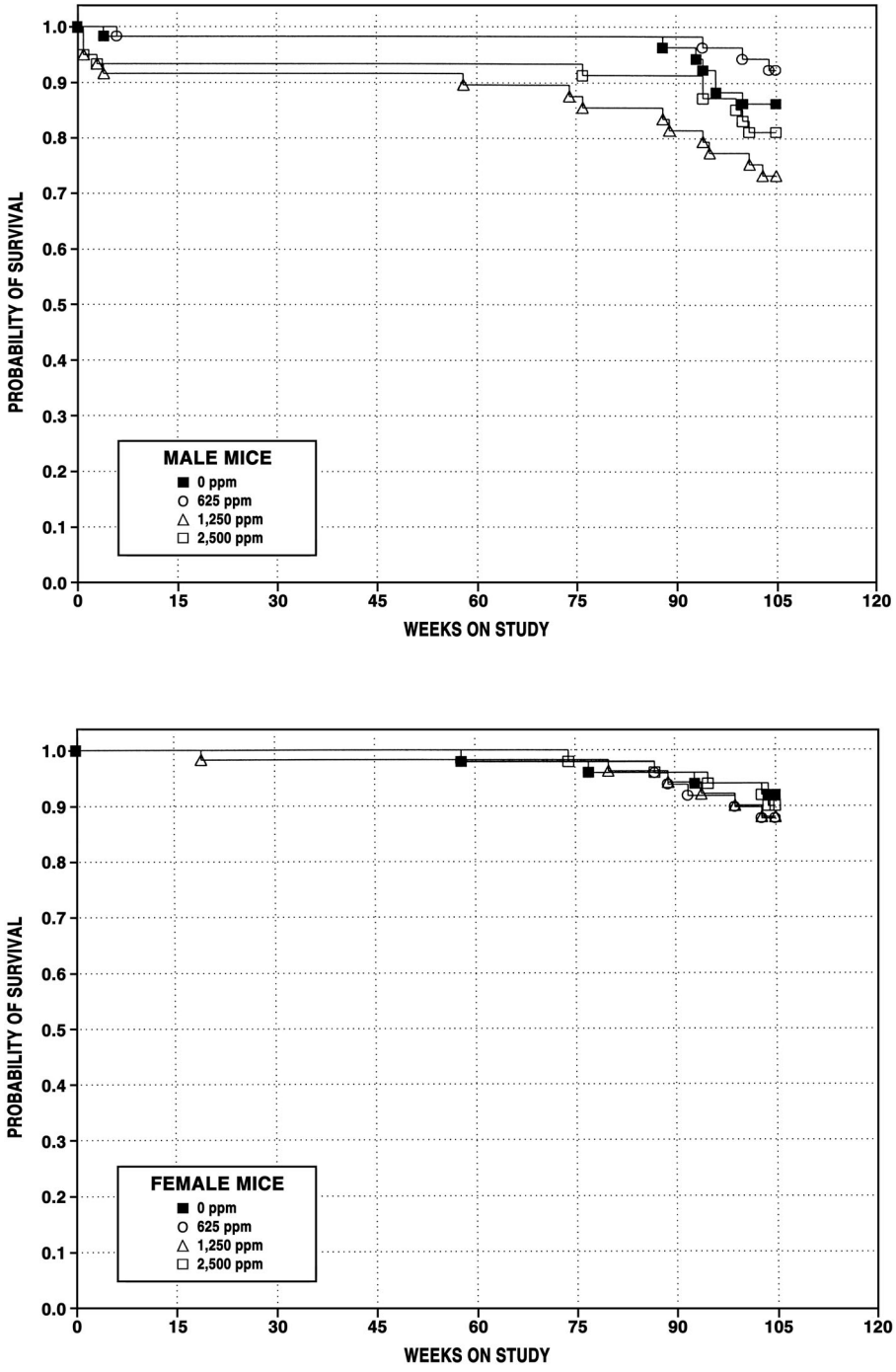


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to 2-Methylimidazole in Feed for 2 Years

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of 1,250 and 2,500 ppm males and 2,500 ppm females were less than those of the controls during most of the study (Figure 4 and Tables 13 and 14). Feed consumption by all exposed groups of mice was similar to that by the control groups (Tables J3 and J4). Dietary concentrations of 625, 1,250, or 2,500 ppm resulted in average daily doses of approximately 75, 150, or 315 mg 2-methylimidazole/kg body weight to males and 80, 150, or 325 mg/kg to females. No clinical findings were attributed to exposure to 2-methylimidazole.

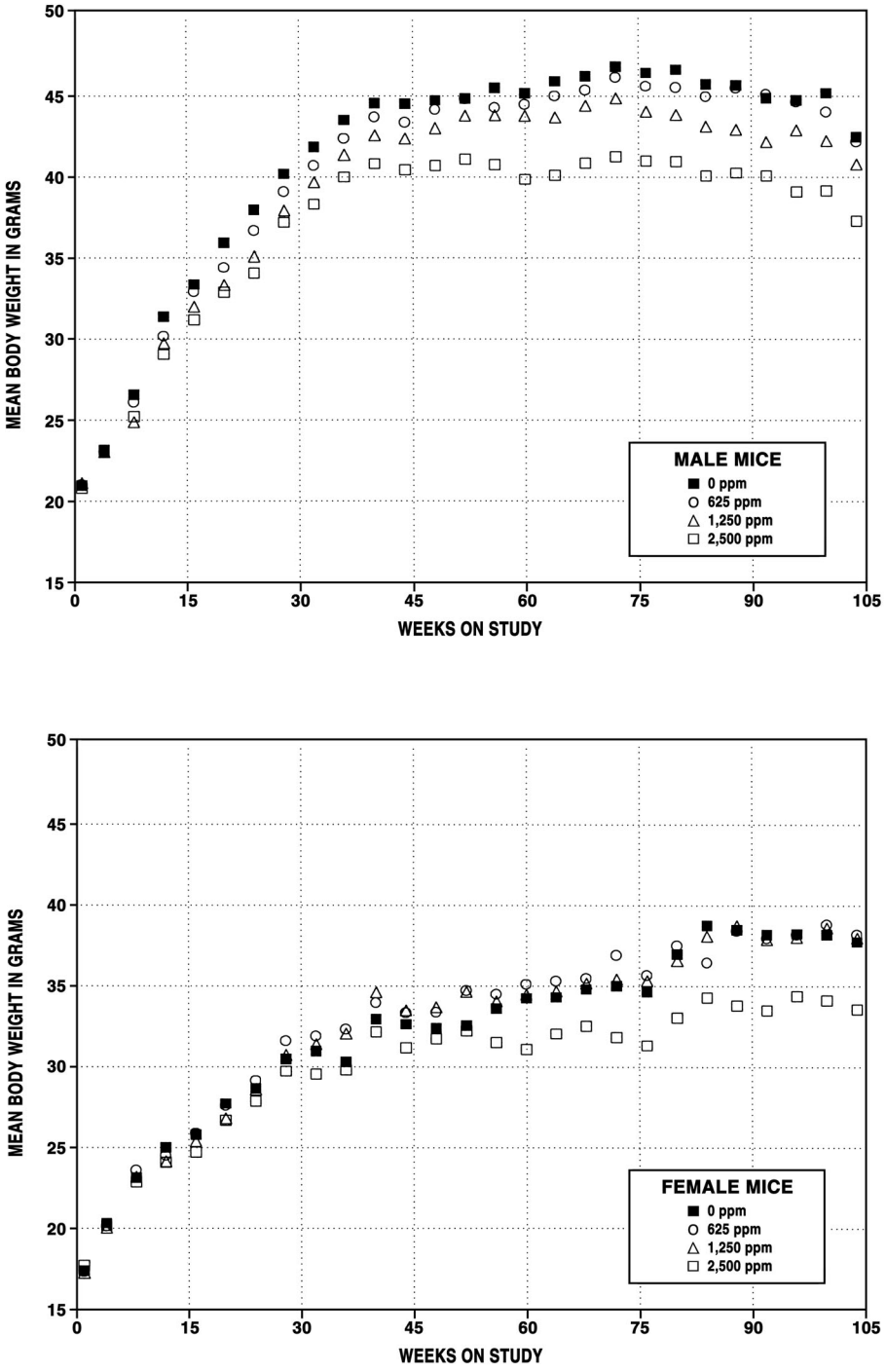


FIGURE 4
Growth Curves for Male and Female Mice
Exposed to 2-Methylimidazole in Feed for 2 Years

TABLE 13
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of 2-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	21.0	60	21.0	100	60	21.1	101	60	20.8	99	60
4	23.1	59	23.0	100	60	23.0	100	55	23.2	100	56
8	26.6	59	26.1	98	59	24.9	94	55	25.2	95	56
12	31.4	59	30.2	96	59	29.7	95	55	29.0	92	56
16	33.4	59	32.9	99	59	32.0	96	55	31.2	93	56
20	35.9	59	34.4	96	59	33.3	93	55	32.9	92	56
24	38.0	59	36.7	97	59	35.1	92	55	34.1	90	56
28[a]	40.2	49	39.1	97	49	37.9	94	45	37.2	93	46
32	41.9	49	40.7	97	49	39.7	95	45	38.3	91	46
36	43.5	49	42.4	98	49	41.4	95	45	40.0	92	46
40	44.5	49	43.7	98	49	42.6	96	45	40.8	92	46
44	44.5	49	43.4	98	49	42.4	95	45	40.4	91	46
48	44.7	49	44.2	99	49	43.0	96	45	40.7	91	46
52	44.8	49	44.8	100	49	43.8	98	45	41.1	92	46
56	45.5	49	44.3	97	49	43.8	96	45	40.8	90	46
60	45.2	49	44.5	99	49	43.8	97	44	39.9	88	46
64	45.9	49	45.0	98	49	43.7	95	44	40.1	87	46
68	46.2	49	45.4	98	49	44.4	96	44	40.9	89	46
72	46.8	49	46.1	99	49	44.9	96	44	41.3	88	46
76	46.4	49	45.6	98	49	44.0	95	43	41.0	88	46
80	46.6	49	45.5	98	49	43.8	94	42	41.0	88	45
84	45.7	49	45.0	99	49	43.1	94	42	40.1	88	45
88	45.7	49	45.5	100	49	42.9	94	42	40.3	88	45
92	44.9	48	45.1	100	49	42.2	94	40	40.1	89	45
96	44.7	46	44.6	100	48	42.9	96	38	39.1	88	43
100	45.2	44	44.0	97	48	42.2	93	38	39.2	87	42
104	42.5	43	42.2	99	47	40.8	96	36	37.3	88	40
Mean for weeks											
1-13	25.5		25.1	99		24.7	98		24.6	97	
14-52	41.1		40.2	98		39.1	95		37.7	92	
53-104	45.5		44.8	99		43.3	95		40.1	88	

[a] Interim evaluation occurred during week 27.

TABLE 14
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of 2-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	17.4	60	17.3	99	60	17.3	99	60	17.7	102	60
4	20.3	60	20.1	99	59	20.0	99	59	20.2	100	60
8	23.1	60	23.6	102	59	23.2	100	59	22.9	99	60
12	25.0	60	24.5	98	59	24.2	97	59	24.1	96	60
16	25.8	60	25.9	100	59	25.4	98	59	24.7	96	60
20	27.7	60	27.6	100	59	26.8	97	58	26.7	96	60
24	28.7	60	29.2	102	59	28.6	100	58	27.9	97	60
28[a]	30.5	50	31.6	104	49	30.7	101	48	29.7	97	50
32	31.0	50	31.9	103	49	31.4	101	48	29.6	96	50
36	30.3	50	32.3	107	49	32.0	106	48	29.8	98	50
40	32.9	50	34.0	103	49	34.6	105	48	32.1	98	50
44	32.6	50	33.4	103	49	33.5	103	48	31.2	96	50
48	32.4	50	33.4	103	49	33.7	104	48	31.7	98	50
52	32.5	50	34.7	107	49	34.6	107	48	32.2	99	50
56	33.6	50	34.5	103	49	34.1	102	48	31.5	94	50
60	34.2	49	35.1	103	48	34.5	101	48	31.1	91	50
64	34.3	49	35.3	103	48	34.7	101	48	32.0	93	50
68	34.8	49	35.5	102	48	35.2	101	48	32.5	93	50
72	35.0	49	36.9	105	48	35.4	101	48	31.8	91	50
76	34.7	49	35.7	103	48	35.3	102	48	31.3	90	49
80	37.0	48	37.5	101	48	36.6	99	48	33.0	89	49
84	38.8	48	36.5	94	48	38.1	98	47	34.3	88	49
88	38.5	48	38.4	100	47	38.7	101	47	33.8	88	48
92	38.2	48	38.0	100	46	37.9	99	46	33.5	88	48
96	38.2	47	38.2	100	45	38.0	100	45	34.4	90	47
100	38.2	47	38.8	102	44	38.6	101	44	34.1	89	47
104	37.8	46	38.2	101	43	38.0	101	43	33.6	89	46
Mean for weeks											
1-13	21.5		21.4	100		21.2	99		21.2	99	
14-52	30.4		31.4	103		31.1	102		29.6	97	
53-104	36.4		36.8	101		36.5	101		32.8	90	

[a] Interim evaluation occurred during week 27.

Clinical Pathology and Organ Weights

The hematology and thyroid hormone data for mice are presented in Tables 15, 16, and F2. 2-Methylimidazole induced a mild to moderate, dose-related, macrocytic, responsive anemia in mice. The anemia was characterized by decreases in automated and manual hematocrit values, hemoglobin concentrations, and erythrocyte counts in 1,250 and 2,500 ppm males and females. An erythropoietic response was indicated by a mild to marked increase in reticulocyte counts. The increase in reticulocyte numbers would also account for the erythrocyte macrocytosis and was indicated by increases in mean cell volume and mean cell hemoglobin values. The erythron effects were usually more severe for male mice. These findings were similar to those observed in the earlier 14-week study of 2-methylimidazole (NTP, 2004). In the 14-week study, exposure to 2-methylimidazole also induced a mild to moderate, dose-related, macrocytic, hyperchromic, responsive anemia in mice. While the cause of the anemia was unknown, the hyperchromia, indicated by increased mean cell hemoglobin concentration values, could be indicative of a hemolytic process in the exposed mice. There was no increase in the mean cell hemoglobin concentration values in the 2-year study and, in fact, there was a decrease in the 2,500 ppm males. An erythropoietic response and an erythrocytic macrocytosis and hypochromia during decreased erythron would be consistent with an anemia in remission, which might be seen with an acute blood loss or hemolytic anemia. Since chronic blood loss would lead to iron deficiency anemia and, since there was indication of a hemolytic process after 6 months of exposure, these findings would support a hemolytic mechanism for the erythron decrease in exposed mice.

There were alterations in thyroxine concentrations in the 2-year study, but the findings were not consistent in males and females. In 2,500 ppm females, thyroxine concentrations were slightly decreased (less than 20%) at 14 weeks and 6 months. Thyroxine concentrations were slightly increased (less than 20%) in the 2,500 ppm males at the same time points. In females, these thyroid hormone findings were similar to those observed in the 14-week study (NTP, 2004). In the 14-week study, thyroxine concentration decreases occurred in 1,250 ppm females. However, the thyroxine concentrations of the males were not affected in the 14-week study. Compared to the controls, there were exposure-related increases in the absolute and relative weights of the thyroid gland in 2,500 ppm males and females at 14 weeks and 6 months (Tables 15, 16, G5, and G6). In addition, the relative weights of the pituitary gland in 1,250 and 2,500 ppm males and 1,250 ppm females were significantly greater than those in the controls at 6 months.

Liver Enzyme Results and Liver Weights

As shown in Tables 17, 18, and H2, in mice exposed to 2-methylimidazole for up to 6 months, hepatic UDP-glucuronosyltransferase activity (calculated as total activity per liver) was significantly increased in a few groups, but none of the increases approached the twofold or greater increases observed in rats. The only significant effect on total hepatic cytochrome P450 in mice was a decrease at 14 weeks associated with a high control value. Absolute and relative liver weights increased in 1,250 and 2,500 ppm males at 14 weeks and 6 months (Tables 17, G5, and G6). In exposed females, liver cytochrome P450 activities were decreased at 8 days and 14 weeks, but the depression in P450 levels was ameliorated at 6 months (Tables 18 and H2). A slight increase in UDP-glucuronosyltransferase activity per gram of liver was observed after 8 days in all groups of exposed female mice. However, absolute and relative liver weights were generally increased in all groups of exposed females at all time points (Tables 18, G4, G5, and G6). Therefore, there was a slight increase in liver enzyme activity per gram of liver in exposed mice.

TABLE 15
Thyroid Hormone Concentrations and Selected Organ Weights of Male Mice in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Thyroid Hormones				
Day 8	8	9	10	7
Week 14	8	9	8	9
Month 6	10	10	9	10
Thyroid-stimulating hormone (TSH) (ng/mL)				
Day 8	224.14 ± 14.23[b]	192.43 ± 5.21[b]	219.63 ± 28.28[c]	236.29 ± 16.66
Week 14	232.83 ± 24.22[d]	208.71 ± 13.42[b]	209.17 ± 8.73[d]	273.56 ± 21.01
Triiodothyronine (T_3) (ng/dL)				
Day 8	74.50 ± 7.37	95.13 ± 9.72[c]	92.86 ± 5.76[b]	90.83 ± 10.49[d]
Week 14	169.38 ± 7.04	153.00 ± 8.01	173.29 ± 7.41[b]	161.33 ± 8.29
Month 6	99.90 ± 6.50	104.60 ± 6.86	105.44 ± 5.92	106.90 ± 7.38
Thyroxine (T_4) (µg/dL)				
Day 8	5.25 ± 0.38[b]	5.71 ± 0.39	6.51 ± 0.95	6.30 ± 0.83
Week 14	5.09 ± 0.35[b]	4.63 ± 0.23	5.44 ± 0.36	5.95 ± 0.21*
Month 6	5.72 ± 0.31	5.10 ± 0.17	5.35 ± 0.19[e]	6.82 ± 0.20*
Organ Weights				
n	10	10	10	10
Pituitary gland				
Day 8				
Absolute	0.0016 ± 0.0002	0.0014 ± 0.0001	0.0016 ± 0.0001	0.0015 ± 0.0001[f]
Relative	0.072 ± 0.008	0.063 ± 0.005	0.082 ± 0.006	0.073 ± 0.005[f]
Week 14				
Absolute	0.0016 ± 0.0001[f]	0.0012 ± 0.0002[f]	0.0009 ± 0.0002▲[f]	0.0016 ± 0.0001
Relative	0.050 ± 0.004[f]	0.039 ± 0.006[f]	0.029 ± 0.006▲[f]	0.050 ± 0.003
Month 6				
Absolute	0.0017 ± 0.0001	0.0019 ± 0.0001	0.0018 ± 0.0001	0.0019 ± 0.0001
Relative	0.042 ± 0.002	0.046 ± 0.002	0.051 ± 0.003▲▲	0.056 ± 0.002▲▲
Thyroid gland				
Day 8				
Absolute	0.003 ± 0.000	0.002 ± 0.000▲▲	0.003 ± 0.000	0.003 ± 0.000[f]
Relative	0.147 ± 0.010	0.105 ± 0.008▲	0.144 ± 0.012	0.127 ± 0.009[f]
Week 14				
Absolute	0.002 ± 0.000	0.002 ± 0.000[c]	0.002 ± 0.000[f]	0.004 ± 0.000▲▲[f]
Relative	0.066 ± 0.006	0.058 ± 0.008[c]	0.062 ± 0.009[f]	0.131 ± 0.009▲▲[f]
Month 6				
Absolute	0.004 ± 0.000	0.004 ± 0.000	0.005 ± 0.000	0.006 ± 0.001▲▲
Relative	0.091 ± 0.007	0.098 ± 0.007	0.130 ± 0.004▲	0.170 ± 0.019▲▲

* Significantly different ($P \le 0.05$) from the control group by Shirley's test
▲ Significantly different ($P \le 0.05$) from the control group by Williams' or Dunnett's test
▲▲ $P \le 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. Organ weights (absolute weights) are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight.
[b] n=7
[c] n=8
[d] n=6
[e] n=10
[f] n=9

TABLE 16
Thyroid Hormone Concentrations and Selected Organ Weights of Female Mice in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Thyroid Hormones				
Day 8	10	9	7	9
Week 14	8	9	9	8
Month 6	10	10	10	10
Thyroid-stimulating hormone (TSH) (ng/mL)				
Day 8	73.50 ± 11.73[b]	67.50 ± 14.92[b]	76.86 ± 15.22	82.20 ± 17.30[c]
Week 14	131.00 ± 8.18[b]	126.29 ± 14.86[d]	110.63 ± 11.29[e]	106.71 ± 9.16[d]
Triiodothyronine (T_3) (ng/dL)				
Day 8	62.20 ± 7.91[c]	63.86 ± 4.65[d]	65.57 ± 3.68	65.17 ± 5.43[b]
Week 14	164.00 ± 11.49[d]	150.25 ± 15.33[e]	154.14 ± 7.36[d]	153.38 ± 10.16
Month 6	72.60 ± 3.84	77.88 ± 6.31[e]	85.70 ± 5.85	82.90 ± 2.04
Thyroxine (T_4) (µg/dL)				
Day 8	4.76 ± 0.23	5.00 ± 0.15	5.43 ± 0.22	4.79 ± 0.16
Week 14	6.51 ± 0.37[e]	5.47 ± 0.51	5.60 ± 0.24	5.21 ± 0.27*[b]
Month 6	6.02 ± 0.37	5.79 ± 0.24	5.36 ± 0.26	4.90 ± 0.23*[f]
Organ Weights				
n	10	10	10	10
Pituitary gland				
Day 8				
Absolute	0.0018 ± 0.0001	0.0014 ± 0.0001	0.0018 ± 0.0002	0.0017 ± 0.0002
Relative	0.106 ± 0.006	0.083 ± 0.006	0.101 ± 0.010	0.096 ± 0.013
Week 14				
Absolute	0.0023 ± 0.0002	0.0025 ± 0.0001	0.0023 ± 0.0001	0.0019 ± 0.0002
Relative	0.085 ± 0.007	0.095 ± 0.006	0.091 ± 0.005	0.074 ± 0.007
Month 6				
Absolute	0.0027 ± 0.0001	0.0027 ± 0.0001	0.0028 ± 0.0001	0.0027 ± 0.0001
Relative	0.081 ± 0.003	0.088 ± 0.004	0.095 ± 0.003▲	0.087 ± 0.003
Thyroid gland				
Day 8				
Absolute	0.003 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.003 ± 0.000
Relative	0.159 ± 0.023	0.133 ± 0.014	0.140 ± 0.032	0.153 ± 0.016
Week 14				
Absolute	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.004 ± 0.000▲▲
Relative	0.096 ± 0.011	0.115 ± 0.006	0.099 ± 0.010	0.171 ± 0.012▲▲
Month 6				
Absolute	0.003 ± 0.000	0.004 ± 0.000	0.004 ± 0.000▲	0.006 ± 0.000▲▲
Relative	0.102 ± 0.006	0.131 ± 0.010▲	0.151 ± 0.008▲▲	0.183 ± 0.013▲▲

* Significantly different ($P \le 0.05$) from the control group by Dunn's or Shirley's test
▲ Significantly different ($P \le 0.05$) from the control group by Williams' or Dunnett's test
▲▲ $P \le 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. Organ weights (absolute weights) are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight.
[b] n=6
[c] n=5
[d] n=7
[e] n=8
[f] n=9

TABLE 17
Liver Enzyme Activities and Liver Weights of Male Mice in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
n	10	10	10	10
Cytochrome P450 (nmol/mg protein)				
Day 8	0.635 ± 0.017	0.664 ± 0.047	0.672 ± 0.045	0.679 ± 0.046
Week 14	0.602 ± 0.030	0.591 ± 0.020	0.626 ± 0.021[b]	0.635 ± 0.009
Month 6	0.534 ± 0.021	0.553 ± 0.023	0.531 ± 0.018	0.508 ± 0.019
Cytochrome P450 (nmol/g liver)				
Day 8	11.914 ± 0.546	10.648 ± 0.656	11.071 ± 0.621	9.963 ± 0.384*
Week 14	13.299 ± 0.686	13.273 ± 0.527	12.144 ± 1.194[b]	11.803 ± 0.296
Month 6	9.959 ± 0.795	9.792 ± 0.729	9.804 ± 0.553	9.301 ± 0.839
Total Cytochrome P450 (nmol/liver)				
Day 8	12.9 ± 0.5	11.6 ± 1.0	11.6 ± 1.3	11.3 ± 1.0
Week 14▼	18.4 ± 0.7	18.1 ± 0.9	19.7 ± 1.0	19.3 ± 0.6
Month 6	15.3 ± 1.2	15.7 ± 1.1	15.8 ± 1.0	15.6 ± 1.4
UDP-Glucuronosyltransferase (nmol/mg protein per minute)				
Day 8	27.510 ± 1.477	30.680 ± 1.889	28.330 ± 1.612	32.200 ± 1.680
Week 14	19.790 ± 0.390	21.610 ± 0.956	21.833 ± 0.897[b]	23.280 ± 0.703**
Month 6	17.410 ± 0.981	18.650 ± 0.756	18.610 ± 0.630	15.980 ± 0.933
UDP-Glucuronosyltransferase (nmol/g liver per minute)				
Day 8	513.920 ± 31.463	485.860 ± 20.565	460.190 ± 12.079	477.180 ± 25.629
Week 14	438.360 ± 11.806	485.360 ± 23.080	418.589 ± 36.016[b]	431.950 ± 7.360
Month 6	325.040 ± 29.216	330.300 ± 24.934	354.420 ± 18.865	294.220 ± 31.680
Total UDP-Glucuronosyltransferase (nmol/minute per liver)				
Day 8	558 ± 32	510 ± 20	464 ± 37	523 ± 29
Week 14▼	612 ± 23	662 ± 37	679 ± 29	706 ± 19**
Month 6▼	499 ± 45	529 ± 34	576 ± 40	491 ± 49
Liver weight				
Day 8				
Absolute	1.090 ± 0.023	1.066 ± 0.053	1.021 ± 0.088	1.125 ± 0.076
Relative	49.914 ± 0.632	49.403 ± 1.691	49.867 ± 2.419	52.382 ± 2.269
Week 14				
Absolute	1.398 ± 0.046	1.362 ± 0.035	1.558 ± 0.056▲[b]	1.635 ± 0.032▲▲
Relative	42.523 ± 0.488	43.725 ± 1.156	49.350 ± 0.779▲▲[b]	52.452 ± 0.606▲▲
Month 6				
Absolute	1.539 ± 0.025	1.633 ± 0.071	1.627 ± 0.077	1.686 ± 0.043
Relative	38.050 ± 0.626	40.402 ± 0.783	44.275 ± 0.969▲▲	48.898 ± 0.949▲▲

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
▼ Significant trend ($P \leq 0.01$) by Jonckheere's test
▲ Significantly different ($P \leq 0.05$) from the control group by Williams' test
▲▲ $P \leq 0.01$
[a] Mean ± standard error. Absolute liver weights are given in grams; relative liver weights are given as mg liver weight/g body weight.
[b] n=9

TABLE 18
Liver Enzyme Activities and Liver Weights of Female Mice in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
n	10	10	10	10
Cytochrome P450 (nmol/mg protein)				
Day 8	0.664 ± 0.046	0.633 ± 0.036	0.562 ± 0.025	0.495 ± 0.019**
Week 14	0.444 ± 0.019	0.389 ± 0.024	0.377 ± 0.032	0.327 ± 0.025**
Month 6	0.336 ± 0.023	0.346 ± 0.020	0.375 ± 0.025	0.325 ± 0.024
Cytochrome P450 (nmol/g liver)				
Day 8	10.390 ± 0.305	9.874 ± 0.427	8.674 ± 0.277**	7.760 ± 0.491**
Week 14	9.922 ± 0.541	7.356 ± 0.521**	6.800 ± 0.523**	6.070 ± 0.195**
Month 6	5.896 ± 0.531	6.431 ± 0.637	7.082 ± 0.438	6.436 ± 0.532
Total Cytochrome P450 (nmol/liver)				
Day 8	7.49 ± 0.33	6.92 ± 0.30	7.27 ± 0.22	6.30 ± 0.37
Week 14	10.6 ± 0.6	8.15 ± 0.58**	7.66 ± 0.68**	6.98 ± 0.33**
Month 6▼	7.33 ± 0.84	7.57 ± 0.64	8.93 ± 0.54	9.23 ± 0.85
UDP-Glucuronosyltransferase (nmol/mg protein per minute)				
Day 8	20.630 ± 1.514	26.110 ± 1.732	24.290 ± 1.957	25.510 ± 1.069
Week 14	14.930 ± 0.716	14.490 ± 1.362	14.680 ± 1.490	14.230 ± 0.570
Month 6	10.550 ± 0.939	11.660 ± 0.791	12.930 ± 1.258	11.580 ± 0.712
UDP-Glucuronosyltransferase (nmol/g liver per minute)				
Day 8	320.560 ± 15.537	401.660 ± 19.004*	370.480 ± 25.163	397.530 ± 28.097*
Week 14	335.600 ± 20.583	276.960 ± 29.682	264.750 ± 19.745	277.960 ± 22.879
Month 6	183.380 ± 18.293	217.100 ± 26.363	242.000 ± 20.613	228.980 ± 16.488
Total UDP-Glucuronosyltransferase (nmol/minute per liver)				
Day 8▼	228 ± 8	280 ± 10**	308 ± 18**	305 ± 20**
Week 14	358 ± 18	304 ± 31	298 ± 25	316 ± 26
Month 6▼	222 ± 23	257 ± 30	304 ± 24*	326 ± 24**
Liver weight				
Day 8				
Absolute	0.721 ± 0.028	0.704 ± 0.021	0.841 ± 0.021▲▲	0.821 ± 0.026▲▲
Relative	42.180 ± 1.126	42.196 ± 0.787	48.573 ± 0.965▲▲	47.820 ± 1.398▲▲
Week 14				
Absolute	1.075 ± 0.026	1.111 ± 0.025	1.122 ± 0.043	1.152 ± 0.045
Relative	39.867 ± 0.376	41.581 ± 0.765	43.623 ± 0.747▲▲	44.756 ± 1.119▲▲
Month 6				
Absolute	1.222 ± 0.054	1.196 ± 0.041	1.268 ± 0.037	1.425 ± 0.035▲▲
Relative	36.445 ± 0.644	38.962 ± 0.998	42.972 ± 1.147▲▲	42.299 ± 1.125▲▲

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
▼ Significant trend (P≤0.01) by Jonckheere's test
▲▲ Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
[a] Mean ± standard error. Absolute liver weights are given in grams; relative liver weights are given as mg liver weight/g body weight.

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 thyroid gland, liver, spleen, bone marrow, kidney, epididymis, and testis. 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.

Thyroid Gland: The incidence of follicular cell adenoma in 2,500 ppm males was significantly greater than that in the control group at 2 years and exceeded the historical range in controls (Tables 19, C3, and C4a). Follicular cell adenomas were also observed in one 625 ppm male and one 2,500 ppm female. The follicular cell adenomas were compressive, nonencapsulated masses of cuboidal epithelial cells, which formed colloid-containing follicles of variable size (Plate 7). The neoplastic epithelial cells generally had an increased nuclear to cytoplasmic ratio, hyperchromatic nuclei, and increased cytoplasmic basophilia compared to normal follicular epithelium. The follicular cell adenomas were mostly unilateral and associated with follicular cell hyperplasia in the thyroid gland.

The incidences of follicular cell hypertrophy were significantly increased in all exposed groups of males and females at 6 months (Tables 19, C5, and D5). The incidences of follicular cell hypertrophy in 1,250 and 2,500 ppm males and females were significantly greater than those in the control groups at 2 years. The findings of follicular cell hypertrophy were similar to those observed in males and females in the 14-week study (NTP, 2004). Follicular cell hypertrophy was locally extensive to diffuse and characterized by an increase in the size of the follicular epithelial cells due to distended cytoplasm (Plates 8 and 9). The cytoplasm of these enlarged cells was foamy and vacuolated. The hypertrophy was usually associated with basophilic floccular colloid. Follicular cell hypertrophy was seen without concomitant hyperplasia at 6 months. However, follicular cell hypertrophy was typically accompanied by follicular cell hyperplasia at the end of the 2-year study. There is no evidence linking follicular cell hypertrophy to neoplastic development. The incidences of follicular cell hyperplasia in 2,500 ppm males and females were significantly greater than those in the control groups at 2 years. Follicular cell hyperplasia is considered a precursor lesion to follicular cell neoplasia; the stronger response in 2,500 ppm males compared to females is supportive of the neoplastic response. Follicular cell hyperplasia was characterized by an increase in the number of follicular cells, frequently resulting in infolding of cells into the follicular lumina (Plate 10). The hyperplastic lesions did not cause compression of the surrounding thyroid parenchyma. Follicular cell hyperplasia occurred focally to multifocally and was often seen bilaterally.

Liver: The incidences of hepatocellular adenoma occurred with positive trends in males and females, and the incidences in 2,500 ppm males and females were significantly increased at 2 years (Tables 20, C3, and D3). The incidence of hepatocellular carcinoma was significantly increased in 1,250 ppm males. The incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in all exposed groups of males. The incidences of hepatocellular adenoma in 2,500 ppm males and females and the incidence of hepatocellular carcinoma in 2,500 ppm males exceeded the historical ranges in controls (Tables 20, C4b, and D4). The incidences of hepatocellular adenoma or carcinoma (combined) in exposed groups of males were at the upper end of the historical control range. Hepatocellular adenomas were discrete masses with distinct borders that caused compression of the surrounding normal hepatic parenchyma. Adenomas were usually composed of hepatocytes that appeared similar to those observed in eosinophilic foci, except that the normal lobular architecture was not apparent and plates of neoplastic hepatocytes intersected the surrounding normal hepatocytes at sharp angles rather than merging with them as in the eosinophilic foci. Carcinomas were discrete masses that generally had irregular borders due to localized areas of growth of neoplastic hepatocytes into the surrounding normal parenchyma. The neoplastic hepatocytes were usually atypical, but the major distinguishing features of carcinomas were abnormal patterns of growth. The most common abnormal growth pattern was formation of trabeculae of neoplastic hepatocytes, which were three or more cell layers thick, while less commonly the neoplastic cells formed glandular structures or solid masses.

The incidence of hepatocyte karyomegaly was significantly increased in 2,500 ppm males at 6 months, and the incidences of this lesion were significantly increased in 1,250 and 2,500 ppm males at 2 years (Tables 20 and

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Mice in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
6-Month Interim Study				
Number Examined Microscopically	10	10	10	10
Follicular Cell Hypertrophy[a]	0	5* (1.0)[b]	9** (1.2)	10** (1.8)
2-Year Study				
Number Examined Microscopically	50	50	50	50
Follicular Cell Hyperplasia	0	2 (1.5)	3 (1.7)	33** (1.9)
Follicular Cell Hypertrophy	1 (1.0)	0	6* (1.2)	25** (1.3)
Follicular Cell Adenoma, Bilateral	0	0	0	1
Follicular Cell Adenoma (includes bilateral)[c]				
Overall rate[d]	0/50 (0%)	1/50 (2%)	0/50 (0%)	7/50 (14%)
Adjusted rate[e]	0.0%	2.1%	0.0%	15.8%
Terminal rate[f]	0/43 (0%)	0/46 (0%)	0/36 (0%)	7/40 (18%)
First incidence (days)	—[h]	657	—[i]	729 (T)
Poly-3 test[g]	P<0.001	P=0.506	—	P=0.006
Female				
6-Month Interim Study				
Number Examined Microscopically	8	10	10	10
Follicular Cell Hypertrophy	0	8** (1.0)	10** (1.1)	10** (1.7)
2-Year Study				
Number Examined Microscopically	49	48	48	50
Follicular Cell Hyperplasia	1 (1.0)	1 (2.0)	1 (1.0)	9** (1.6)
Follicular Cell Hypertrophy	6 (1.2)	3 (1.0)	23** (1.1)	46** (1.7)
Follicular Cell Adenoma	1	0	0	1

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (interim evaluation) or the Poly-3 test (2-year study)
** $P \leq 0.01$
(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 studies with controls given NTP-2000 diet (mean ± standard deviation): 3/309 (1.0% ± 1.0%), range 0%-2%
[d] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Not applicable; no neoplasms in animal group
[i] Value of statistic cannot be computed.

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
6-Month Interim Study				
Number Examined Microscopically	10	10	10	10
Hepatocyte, Karyomegaly[a]	0	0	0	5* (1.2)[b]
Hepatocyte, Vacuolization Cytoplasmic	0	0	0	4* (2.0)
2-Year Study				
Number Examined Microscopically	50	50	50	50
Hepatocyte, Karyomegaly	0	0	10**(1.0)	29**(1.3)
Hepatocyte, Cytoplasmic Alteration	0	0	11**(1.2)	37**(1.8)
Kupffer Cell, Pigmentation	0	1 (3.0)	1 (2.0)	19**(1.5)
Hepatocellular Adenoma, Multiple	2	3	4	3
Hepatocellular Adenoma (includes multiple)[c]				
Overall rate[d]	7/50 (14%)	14/50 (28%)	13/50 (26%)	18/50 (36%)
Adjusted rate[e]	14.8%	28.8%	30.8%	40.3%
Terminal rate[f]	7/43 (16%)	14/46 (30%)	10/36 (28%)	17/40 (43%)
First incidence (days)	729 (T)	729 (T)	611	656
Poly-3 test[g]	P=0.006	P=0.077	P=0.058	P=0.005
Hepatocellular Carcinoma, Multiple	0	3	6*	1
Hepatocellular Carcinoma (includes multiple)[h]				
Overall rate	4/50 (8%)	8/50 (16%)	14/50 (28%)	6/50 (12%)
Adjusted rate	8.4%	16.3%	32.7%	13.4%
Terminal rate	3/43 (7%)	5/46 (11%)	9/36 (25%)	4/40 (10%)
First incidence (days)	611	657	531	656
Poly-3 test	P=0.261	P=0.190	P=0.003	P=0.330
Hepatocellular Adenoma or Carcinoma[i]				
Overall rate	10/50 (20%)	22/50 (44%)	22/50 (44%)	22/50 (44%)
Adjusted rate	20.9%	44.9%	50.9%	49.0%
Terminal rate	9/43 (21%)	19/46 (41%)	16/36 (44%)	20/40 (50%)
First incidence (days)	611	657	531	656
Poly-3 test	P=0.007	P=0.009	P=0.002	P=0.003

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Female				
6-Month Interim Study				
Number Examined Microscopically	10	10	10	10
Hepatocyte, Vacuolization Cytoplasmic	0	1 (2.0)	2 (2.0)	4* (2.0)
2-Year Study				
Number Examined Microscopically	50	49	49	50
Hepatocellular Adenoma, Multiple	1	1	0	1
Hepatocellular Adenoma (includes multiple)[j]				
Overall rate	3/50 (6%)	4/49 (8%)	6/49 (12%)	10/50 (20%)
Adjusted rate	6.2%	8.5%	12.9%	20.5%
Terminal rate	2/46 (4%)	3/43 (7%)	6/43 (14%)	9/45 (20%)
First incidence (days)	723	688	729 (T)	664
Poly-3 test	P=0.015	P=0.485	P=0.226	P=0.037

* Significantly different (P≤0.05) from the control group by the Fisher exact test (interim evaluation) or the Poly-3 test (2-year study)
** P≤0.01
(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 studies with controls given NTP-2000 diet (mean ± standard deviation): 60/310 (19.0% ± 8.5%), range 10%-30%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence is the P value corresponding to pairwise comparison between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence: 43/310 (13.7% ± 5.1%), range 8%-20%
[i] Historical incidence: 95/310 (30.2% ± 10.4%), range 20%-45%
[j] Historical incidence: 29/309 (9.3% ± 2.4%), range 6%-12%

C5). Karyomegaly was characterized by enlargement of the nuclei of hepatocytes. Hepatocyte karyomegaly was seen in the centrilobular regions and was usually associated with cytoplasmic alteration. The incidences of hepatocyte cytoplasmic vacuolization were significantly increased in 2,500 ppm males and females at 6 months (Tables 20, C5, and D5). The incidences of cytoplasmic alteration in 1,250 and 2,500 ppm males were significantly greater than that in the controls at 2 years, and the lesion was characterized by increased cytoplasmic eosinophilia and a loss of normal glycogenic vacuolization of hepatocytes in the centrilobular and midzonal areas of the hepatic lobules. The incidence of Kupffer cell pigmentation in 2,500 ppm males was significantly increased at 2 years. The pigmentation, consistent with hemosiderin, was globular and golden brown.

Spleen: Spleen weights were significantly increased in 1,250 and 2,500 ppm males and 2,500 ppm females at 14 weeks and 6 months (Tables G5 and G6). The incidences of hematopoietic cell proliferation in all exposed groups of males and in 2,500 ppm females were significantly greater than those in the controls at 6 months and 2 years (Tables 21, C5, and D5). The severity of the hematopoietic cell proliferation was minimal to marked in exposed mice at 2 years. This lesion was histologically similar to the hematopoietic cell proliferation described in the 14-week toxicity study of 2-methylimidazole (NTP, 2004). Hematopoietic cell proliferation is a typical regenerative response to the anemia observed in exposed mice. The incidences of hemosiderin pigmentation within macrophages in the red pulp of the spleen were significantly increased in 1,250 and 2,500 ppm males and females at 6 months and all groups of exposed males and 1,250 and 2,500 ppm females at 2 years. The incidences of lymphoid follicular atrophy were significantly increased in 1,250 and 2,500 ppm males at 6 months and 2 years and in 2,500 ppm females at 6 months. This lesion was minimal to mild and was characterized by a smaller size of the periarteriolar lymphoid sheaths with an occasionally decreased cell density, a thin or indiscernible pale mantle zone, and a reduction in the number of germinal centers compared to controls.

Bone Marrow: The incidences of hyperplasia were significantly increased in 1,250 and 2,500 ppm males at 2 years (Tables 21 and C5). Hyperplasia was mild to marked and characterized by an overall increase in all cell types. The bone marrow hyperplasia was a regenerative response to the anemia in mice.

Kidney: The incidences of pigmentation in the proximal convoluted tubules were increased in 2,500 ppm males at 6 months and at 2 years (Tables 21 and C5). The pigmentation, consistent with hemosiderin, was globular and golden brown. The pigmentation was similar to that found in the spleen and liver and was consistent with treatment-related hemolysis/anemia. The observation of pigment in the males but not the females was consistent with the finding of more hemosiderin pigment in male liver and spleen compared to females and an increased severity and incidence of bone marrow hyperplasia in males compared to females. Pigmentation of the proximal convoluted tubules was histologically similar to that described in mice in the 14-week study (NTP, 2004).

Epididymis and Testis: The incidences and severities of chronic active inflammation of the epididymis were increased in 1,250 and 2,500 ppm males at 2 years, and the incidence of sperm granuloma was increased in 2,500 ppm males at 2 years (Tables 21 and C5). Inflammation was generally unilateral and characterized by focal to locally extensive infiltrates of mononuclear inflammatory cells, engorgement of ducts with spermatozoa, multinucleated spermatozoa in duct lumina, marked vacuolization and disruption of the cells lining the epididymal ducts, and ducts contained coagulated protein and cell debris. Interstitial fibrosis and edema were seen to variable degrees. The inflammatory lesion was commonly seen with sperm granuloma. In sperm granuloma, there was rupture of the epididymal ducts with a focal aggregation of macrophages in the interstitium. The incidences of germinal epithelial atrophy of the testis were increased in 1,250 and 2,500 ppm males at 2 years (Tables 21 and C5). Germinal epithelial atrophy was commonly associated with ipsilateral epididymal inflammation and/or sperm granuloma. The atrophy was locally extensive to diffuse and characterized by a minimal to moderate decrease in the numbers of spermatogenic epithelium. Many tubules were lined only by Sertoli cells. The unilateral testicular atrophy was considered a secondary response to the epididymal inflammation.

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
6-Month Interim Study				
Spleen[a]	10	10	10	10
Hematopoietic Cell Proliferation[b]	0	5* (1.0)[c]	10**(2.1)	10**(3.0)
Pigmentation	0	1 (1.0)	10**(1.1)	10**(2.2)
Lymphoid Follicle, Atrophy	0	0	5* (1.2)	10**(1.0)
Kidney	10	10	10	10
Renal Tubule, Pigmentation	0	0	1 (1.0)	10**(2.2)
2-Year Study				
Spleen	50	50	49	50
Hematopoietic Cell Proliferation	10 (2.2)	21* (2.3)	38**(2.5)	45**(2.9)
Pigmentation	1 (2.0)	16**(1.1)	33**(1.6)	43**(2.2)
Lymphoid Follicle, Atrophy	0	4 (3.3)	14**(1.6)	30**(1.8)
Bone Marrow	50	50	50	50
Hyperplasia	4 (3.0)	10 (2.8)	20**(2.1)	42**(2.5)
Kidney	50	50	50	50
Renal Tubule, Pigmentation	1 (2.0)	0	2 (1.5)	45**(2.5)
Epididymis	50	50	50	50
Granuloma, Sperm	0	0	2 (3.0)	5* (2.6)
Inflammation, Chronic Active	1 (1.0)	3 (1.3)	7* (2.0)	8* (2.9)
Testes	50	50	50	50
Germinal Epithelium, Atrophy	1 (2.0)	4 (2.0)	8**(2.3)	14**(2.0)
Female				
6-Month Interim Study				
Spleen	10	10	10	10
Hematopoietic Cell Proliferation	2 (1.0)	5 (1.2)	6 (1.5)	10**(2.6)
Pigmentation	1 (1.0)	3 (1.0)	7**(1.3)	10**(1.9)
Lymphoid Follicle, Atrophy	0	0	0	4* (1.5)
2-Year Study				
Spleen	50	49	49	50
Hematopoietic Cell Proliferation	15 (2.1)	20 (2.2)	24 (2.5)	39**(2.4)
Pigmentation	0	4 (1.3)	11**(1.5)	34**(1.7)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (interim evaluation) or the Poly-3 test (2-year evaluation)
** $P \leq 0.01$
[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

Physiologically Based Pharmacokinetic Model

A physiologically based pharmacokinetic (PBPK) model representing the uptake, distribution, and metabolism of 2-methylimidazole in rats and mice was developed to describe the processes involved in 2-methylimidazole toxicokinetics (Appendix N). The PBPK model has separate compartments representing liver, kidney, muscle, skin, adipose, brain, thyroid gland, and other aggregated tissues. Most of the model parameters were obtained from estimates in the literature, while a few were estimated from single-dose toxicokinetic data (Appendix M). The toxicokinetic data include 2-methylimidazole plasma concentrations for up to 24 hours following intravenous (10 mg/kg) and oral (25, 50, or 100 mg/kg) dosing for male and female rats and mice. Overall, the model predictions fit the data. The model was used to show there are statistically significant differences in the toxicokinetic data for rats and mice, and for males and females within each species. Urinary clearance is the primary route of elimination of the parent compound with the rate of urinary elimination higher in rats than in mice. A difference in urinary clearance alone can account for the statistically significant differences between genders and species.

Genetic Toxicology

2-Methylimidazole (100 to 10,000 µg/plate) was negative in the *Salmonella typhimurium* gene mutation assay when tested in strains TA97, TA98, TA100, and TA1535, with and without S9 metabolic activation enzymes (Table E1). 2-Methylimidazole was also tested in three *in vivo* assays for induction of chromosomal damage as measured by micronucleated erythrocyte frequency, and the results were mixed (Tables E2, E3, and E4). 2-Methylimidazole, administered to male mice by intraperitoneal injection three times at 24-hour intervals, produced small increases in the frequency of micronucleated polychromatic erythrocytes (PCEs) in bone marrow, but these increases were not significant and the results of the assay were concluded to be negative. Results of a three-injection bone marrow micronucleus test in male rats were also negative. In contrast to the results obtained in these two short-term studies, results of the 14-week 2-methylimidazole study in mice showed significant exposure-related increases in the frequencies of micronucleated normochromatic erythrocytes in peripheral blood samples of males and females. The increases in the frequencies of micronuclei noted in female mice were greater than those observed in male mice (the three highest doses tested in females induced micronucleated frequencies that were significantly elevated above the control frequency), but the overall magnitudes of the responses in males and females were similar. An exposure concentration-related increase in the percentage of micronucleated PCEs in peripheral blood was seen in male and female mice in the 14-week study. It is possible that an increase in the rate of hematopoiesis, evidenced by the increase in percentage of PCEs, may have contributed to an enhancement of the micronucleus frequencies in mice treated with 2-methylimidazole for 14 weeks.

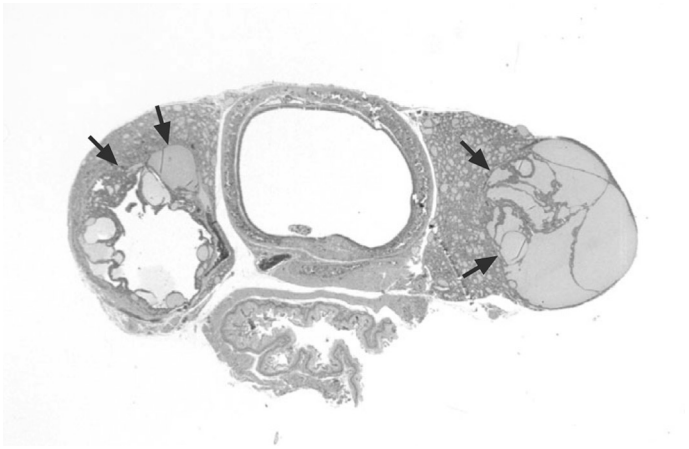


PLATE 1
Bilateral follicular cell adenomas (arrows) in the thyroid gland of a female F344/N rat exposed to 5,000 ppm 2-methylimidazole for 2 years. H&E; 3.3x

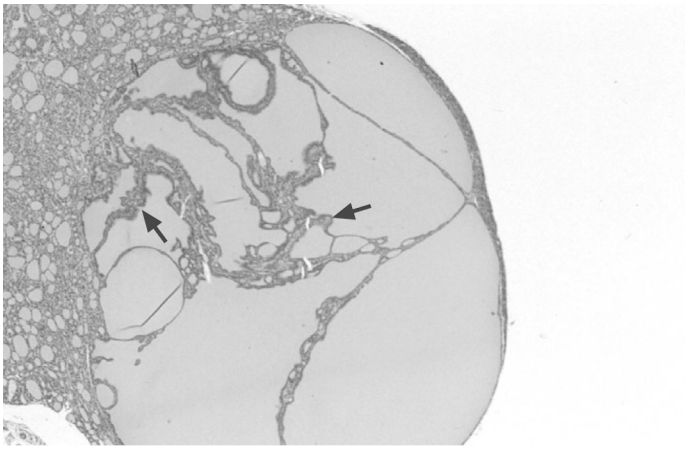


PLATE 2
Higher magnification of a follicular cell adenoma shown in Plate 1. Note the cystic papillary pattern (arrow). H&E; 10x

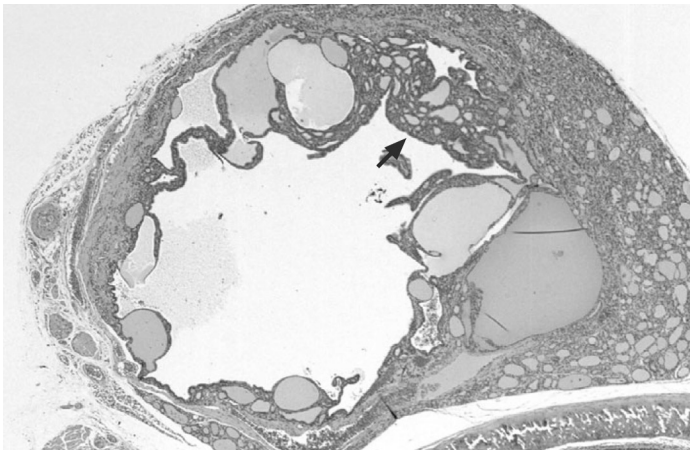


PLATE 3
Higher magnification of a follicular cell adenoma shown in Plate 1. Note the complex papillary growth (arrow). H&E; 10x

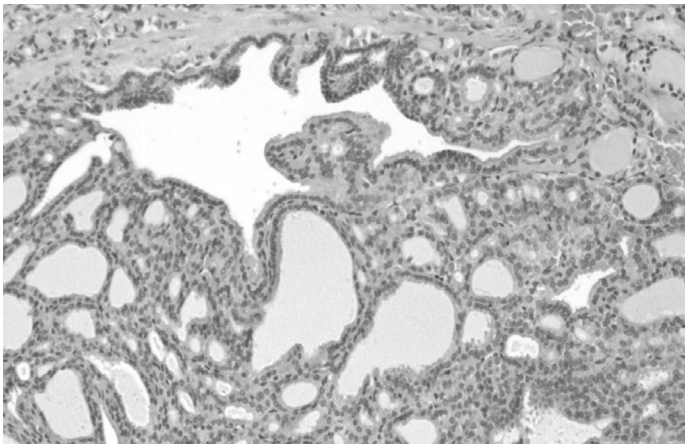


PLATE 4
Higher magnification of the complex papillary growth shown in Plate 3 showing numerous small irregular follicules containing little or no colloid. H&E; 50x

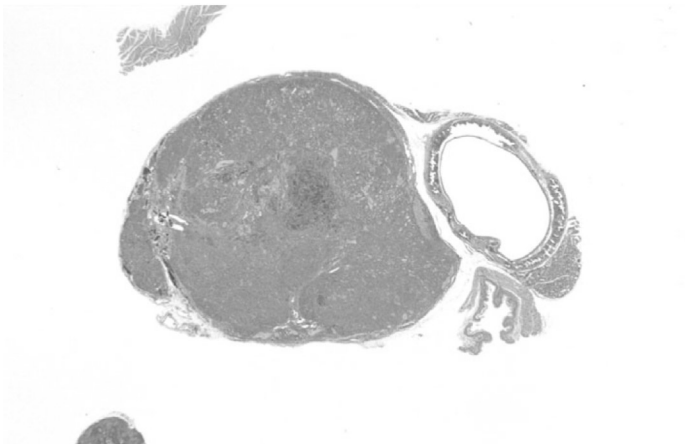


PLATE 5
Follicular cell carcinoma in the thyroid gland of a female F344/N rat exposed to 5,000 ppm 2-methylimidazole in feed for 2 years. Note the carcinoma obliterated the entire thyroid gland. H&E; 2.5x

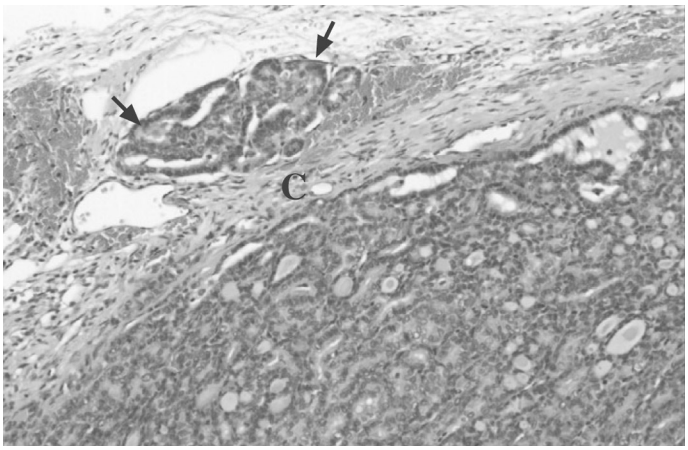


PLATE 6
Higher magnification of the follicular cell carcinoma shown in Plate 5 showing extension (arrows) outside the capsule (C). H&E; 50x

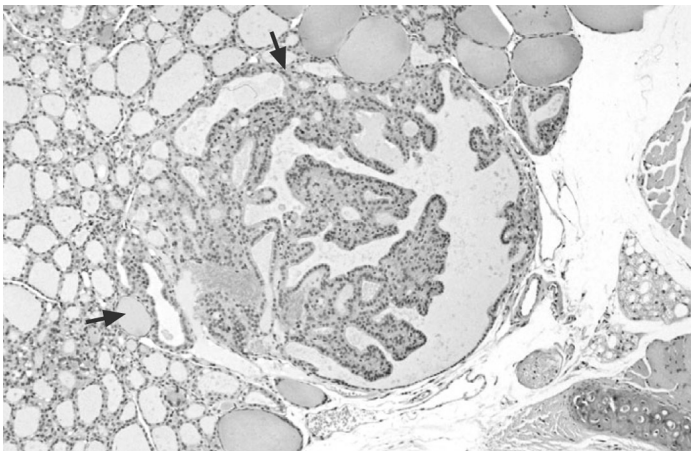


PLATE 7
Follicular cell adenoma (arrows) in the thyroid gland of a male $B6C3F_1$ mouse exposed to 2,500 ppm 2-methylimidazole in feed for 2 years. Note the papillary pattern. H&E; 25x

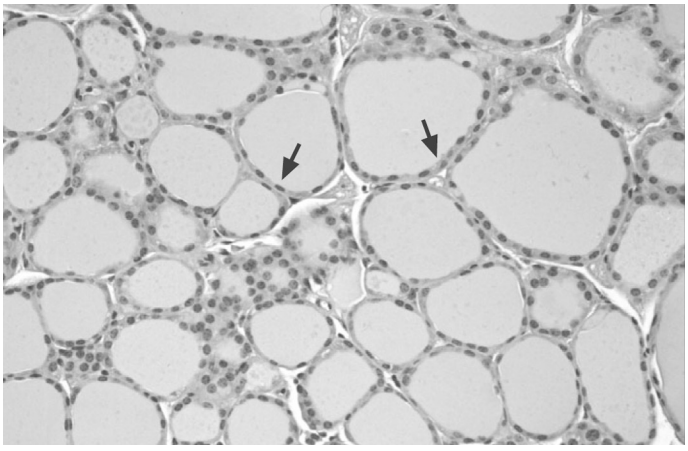


PLATE 8
Thyroid gland follicular cell epithelium (arrows) of a control male $B6C3F_1$ mouse in the 2-year feed study of 2-methylimidazole. H&E; 80x

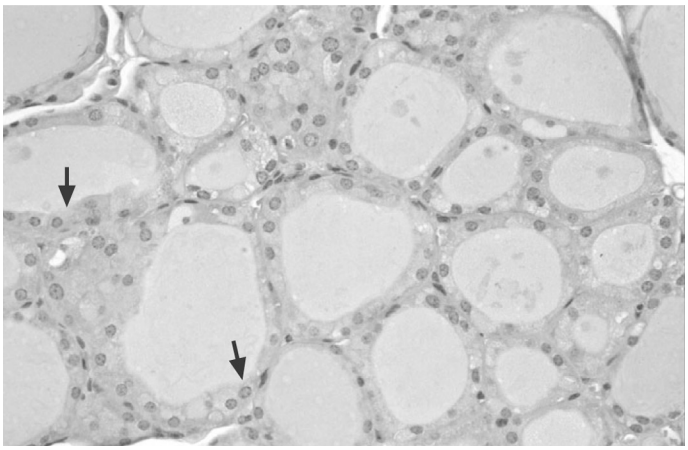


PLATE 9
Follicular cell hypertrophy (arrows) in the thyroid gland of a 2,500 ppm male $B6C3F_1$ mouse in the 2-year feed study of 2-methylimidazole. Compare the increased size of the follicular epithelial cells due to the distended cytoplasm to the normal follicular epithelium in Plate 8. H&E; 80x

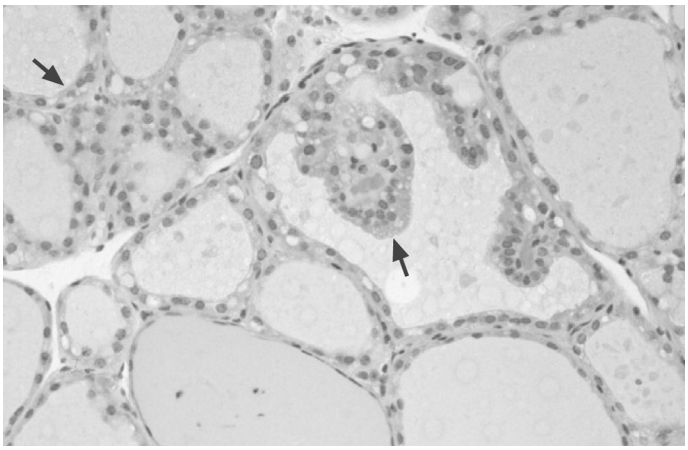


PLATE 10
Follicular cell hyperplasia (arrows) in the thyroid gland of a 2,500 ppm male $B6C3F_1$ mouse in the 2-year feed study of 2-methylimidazole. Note the increase in the number of follicular epithelial cells and infolding of cells into follicular lumina. H&E; 80x

DISCUSSION AND CONCLUSIONS

2-Methylimidazole and 4-methylimidazole were nominated for toxicity and carcinogenicity studies because of their widespread use in the electronics and pharmaceutical industries, their presence as contaminants in food products and in the environment, the high potential for human exposure, a lack of carcinogenicity data in the literature, and a suspicion of carcinogenicity based on structural similarity with other substituted imidazoles.

Some observations on disposition and metabolism may be relevant to the interpretation of the toxicity of 2-methylimidazole. Orally administered 2-methylimidazole is well absorbed (Sanders *et al.*, 1998) and cleared rapidly from most tissues. However, 2-methylimidazole-derived radioactivity persisted in several tissues including the thyroid gland, skin, and muscle. In rats, most of the 2-methylimidazole was excreted unchanged in urine. Sanders *et al.* (1998) indicated that mice excrete 2-methylimidazole-derived radioactivity in urine more slowly than rats and less of the excreted radioactivity is parent chemical. In toxicokinetic studies, significant species and sex differences in clearance of 2-methylimidazole were seen (Johnson *et al.*, 2002; Appendix M). Male rats cleared 2-methylimidazole faster than did female rats and mice cleared 2-methylimidazole faster than did rats. Ohta *et al.* (1998) identified the primary urinary metabolite of 2-methylimidazole as 2-methylimidazolone. Formation of 2-methylimidazolone may not be exclusively from oxidation by cytochrome P450 enzymes. While Miyachi and Nagatsu (2002) were able to oxidize 2-methylimidazole to 2-methylimidazolone using a cytochrome P450 model system, *in vivo* studies with cytochrome P450 inhibitors SKF525-A and cimetidine had no effect on the amount of 2-methylimidazolone excreted in urine, although the amount of irreversibly bound radioactivity in tissue was doubled (Ohta *et al.*, 1996). The present data indicate that there was no increase in total hepatic cytochrome P450 content in either rats or mice exposed to 2-methylimidazole for up to 6 months, although changes in specific isozyme activities could have occurred without a measurable change in total cytochrome P450. While there was no change in cytochrome P450 content, there was a sustained exposure-related increase in hepatic UDP-glucuronosyltransferase in rats, but not mice, during at least the first 6 months of the study.

In the 14-week toxicity studies, rats and mice were exposed to concentrations of up to 10,000 ppm 2-methylimidazole in feed (NTP, 2004). The primary toxic response to 2-methylimidazole in male and female rats was exposure-related increase of thyroid gland follicular cell hyperplasia. Two of 10 male rats in the 10,000 ppm group developed thyroid gland follicular cell adenomas. These changes were accompanied by decreases in thyroxine (T_4) and increases in circulating thyroid-stimulating hormone (TSH), suggesting that the follicular cell hyperplasia was a response to persistent TSH stimulation of the thyroid gland to compensate for low circulating T_4. Male and female mice exposed to 2,500 ppm or more also had increased incidences of thyroid gland follicular cell hypertrophy, although T_4 concentrations were decreased only in the 10,000 ppm group of females and TSH levels were unaffected. Other changes produced by 2-methylimidazole included a minimal anemia in rats and a more severe regenerative anemia in mice.

Liver weights were increased in mice in the 14-week study (NTP, 2004) and were marginally increased in female rats. Sanders *et al.* (1998) reported that liver UDP-glucuronosyltransferase (UDPGT) activity was significantly increased in male rats receiving 2-methylimidazole at 10,000 ppm in feed for 29 days. These data suggested that thyroid gland neoplasms in rats may follow persistent TSH stimulation of the thyroid gland resulting from enhanced glucuronidation and elimination of T_4. Additional studies were carried out during the 2-year study of 2-methylimidazole to further examine this hypothesis.

In the 2-year study, the mean body weights of rats exposed to 2,500 ppm or more 2-methylimidazole were less than those of the controls. This body weight gain

deficit became significant in female rats receiving 5,000 ppm and may have contributed to the observed marked decreases in the incidences of mammary gland, pituitary gland, and clitoral gland neoplasms in this group. All three of these neoplasm types have been shown previously to be associated with body weight changes in control F344/N rats fed the NIH-07 diet (Haseman *et al.*, 1997; Haseman, 1998). The greater reduction in female rats was related to the higher top dose of 2-methylimidazole administered to female rats (5,000 ppm) than that administered to male rats (3,000 ppm).

The major target of 2-methylimidazole toxicity in the 2-year rat study was again the thyroid gland. At 8 days, 14 weeks, and 6 months, serum thyroid hormone levels were decreased, TSH levels were increased, and liver UDPGT activity was increased. At the 6-month interim evaluation, the thyroid gland weights of 3,000 ppm males and of 2,500 and 5,000 ppm females were significantly increased. Thyroid gland follicular cell hyperplasia was observed at 6 months in all exposed groups of rats. Follicular cell adenomas occurred in two 5,000 ppm females at 6 months. At 2 years, the incidences of thyroid gland follicular cell hyperplasia were significantly increased in all exposed groups of males and females. At 2 years, the incidence of thyroid gland follicular cell adenoma or carcinoma (combined) in males and females occurred with a positive trend, and incidences in the 3,000 ppm (males) and 5,000 ppm (females) groups exceeded the historical ranges in controls. Thus, in rats, 2-methylimidazole likely induced the glucuronidation and excretion of thyroxine which in turn stimulated higher TSH synthesis and secretion. Under the influence of persistent TSH stimulation, the thyroid gland follicular cells first became hyperplastic and eventually developed into follicular cell adenomas and carcinomas, confirming the finding in the NTP 14-week toxicity study (NTP, 2004).

Haseman and Lockhart (1993) reported in NTP studies in rats, a significant correspondence between males and females with respect to thyroid gland follicular cell carcinogenic responses. 2-Methylimidazole appears to have similar effects on male and female rat thyroid gland neoplasms although the toxicokinetic data showed that male rats clear 2-methylimidazole faster than do females (Appendix M).

Capen (1997) reviewed the mechanisms of toxic effects of xenobiotics that interfere with thyroid homeostasis. Theoretically, 2-methylimidazole may disrupt one or more of the possible steps in the biosynthesis, secretion, or metabolism of thyroid hormones. 2-Methylimidazole may inhibit thyroid hormone synthsis as does the related compound methimazole, (1-methylimidazole-2-thiol), which is known to act by inhibiting the incorporation of iodine, thereby effectively inhibiting thyroid hormone synthesis (Taurog, 1976). 2-Mercaptoimidazole, 1-methyl-2-mercaptoimidazole (Nagasaka and Hidaka, 1976), and 1-methyl-3-propylimidazole-2-thione (Biegel *et al*., 1995) have been shown to inhibit thyroxine synthesis by inhibiting thyroid peroxidase. Mercaptobenzimidazole has been shown to reduce serum triiodothyronine (T_3) and T_4 concentrations by an unknown mechanism (Gaworski *et al*., 1991). The components of thyroid hormone synthesis and secretion, including uptake of inorganic iodine, oxidation of iodide by thyroid peroxidase, iodination of tyrosyl groups of thyroglobulin, coupling iodotyrosines to form iodothyronines and release of T_4 and T_3 (Paynter *et al.*, 1988, McClain, 1992), have not been investigated specifically for effects of 2-methylimidazole, although the observation that 2-methylimidazole derived radioactivity was concentrated actively in rat thyroid (Sanders *et al.*, 1998) suggests the possibility of an additional direct action on one or more of these processes.

Investigations with another related compound, imidazole SC-37211, showed that decreases in thyroid hormone levels could be attributed to an increase in glucuronidation and excretion of the hormone in rats (Comer *et al.*, 1985), rather than a direct effect of the imidazole on the thyroxine synthesis and/or release. T_4 is also converted to T_3 by microsomal 5′-monodeiodinases in peripheral tissues and is sulfated before being excreted (Thomas and Williams, 1991; 1999). A decrease in serum T_3 levels can also cause a compensatory increase in TSH (McClain, 1992). However, the effects of 2-methylimidazole on 5′-monodeiodinase and T_3 sulfation have not been investigated.

In the 2-year mouse study, exposure to 2-methylimidazole had no significant effect on survival. Mean body weights of 1,250 and 2,500 ppm males and of 2,500 ppm females were less than those of the controls during most of the study. The reduction in body weight gain may be related to the toxic effects of 2-methylimidazole, because feed consumption by all exposed groups was similar to that by the controls. 2-Methylimidazole exposure caused a mild to moderate macrocytic regenerative anemia as was seen in the 14-week studies

(NTP, 2004). There were also increases in thyroid gland, liver, pituitary gland, and spleen weights of exposed male and female mice.

The incidence of thyroid gland follicular cell adenoma in male mice exposed to 2,500 ppm 2-methylimidazole for 2 years was significantly increased; no increased incidences of thyroid gland follicular cell neoplasms occurred in exposed females. The incidences of thyroid gland follicular cell hypertrophy were significantly increased in all exposed groups of male and female mice at 6 months and in the 1,250 and 2,500 ppm groups at 2 years, and the incidences of follicular cell hyperplasia were significantly increased in 2,500 ppm males and females at 2 years. In contrast to the rat results, there were no changes in circulating T_3, T_4, or TSH levels in mice at 8 days, 14 weeks, or 6 months in the 2-year study. Consistent with these observations was the lack of any significant or consistent change in hepatic UDPGT activities in mice. Possibly the mechanism accounting for the thyroid gland follicular cell neoplasm response in rats was not operative in mice, or the hormone changes in mice were too small to be detected. At this point there is little information available that would suggest an alternative mechanism from the list of possibilities outlined by Capen (1997). On the other hand, it is plausible that a combination of increased hepatic clearance and decreased synthesis of T_3 and T_4 are attributable to the actions of 2-methylimidazole on the thyroid gland.

Increased incidences of hepatocellular neoplasms occurred in male and female rats and mice exposed to 2-methylimidazole for 2 years. In rats, which have lower background rates of hepatocellular neoplasms, the slight increases in hepatocellular neoplasms were not statistically significant, but the incidences in the highest two exposure groups of males and females exceeded their historical control ranges. These marginal increases may have been related to 2-methylimidazole exposure. In male mice, increased incidences of adenoma or carcinoma (combined) occurred in all exposed groups, while female mice had a positive exposure-related trend in the incidence of adenomas and the increase was significant at 2,500 ppm. These increases occurred despite lower body weights of 1,250 and 2,500 ppm males and 2,500 ppm females and were considered "some evidence of carcinogenic activity." There were no increases in multiplicity or preneoplastic foci.

Hepatocellular neoplasms and thyroid gland follicular cell neoplasms often occur together in rodent carcinogenicity studies (Huff et al., 1991, McConnell, 1992, Haseman and Lockhart, 1993). For chemicals that produce neoplasms in both organs, microsomal enzyme induction has been suggested to be a mechanistic link that connects the pathogenesis of thyroid gland follicular neoplasms with hepatocellular neoplasms (McClain, 1989, McClain and Rice, 1999). The present study demonstrated that 2-methylimidazole induced increases in liver weights in mice and liver microsomal UDPGT activity in rats. However, the increases were not accompanied by changes in liver cytochrome P450, and microsomal enzyme induction alone appears to be insufficient to account for the findings in these studies.

At 1,250 and 2,500 ppm, 2-methylimidazole induced testicular atrophy in male mice. The effect may be indirect as Adams *et al*. (1998) reported that 2-methylimidazole decreased luteinizing hormone and tissue interstitial fluid testosterone concentrations in rats.

2-methylimidazole did not induce mutations in Salmonella but subchronic administration in feed resulted in significantly increased numbers of micronucleated erythrocytes in mice (Table E4). Hematopoietic cell proliferation, reflected in the increased percentages of immature erythrocytes (% PCEs) in peripheral blood, in response to 2-methylimidazole-induced anemia in male and female mice may have contributed to an enhancement of the micronucleus frequencies observed in the 14-week study (NTP, 2004). Micronuclei are indicators of structural or numerical chromosomal alterations. Rapid cell turnover in the absence of exposure to a chromosome damaging agent may increase the rate of mitotic error and thus, the frequency of damaged cells (Hirai et al., 1991; Suzuki et al., 1989). This may be one reason for the discordant results between the acute bone marrow and subchronic peripheral blood micronucleus tests reported in Appendix E. However, a comparison of individual exposure groups in the 14-week study shows significant changes in micronucleus frequencies without an accompanying change in % PCEs (for example, female mice, the 5,000 and 10,000 ppm dose groups). Thus, the increased frequencies of micronucleated erythrocytes observed in 2-methylimidazole-treated mice are probably not due to increased rates of cell proliferation alone. Positive results in subchronic peripheral blood micronucleus tests have been shown to strongly correlate with rodent carcinogenicity (Witt *et al*., 2000), and the results with 2-methylimidazole are consistent with that observation.

CONCLUSIONS

Under the conditions of this 2-year feed study, there was *some evidence of carcinogenic activity** of 2-methylimidazole in male F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. The increased incidences of hepatocellular neoplasms in males may have been related to exposure. There was *clear evidence of carcinogenic activity* of 2-methylimidazole in female F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. The increased incidences of hepatocellular adenoma in females may have been related to exposure. There was *some evidence of carcinogenic activity* in male $B6C3F_1$ mice based on increased incidences of thyroid gland follicular cell adenoma and hepatocellular neoplasms. There was *some evidence of carcinogenic activity* in female $B6C3F_1$ mice based on increased incidences of hepatocellular adenoma.

Exposure to 2-methylimidazole resulted in nonneoplastic lesions in the thyroid gland and liver of male rats; the thyroid gland, liver, and spleen of female rats; the thyroid gland, liver, spleen, bone marrow, kidney, epididymis and testes of male mice; and the thyroid gland and spleen of female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

NOTE: An erratum is posted on pages 301-302 that updates information on pages 7, 15-16, and 67.

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

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

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
6-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	13	6	7	13
Natural deaths	2	4	7	2
Survivors				
Died last week of study	1	1	1	
Terminal sacrifice	34	39	35	35
Animals examined microscopically	60	60	60	60
Systems Examined at 6 Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Intestine large, colon	(50)	(49)	(48)	(50)
Intestine large, rectum	(49)	(48)	(48)	(50)
Intestine large, cecum	(48)	(45)	(45)	(49)
Lipoma			1 (2%)	
Intestine small, duodenum	(49)	(49)	(48)	(50)
Intestine small, jejunum	(47)	(45)	(44)	(48)
Leiomyosarcoma			1 (2%)	
Intestine small, ileum	(47)	(46)	(45)	(48)
Liver	(50)	(50)	(50)	(50)
Cholangiocarcinoma	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Cholangioma		2 (4%)		
Hemangiosarcoma		1 (2%)		
Hepatocellular carcinoma			1 (2%)	2 (4%)
Hepatocellular adenoma		1 (2%)	3 (6%)	1 (2%)
Hepatocellular adenoma, multiple				1 (2%)
Mesentery	(26)	(46)	(35)	(20)
Hemangiosarcoma	1 (4%)	1 (2%)		
Oral mucosa			(1)	
Squamous cell papilloma			1 (100%)	
Pancreas	(49)	(49)	(50)	(50)
Acinus, adenoma	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(49)
Stomach, glandular	(50)	(50)	(50)	(49)
Adenoma				1 (2%)

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

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
2-Year Study (continued)				
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Pericardium, epicardium, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant		1 (2%)	3 (6%)	1 (2%)
Pheochromocytoma benign	7 (14%)	2 (4%)	6 (12%)	6 (12%)
Schwannoma malignant, metastatic, adrenal medulla			1 (2%)	
Bilateral, pheochromocytoma benign	1 (2%)	1 (2%)		
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma			1 (2%)	3 (6%)
Carcinoma		3 (6%)		
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	9 (18%)	6 (12%)	8 (16%)	8 (16%)
Pars distalis, adenoma, multiple				1 (2%)
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(48)	(46)	(43)	(50)
Bilateral, C-cell, adenoma	1 (2%)			
Bilateral, C-cell, adenoma, multiple				1 (2%)
C-cell, adenoma	8 (17%)	5 (11%)	9 (21%)	6 (12%)
C-cell, carcinoma	1 (2%)			3 (6%)
Follicular cell, adenoma	1 (2%)		1 (2%)	3 (6%)
Follicular cell, carcinoma		2 (4%)		2 (4%)
General Body System				
Peritoneum	(1)	(3)		
Tissue NOS	(7)	(2)	(6)	(4)
Sarcoma	1 (14%)			
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung			1 (17%)	
Thoracic, alveolar/bronchiolar carcinoma, metastatic, lung			1 (17%)	
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	2 (4%)	
Adenoma, cystic			1 (2%)	
Carcinoma		1 (2%)	2 (4%)	3 (6%)
Bilateral, carcinoma		1 (2%)		
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	2 (4%)			
Bilateral, interstitial cell, adenoma, multiple	42 (84%)	36 (72%)	34 (68%)	28 (56%)
Interstitial cell, adenoma	3 (6%)	2 (4%)	6 (12%)	7 (14%)
Interstitial cell, adenoma, multiple	1 (2%)	7 (14%)	2 (4%)	8 (16%)

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

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
2-Year Study (continued)				
Hematopoietic System				
Bone marrow	(50)	(50)	(47)	(50)
Lymph node	(39)	(49)	(42)	(38)
Deep cervical, carcinoma, metastatic, Zymbal's gland		1 (2%)		
Lymph node, mesenteric	(47)	(50)	(50)	(50)
Spleen	(49)	(49)	(50)	(50)
Thymus	(48)	(48)	(44)	(49)
Integumentary System				
Mammary gland	(48)	(47)	(45)	(42)
Fibroadenoma				2 (5%)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma				2 (4%)
Keratoacanthoma	1 (2%)	2 (4%)	1 (2%)	4 (8%)
Melanoma benign			1 (2%)	
Osteosarcoma, metastatic, uncertain primary site	1 (2%)			
Trichoepithelioma	1 (2%)		1 (2%)	1 (2%)
Dermis, fibroma		1 (2%)		
Pinna, schwannoma malignant	1 (2%)			
Sebaceous gland, adenoma				1 (2%)
Subcutaneous tissue, fibroma	1 (2%)	3 (6%)		2 (4%)
Subcutaneous tissue, fibroma, multiple	1 (2%)			
Subcutaneous tissue, fibrosarcoma			1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma				1 (2%)
Cranium, carcinoma, metastatic, Zymbal's gland		1 (2%)		
Cranium, osteoma	2 (4%)			
Cranium, osteosarcoma				1 (2%)
Skeletal muscle	(2)	(1)	(1)	(4)
Hemangiosarcoma	1 (50%)			
Rhabdomyosarcoma				1 (25%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant				1 (2%)
Spinal cord	(1)		(1)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Alveolar/bronchiolar carcinoma	2 (4%)	1 (2%)	1 (2%)	
Alveolar/bronchiolar carcinoma, multiple			1 (2%)	
Carcinoma, metastatic, preputial gland				1 (2%)
Carcinoma, metastatic, Zymbal's gland		1 (2%)		
Osteosarcoma, metastatic, bone				1 (2%)
Alveolar epithelium, carcinoma			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Glands, adenoma	1 (2%)			

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

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
2-Year Study (continued)				
Special Senses System				
Harderian gland	(50)	(50)	(49)	(50)
Zymbal's gland		(1)	(2)	
Carcinoma		1 (100%)	2 (100%)	
Urinary System				
Kidney	(49)	(49)	(49)	(50)
Urinary bladder	(50)	(50)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	15 (30%)	14 (28%)	21 (42%)	10 (20%)
Lymphoma malignant				1 (2%)
Mesothelioma malignant	2 (4%)	4 (8%)	2 (4%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	49	49	48
Total primary neoplasms	111	104	118	115
Total animals with benign neoplasms	49	46	47	45
Total benign neoplasms	86	72	80	85
Total animals with malignant neoplasms	22	26	30	25
Total malignant neoplasms	25	32	38	30
Total animals with metastatic neoplasms	1	2	2	2
Total metastatic neoplasms	1	4	4	2
Total animals with malignant neoplasms of uncertain primary site	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 2-Methylimidazole: 0 ppm

Number of Days on Study	315	517	602	638	643	651	668	680	689	699	704	709	716	722	726	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	01 10	00 08	03 04	02 03	00 09	01 06	00 02	02 06	03 01	02 12	05 04	04 48	05 09	04 03	04 02	01 11	01 12	01 27	01 18	04 44	04 45	04 59	05 00	05 01	05 18
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																	X								
Mesentery		+	+	+	+	+	+	+	+				+	+	+					+	+	+	+	+	+
Hemangiosarcoma															X										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue		+																							
Tooth				+																					
Cardiovascular System																									
Blood vessel							+													+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine Sytem																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign									X		X														
Bilateral, pheochromocytoma benign												X													
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X	X									X		X		X	X
Pars intermedia, adenoma																									
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma																	X						X		
C-cell, carcinoma																		X							
Follicular cell, adenoma																		X							
General Body System																									
Peritoneum																									
Tissue NOS	+		+		+				+				+	+											
Sarcoma	X																								

+: 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 2-Methylimidazole: 0 ppm

Number of Days on Study	729	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	734	734	734	734	734	734	734	
Carcass ID Number	06 10	06 11 3	06 11 4	06 11 5	06 11 9	06 12 0	06 12 1	06 12 5	06 12 7	06 13 2	06 13 3	06 13 5	06 13 6	06 14 1	06 14 3	06 14 4	06 15 1	06 15 2	06 15 7	06 16 8	06 16 9	06 17 0	06 17 6	06 17 7	06 17 3	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma																										1
Mesentery	+										+	+	+				+				+		+	+	+	26
Hemangiosarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinus, adenoma																					X					1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Tooth																										1
Cardiovascular System																										
Blood vessel	+						+				+	+	+				+						+	+	+	16
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign			X					X						X				X			X					7
Bilateral, pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma					X									X											X	9
Pars intermedia, adenoma											X															1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bilateral, C-cell, adenoma															X											1
C-cell, adenoma										X								X			X	X		X	X	8
C-cell, carcinoma																										1
Follicular cell, adenoma																										1
General Body System																										
Peritoneum			+																							1
Tissue NOS									+																	7
Sarcoma																										1

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

Number of Days on Study	315	572	603	638	643	651	668	680	689	699	704	716	722	726	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	01 10	00 08	00 34	00 23	00 39	00 16	00 02	00 26	00 31	00 12	00 54	00 48	00 59	00 43	00 42	00 11	00 12	00 17	00 18	00 44	00 45	00 49	00 50	00 51	00 58
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis																									
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X																					X	
Bilateral, interstitial cell, adenoma, multiple				X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X		X
Interstitial cell, adenoma																									
Interstitial cell, adenoma, multiple		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	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	I	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Osteosarcoma, metastatic, uncertain primary site																									
Trichoepithelioma																	X								
Pinna, schwannoma malignant																									
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibroma, multiple							X																		
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranium, osteoma															X			X							
Skeletal muscle												+			+										
Hemangiosarcoma															X										
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve		+																							
Spinal cord		+																							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																								X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glands, adenoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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

Number of Days on Study	72	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	
	29	30	30	30	30	30	30	30	30	30	30	30	30	30	31	31	31	31	31	34	34	34	34	34	34	34	
Carcass ID Number	06	01	01	01	01	02	02	02	02	02	03	03	03	03	00	00	02	04	05	00	02	02	03	04	04	05	Total Tissues/ Tumors
	60	63	64	65	69	70	71	75	77	72	73	73	75	76	01	03	44	41	52	07	28	29	30	46	47	53	
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																											1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma																											2
Bilateral, interstitial cell, adenoma, multiple	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	42
Interstitial cell, adenoma					X		X											X									3
Interstitial cell, adenoma, multiple																											1
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+			+		+		+	+		+	+	+		+	+	+	+	+	+	+	+		+	+	+	39
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	+	2
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	M		47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Integumentary System																											
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Keratoacanthoma																									X		1
Osteosarcoma, metastatic, uncertain primary site											X																1
Trichoepithelioma																											1
Pinna, schwannoma malignant							X																				1
Subcutaneous tissue, fibroma																X											1
Subcutaneous tissue, fibroma, multiple																											1
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Cranium, osteoma																											2
Skeletal muscle																											2
Hemangiosarcoma																											1
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Peripheral nerve																											1
Spinal cord																											1
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar adenoma																		X		X							2
Alveolar/bronchiolar carcinoma												X															2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Glands, adenoma																						X					1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50

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

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

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

Number of Days on Study	729	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	06 0	11 3	11 4	11 5	11 9	12 0	12 1	12 5	12 7	13 2	13 3	13 5	13 6	00 1	00 3	24 4	41 1	52 2	07 7	28 8	29 9	30 0	46 6	47 7	53 3	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X							X							X								15
Mesothelioma malignant																										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 300 ppm

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 300 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	1 0 0 6	1 0 0 7	1 0 0 8	0 0 6 3	0 0 7 3	0 0 7 4	0 0 7 6	0 0 7 7	0 0 7 8	0 0 7 9	0 0 8 0	0 0 8 1	0 0 8 3	0 0 8 4	0 1 0 3	1 1 0 4	1 1 1 5	1 1 1 7	1 0 7 0	0 0 7 1	0 0 9 1	0 0 9 2	0 0 9 3	0 1 0 9	1 1 1 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma													X							X						2
Cholangioma																									X	2
Hemangiosarcoma																				X						1
Hepatocellular adenoma											X															1
Mesentery	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Hemangiosarcoma																				X						1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										2
Tooth																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant													X													1
Pheochromocytoma benign										X											X					2
Bilateral, pheochromocytoma benign															X											1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma						X																				3
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma									X	X					X				X							6
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
C-cell, adenoma										X		X					X					X				5
Follicular cell, carcinoma																								X		2
General Body System																										
Peritoneum																										3
Tissue NOS																										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 300 ppm

Number of Days on Study	5 0 7	5 2 1	5 4 8	5 7 2	6 0 5	6 0 8	6 6 0	6 6 8	6 7 5	6 9 3	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 8 8	1 0 0	1 1 0	0 9 0	0 7 5	0 7 2	1 1 6	1 0 1	0 6 1	1 0 5	0 6 2	0 6 7	0 6 8	0 6 9	0 8 5	0 8 6	0 8 7	0 8 9	0 6 4	0 6 5	0 6 6	0 9 7	0 9 8	0 9 9	1 0 2
Genital System																									
Coagulating gland																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis										+															
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X													
Carcinoma																									
Bilateral, carcinoma			X																						
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma, multiple						X	X	X		X	X	X	X		X	X	X	X	X	X		X	X	X	
Interstitial cell, adenoma			X						X																
Interstitial cell, adenoma, multiple				X										X							X				X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deep cervical, carcinoma, metastatic, Zymbal's gland	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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma													X								X				
Dermis, fibroma																									
Subcutaneous tissue, fibroma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranium, carcinoma, metastatic, Zymbal's gland	X																								
Skeletal muscle																					+				
Mesothelioma malignant, metastatic, mesentery																					X				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve										+															

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 300 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	1 0 0 6	1 0 0 7	1 0 0 8	0 6 3 3	0 7 3 3	0 7 7 4	0 7 7 6	0 7 7 7	0 7 7 8	0 7 7 9	0 7 8 0	0 8 8 1	0 8 8 3	0 8 8 4	0 8 0 3	1 0 0 4	1 1 1 5	1 1 1 7	1 0 7 0	0 0 7 1	0 0 9 1	0 9 9 2	0 9 9 3	1 0 9 9	1 1 1 1	Total Tissues/ Tumors
Genital System																										
Coagulating gland																								+		1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma	X																									1
Bilateral, carcinoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma, multiple	X	X	X		X	X	X	X			X	X	X	X	X	X	X	X	X	X		X	X		X	36
Interstitial cell, adenoma																										2
Interstitial cell, adenoma, multiple									X												X			X		7
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Deep cervical, carcinoma, metastatic, Zymbal's gland																										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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	M	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																										2
Dermis, fibroma											X															1
Subcutaneous tissue, fibroma					X		X		X																	3
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cranium, carcinoma, metastatic, Zymbal's gland																										1
Skeletal muscle																										1
Mesothelioma malignant, metastatic, mesentery																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 300 ppm

Number of Days on Study	5 0 7	5 2 1	5 4 8	5 7 2	6 0 5	6 0 8	6 6 0	6 6 8	6 7 5	6 9 3	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 8 8	1 0 0	1 1 0	0 9 0	0 7 5	0 7 2	1 1 6	1 0 1	0 6 1	1 0 5	0 6 2	0 6 7	0 6 8	0 6 9	0 8 5	0 8 6	0 8 7	0 8 9	0 6 4	0 6 5	0 6 6	0 9 7	0 9 8	0 9 9	1 0 2
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X																					
Alveolar/bronchiolar carcinoma															X										
Carcinoma, metastatic, Zymbal's gland	X																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland	+																								
Carcinoma	X																								
Urinary System																									
Kidney	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X	X			X	X	X	X						X								
Mesothelioma malignant						X	X														X				

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 300 ppm

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	1 0 0 6	1 0 0 7	1 0 0 8	0 0 6 3	0 0 7 3	0 0 7 4	0 0 7 6	0 0 7 7	0 0 7 8	0 0 7 9	0 0 8 0	0 0 8 1	0 0 8 3	0 0 8 4	0 1 0 3	1 1 0 4	1 1 1 5	1 1 1 7	0 0 7 0	0 0 7 1	0 0 9 1	0 0 9 2	0 0 9 3	1 0 9	1 1 1	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																							X			2
Alveolar/bronchiolar carcinoma																										1
Carcinoma, metastatic, Zymbal's gland																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X									X		X				X		X			X					14
Mesothelioma malignant													X													4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 1,000 ppm

Number of Days on Study	3 9 2	4 3 9	4 7 1	5 3 8	5 4 8	5 8 3	6 4 1	6 4 4	6 6 8	6 7 3	6 9 4	6 9 6	7 0 3	7 1 7	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	1 6 2	1 6 3	1 7 2	1 6 4	1 3 2	1 8 0	1 5 0	1 2 3	1 7 3	1 5 6	1 6 8	1 6 7	1 5 1	1 6 6	1 3 3	1 3 4	1 3 5	1 7 4	1 5 4	1 5 5	1 6 0	1 6 1	1 6 5	1 6 9	1 7 0
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	A	+	+	A	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									
Intestine small, duodenum	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	A	+	+	A	+	A	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
Intestine small, ileum	A	A	+	+	A	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																									
Hepatocellular carcinoma																									
Hepatocellular adenoma												X													
Mesentery	+					+		+	+	+		+	+	+	+	+	+	+	+	+			+	+	+
Oral mucosa																									
Squamous cell papilloma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
Cardiovascular System																									
Blood vessel															+			+	+	+			+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pericardium, epicardium, alveolar/bronchiolar carcinoma, metastatic, lung			X																						
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign						X										X	X							X	
Schwannoma malignant, metastatic, adrenal medulla																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X				X						X						X	X							X
Thyroid gland	A	A	+	+	A	+	A	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma															X						X				X
Follicular cell, adenoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 1,000 ppm

Number of Days on Study	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	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	1 7 1	1 4 2	1 4 3	1 4 4	1 4 8	1 4 9	1 5 2	1 5 3	1 2 2	1 2 4	1 2 5	1 2 6	1 2 7	1 2 8	1 2 9	1 3 0	1 3 1	1 5 7	1 5 8	1 5 9	1 7 5	1 7 6	1 7 7	1 7 8	1 7 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Lipoma		X																								1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Leiomyosarcoma																									X	1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma																			X						X	2
Hepatocellular carcinoma	X																									1
Hepatocellular adenoma	X													X												3
Mesentery	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+				+			+	35
Oral mucosa				+																						1
Squamous cell papilloma				X																						1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth					+																					1
Cardiovascular System																										
Blood vessel	+				+	+	+		+	+	+	+	+	+	+	+	+									20
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pericardium, epicardium, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant						X	X		X																	3
Pheochromocytoma benign										X													X			6
Schwannoma malignant, metastatic, adrenal medulla									X																	1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																							X			1
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma						X					X															8
Thyroid gland	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
C-cell, adenoma	X						X		X		X		X						X							9
Follicular cell, adenoma			X																							1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 1,000 ppm

Number of Days on Study	3 9 2	4 3 9	4 7 1	5 3 8	5 4 8	5 8 3	6 4 1	6 4 4	6 6 8	6 7 3	6 9 4	6 9 6	7 0 3	7 1 7	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	1 6 2	1 6 3	1 7 2	1 6 4	1 3 2	1 8 0	1 5 0	1 2 3	1 7 3	1 5 6	1 6 8	1 6 7	1 5 1	1 6 6	1 3 3	1 3 4	1 3 5	1 7 4	1 5 4	1 5 5	1 6 0	1 6 1	1 6 5	1 6 9	1 7 0
General Body System																									
Tissue NOS			+	+									+	+			+								
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung			X																						
Thoracic, alveolar/bronchiolar carcinoma, metastatic, lung			X																						
Genital System																									
Coagulating gland																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma										X															
Adenoma, cystic														X											
Carcinoma											X														
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma, multiple							X	X		X		X		X	X	X				X		X	X	X	X
Interstitial cell, adenoma				X									X				X				X				
Interstitial cell, adenoma, multiple									X									X							
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	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	A	I	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Malanoma benign								X																	
Trichoepithelioma								X																	
Subcutaneous tissue, fibrosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																		+							
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve						+																			
Spinal cord						+																			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 1,000 ppm

Number of Days on Study	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	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	1 7 1	1 4 2	1 4 3	1 4 4	1 4 8	1 4 9	1 5 2	1 5 3	1 2 2	1 2 4	1 2 5	1 2 6	1 2 7	1 2 8	1 2 9	1 3 0	1 3 1	1 5 7	1 5 8	1 5 9	1 7 5	1 7 6	1 7 7	1 7 8	1 7 9	Total Tissues/ Tumors
General Body System																										
Tissue NOS														+												6
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Thoracic, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Genital System																										
Coagulating gland											+															1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma															X											2
Adenoma, cystic																										1
Carcinoma													X													2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma, multiple	X		X	X	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					6
Interstitial cell, adenoma, multiple																										2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+			+	42
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	2
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	M	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	44
Integumentary System																										
Mammary gland	+	I	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma												X														1
Melanoma benign																										1
Trichoepithelioma																										1
Subcutaneous tissue, fibrosarcoma														X												1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 1,000 ppm

Number of Days on Study	3 9 2	4 3 9	4 7 1	5 3 8	5 4 8	5 8 3	6 4 1	6 4 4	6 6 8	6 7 3	6 9 4	6 9 6	7 0 3	7 1 7	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	1 6 2	1 6 3	1 7 2	1 6 4	1 3 2	1 8 0	1 5 0	1 2 3	1 7 3	1 5 6	1 6 8	1 6 7	1 5 1	1 6 6	1 3 3	1 3 4	1 3 5	1 7 4	1 5 4	1 5 5	1 6 0	1 6 1	1 6 5	1 6 9	1 7 0
Respiratory System																									
Larynx																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																				X					
Alveolar/bronchiolar carcinoma, multiple			X																						
Alveolar epithelium, carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	A	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland										+															
Carcinoma										X															
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		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 2-Methylimidazole: 1,000 ppm

Number of Days on Study	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	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	1 7 1	1 4 2	1 4 3	1 4 4	1 4 8	1 4 9	1 5 2	1 5 3	1 2 2	1 2 4	1 2 5	1 2 6	1 2 7	1 2 8	1 2 9	1 3 0	1 3 1	1 5 7	1 5 8	1 5 9	1 7 5	1 7 6	1 7 7	1 7 8	1 7 9	Total Tissues/ Tumors
Respiratory System																											
Larynx																					+					1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X																							X	2
Alveolar/bronchiolar carcinoma																										1
Alveolar/bronchiolar carcinoma, multiple																										1
Alveolar epithelium, carcinoma					X																					1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																											
Eye	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Zymbal's gland																	+									2
Carcinoma																	X									2
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X			X	X			X	X			X		X								X				21
Mesothelioma malignant																						X				2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 3,000 ppm

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 3,000 ppm

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 3,000 ppm

Number of Days on Study	24 29	39 51	49 91	56 12	57 24	59 38	59 88	60 25	61 88	68 73	68 89	68 99	68 99	70 22	70 26	72 29	72 99	72 99	72 99	72 99	72 99	72 99	72 99	72 99	72 99
Carcass ID Number	22 21	21 29	22 20	20 04	23 44	20 33	22 24	22 22	23 23	18 33	19 99	20 22	22 26	20 25	18 11	19 16	19 97	19 98	20 00	20 01	20 06	20 07	22 77	22 78	23 35
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma										X															
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma, multiple								X		X				X	X		X	X		X	X		X		
Interstitial cell, adenoma				X	X	X	X									X								X	X
Interstitial cell, adenoma, multiple									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	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	M	+	+	+	M	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	I
Fibroadenoma																			X						
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																X									
Keratoacanthoma						X				X		X													
Trichoepithelioma																									
Sebaceous gland, adenoma																									
Subcutaneous tissue, fibroma																X									
Subcutaneous tissue, fibrosarcoma																			X						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma			X																						
Cranium, osteosarcoma																									
Skeletal muscle					+											+									
Rhabdomyosarcoma																X									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant						X																			
Respiratory System																									
Larynx																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																	X								
Carcinoma, metastatic, preputial gland										X															
Osteosarcoma, metastatic, bone			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 3,000 ppm

Number of Days on Study	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	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	
Carcass ID Number	2 3 6	2 3 7	2 3 8	2 3 9	2 4 0	1 8 4	1 8 5	1 8 6	1 9 0	1 9 1	1 9 2	2 0 8	2 0 9	2 1 0	2 1 1	2 1 2	2 1 3	2 1 4	2 1 5	2 1 6	2 1 7	2 1 8	1 8 2	2 2 3	2 2 5	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma						X															X					3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma, multiple	X	X	X	X	X	X			X	X	X	X	X		X	X			X	X	X	X	X		X	28
Interstitial cell, adenoma																										7
Interstitial cell, adenoma, multiple							X	X										X								8
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+		+		+	+	+	+	+	+		+	+	+	+	+		+	+	+			+	+	+	38
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	2
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	42
Fibroadenoma																						X				2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma				X																						2
Keratoacanthoma								X																		4
Trichoepithelioma												X														1
Sebaceous gland, adenoma										X																1
Subcutaneous tissue, fibroma				X																						2
Subcutaneous tissue, fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Cranium, osteosarcoma																					X					1
Skeletal muscle																			+	+						4
Rhabdomyosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Respiratory System																										
Larynx																						+				1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Carcinoma, metastatic, preputial gland																										1
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 3,000 ppm

Number of Days on Study	2 4 9	3 9 5	4 9 1	5 6 2	5 7 4	5 9 8	5 9 8	6 0 5	6 1 8	6 8 7	6 8 9	6 8 9	6 8 9	6 8 9	7 0 2	7 0 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
Carcass ID Number	2 2 1	2 1 9	2 2 0	2 0 4	2 3 4	2 0 3	2 2 4	2 2 2	2 3 3	1 8 3	1 9 9	2 0 2	2 2 6	2 0 5	1 8 1	1 9 6	1 9 7	1 9 8	2 0 0	2 0 1	2 0 6	2 0 7	2 2 7	2 2 8	2 3 5
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X	X				X			X	X								X			
Lymphoma malignant																		X								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 2-Methylimidazole: 3,000 ppm

Number of Days on Study	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	
Carcass ID Number	23 6	23 7	23 8	23 9	24 0	18 4	18 5	18 6	19 0	19 1	19 2	20 8	20 9	21 0	21 1	21 2	21 3	21 4	21 5	21 6	21 7	21 8	18 2	22 3	22 5	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lacrimal gland													+													1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X		X																X			10
Lymphoma malignant																										1

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

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	8/50 (16%)	3/50 (6%)	6/50 (12%)	6/50 (12%)
Adjusted rate[b]	17.2%	6.5%	13.3%	13.6%
Terminal rate[c]	5/35 (14%)	3/40 (8%)	5/36 (14%)	4/35 (11%)
First incidence (days)	689	729 (T)	583	689
Poly-3 test[d]	P=0.526	P=0.102N	P=0.413N	P=0.428N
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	2.2%	6.7%	2.3%
Terminal rate	0/35 (0%)	1/40 (3%)	3/36 (8%)	1/35 (3%)
First incidence (days)	—[e]	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.483	P=0.499	P=0.112	P=0.490
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	8/50 (16%)	4/50 (8%)	9/50 (18%)	7/50 (14%)
Adjusted rate	17.2%	8.7%	20.0%	15.9%
Terminal rate	5/35 (14%)	4/40 (10%)	8/36 (22%)	5/35 (14%)
First incidence (days)	689	729 (T)	583	689
Poly-3 test	P=0.448	P=0.183N	P=0.469	P=0.544N
Liver: Hepatocellular Adenoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	0.0%	2.2%	6.7%	4.5%
Terminal rate	0/35 (0%)	1/40 (3%)	2/36 (6%)	1/35 (3%)
First incidence (days)	—	729 (T)	696	702
Poly-3 test	P=0.235	P=0.499	P=0.113	P=0.227
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	0.0%	2.2%	6.7%	6.8%
Terminal rate	0/35 (0%)	1/40 (3%)	2/36 (6%)	2/35 (6%)
First incidence (days)	—	729 (T)	696	702
Poly-3 test	P=0.095	P=0.499	P=0.113	P=0.110
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.3%	2.2%	6.6%	0.0%
Terminal rate	2/35 (6%)	1/40 (3%)	2/36 (6%)	0/35 (0%)
First incidence (days)	729 (T)	729 (T)	471	—
Poly-3 test	P=0.240N	P=0.502N	P=0.491	P=0.248N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	5/50 (10%)	1/50 (2%)
Adjusted rate	8.7%	6.5%	11.1%	2.3%
Terminal rate	4/35 (11%)	2/40 (5%)	4/36 (11%)	1/35 (3%)
First incidence (days)	729 (T)	572	471	729 (T)
Poly-3 test	P=0.180N	P=0.496N	P=0.488	P=0.194N
Pancreatic Islets: Adenoma				
Overall rate	0/50 (0%)	0/49 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	2.3%	6.8%
Terminal rate	0/35 (0%)	0/40 (0%)	1/36 (3%)	3/35 (9%)
First incidence (days)	—	—[f]	729 (T)	729 (T)
Poly-3 test	P=0.019		P=0.493	P=0.110

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

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Pancreatic Islets: Carcinoma				
Overall rate	0/50 (0%)	3/49 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	6.6%	0.0%	0.0%
Terminal rate	0/35 (0%)	3/40 (8%)	0/36 (0%)	0/35 (0%)
First incidence (days)	—	729 (T)	—	—
Poly-3 test	P=0.257N	P=0.116	—	—
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	0/50 (0%)	3/49 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	6.6%	2.3%	6.8%
Terminal rate	0/35 (0%)	3/40 (8%)	1/36 (3%)	3/35 (9%)
First incidence (days)	—	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.203	P=0.116	P=0.493	P=0.110
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	9/50 (18%)	6/50 (12%)	8/50 (16%)	9/50 (18%)
Adjusted rate	19.4%	13.1%	17.4%	20.4%
Terminal rate	7/35 (20%)	6/40 (15%)	5/36 (14%)	8/35 (23%)
First incidence (days)	694	729 (T)	392	689
Poly-3 test	P=0.363	P=0.294N	P=0.507N	P=0.556
Preputial Gland: Adenoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	2.2%	6.7%	0.0%
Terminal rate	0/35 (0%)	1/40 (3%)	1/36 (3%)	0/35 (0%)
First incidence (days)	—	729 (T)	673	—
Poly-3 test	P=0.524N	P=0.499	P=0.113	—
Preputial Gland: Carcinoma				
Overall rate	0/50 (0%)	2/50 (4%)	2/50 (4%)	3/50 (6%)
Adjusted rate	0.0%	4.3%	4.5%	6.8%
Terminal rate	0/35 (0%)	1/40 (3%)	1/36 (3%)	2/35 (6%)
First incidence (days)	—	548	694	687
Poly-3 test	P=0.142	P=0.239	P=0.230	P=0.111
Preputial Gland: Adenoma or Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	5/50 (10%)	3/50 (6%)
Adjusted rate	0.0%	6.5%	11.1%	6.8%
Terminal rate	0/35 (0%)	2/40 (5%)	2/36 (6%)	2/35 (6%)
First incidence (days)	—	548	673	687
Poly-3 test	P=0.259	P=0.120	P=0.028	P=0.111
Skin: Keratoacanthoma				
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)	4/50 (8%)
Adjusted rate	2.2%	4.4%	2.3%	9.0%
Terminal rate	1/35 (3%)	2/40 (5%)	1/36 (3%)	1/35 (3%)
First incidence (days)	729 (T)	729 (T)	729 (T)	598
Poly-3 test	P=0.102	P=0.498	P=0.753	P=0.169
Skin: Trichoepithelioma or Basal Cell Carcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.2%	0.0%	2.2%	6.8%
Terminal rate	1/35 (3%)	0/40 (0%)	0/36 (0%)	3/35 (9%)
First incidence (days)	729 (T)	—	644	729 (T)
Poly-3 test	P=0.073	P=0.501N	P=0.754	P=0.288

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

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Skin: Keratoacanthoma, Trichoepithelioma, or Basal Cell Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	2/50 (4%)	7/50 (14%)
Adjusted rate	4.3%	4.4%	4.5%	15.7%
Terminal rate	2/35 (6%)	2/40 (5%)	1/36 (3%)	4/35 (11%)
First incidence (days)	729 (T)	729 (T)	644	598
Poly-3 test	P=0.015	P=0.692	P=0.683	P=0.071
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	2/50 (4%)	4/50 (8%)	0/50 (0%)	2/50 (4%)
Adjusted rate	4.3%	8.7%	0.0%	4.6%
Terminal rate	1/35 (3%)	4/40 (10%)	0/36 (0%)	2/35 (6%)
First incidence (days)	668	729 (T)	—	729 (T)
Poly-3 test	P=0.467N	P=0.333	P=0.246N	P=0.674
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.3%	8.7%	2.3%	6.8%
Terminal rate	1/35 (3%)	4/40 (10%)	1/36 (3%)	3/35 (9%)
First incidence (days)	668	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.536	P=0.333	P=0.514N	P=0.475
Testes: Adenoma				
Overall rate	48/50 (96%)	45/50 (90%)	42/50 (84%)	43/50 (86%)
Adjusted rate	98.1%	93.2%	90.4%	90.5%
Terminal rate	35/35 (100%)	38/40 (95%)	34/36 (94%)	31/35 (89%)
First incidence (days)	572	548	538	562
Poly-3 test	P=0.144N	P=0.225N	P=0.090N	P=0.109N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	1/48 (2%)	0/46 (0%)	1/43 (2%)	3/50 (6%)
Adjusted rate	2.2%	0.0%	2.5%	6.8%
Terminal rate	1/35 (3%)	0/39 (0%)	1/35 (3%)	3/35 (9%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P=0.083	P=0.507N	P=0.736	P=0.296
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/48 (2%)	2/46 (4%)	1/43 (2%)	5/50 (10%)
Adjusted rate	2.2%	4.6%	2.5%	11.2%
Terminal rate	1/35 (3%)	1/39 (3%)	1/35 (3%)	4/35 (11%)
First incidence (days)	729 (T)	693	729 (T)	395
Poly-3 test	P=0.046	P=0.489	P=0.736	P=0.099
Thyroid Gland (C-cell): Adenoma				
Overall rate	9/48 (19%)	5/46 (11%)	9/43 (21%)	7/50 (14%)
Adjusted rate	20.0%	11.5%	22.3%	15.9%
Terminal rate	9/35 (26%)	5/39 (13%)	9/35 (26%)	5/35 (14%)
First incidence (days)	729 (T)	729 (T)	729 (T)	689
Poly-3 test	P=0.521N	P=0.211N	P=0.499	P=0.409N
Thyroid Gland (C-cell): Carcinoma				
Overall rate	1/48 (2%)	0/46 (0%)	0/43 (0%)	3/50 (6%)
Adjusted rate	2.2%	0.0%	0.0%	6.8%
Terminal rate	1/35 (3%)	0/39 (0%)	0/35 (0%)	2/35 (6%)
First incidence (days)	729 (T)	—	—	689
Poly-3 test	P=0.059	P=0.507N	P=0.522N	P=0.297

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

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	10/48 (21%)	5/46 (11%)	9/43 (21%)	10/50 (20%)
Adjusted rate	22.2%	11.5%	22.3%	22.6%
Terminal rate	10/35 (29%)	5/39 (13%)	9/35 (26%)	7/35 (20%)
First incidence (days)	729 (T)	729 (T)	729 (T)	689
Poly-3 test	P=0.318	P=0.144N	P=0.596	P=0.584
All Organs: Malignant Mesothelioma				
Overall rate	2/50 (4%)	4/50 (8%)	2/50 (4%)	0/50 (0%)
Adjusted rate	4.3%	8.6%	4.5%	0.0%
Terminal rate	1/35 (3%)	2/40 (5%)	2/36 (6%)	0/35 (0%)
First incidence (days)	643	608	729 (T)	—
Poly-3 test	P=0.097N	P=0.339	P=0.678	P=0.250N
All Organs: Mononuclear Cell Leukemia				
Overall rate	15/50 (30%)	14/50 (28%)	21/50 (42%)	10/50 (20%)
Adjusted rate	30.8%	29.1%	44.1%	21.9%
Terminal rate	4/35 (11%)	8/40 (20%)	11/36 (31%)	4/35 (11%)
First incidence (days)	572	521	439	574
Poly-3 test	P=0.193N	P=0.516N	P=0.127	P=0.228N
All Organs: Benign Neoplasms				
Overall rate	49/50 (98%)	46/50 (92%)	47/50 (94%)	45/50 (90%)
Adjusted rate	99.8%	95.3%	96.9%	94.7%
Terminal rate	35/35 (100%)	39/40 (98%)	35/36 (97%)	33/35 (94%)
First incidence (days)	572	548	392	562
Poly-3 test	P=0.220N	P=0.165N	P=0.347N	P=0.154N
All Organs: Malignant Neoplasms				
Overall rate	22/50 (44%)	26/50 (52%)	30/50 (60%)	25/50 (50%)
Adjusted rate	44.3%	52.0%	61.8%	52.6%
Terminal rate	9/35 (26%)	16/40 (40%)	18/36 (50%)	15/35 (43%)
First incidence (days)	315	507	439	395
Poly-3 test	P=0.335	P=0.287	P=0.062	P=0.270
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	49/50 (98%)	49/50 (98%)	48/50 (96%)
Adjusted rate	100.0%	98.0%	98.0%	97.9%
Terminal rate	35/35 (100%)	39/40 (98%)	35/36 (97%)	34/35 (97%)
First incidence (days)	315	507	392	395
Poly-3 test	P=0.463N	P=0.500N	P=0.500N	P=0.480N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal medulla, liver, lung, pancreatic islets, pituitary gland, preputial 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 A4a
Historical Incidence of Thyroid Gland (Follicular Cell) Neoplasms in Control Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Feed Studies			
trans-Cinnamaldehyde	1/50	1/50	2/50
Citral	3/50	1/50	4/50
p,p'-Dichlorodiphenyl sulfone	0/50	0/50	0/50
2-Methylimidazole	1/48	0/48	1/48
o-Nitrotoluene	0/59	1/59	1/59
p-Nitrotoluene	0/50	0/50	0/50
Overall Historical Incidence: Feed Studies			
Total (%)	5/307 (1.6%)	3/307 (1.0%)	8/307 (2.6%)
Mean ± standard deviation	1.7% ± 2.3%	1.0% ± 1.1%	2.6% ± 3.0%
Range	0%-6%	0%-2%	0%-8%
Overall Historical Incidence			
Total (%)	9/1,043 (0.9%)	11/1,043 (1.1%)	20/1,043 (1.9%)
Mean ± standard deviation	0.8% ± 1.1%	1.0% ± 1.3%	1.7% ± 1.5%
Range	0%-3%	0%-4%	0%-4%

[a] Data as of March 3, 2003

TABLE A4b
Historical Incidence of Liver Neoplasms in Control Male F344/N Rats[a]

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence: Feed Studies			
trans-Cinnamaldehyde	1/50	0/50	1/50
Citral	0/50	0/50	0/50
p,p'-Dichlorodiphenyl sulfone	0/50	2/50	2/50
2-Methylimidazole	0/50	0/50	0/50
o-Nitrotoluene	2/60	1/60	3/60
p-Nitrotoluene	0/50	1/50	1/50
Overall Historical Incidence: Feed Studies			
Total (%)	3/310 (1.0%)	4/310 (1.3%)	7/310 (2.3%)
Mean ± standard deviation	0.9% ± 1.4%	1.3% ± 1.6%	2.2% ± 2.0%
Range	0%-3%	0%-4%	0%-5%
Overall Historical Incidence			
Total (%)	10/1,059 (0.9%)	5/1,059 (0.5%)	15/1,059 (1.4%)
Mean ± standard deviation	0.9% ± 1.6%	0.5% ± 1.1%	1.5% ± 2.0%
Range	0%-6%	0%-4%	0%-6%

[a] Data as of March 3, 2003

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
6-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	13	6	7	13
Natural deaths	2	4	7	2
Survivors				
Died last week of study	1	1	1	
Terminal sacrifice	34	39	35	35
Animals examined microscopically	60	60	60	60
6-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	2 (20%)		1 (10%)	
Hyperplasia, focal, mast cell			1 (10%)	
Infiltration cellular, focal, polymorphonuclear			1 (10%)	
Inflammation, granulomatous	9 (90%)	7 (70%)	10 (100%)	10 (100%)
Mesentery			(1)	
Fat, necrosis			1 (100%)	
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule				2 (20%)
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, cyst	1 (10%)	2 (20%)		1 (10%)
Pars distalis, mitotic alteration		1 (10%)	1 (10%)	
Pars distalis, vacuolization cytoplasmic, focal				1 (10%)
Pars intermedia, cyst				2 (20%)
Thyroid gland	(10)	(10)	(10)	(10)
Follicle, mineralization, focal	1 (10%)	4 (40%)	9 (90%)	9 (90%)
Follicular cell, hyperplasia		7 (70%)	10 (100%)	10 (100%)
Hematopoietic System				
Lymph node		(1)	(1)	(1)
Mediastinal, hemorrhage		1 (100%)		1 (100%)
Pancreatic, hemorrhage			1 (100%)	
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Hemorrhage				1 (10%)
Spleen	(10)	(10)	(10)	(10)
Accessory spleen		2 (20%)		
Thymus	(10)	(10)	(10)	(10)
Hemorrhage		1 (10%)	1 (10%)	
Special Senses System				
Harderian gland	(10)	(10)	(10)	(10)
Inflammation, focal, granulomatous	1 (10%)			
Necrosis, focal	1 (10%)			

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

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Systems Examined at 6 Months with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Genital System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Urinary System				
2-Year Study				
Alimentary System				
Intestine large, rectum	(49)	(48)	(48)	(50)
Angiectasis, focal			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis, focal			1 (2%)	
Basophilic focus	23 (46%)	23 (46%)	8 (16%)	2 (4%)
Cholangiofibrosis	1 (2%)			1 (2%)
Congestion			2 (4%)	1 (2%)
Degeneration, cystic, focal	4 (8%)	3 (6%)	2 (4%)	7 (14%)
Eosinophilic focus	3 (6%)		6 (12%)	1 (2%)
Fibrosis, focal			1 (2%)	
Hematopoietic cell proliferation				2 (4%)
Hepatodiaphragmatic nodule	6 (12%)	5 (10%)	4 (8%)	9 (18%)
Hyperplasia, focal, lymphoid	1 (2%)		2 (4%)	1 (2%)
Infiltration cellular, mixed cell	25 (50%)	30 (60%)	27 (54%)	36 (72%)
Inflammation, granulomatous	12 (24%)	11 (22%)	4 (8%)	11 (22%)
Mixed cell focus	14 (28%)	16 (32%)	23 (46%)	26 (52%)
Necrosis, focal	2 (4%)		2 (4%)	1 (2%)
Pigmentation, focal			2 (4%)	
Bile duct, cyst				1 (2%)
Bile duct, hyperplasia	49 (98%)	49 (98%)	47 (94%)	49 (98%)
Centrilobular, congestion			1 (2%)	
Hepatocyte, necrosis, focal	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Hepatocyte, vacuolization cytoplasmic, diffuse		1 (2%)		1 (2%)
Hepatocyte, vacuolization cytoplasmic, focal	23 (46%)	30 (60%)	26 (52%)	23 (46%)
Hepatocyte, centrilobular, necrosis		2 (4%)	2 (4%)	
Hepatocyte, centrilobular, vacuolization cytoplasmic	5 (10%)	4 (8%)		3 (6%)
Hepatocyte, midzonal, vacuolization cytoplasmic	1 (2%)	1 (2%)	2 (4%)	
Oval cell, hyperplasia				1 (2%)
Serosa, fibrosis, focal		2 (4%)		
Mesentery	(26)	(46)	(35)	(20)
Fibrosis, focal				1 (5%)
Hemorrhage				2 (10%)
Necrosis, focal				1 (5%)
Artery, inflammation, chronic				1 (5%)
Fat, necrosis	1 (4%)	1 (2%)	2 (6%)	
Fat, necrosis, focal	8 (31%)	10 (22%)	16 (46%)	10 (50%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Pancreas	(49)	(49)	(50)	(50)
Atrophy, diffuse			1 (2%)	
Atrophy, focal			1 (2%)	1 (2%)
Fibrosis, focal				1 (2%)
Acinus, atrophy, diffuse		1 (2%)		1 (2%)
Acinus, atrophy, focal	22 (45%)	27 (55%)	20 (40%)	36 (72%)
Acinus, hyperplasia, focal			1 (2%)	
Duct, cyst	1 (2%)			
Duct, cyst, focal		1 (2%)		
Duct, cyst, focal, multiple	6 (12%)	2 (4%)	5 (10%)	12 (24%)
Duct, hyperplasia, cystic		1 (2%)	1 (2%)	
Duct, hyperplasia, cystic, focal		2 (4%)	3 (6%)	
Duct, hyperplasia, focal		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Atrophy, focal				1 (2%)
Inflammation, chronic				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(49)
Diverticulum	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Edema	1 (2%)			
Erosion		1 (2%)		1 (2%)
Inflammation, focal	1 (2%)			1 (2%)
Ulcer	2 (4%)	1 (2%)	1 (2%)	4 (8%)
Epithelium, hyperplasia	2 (4%)	3 (6%)		6 (12%)
Epithelium, hyperplasia, focal				1 (2%)
Serosa, foreign body				1 (2%)
Serosa, inflammation, chronic, focal				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(49)
Erosion	4 (8%)	1 (2%)	1 (2%)	
Erosion, focal	1 (2%)		1 (2%)	
Inflammation, focal	1 (2%)			
Ulcer	2 (4%)			
Epithelium, necrosis, focal				1 (2%)
Glands, hyperplasia, cystic, focal		1 (2%)		
Glands, hyperplasia, focal	1 (2%)			
Tongue	(1)	(2)		(1)
Epithelium, hyperplasia, focal				1 (100%)
Tooth	(1)	(1)	(1)	(2)
Malformation		1 (100%)		
Peridontal tissue, inflammation	1 (100%)			
Peridontal tissue, inflammation, chronic				1 (50%)
Peridontal tissue, inflammation, focal, suppurative			1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	3 (6%)	5 (10%)	10 (20%)	5 (10%)
Cardiomyopathy, focal			2 (4%)	1 (2%)
Congestion			1 (2%)	
Fibrosis				1 (2%)
Infiltration cellular, mixed cell		1 (2%)		
Thrombosis	1 (2%)	1 (2%)		
Endocardium, myocardium, fibrosis, focal	1 (2%)			

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
2-Year Study (continued)				
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)	4 (8%)	9 (18%)	5 (10%)
Cytoplasmic alteration, focal	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Degeneration, cystic, focal			1 (2%)	
Hemorrhage	2 (4%)			
Vacuolization cytoplasmic, focal	6 (12%)	9 (18%)	10 (20%)	10 (20%)
Adrenal medulla	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Hyperplasia	1 (2%)	1 (2%)		1 (2%)
Hyperplasia, focal	7 (14%)	5 (10%)	3 (6%)	7 (14%)
Infiltration cellular, lymphoid		1 (2%)		
Vacuolization cytoplasmic, focal		1 (2%)		
Islets, pancreatic	(50)	(49)	(50)	(50)
Atrophy		1 (2%)		
Hyperplasia, focal		2 (4%)	1 (2%)	1 (2%)
Parathyroid gland	(47)	(49)	(48)	(49)
Cyst		1 (2%)		
Hyperplasia				1 (2%)
Hyperplasia, focal	1 (2%)		1 (2%)	
Bilateral, hyperplasia, focal	1 (2%)			
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)		3 (6%)	
Pars distalis, angiectasis	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Pars distalis, cyst	1 (2%)	4 (8%)	2 (4%)	3 (6%)
Pars distalis, cytoplasmic alteration, focal	1 (2%)	4 (8%)	2 (4%)	5 (10%)
Pars distalis, degeneration, cystic, focal	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Pars distalis, hyperplasia, focal	2 (4%)	1 (2%)		2 (4%)
Pars distalis, inflammation, chronic, focal, granulomatous			1 (2%)	
Pars distalis, pars intermedia, angiectasis		1 (2%)		
Pars intermedia, cyst		2 (4%)		1 (2%)
Pars intermedia, hemorrhage			1 (2%)	
Pars intermedia, hyperplasia, focal		1 (2%)		
Pars nervosa, infiltration cellular, focal, mixed cell			1 (2%)	
Thyroid gland	(48)	(46)	(43)	(50)
Cyst		1 (2%)		
C-cell, hyperplasia	41 (85%)	43 (93%)	38 (88%)	34 (68%)
Follicle, cyst	1 (2%)			1 (2%)
Follicle, mineralization, focal	48 (100%)	45 (98%)	43 (100%)	49 (98%)
Follicular cell, atrophy, focal	1 (2%)			
Follicular cell, hyperplasia		17 (37%)	37 (86%)	43 (86%)
Follicular cell, hyperplasia, cystic, focal	1 (2%)			1 (2%)
General Body System				
Tissue NOS	(7)	(2)	(6)	(4)
Mediastinum, hemorrhage				1 (25%)
Thoracic, hemorrhage				1 (25%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
2-Year Study (continued)				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		1 (2%)
Granuloma sperm		1 (2%)	1 (2%)	1 (2%)
Inflammation, chronic		1 (2%)	1 (2%)	
Preputial gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Degeneration, cystic				2 (4%)
Hyperplasia			1 (2%)	
Hyperplasia, cystic	2 (4%)		2 (4%)	1 (2%)
Hyperplasia, focal	1 (2%)			
Inflammation, chronic	4 (8%)	10 (20%)	10 (20%)	11 (22%)
Inflammation, chronic, focal		1 (2%)		
Inflammation, suppurative		1 (2%)	1 (2%)	2 (4%)
Prostate	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Hyperplasia, focal		1 (2%)		
Inflammation, chronic	15 (30%)	10 (20%)	17 (34%)	19 (38%)
Epithelium, degeneration, focal, mucoid	1 (2%)			
Epithelium, hyperplasia, focal	4 (8%)	2 (4%)		5 (10%)
Testes	(50)	(50)	(50)	(50)
Atrophy	5 (10%)	4 (8%)	13 (26%)	13 (26%)
Mineralization, focal	1 (2%)			
Bilateral, atrophy	2 (4%)	3 (6%)	2 (4%)	
Bilateral, interstitial cell, hyperplasia, focal				2 (4%)
Germinal epithelium, atrophy	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Interstitial cell, hyperplasia		2 (4%)	1 (2%)	1 (2%)
Interstitial cell, hyperplasia, focal	1 (2%)	4 (8%)	4 (8%)	9 (18%)
Hematopoietic System				
Bone marrow	(50)	(50)	(47)	(50)
Fibrosis			1 (2%)	
Hyperplasia		1 (2%)		
Hyperplasia, focal, histiocytic		1 (2%)	1 (2%)	
Myeloid cell, hyperplasia		1 (2%)		5 (10%)
Lymph node	(39)	(49)	(42)	(38)
Hemorrhage				1 (3%)
Hyperplasia, lymphoid			1 (2%)	
Mediastinal, angiectasis		1 (2%)		
Mediastinal, congestion			1 (2%)	
Mediastinal, ectasia	1 (3%)		3 (7%)	1 (3%)
Mediastinal, hemorrhage	1 (3%)	1 (2%)	5 (12%)	1 (3%)
Mediastinal, hyperplasia, histiocytic	2 (5%)	1 (2%)		4 (11%)
Mediastinal, hyperplasia, lymphoid	1 (3%)	1 (2%)	1 (2%)	1 (3%)
Mediastinal, pigmentation		1 (2%)		1 (3%)
Pancreatic, angiectasis	1 (3%)			1 (3%)
Pancreatic, ectasia	1 (3%)	3 (6%)	3 (7%)	2 (5%)
Pancreatic, hemorrhage	1 (3%)		1 (2%)	4 (11%)
Pancreatic, hyperplasia, focal, histiocytic	1 (3%)			
Pancreatic, hyperplasia, histiocytic	13 (33%)	12 (24%)	15 (36%)	19 (50%)
Pancreatic, hyperplasia, lymphoid			1 (2%)	1 (3%)
Pancreatic, pigmentation	1 (3%)			1 (3%)
Lymph node, mandibular	(2)	(1)	(2)	(2)
Hyperplasia, plasma cell			1 (50%)	

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node, mesenteric	(47)	(50)	(50)	(50)
Ectasia		2 (4%)		2 (4%)
Hemorrhage			1 (2%)	1 (2%)
Hyperplasia, histiocytic	1 (2%)	2 (4%)	1 (2%)	4 (8%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)		
Spleen	(49)	(49)	(50)	(50)
Angiectasis, focal	1 (2%)		1 (2%)	
Congestion				1 (2%)
Cyst		1 (2%)		
Fibrosis, focal	2 (4%)		2 (4%)	1 (2%)
Hematopoietic cell proliferation	4 (8%)	4 (8%)	1 (2%)	6 (12%)
Hemorrhage			1 (2%)	
Hemorrhage, focal				1 (2%)
Hyperplasia, lymphoid		1 (2%)	2 (4%)	
Infarct	1 (2%)	1 (2%)		
Inflammation, granulomatous			2 (4%)	3 (6%)
Necrosis, focal	1 (2%)		1 (2%)	1 (2%)
Pigmentation			1 (2%)	
Capsule, fibrosis, focal	1 (2%)			
Thymus	(48)	(48)	(44)	(49)
Atrophy	1 (2%)	1 (2%)		
Cyst, multiple	1 (2%)			
Hemorrhage	1 (2%)			1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	1 (2%)	
Epithelial cell, cyst, multiple		1 (2%)		
Epithelial cell, hyperplasia	1 (2%)			
Integumentary System				
Mammary gland	(48)	(47)	(45)	(42)
Dilatation	7 (15%)	1 (2%)	3 (7%)	2 (5%)
Ectasia			1 (2%)	
Hyperplasia	2 (4%)			
Hyperplasia, cystic				1 (2%)
Epithelium, pigmentation		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Angiectasis, focal		1 (2%)		
Cyst epithelial inclusion			1 (2%)	1 (2%)
Fibrosis, focal	1 (2%)			
Hemorrhage, focal	1 (2%)			1 (2%)
Inflammation, chronic, focal	1 (2%)		1 (2%)	1 (2%)
Necrosis, focal				1 (2%)
Pinna, necrosis, focal	1 (2%)			
Subcutaneous tissue, angiectasis, focal				1 (2%)
Subcutaneous tissue, cyst				1 (2%)
Subcutaneous tissue, foreign body				1 (2%)
Subcutaneous tissue, hemorrhage, focal				1 (2%)
Musculoskeletal System				
Skeletal muscle	(2)	(1)	(1)	(4)
Fibrosis, focal				1 (25%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
2-Year Study (continued)				
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression, focal	4 (8%)	2 (4%)	4 (8%)	1 (2%)
Hemorrhage, focal	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Necrosis, focal	1 (2%)	1 (2%)		
Spinal cord	(1)		(1)	
Hemorrhage, focal			1 (100%)	
Mineralization, focal			1 (100%)	
Necrosis, focal			1 (100%)	
Respiratory System				
Larynx			(1)	(1)
Glands, hyperplasia, focal				1 (100%)
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)		1 (2%)	1 (2%)
Hemorrhage, focal	1 (2%)		1 (2%)	1 (2%)
Hyperplasia, focal, histiocytic	4 (8%)	1 (2%)		
Hyperplasia, histiocytic	3 (6%)	3 (6%)	2 (4%)	2 (4%)
Infiltration cellular, focal, mixed cell				1 (2%)
Infiltration cellular, mixed cell	2 (4%)	1 (2%)	1 (2%)	
Inflammation, focal				1 (2%)
Metaplasia, focal, osseous		2 (4%)	1 (2%)	2 (4%)
Metaplasia, osseous				2 (4%)
Alveolar epithelium, hyperplasia, focal	2 (4%)	9 (18%)	6 (12%)	5 (10%)
Alveolus, foreign body, focal				1 (2%)
Interstitium, edema			1 (2%)	1 (2%)
Nose	(50)	(50)	(50)	(50)
Foreign body	1 (2%)			
Inflammation, suppurative	2 (4%)		1 (2%)	
Nasolacrimal duct, inflammation		2 (4%)	1 (2%)	2 (4%)
Nasolacrimal duct, inflammation, chronic		1 (2%)		
Nasolacrimal duct, inflammation, suppurative		3 (6%)		
Sinus, foreign body		1 (2%)		
Sinus, inflammation, suppurative		1 (2%)		
Special Senses System				
Eye	(49)	(50)	(46)	(50)
Atrophy		1 (2%)		
Cataract	1 (2%)		1 (2%)	
Synechia			1 (2%)	
Cornea, inflammation, focal		1 (2%)		
Cornea, necrosis, focal		1 (2%)		
Iris, hyperplasia	1 (2%)			
Lens, cataract		1 (2%)		
Retina, degeneration			1 (2%)	
Retrobulbar, inflammation, focal		1 (2%)		
Harderian gland	(50)	(50)	(49)	(50)
Hyperplasia, focal		1 (2%)		2 (4%)
Hyperplasia, focal, histiocytic				1 (2%)
Hyperplasia, histiocytic			1 (2%)	
Inflammation, chronic, focal	1 (2%)		2 (4%)	3 (6%)
Inflammation, focal, granulomatous				1 (2%)
Epithelium, hyperplasia, focal	3 (6%)			1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
2-Year Study (continued)				
Urinary System				
Kidney	(49)	(49)	(49)	(50)
Accumulation, hyaline droplet	1 (2%)			
Atrophy, focal				1 (2%)
Congestion	1 (2%)	1 (2%)		
Cyst		2 (4%)		2 (4%)
Fibrosis, focal				1 (2%)
Infarct		1 (2%)		
Inflammation	1 (2%)			
Nephropathy	43 (88%)	43 (88%)	41 (84%)	46 (92%)
Pelvis, inflammation, chronic			1 (2%)	
Pelvis, transitional epithelium, hyperplasia			1 (2%)	
Renal tubule, accumulation, hyaline droplet	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Renal tubule, necrosis, focal	1 (2%)			
Renal tubule, pigmentation	5 (10%)	3 (6%)	2 (4%)	3 (6%)
Urinary bladder	(50)	(50)	(49)	(50)
Hemorrhage			1 (2%)	

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

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
6-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	6	8	12	7
Natural deaths	4	3	10	1
Survivors				
Died last week of study				1
Terminal sacrifice	40	39	28	41
Animals examined microscopically	60	60	60	60
6-Month Interim Evaluation				
Endocrine System				
Thyroid gland	(10)	(10)	(10)	(10)
Follicular cell adenoma				2 (20%)
Systems Examined at 6 Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Intestine large, colon	(48)	(49)	(48)	(50)
Intestine large, rectum	(47)	(48)	(50)	(49)
Intestine large, cecum	(48)	(47)	(44)	(50)
Intestine small, duodenum	(48)	(49)	(49)	(50)
Intestine small, jejunum	(47)	(46)	(44)	(49)
Intestine small, ileum	(48)	(46)	(44)	(50)
Liver	(50)	(49)	(50)	(50)
Cholangiocarcinoma	1 (2%)			1 (2%)
Hepatocellular adenoma	1 (2%)		2 (4%)	3 (6%)
Hepatocellular adenoma, multiple				1 (2%)
Mesentery	(8)	(10)	(12)	(9)
Nerve, schwannoma malignant				1 (11%)
Pancreas	(50)	(49)	(49)	(50)
Salivary glands	(50)	(49)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)	
Stomach, glandular	(49)	(49)	(48)	(50)
Tongue	(3)		(1)	
Liposarcoma	1 (33%)			
Squamous cell papilloma			1 (100%)	

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Cardiovascular System				
Heart	(50)	(49)	(50)	(50)
Squamous cell carcinoma, metastatic, skin			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Adenoma	5 (10%)			
Adrenal medulla	(49)	(49)	(50)	(50)
Pheochromocytoma malignant			1 (2%)	
Pheochromocytoma benign		2 (4%)		
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma			1 (2%)	1 (2%)
Carcinoma	2 (4%)			
Pituitary gland	(50)	(48)	(50)	(50)
Pars distalis, adenoma	23 (46%)	24 (50%)	21 (42%)	13 (26%)
Pars distalis, carcinoma	2 (4%)	1 (2%)	3 (6%)	
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(49)	(48)	(42)	(48)
Bilateral, C-cell, adenoma	1 (2%)			
Bilateral, C-cell, adenoma, multiple	1 (2%)			
Bilateral, follicular cell, adenoma				1 (2%)
Bilateral, follicular cell, carcinoma				1 (2%)
C-cell, adenoma	7 (14%)	8 (17%)	8 (19%)	3 (6%)
C-cell, adenoma, multiple		1 (2%)		
C-cell, carcinoma		1 (2%)		1 (2%)
Follicular cell, adenoma				4 (8%)
Follicular cell, carcinoma	1 (2%)	1 (2%)	1 (2%)	6 (13%)
General Body System				
Tissue NOS	(3)		(4)	(2)
Mediastinum, squamous cell carcinoma, metastatic, skin			1 (25%)	
Thoracic, fibrosarcoma	1 (33%)			
Thoracic, rhabdomyosarcoma			1 (25%)	
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Adenoma	5 (10%)	4 (8%)	5 (10%)	
Carcinoma	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Squamous cell papilloma	1 (2%)			
Bilateral, adenoma			1 (2%)	
Bilateral, carcinoma, multiple		1 (2%)		
Ovary	(50)	(49)	(50)	(50)
Granulosa cell tumor benign				1 (2%)
Granulosa-theca tumor benign			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Endometrium, polyp stromal	7 (14%)	6 (12%)	7 (14%)	3 (6%)
Endometrium, polyp stromal, multiple	1 (2%)			
Vagina	(2)		(2)	

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Lymph node	(32)	(31)	(34)	(41)
Lymph node, mesenteric	(49)	(49)	(49)	(50)
Spleen	(50)	(49)	(48)	(50)
Thymus	(48)	(45)	(49)	(49)
Thymoma malignant			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	4 (8%)	1 (2%)	3 (6%)	1 (2%)
Fibroadenoma	24 (48%)	18 (36%)	18 (36%)	5 (10%)
Fibroadenoma, multiple	2 (4%)	5 (10%)	6 (12%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)			
Basal cell carcinoma	1 (2%)			
Keratoacanthoma				1 (2%)
Squamous cell carcinoma, multiple			1 (2%)	
Subcutaneous tissue, fibroma	1 (2%)		2 (4%)	2 (4%)
Musculoskeletal System				
Skeletal muscle	(1)	(1)	(2)	
Rhabdomyosarcoma		1 (100%)	1 (50%)	
Nervous System				
Brain	(50)	(49)	(50)	(50)
Glioma malignant, mixed cell				1 (2%)
Spinal cord	(3)	(1)	(4)	
Respiratory System				
Lung	(50)	(49)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)	1 (2%)	1 (2%)
Rhabdomyosarcoma, metastatic, tissue NOS			1 (2%)	
Squamous cell carcinoma, metastatic, skin			1 (2%)	
Special Senses System				
Eye	(50)	(49)	(49)	(50)
Harderian gland	(50)	(49)	(50)	(50)
Urinary System				
Kidney	(50)	(48)	(47)	(50)
Sarcoma				1 (2%)
Squamous cell carcinoma, metastatic, skin			1 (2%)	
Urinary bladder	(50)	(49)	(49)	(50)
Transitional epithelium, papilloma	1 (2%)	1 (2%)		

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	6 (12%)	4 (8%)	9 (18%)	10 (20%)
Neoplasm Summary				
Total animals with primary neoplasms[c]				
6-Month interim evaluation				2
2-Year study	45	44	48	38
Total primary neoplasms				
6-Month interim evaluation				2
2-Year study	103	82	97	64
Total animals with benign neoplasms				
6-Month interim evaluation				2
2-Year study	43	43	40	27
Total benign neoplasms				
6-Month interim evaluation				2
2-Year study	81	71	75	40
Total animals with malignant neoplasms				
2-Year study	19	10	21	20
Total malignant neoplasms				
2-Year study	22	11	22	24
Total animals with metastatic neoplasms				
2-Year study			2	
Total metastatic neoplasms				
2-Year study			5	

[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 2-Methylimidazole: 0 ppm

Number of Days on Study	3 2 8	5 8 3	5 8 8	6 0 2	6 0 5	6 1 6	6 5 3	6 5 8	6 7 1	7 0 3	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	2 6 7	2 4 2	2 5 7	2 9 0	2 6 9	2 9 7	2 7 5	2 6 8	2 7 4	2 9 8	2 5 6	2 5 8	2 5 9	2 6 0	2 9 6	2 9 9	3 0 0	2 6 6	2 7 0	2 7 1	2 7 2	2 7 3	2 8 6	2 8 7	2 8 8
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	A	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																								
Hepatocellular adenoma																								
Mesentery		+							+	+	+		+									+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue			+			+															+			
Liposarcoma			X																					
Tooth						+																		
Cardiovascular System																								
Blood vessel			+																					
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X			X					X										X	X		
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma									X															
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma								X	X		X	X	X	X	X		X		X	X			X	X
Pars distalis, carcinoma				X			X																	
Thyroid gland	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																								
Bilateral, C-cell, adenoma, multiple													X											
C-cell, adenoma				X							X										X			
Follicular cell, carcinoma		X																						
General Body System																								
Tissue NOS	+					+																		
Thoracic, fibrosarcoma	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 2-Methylimidazole: 0 ppm

Number of Days on Study	735	735	735	735	735	735	736	736	736	736	736	737	737	737	737	737	737	737	737	737	737	737	737	737	737	
Carcass ID Number	28 89	29 91	29 92	29 93	29 94	29 95	27 76	27 77	27 78	27 79	28 80	24 41	24 43	24 44	24 45	24 46	24 47	24 48	24 49	25 00	28 01	28 02	28 03	28 04	28 05	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma										X																1
Hepatocellular adenoma			X																							1
Mesentery						+													+							8
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue																										3
Liposarcoma																										1
Tooth																										1
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal Cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										5
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma														X												2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X			X					X		X				X	X				X	X	X		X	X	23
Pars distalis, carcinoma																										2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Bilateral, C-cell, adenoma						X																				1
Bilateral, C-cell, adenoma, multiple																										1
C-cell, adenoma		X										X		X								X				7
Follicular cell, carcinoma																										1
General Body System																										
Tissue NOS																			+							3
Thoracic, fibrosarcoma																										1

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

Number of Days on Study	3 2 8	5 8 3	5 8 8	6 0 2	6 0 5	6 1 6	6 5 3	6 5 8	6 7 1	7 0 3	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	2 6 7	2 4 2	2 5 7	2 9 0	2 6 9	2 9 7	2 7 5	2 6 8	2 7 4	2 9 8	2 5 6	2 5 8	2 5 9	2 6 0	2 9 6	2 9 9	3 0 0	2 6 6	2 7 0	2 7 1	2 7 2	2 7 3	2 8 6	2 8 7	2 8 8
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X	X												X	
Carcinoma														X											
Squamous cell papilloma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal																									
Endometrium, polyp stromal, multiple																					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
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma					X																				
Fibroadenoma				X	X		X	X	X			X		X					X		X	X		X	X
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma											X														
Basal cell carcinoma									X																
Subcutaneous tissue, fibroma																	X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+					+																			
Spinal cord	+					+																			
Respiratory System																									
Larynx																						+			
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 2-Methylimidazole: 0 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 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	
Carcass ID Number	2 8 9	2 9 1	2 9 2	2 9 3	2 9 4	2 9 5	2 7 6	2 7 7	2 7 8	2 7 9	2 8 0	2 4 1	2 4 3	2 4 4	2 4 5	2 4 6	2 4 7	2 4 8	2 4 9	2 5 0	2 8 1	2 8 2	2 8 3	2 8 4	2 8 5	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma							X														X					5
Carcinoma											X									X						3
Squamous cell papilloma																					X					1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal		X	X		X									X	X				X						X	7
Endometrium, polyp stromal, multiple																										1
Vagina																			+							2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+				+	+		+		+		+		+	+	+			+	+	+	32
Lymph node, nandibular	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	5
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma	X								X						X											4
Fibroadenoma	X				X					X	X			X			X	X		X		X	X	X	X	24
Fibroadenoma, multiple						X															X					2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																										1
Basal cell carcinoma																										1
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										2
Spinal cord																								+		3
Respiratory System																										
Larynx																								+		2
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

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

Number of Days on Study	3 2 8	5 8 3	5 8 8	6 0 2	6 0 5	6 1 6	6 5 3	6 5 8	6 7 1	7 0 3	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	2 6 7	2 4 2	2 5 7	2 9 0	2 6 9	2 9 7	2 7 5	2 6 8	2 7 4	2 9 8	2 5 6	2 5 8	2 5 9	2 6 0	2 9 6	2 9 9	3 0 0	2 6 6	2 7 0	2 7 1	2 7 2	2 7 3	2 8 6	2 8 7	2 8 8
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimal gland																			+						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																		X							
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 2-Methylimidazole: 0 ppm

Number of Days on Study	735	735	735	735	735	735	736	736	736	736	736	737	737	737	737	737	737	737	737	737	737	737	737	737	737	737	
Carcass ID Number	28891	29912	29923	29934	29945	29955	27766	27777	27788	27799	28800	24411	24433	24447	24455	24466	24477	24488	24499	25500	28811	28822	28833	28844	28855	Total Tissues/ Tumors
Special Senses System																											
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Lacrimal gland														+													2
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Transitional epithelium, papilloma																											1
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia mononuclear																			X								6

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 2-Methylimidazole: 1,000 ppm

Number of Days on Study	4 2 5	4 8 3	4 8 3	5 3 8	5 3 8	5 9 8	6 3 8	6 3 8	6 8 0	6 8 0	7 0 2	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
Carcass ID Number	3 3 5	3 0 9	3 5 6	3 3 7	3 3 9	3 3 2	3 3 0	3 5 9	3 1 9	3 4 9	3 2 0	3 5 7	3 5 8	3 6 0	3 0 1	3 0 2	3 0 3	3 0 4	3 0 5	3 2 1	3 2 2	3 2 3	3 2 4	3 2 5	3 4 6
Alimentary System																									
Esophagus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	A	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	A	+	+	+	+	I	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery						+		+		+										+				+	+
Pancreas	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel				+				+		+															
Heart	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X				X	X	X		X		X	X	X	X		X		X		X		X	X	X	X
Pars distalis, carcinoma																									
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma								X			X				X									X	
C-cell, adenoma, multiple																									
C-cell, carcinoma							X																		
Follicular cell, carcinoma					X																				
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X		
Carcinoma					X																				
Bilateral, carcinoma, multiple																									
Ovary	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal			X														X								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 2-Methylimidazole: 1,000 ppm

Carcass ID Number	73535	73545	73550	73606	73606	73606	73606	73606	73606	73606	73606	73606	73606	73607	73607	73607	73607	73607	73607	73607	73607	73607	73607	73607	73607	73607	Total Tissues/ Tumors
Carcass ID Number	34707	34808	35000	30606	00607	00608	10700	13301	33313	33304	33606	33808	34000	11606	11707	11808	22606	22707	22808	22909	35101	35202	35303	35404	35505		
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Intestine small, lleum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Mesentery				+												+	+	+									10
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Cardiovascular System																											
Blood vessel																											3
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Pheochromocytoma benign								X															X				2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Pars distalis, adenoma		X	X				X	X					X	X	X		X										24
Pars distalis, carcinoma						X																					1
Pars intermedia, adenoma					X																						1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
C-cell, adenoma				X						X			X													X	8
C-cell, adenoma, multiple																		X									1
C-cell, carcinoma																											1
Follicular cell, carcinoma																											1
General Body System																											
None																											
Genital System																											
Clitoral gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Adenoma														X						X		X					4
Carcinoma																											1
Bilateral, carcinoma, multiple				X																							1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Endometrium, polyp stromal						X				X									X						X		6

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 2-Methylimidazole: 1,000 ppm

Number of Days on Study	42 35	44 83	48 33	53 38	53 38	53 98	63 88	63 88	63 80	68 00	70 02	73 34	73 44	73 46	73 55	73 55	73 55	73 55	73 55	73 55	73 55	73 55	73 55	73 55	73 55
Carcass ID Number	33 35	30 59	35 06	33 67	33 39	33 92	33 20	35 09	31 99	34 99	32 00	35 77	35 78	36 60	30 01	30 02	30 03	30 04	30 05	32 11	32 12	32 23	32 24	32 25	34 46
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	+	M
Lymph mode, mesenteric	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	M	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma		X	X			X						X		X	X		X			X		X		X	
Fibroadenoma, multiple							X												X		X		X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal Sytem																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle											+														
Rhabdomyosarcoma											X														
Nervous System																									
Brain	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve										+															
Spinal cord										+															
Respiratory System																									
Larynx																	+			+					
Lung	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																X									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X				X					X										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 2-Methylimidazole: 1,000 ppm

Carcass ID Number	73535	73555	73556	73566	73576	73586	73596	73606	73616	73626	73636	73646	73656	73667	73677	73687	73697	73707	73717	73727	73737	73747	73757	73767	73777	
Carcass ID Number	34747	34748	35050	30306	30007	30008	31310	31331	33313	33334	33336	33338	34140	11616	11617	11618	12626	22707	22808	22909	35101	35512	35523	35534	35545	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+		+	+	+		+	+	+	+				+	+		+	+	+				+	31
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	45
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																	X									1
Fibroadenoma	X	X				X	X	X											X		X		X			18
Fibroadenoma, multiple																									X	5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Rhabdomyosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Larynx																										2
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma																									X	1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Transitional epithelium, papilloma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X																4

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

Number of Days on Study	2 4 4	3 3 2	4 9 6	5 2 6	5 3 2	5 4 8	5 4 8	5 4 8	5 8 0	5 9 8	6 0 5	6 3 8	6 3 8	6 4 1	6 4 6	6 6 5	6 6 8	6 8 0	7 0 1	7 0 2	7 0 2	7 1 0	7 3 4	7 3 4	7 3 4
Carcass ID Number	3 6 4	4 0 5	3 8 5	3 9 4	3 9 0	3 7 2	4 0 4	4 1 4	3 7 4	3 8 1	3 6 5	3 8 7	3 9 6	4 1 6	3 7 6	4 1 3	3 6 1	3 7 5	3 6 3	4 0 0	4 0 3	3 7 1	3 6 2	3 7 3	4 0 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	A	+	+	+	+	+	A	+	A	+	A	+	A	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	+	+	A	+	+	+	+	+	A	+	A	+	A	+	A	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	+	+	A	+	+	+	+	+	A	+	A	+	A	+	A	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery						+	+	+	+					+		+			+	+					
Pancreas	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma															X										
Stomach, glandular	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Tongue																							+		
Squamous cell papilloma																							X		
Cardiovascular System																									
Blood vessel							+																		
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, skin									X																
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
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	+	A	A	A	+	+	+	+	+
C-cell, adenoma												X	X									X			X
Follicular cell, carcinoma																									
General Body System																									
Tissue NOS	+						+		+							+									
Mediastinum, squamous cell carcinoma, metastatic, skin									X																
Thoracic, rhabdomyosarcoma	X																								

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

Number of Days on Study	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 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	4 0 2	3 7 7	3 7 8	3 7 9	3 8 0	3 8 6	3 8 8	3 8 9	3 9 1	3 9 2	3 9 3	3 9 5	3 8 2	3 8 3	3 8 4	3 9 7	3 9 8	3 9 9	4 1 1	4 1 2	4 1 5	4 1 7	4 1 8	4 1 9	4 2 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma				X																X						2
Mesentery			+							+		+											+			12
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue																										1
Squamous cell papilloma																										1
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, skin																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant					X																					1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																									X	1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X				X						X			X		X						X	X	X	X	21
Pars distalis, carcinoma							X								X											3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
C-cell, adenoma	X	X			X														X							8
Follicular cell, carcinoma						X																				1
General Body System																										
Tissue NOS																										4
Mediastinum, squamous cell carcinoma, metastatic, skin																										1
Thoracic, rhabdomyosarcoma																										1

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

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

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

Number of Days on Study	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 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	4 0 2	3 7 7	3 7 8	3 7 9	3 8 0	3 8 6	3 8 8	3 8 9	3 9 1	3 9 2	3 9 3	3 9 5	3 8 2	3 8 3	3 8 4	3 9 7	3 9 8	3 9 9	4 1 1	4 1 2	4 1 5	4 1 7	4 1 8	4 1 9	4 2 0	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X		X	X				X								X			5
Carcinoma																	X									1
Bilateral, adenoma		X																								1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa-theca tumor benign																							X			1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal				X			X				X	X														7
Vagina								+																		2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+			+			+	+	+	+	+	+	+	+	+		+	+	+	+	+	+		+	34
Lymph node, mandibular	M	M	M	M	M	M	M	M	+	M	M	M	M	M	+	M	M	M	M	M	M	M	+	M	+	5
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymoma malignant																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																			X							3
Fibroadenoma		X			X			X	X					X		X		X			X	X		X		18
Fibroadenoma, multiple			X							X					X				X						X	6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, multiple																										1
Subcutaneous tissue, fibroma																										2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle										+																2
Rhabdomyosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										4
Spinal cord																										4
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma											X															1
Rhabdomyosarcoma, metastatic, tissue NOS																										1
Squamous cell carcinoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

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

Number of Days on Study	2 4 4	3 3 2	4 9 6	5 2 6	5 3 2	5 4 8	5 4 8	5 4 8	5 8 0	5 9 8	6 0 5	6 3 8	6 3 8	6 4 1	6 4 6	6 6 5	6 6 8	6 8 0	7 0 1	7 0 2	7 0 2	7 1 0	7 3 4	7 3 4	7 3 4
Carcass ID Number	3 6 4	4 0 5	3 8 5	3 9 4	3 9 0	3 7 2	4 0 4	4 1 4	3 7 4	3 8 1	3 6 5	3 8 7	3 9 6	4 1 6	3 7 6	4 1 3	3 6 1	3 7 5	3 6 3	4 0 0	4 0 3	3 7 1	3 6 2	3 7 3	4 0 1
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, skin									X																
Urinary bladder	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X	X						X	X	X							X			

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

Number of Days on Study	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 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	4 0 2	3 7 7	3 7 8	3 7 9	3 8 0	3 8 6	3 8 8	3 8 9	3 9 1	3 9 2	3 9 3	3 9 5	3 8 2	3 8 3	3 8 4	3 9 7	3 9 8	3 9 9	4 1 1	4 1 2	4 1 5	4 1 7	4 1 8	4 1 9	4 2 0	Total Tissues/ Tumors
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Squamous cell carcinoma, metastatic, skin																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X								X								X						9

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

Number of Days on Study	4 3 4	6 3 8	6 4 4	6 7 5	6 8 9	6 9 6	7 2 2	7 2 2	7 2 9	7 2 9	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	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6
Carcass ID Number	4 6 2	4 3 6	4 6 0	4 4 4	4 3 2	4 5 1	4 5 2	4 6 1	4 4 1	4 4 2	4 4 3	4 4 5	4 5 3	4 5 4	4 5 5	4 3 7	4 3 8	4 3 9	4 4 0	4 6 3	4 6 4	4 6 5	4 6 9	4 2 6	4 2 7
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma										X															
Hepatocellular adenoma											X														
Hepatocellular adenoma, multiple																									
Mesentery	+													+				+	+						
Nerve, schwannoma malignant																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+				+														+						
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X		
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X					X								X						X			
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Bilateral, follicular cell, adenoma													X												
Bilateral, follicular cell, carcinoma																									
C-Cell, adenoma															X										X
C-Cell, carcinoma																									
Follicular cell, adenoma		X																							
Follicular cell, carcinoma									X					X									X		
General Body System																									
Tissue NOS									+																
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal																X				X					X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 2-Methylimidazole: 5,000 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	4 2 8	4 2 9	4 3 0	4 3 1	4 3 3	4 3 4	4 3 5	4 5 6	4 5 7	4 5 8	4 5 9	4 7 6	4 7 7	4 7 8	4 7 9	4 8 0	4 4 6	4 4 7	4 4 8	4 4 9	4 5 0	4 6 6	4 6 7	4 6 8	4 7 0	Total Tissue/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma																										1
Hepatocellular adenoma			X															X								3
Hepatocellular adenoma, multiple	X																									1
Mesentery					+			+	+												+	+				9
Nerve, schwannoma malignant					X																					1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel		+																								4
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X										X			X	X		X	X		X		X		X	13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Bilateral, follicular cell, adenoma																										1
Bilateral, follicular cell, carcinoma							X																			1
C-cell, adenoma																									X	3
C-cell, carcinoma																									X	1
Follicular cell, adenoma						X			X												X					4
Follicular cell, carcinoma						X				X														X		6
General Body System																										
Tissue NOS						+																				2
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma			X																							1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor benign																				X						1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal																										3

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

Number of Days on Study	4 3 3 4	6 3 3 8	6 4 4 4	6 7 5 5	6 8 9 9	6 9 6 6	7 2 2 2	7 2 2 2	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 3 9	7 3 3 5	7 3 3 5	7 3 3 5	7 3 3 5	7 3 3 5	7 3 3 5	7 3 3 5	7 3 3 5	7 3 3 6	7 3 3 6
Carcass ID Number	4 6 2 2	4 3 6 6	4 6 0 0	4 4 4 4	4 3 4 2	4 5 1 1	4 5 5 2	4 6 1 1	4 4 1 1	4 4 2 2	4 4 3 3	4 4 5 5	4 5 3 3	4 5 5 4	4 5 5 5	4 3 3 7	4 3 3 8	4 3 3 9	4 4 4 0	4 6 0 3	4 6 6 4	4 6 6 5	4 6 6 9	4 6 2 6	4 2 2 7
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	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma								X																	
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue, fibroma		X																							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant, mixed cell																							X		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Urethra																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X			X		X		X		X	X														

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 2-Methylimidazole: 5,000 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	
Carcass ID Number	4 2 8	4 2 9	4 3 0	4 3 1	4 3 3	4 3 4	4 3 5	4 5 6	4 5 7	4 5 8	4 5 9	4 7 6	4 7 7	4 7 8	4 7 9	4 8 0	4 4 6	4 4 7	4 4 8	4 4 9	4 5 0	4 6 6	4 6 7	4 6 8	4 7 0	Total Tissue/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+		+	+	+	+		41
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma									X																	1
Fibroadenoma																	X	X	X	X						5
Fibroadenoma, multiple									X																	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																									X	1
Subcutaneous tissue, fibroma	X																									2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma malignant, mixed cell																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma								X																		1
Urethra						+												+								2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X						X	X																	10

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	5/50 (10%)	0/49 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate[b]	10.7%	0.0%	0.0%	0.0%
Terminal rate[c]	3/40 (8%)	0/39 (0%)	0/28 (0%)	0/42 (0%)
First incidence (days)	588	—[e]	—	—
Poly-3 test[d]	P=0.010N	P=0.035N	P=0.044N	P=0.029N
Clitoral Gland: Adenoma				
Overall rate	5/50 (10%)	4/49 (8%)	6/50 (12%)	0/50 (0%)
Adjusted rate	10.9%	9.1%	14.7%	0.0%
Terminal rate	4/40 (10%)	4/38 (11%)	6/28 (21%)	0/42 (0%)
First incidence (days)	703	729 (T)	729 (T)	—
Poly-3 test	P=0.037N	P=0.529N	P=0.414	P=0.027N
Clitoral Gland: Carcinoma				
Overall rate	3/50 (6%)	2/49 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.5%	4.5%	2.5%	2.1%
Terminal rate	3/40 (8%)	1/38 (3%)	1/28 (4%)	1/42 (2%)
First incidence (days)	729 (T)	538	729 (T)	729 (T)
Poly-3 test	P=0.194N	P=0.514N	P=0.350N	P=0.290N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	6/49 (12%)	7/50 (14%)	1/50 (2%)
Adjusted rate	17.4%	13.5%	17.2%	2.1%
Terminal rate	7/40 (18%)	5/38 (13%)	7/28 (25%)	1/42 (2%)
First incidence (days)	703	538	729 (T)	729 (T)
Poly-3 test	P=0.015N	P=0.412N	P=0.603N	P=0.013N
Liver: Hepatocellular Adenoma				
Overall rate	1/50 (2%)	0/49 (0%)	2/50 (4%)	4/50 (8%)
Adjusted rate	2.2%	0.0%	4.9%	8.3%
Terminal rate	1/40 (3%)	0/39 (0%)	2/28 (7%)	4/42 (10%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P=0.039	P=0.506N	P=0.458	P=0.192
Mammary Gland: Fibroadenoma				
Overall rate	26/50 (52%)	23/50 (46%)	24/50 (48%)	6/50 (12%)
Adjusted rate	54.6%	48.7%	56.7%	12.5%
Terminal rate	21/40 (53%)	19/39 (49%)	16/28 (57%)	5/42 (12%)
First incidence (days)	602	483	598	722
Poly-3 test	P<0.001N	P=0.357N	P=0.506	P<0.001N
Mammary Gland: Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	8.6%	2.2%	7.2%	2.1%
Terminal rate	3/40 (8%)	1/39 (3%)	1/28 (4%)	1/42 (2%)
First incidence (days)	605	729 (T)	332	729 (T)
Poly-3 test	P=0.198N	P=0.188N	P=0.555N	P=0.169N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	28/50 (56%)	24/50 (48%)	26/50 (52%)	6/50 (12%)
Adjusted rate	58.8%	50.9%	59.7%	12.5%
Terminal rate	23/40 (58%)	20/39 (51%)	16/28 (57%)	5/42 (12%)
First incidence (days)	602	483	332	722
Poly-3 test	P<0.001N	P=0.283N	P=0.551	P<0.001N

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	23/50 (46%)	24/48 (50%)	21/50 (42%)	13/50 (26%)
Adjusted rate	49.5%	51.9%	47.3%	26.9%
Terminal rate	21/40 (53%)	18/39 (46%)	10/28 (36%)	11/42 (26%)
First incidence (days)	658	425	532	644
Poly-3 test	P=0.006N	P=0.490	P=0.500N	P=0.018N
Pituitary Gland (Pars Distalis): Carcinoma				
Overall rate	2/50 (4%)	1/48 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.3%	2.3%	7.4%	0.0%
Terminal rate	0/40 (0%)	1/39 (3%)	3/28 (11%)	0/42 (0%)
First incidence (days)	602	729 (T)	729 (T)	—
Poly-3 test	P=0.236N	P=0.523N	P=0.438	P=0.231N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	25/50 (50%)	25/48 (52%)	24/50 (48%)	13/50 (26%)
Adjusted rate	53.0%	54.1%	54.0%	26.9%
Terminal rate	21/40 (53%)	19/39 (49%)	13/28 (46%)	11/42 (26%)
First incidence (days)	602	425	532	644
Poly-3 test	P=0.002N	P=0.541	P=0.543	P=0.007N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/49 (0%)	0/48 (0%)	0/42 (0%)	5/48 (10%)
Adjusted rate	0.0%	0.0%	0.0%	10.6%
Terminal rate	0/40 (0%)	0/39 (0%)	0/28 (0%)	4/41 (10%)
First incidence (days)	—	—	—	638
Poly-3 test	P<0.001	—[f]	—	P=0.034
Thyroid Gland (Follicular Cell): Carcinoma				
Overall rate	1/49 (2%)	1/48 (2%)	1/42 (2%)	7/48 (15%)
Adjusted rate	2.2%	2.3%	2.8%	14.9%
Terminal rate	0/40 (0%)	0/39 (0%)	1/28 (4%)	6/41 (15%)
First incidence (days)	583	538	729 (T)	722
Poly-3 test	P=0.003	P=0.754	P=0.703	P=0.033
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/49 (2%)	1/48 (2%)	1/42 (2%)	11/48 (23%)
Adjusted rate	2.2%	2.3%	2.8%	23.3%
Terminal rate	0/40 (0%)	0/39 (0%)	1/28 (4%)	9/41 (22%)
First incidence (days)	583	538	729 (T)	638
Poly-3 test	P<0.001	P=0.754	P=0.703	P=0.002
Thyroid Gland (C-cell): Adenoma				
Overall rate	9/49 (18%)	9/48 (19%)	8/42 (19%)	3/48 (6%)
Adjusted rate	19.7%	20.4%	22.0%	6.4%
Terminal rate	8/40 (20%)	7/39 (18%)	5/28 (18%)	3/41 (7%)
First incidence (days)	602	638	638	729 (T)
Poly-3 test	P=0.036N	P=0.573	P=0.505	P=0.054N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	9/49 (18%)	10/48 (21%)	8/42 (19%)	3/48 (6%)
Adjusted rate	19.7%	22.5%	22.0%	6.4%
Terminal rate	8/40 (20%)	7/39 (18%)	5/28 (18%)	3/41 (7%)
First incidence (days)	602	638	638	729 (T)
Poly-3 test	P=0.030N	P=0.475	P=0.505	P=0.054N

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
Uterus: Stromal Polyp				
Overall rate	8/50 (16%)	6/50 (12%)	7/50 (14%)	3/50 (6%)
Adjusted rate	17.4%	13.1%	16.7%	6.3%
Terminal rate	8/40 (20%)	5/39 (13%)	5/28 (18%)	3/42 (7%)
First incidence (days)	729 (T)	483	332	729 (T)
Poly-3 test	P=0.082N	P=0.391N	P=0.579N	P=0.086N
All Organs: Mononuclear Cell Leukemia				
Overall rate	6/50 (12%)	4/50 (8%)	9/50 (18%)	10/50 (20%)
Adjusted rate	12.7%	8.8%	21.0%	20.3%
Terminal rate	2/40 (5%)	2/39 (5%)	3/28 (11%)	6/42 (14%)
First incidence (days)	583	598	548	434
Poly-3 test	P=0.090	P=0.391N	P=0.222	P=0.233
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	43/50 (86%)	40/50 (80%)	27/50 (54%)
Adjusted rate	88.5%	87.4%	87.3%	55.5%
Terminal rate	35/40 (88%)	34/39 (87%)	25/28 (89%)	24/42 (57%)
First incidence (days)	588	425	332	638
Poly-3 test	P<0.001N	P=0.558N	P=0.557N	P<0.001N
All Organs: Malignant Neoplasms				
Overall rate	19/50 (38%)	10/50 (20%)	21/50 (42%)	20/50 (40%)
Adjusted rate	38.2%	21.4%	45.0%	40.7%
Terminal rate	10/40 (25%)	5/39 (13%)	10/28 (36%)	16/42 (38%)
First incidence (days)	328	538	244	434
Poly-3 test	P=0.172	P=0.056N	P=0.318	P=0.482
All Organs: Benign or Malignant Neoplasms				
Overall rate	45/50 (90%)	44/50 (88%)	48/50 (96%)	38/50 (76%)
Adjusted rate	90.0%	89.1%	96.5%	76.3%
Terminal rate	35/40 (88%)	34/39 (87%)	27/28 (96%)	32/42 (76%)
First incidence (days)	328	425	244	434
Poly-3 test	P=0.024N	P=0.570N	P=0.185	P=0.057N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal cortex, clitoral gland, liver, 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
[f] Value of statistic cannot be computed.

TABLE B4a
Historical Incidence of Thyroid Gland (Follicular Cell) Neoplasms in Control Female F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Feed Studies			
trans-Cinnamaldehyde	0/50	0/50	0/50
Citral	0/50	0/50	0/50
p,p'-Dichlorodiphenyl sulfone	1/50	0/50	1/50
2-Methylimidazole	0/49	1/49	1/49
o-Nitrotoluene	0/60	0/60	0/60
p-Nitrotoluene	0/50	1/50	1/50
Overall Historical Incidence: Feed Studies			
Total (%)	1/309 (0.3%)	2/309 (0.7%)	3/309 (1.0%)
Mean ± standard deviation	0.3% ± 0.8%	0.7% ± 1.0%	1.0% ± 1.1%
Range	0%-2%	0%-2%	0%-2%
Overall Historical Incidence			
Total (%)	3/1,095 (0.3%)	12/1,095 (1.1%)	15/1,095 (1.4%)
Mean ± standard deviation	0.3% ± 0.7%	1.2% ± 1.2%	1.5% ± 1.3%
Range	0%-2%	0%-4%	0%-4%

[a] Data as of March 3, 2003

TABLE B4b
Historical Incidence of Liver Neoplasms in Control Female F344/N Rats[a]

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence: Feed Studies			
trans-Cinnamaldehyde	0/50	0/50	0/50
Citral	0/50	0/50	0/50
p,p'-Dichlorodiphenyl sulfone	0/50	0/50	0/50
2-Methylimidazole	1/50	0/50	1/50
o-Nitrotoluene	1/60	0/60	1/60
p-Nitrotoluene	0/50	0/50	0/50
Overall Historical Incidence: Feed Studies			
Total (%)	2/310 (0.7%)	0/310 (0%)	2/310 (0.7%)
Mean ± standard deviation	0.6% ± 1.0%		0.6% ± 1.0%
Range	0%-2%		0%-2%
Overall Historical Incidence			
Total (%)	6/1,107 (0.5%)	1/1,107 (0.1%)	7/1,107 (0.6%)
Mean ± standard deviation	0.6% ± 0.9%	0.1% ± 0.5%	0.7% ± 1.0%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of March 3, 2003

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
6-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	6	8	12	7
Natural deaths	4	3	10	1
Survivors				
Died last week of study				1
Terminal sacrifice	40	39	28	41
Animals examined microscopically	60	60	60	60
6-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	1 (10%)		1 (10%)	
Inflammation, granulomatous	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule				1 (10%)
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, cyst	1 (10%)	2 (20%)		1 (10%)
Pars distalis, mitotic alteration	1 (10%)	2 (20%)	2 (20%)	2 (20%)
Pars intermedia, cyst		1 (10%)	1 (10%)	
Thyroid gland	(10)	(10)	(10)	(10)
Follicle, mineralization, focal	1 (10%)	6 (60%)	10 (100%)	10 (100%)
Follicular cell, hyperplasia		5 (50%)	10 (100%)	10 (100%)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Cyst	1 (10%)		1 (10%)	
Periovarian tissue, cyst	1 (10%)			
Uterus	(10)	(10)	(10)	(10)
Hydrometra	1 (10%)			
Hematopoietic System				
Lymph node	(3)	(4)	(1)	(3)
Mediastinal, hemorrhage	1 (33%)	1 (25%)		2 (67%)
Pancreatic, hemorrhage		2 (50%)		1 (33%)
Pancreatic, hyperplasia, lymphoid	2 (67%)	1 (25%)	1 (100%)	
Spleen	(10)	(10)	(10)	(10)
Accessory spleen			1 (10%)	
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Cyst epithelial inclusion			1 (10%)	

[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 2-Methylimidazole

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
6-Month Interim Evaluation (continued)				
Special Senses System				
Eye	(10)	(10)	(10)	(10)
Retrobulbar, inflammation, focal		1 (10%)		
Harderian gland	(10)	(10)	(10)	(10)
Hyperplasia, focal, lymphoid				1 (10%)
Inflammation, focal, granulomatous			1 (10%)	5 (50%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Cyst		1 (10%)		
Systems Examined at 6 Months with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Respiratory System				
2-Year Study				
Alimentary System				
Intestine large, cecum	(48)	(47)	(44)	(50)
Edema				1 (2%)
Intestine small, jejunum	(47)	(46)	(44)	(49)
Necrosis, focal				1 (2%)
Liver	(50)	(49)	(50)	(50)
Angiectasis, focal	2 (4%)	1 (2%)	2 (4%)	
Basophilic focus	42 (84%)	44 (90%)	43 (86%)	39 (78%)
Cholangiofibrosis				1 (2%)
Congestion			2 (4%)	1 (2%)
Congestion, focal			1 (2%)	
Degeneration, cystic, focal		1 (2%)		2 (4%)
Eosinophilic focus			1 (2%)	
Fatty change		2 (4%)	1 (2%)	
Hemorrhage	2 (4%)			2 (4%)
Hepatodiaphragmatic nodule	6 (12%)	11 (22%)	14 (28%)	13 (26%)
Hyperplasia, focal, lymphoid			1 (2%)	1 (2%)
Infarct				2 (4%)
Infiltration cellular, mixed cell	42 (84%)	44 (90%)	34 (68%)	38 (76%)
Inflammation, granulomatous	18 (36%)	23 (47%)	22 (44%)	42 (84%)
Mixed cell focus	15 (30%)	14 (29%)	11 (22%)	26 (52%)
Necrosis, focal		1 (2%)		1 (2%)
Bile duct, cholangiofibrosis, focal			1 (2%)	
Bile duct, cyst	2 (4%)			
Bile duct, cyst, multiple			1 (2%)	1 (2%)
Bile duct, hyperplasia	20 (40%)	29 (59%)	20 (40%)	40 (80%)
Hepatocyte, necrosis, focal			1 (2%)	2 (4%)
Hepatocyte, vacuolization cytoplasmic, focal	9 (18%)	10 (20%)	11 (22%)	9 (18%)
Hepatocyte, periportal, vacuolization cytoplasmic			1 (2%)	
Hepatocyte, centrilobular, necrosis			1 (2%)	1 (2%)
Hepatocyte, centrilobular, vacuolization cytoplasmic	1 (2%)		4 (8%)	1 (2%)

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Mesentery	(8)	(10)	(12)	(9)
Fibrosis		1 (10%)		
Hemorrhage, focal				1 (11%)
Infiltration cellular, mixed cell			1 (8%)	
Inflammation, chronic		1 (10%)		
Artery, inflammation, chronic, focal				1 (11%)
Fat, necrosis		1 (10%)	2 (17%)	
Fat, necrosis, focal	5 (63%)	7 (70%)	6 (50%)	3 (33%)
Pancreas	(50)	(49)	(49)	(50)
Infiltration cellular, diffuse, mixed cell		1 (2%)		
Acinus, atrophy, diffuse	1 (2%)	1 (2%)		1 (2%)
Acinus, atrophy, focal	13 (26%)	14 (29%)	13 (27%)	11 (22%)
Duct, cyst, focal	1 (2%)			1 (2%)
Duct, cyst, focal, multiple	9 (18%)	12 (24%)	4 (8%)	5 (10%)
Salivary glands	(50)	(49)	(50)	(50)
Duct, mineralization, focal				2 (4%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum	1 (2%)	1 (2%)		2 (4%)
Edema				1 (2%)
Erosion			1 (2%)	
Inflammation, chronic		1 (2%)	1 (2%)	
Inflammation, focal			1 (2%)	
Ulcer		2 (4%)	1 (2%)	1 (2%)
Epithelium, hyperplasia	3 (6%)	1 (2%)	4 (8%)	1 (2%)
Stomach, glandular	(49)	(49)	(48)	(50)
Erosion		1 (2%)	1 (2%)	
Inflammation, chronic			1 (2%)	
Necrosis, focal			1 (2%)	
Ulcer	1 (2%)			
Epithelium, hyperplasia, focal	1 (2%)			
Glands, degeneration, cystic, focal			1 (2%)	
Glands, ectasia, focal	1 (2%)			
Glands, hyperplasia, focal				2 (4%)
Tongue	(3)		(1)	
Epithelium, hyperplasia, focal	1 (33%)			
Tooth	(1)			
Peridontal tissue, inflammation, chronic	1 (100%)			
Cardiovascular System				
Heart	(50)	(49)	(50)	(50)
Cardiomyopathy	5 (10%)	8 (16%)	7 (14%)	6 (12%)
Infiltration cellular, mixed cell		4 (8%)	3 (6%)	2 (4%)
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Accessory adrenal cortical nodule	2 (4%)	2 (4%)	5 (10%)	5 (10%)
Angiectasis	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Cytoplasmic alteration, focal	3 (6%)		1 (2%)	5 (10%)
Hematopoietic cell proliferation		1 (2%)		
Hemorrhage		1 (2%)		
Hemorrhage, focal		1 (2%)		
Vacuolization cytoplasmic, focal	8 (16%)	3 (6%)	7 (14%)	12 (24%)

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Endocrine System (continued)				
Adrenal medulla	(49)	(49)	(50)	(50)
Hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Pituitary gland	(50)	(48)	(50)	(50)
Angiectasis	5 (10%)	9 (19%)	7 (14%)	2 (4%)
Cyst		1 (2%)		
Hemorrhage		1 (2%)		
Metaplasia, focal, lipocyte			1 (2%)	
Pars distalis, angiectasis				1 (2%)
Pars distalis, cyst	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Pars distalis, cyst, multiple		2 (4%)		
Pars distalis, cytoplasmic alteration, focal	4 (8%)	4 (8%)	1 (2%)	3 (6%)
Pars distalis, degeneration, cystic	1 (2%)			
Pars distalis, degeneration, cystic, focal	9 (18%)	14 (29%)	8 (16%)	5 (10%)
Pars distalis, hemorrhage, focal	1 (2%)	3 (6%)	4 (8%)	6 (12%)
Pars distalis, hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Pars distalis, pigmentation, focal			1 (2%)	
Pars intermedia, cyst			1 (2%)	3 (6%)
Pars intermedia, degeneration, cystic, focal				1 (2%)
Pars intermedia, hemorrhage				1 (2%)
Pars intermedia, pars nervosa, atypia cellular, focal		1 (2%)		
Rathke's cleft, cyst	1 (2%)		1 (2%)	
Rathke's cleft, hemorrhage				2 (4%)
Thyroid gland	(49)	(48)	(42)	(48)
Vacuolization cytoplasmic, focal		1 (2%)		
C-cell, hyperplasia	48 (98%)	46 (96%)	40 (95%)	25 (52%)
Follicle, cyst		2 (4%)	1 (2%)	
Follicle, mineralization, focal	42 (86%)	47 (98%)	41 (98%)	48 (100%)
Follicular cell, hyperplasia		41 (85%)	34 (81%)	46 (96%)
General Body System				
Tissue NOS	(3)		(4)	(2)
Mediastinum, infiltration cellular, mixed cell			1 (25%)	
Pelvic, hemorrhage, focal				1 (50%)
Thoracic, infiltration cellular, focal, mixed cell			1 (25%)	
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Degeneration, cystic	6 (12%)	4 (8%)	1 (2%)	1 (2%)
Fibrosis, focal		1 (2%)		
Hyperplasia, cystic	1 (2%)	4 (8%)	3 (6%)	1 (2%)
Inflammation, chronic	2 (4%)	5 (10%)	4 (8%)	
Ovary	(50)	(49)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)		
Atrophy		1 (2%)		
Congestion			1 (2%)	
Cyst	5 (10%)	5 (10%)	1 (2%)	
Bilateral, cyst, multiple			1 (2%)	
Periovarian tissue, cyst	4 (8%)	5 (10%)	4 (8%)	6 (12%)
Periovarian tissue, cyst, multiple	1 (2%)			

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Genital System (continued)				
Uterus	(50)	(50)	(50)	(50)
Cyst	3 (6%)		1 (2%)	1 (2%)
Hemorrhage			2 (4%)	
Inflammation, chronic			1 (2%)	
Cervix, hyperplasia			1 (2%)	
Endometrium, hyperplasia, cystic	10 (20%)	14 (28%)	15 (30%)	15 (30%)
Vagina	(2)		(2)	
Inflammation, chronic			1 (50%)	
Epithelium, cyst	1 (50%)			
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Atrophy	1 (2%)			
Fibrosis				1 (2%)
Hyperplasia, focal, histiocytic		1 (2%)		4 (8%)
Myelofibrosis				1 (2%)
Necrosis				1 (2%)
Myeloid cell, hyperplasia	2 (4%)	3 (6%)	5 (10%)	2 (4%)
Myeloid cell, erythroid cell, hyperplasia		2 (4%)	1 (2%)	
Lymph node	(32)	(31)	(34)	(41)
Deep cervical, hemorrhage				2 (5%)
Mediastinal, angiectasis	1 (3%)			
Mediastinal, congestion			1 (3%)	
Mediastinal, ectasia	5 (16%)	3 (10%)	2 (6%)	7 (17%)
Mediastinal, hemorrhage	2 (6%)	3 (10%)	9 (26%)	8 (20%)
Mediastinal, hyperplasia		1 (3%)	2 (6%)	
Mediastinal, hyperplasia, histiocytic	8 (25%)	8 (26%)	5 (15%)	12 (29%)
Mediastinal, hyperplasia, lymphoid		1 (3%)	1 (3%)	1 (2%)
Mediastinal, pigmentation	1 (3%)		2 (6%)	
Pancreatic, atrophy		1 (3%)		
Pancreatic, ectasia	2 (6%)	2 (6%)		11 (27%)
Pancreatic, hemorrhage	5 (16%)	3 (10%)	2 (6%)	9 (22%)
Pancreatic, hyperplasia, histiocytic	16 (50%)	24 (77%)	17 (50%)	18 (44%)
Pancreatic, hyperplasia, plasma cell	1 (3%)			
Pancreatic, infiltration cellular, mixed cell				1 (2%)
Pancreatic, pigmentation	4 (13%)	2 (6%)		1 (2%)
Lymph node, mandibular	(5)	(4)	(5)	(1)
Ectasia	1 (20%)			
Lymph node, mesenteric	(49)	(49)	(49)	(50)
Angiectasis			1 (2%)	
Ectasia	1 (2%)			5 (10%)
Hemorrhage	1 (2%)		2 (4%)	2 (4%)
Hyperplasia, histiocytic	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Hyperplasia, lymphoid		1 (2%)		
Pigmentation	2 (4%)			

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Spleen	(50)	(49)	(48)	(50)
Accessory spleen		1 (2%)	1 (2%)	
Angiectasis, focal		1 (2%)		
Congestion		1 (2%)		
Fibrosis, focal		1 (2%)	1 (2%)	
Hematopoietic cell proliferation	6 (12%)	8 (16%)	5 (10%)	4 (8%)
Hemorrhage			1 (2%)	
Infarct				1 (2%)
Inflammation, granulomatous	3 (6%)	2 (4%)	4 (8%)	27 (54%)
Pigmentation		1 (2%)		
Pigmentation, focal	1 (2%)	1 (2%)	1 (2%)	
Thymus	(48)	(45)	(49)	(49)
Angiectasis			2 (4%)	
Hyperplasia, lymphoid	1 (2%)	1 (2%)		2 (4%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Dilatation	33 (66%)	35 (70%)	22 (44%)	19 (38%)
Ectasia	2 (4%)	3 (6%)	7 (14%)	
Fibrosis		1 (2%)		
Hyperplasia	3 (6%)	5 (10%)	4 (8%)	3 (6%)
Hyperplasia, cystic	1 (2%)		2 (4%)	
Inflammation, focal		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Necrosis, focal				4 (8%)
Ulcer			1 (2%)	1 (2%)
Subcutaneous tissue, angiectasis, focal				1 (2%)
Subcutaneous tissue, hemorrhage, focal				1 (2%)
Subcutaneous tissue, inflammation, focal, suppurative				1 (2%)
Musculoskeletal System				
None				
Nervous System				
Brain	(50)	(49)	(50)	(50)
Compression, focal	10 (20%)	8 (16%)	12 (24%)	4 (8%)
Hemorrhage, focal	2 (4%)	1 (2%)	2 (4%)	
Necrosis, focal			2 (4%)	
Pigmentation, focal			1 (2%)	
Cerebellum, developmental malformation			1 (2%)	
Spinal cord	(3)	(1)	(4)	
Hemorrhage, focal	1 (33%)			
Necrosis, focal	1 (33%)			

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

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
2-Year Study (continued)				
Respiratory System				
Lung	(50)	(49)	(50)	(50)
Congestion	2 (4%)	4 (8%)	2 (4%)	2 (4%)
Emphysema			1 (2%)	
Hemorrhage	1 (2%)			
Hemorrhage, focal	1 (2%)			2 (4%)
Hyperplasia, focal, histiocytic			1 (2%)	1 (2%)
Hyperplasia, histiocytic	6 (12%)	6 (12%)	7 (14%)	1 (2%)
Infiltration cellular, focal, mixed cell				1 (2%)
Infiltration cellular, mixed cell		3 (6%)	1 (2%)	6 (12%)
Inflammation, chronic, focal	1 (2%)	2 (4%)	1 (2%)	
Inflammation, focal, granulomatous	1 (2%)			1 (2%)
Pigmentation, focal	1 (2%)			
Alveolar epithelium, hyperplasia, focal	7 (14%)	5 (10%)	3 (6%)	3 (6%)
Alveolus, inflammation, focal, granulomatous				1 (2%)
Interstitium, edema			1 (2%)	
Nose	(50)	(49)	(50)	(50)
Foreign body		1 (2%)		1 (2%)
Inflammation, suppurative	1 (2%)	1 (2%)		2 (4%)
Nasolacrimal duct, inflammation	4 (8%)	2 (4%)	4 (8%)	
Special Senses System				
Eye	(50)	(49)	(49)	(50)
Atrophy	1 (2%)	1 (2%)	1 (2%)	
Cataract	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Iris, synechia			1 (2%)	
Retina, degeneration		2 (4%)	1 (2%)	1 (2%)
Harderian gland	(50)	(49)	(50)	(50)
Hyperplasia, cystic, focal	1 (2%)			
Hyperplasia, focal	2 (4%)		1 (2%)	1 (2%)
Hyperplasia, focal, histiocytic	1 (2%)	2 (4%)	1 (2%)	4 (8%)
Inflammation, chronic, focal			3 (6%)	1 (2%)
Inflammation, focal, granulomatous			1 (2%)	
Epithelium, hyperplasia, focal		2 (4%)	2 (4%)	1 (2%)
Urinary System				
Kidney	(50)	(48)	(47)	(50)
Atrophy, focal		2 (4%)	1 (2%)	
Congestion			2 (4%)	
Cyst				1 (2%)
Infarct			1 (2%)	
Nephropathy	38 (76%)	40 (83%)	38 (81%)	40 (80%)
Glomerulus, amyloid deposition, diffuse				1 (2%)
Pelvis, inflammation			1 (2%)	
Renal tubule, accumulation, hyaline droplet	11 (22%)	7 (15%)	3 (6%)	4 (8%)
Renal tubule, pigmentation	8 (16%)	4 (8%)	5 (11%)	
Urethra				(2)
Transitional epithelium, hyperplasia, diffuse				1 (50%)
Urinary bladder	(50)	(49)	(49)	(50)
Transitional epithelium, hyperplasia				2 (4%)

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

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
6-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	4	1	5	5
Natural deaths	3	3	9	5
Survivors				
Terminal sacrifice	43	46	36	40
Animals examined microscopically	60	60	60	60
6-Month Interim Evaluation				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma			1 (10%)	
Systems Examined at 6 Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Intestine large, cecum	(49)	(49)	(47)	(50)
Intestine small, duodenum	(49)	(49)	(45)	(50)
Polyp adenomatous	1 (2%)		1 (2%)	
Intestine small, jejunum	(50)	(49)	(46)	(50)
Carcinoma	1 (2%)	1 (2%)	1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hemangioma				1 (2%)
Hemangiosarcoma	1 (2%)			2 (4%)
Hepatocellular carcinoma	4 (8%)	5 (10%)	8 (16%)	5 (10%)
Hepatocellular carcinoma, multiple		3 (6%)	6 (12%)	1 (2%)
Hepatocellular adenoma	5 (10%)	11 (22%)	9 (18%)	15 (30%)
Hepatocellular adenoma, multiple	2 (4%)	3 (6%)	4 (8%)	3 (6%)
Histiocytic sarcoma	1 (2%)		1 (2%)	
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Osteosarcoma, metastatic, bone				1 (2%)
Pancreas	(50)	(50)	(49)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(49)	(50)	(50)
Squamous cell papilloma		1 (2%)		1 (2%)

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
2-Year Study (continued)				
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Capsule, adenoma		2 (4%)		1 (2%)
Islets, pancreatic	(50)	(50)	(49)	(50)
Adenoma				1 (2%)
Pituitary gland	(49)	(48)	(48)	(50)
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, follicular cell, adenoma				1 (2%)
Follicular cell, adenoma		1 (2%)		
Unilateral, follicular cell, adenoma				6 (12%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Sertoli cell tumor malignant	1 (2%)			
Interstitial cell, adenoma			1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)		
Histiocytic sarcoma	1 (2%)			
Lymph node	(2)	(3)		(1)
Inguinal, histiocytic sarcoma	1 (50%)			
Inguinal, osteosarcoma, metastatic, bone				1 (100%)
Mediastinal, osteosarcoma, metastatic, bone				1 (100%)
Lymph node, mandibular	(48)	(46)	(45)	(50)
Histiocytic sarcoma	1 (2%)			
Lymph node, mesenteric	(47)	(47)	(46)	(48)
Histiocytic sarcoma	1 (2%)			
Spleen	(50)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)		1 (2%)	
Histiocytic sarcoma	1 (2%)			
Thymus	(47)	(46)	(46)	(47)
Histiocytic sarcoma	1 (2%)			
Osteosarcoma, metastatic, bone				1 (2%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Subcutaneous tissue, sarcoma	1 (2%)			
Subcutaneous tissue, schwannoma benign			1 (2%)	

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
2-Year Study (continued)				
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Osteoma		1 (2%)		
Osteosarcoma				1 (2%)
Skeletal muscle	(1)	(1)		(2)
Hemangiosarcoma		1 (100%)		
Liposarcoma, metastatic, uncertain primary site				1 (50%)
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	11 (22%)	7 (14%)	6 (12%)	4 (8%)
Alveolar/bronchiolar adenoma, multiple				1 (2%)
Alveolar/bronchiolar carcinoma	5 (10%)	2 (4%)	4 (8%)	4 (8%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Carcinoma, metastatic, harderian gland	1 (2%)			
Carcinoma, metastatic, kidney			1 (2%)	
Hemangiosarcoma, metastatic, liver				1 (2%)
Hepatocellular carcinoma, metastatic, liver		3 (6%)	6 (12%)	2 (4%)
Histiocytic sarcoma	1 (2%)			
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Osteosarcoma, metastatic, bone				1 (2%)
Special Senses System				
Harderian gland	(50)	(50)	(48)	(50)
Adenoma	9 (18%)	6 (12%)	5 (10%)	3 (6%)
Carcinoma	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Histiocytic sarcoma	1 (2%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Liposarcoma, metastatic, uncertain primary site				1 (2%)
Renal tubule, adenoma				1 (2%)
Renal tubule, carcinoma	1 (2%)		1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)		1 (2%)	
Leukemia granulocytic			1 (2%)	
Lymphoma malignant	2 (4%)	3 (6%)		1 (2%)

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]				
6-Month interim evaluation			1	
2-Year study	32	34	34	38
Total primary neoplasms				
6-Month interim evaluation			1	
2-Year study	50	53	52	56
Total animals with benign neoplasms				
6-Month interim evaluation			1	
2-Year study	23	25	24	30
Total benign neoplasms				
6-Month interim evaluation			1	
2-Year study	28	32	27	39
Total animals with malignant neoplasms				
2-Year study	20	16	21	14
Total malignant neoplasms				
2-Year study	22	21	25	17
Total animals with metastatic neoplasms				
2-Year study	1	3	7	5
Total metastatic neoplasm				
2-Year study	1	3	7	14
Total animals with malignant neoplasms of uncertain primary site				
2-Year study				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 C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-Methylimidazole: 0 ppm

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

+: 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 2-Methylimidazole: 0 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	732	
Carcass ID Number	02525	03537	03538	03539	04042	04445	04746	04847	05050	05551	05757	06001	06006	06116	06629	06936	07340	07641	07744	07848	08053	08554	08555	08756	09001	09207	Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp adenomatous																											1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																											1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																											1
Hepatocellular carcinoma	X																								X		4
Hepatocellular adenoma							X			X				X												X	5
Hepatocellular adenoma, multiple	X																X										2
Histiocytic sarcoma																											1
Mesentery			+										+														6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																											
None																											
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																											1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sertoli cell tumor malignant									X																		1

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

Number of Days on Study	0 2 4	6 1 1	6 5 0	6 5 7	6 6 8	6 6 8	6 9 4	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
Carcass ID Number	0 5 8	0 1 8	0 5 2	0 0 9	0 0 3	0 0 6	0 2 3	0 0 4	0 0 5	0 0 8	0 1 2	0 1 4	0 2 2	0 2 4	0 2 8	0 3 0	0 3 1	0 3 2	0 3 3	0 4 9	0 0 7	0 1 5	0 1 7	0 1 9	0 2 0
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Histiocytic sarcoma			X																						
Lymph node			+																						
Inguinal, histiocytic sarcoma			X																						
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Lymph Node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Histiocytic sarcoma			X																						
Thymus	+	+	+	M	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma																									
Subcutaneous tissue, sarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle						+																			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve						+																			
Spinal cord						+																			
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X	X									X								X			
Alveolar/bronchiolar carcinoma										X	X											X		X	
Alveolar/bronchiolar carcinoma, multiple																									
Carcinoma, metastatic, harderian gland				X																					
Histiocytic sarcoma			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X					
Carcinoma				X																					
Histiocytic sarcoma			X																						
Lacrimal gland																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-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 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	
Carcass ID Number	0 2 5	0 3 7	0 3 8	0 3 9	0 4 2	0 4 5	0 4 6	0 4 7	0 5 0	0 5 1	0 5 7	0 0 1	0 0 6	0 1 6	0 2 9	0 3 6	0 4 0	0 4 1	0 4 4	0 4 8	0 5 3	0 5 4	0 5 5	0 5 6	0 2 7	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Histiocytic sarcoma																										1
Lymph node					+																					2
Inguinal, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Lymph node, mesenteric	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	47
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																	X									1
Subcutaneous tissue, sarcoma																		X								1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X	X					X	X			X	X		X										11
Alveolar/bronchiolar carcinoma													X													5
Alveolar/bronchiolar carcinoma, multiple										X																1
Carcinoma, metastatic, harderian gland																										1
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear												+														1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma					X			X				X		X		X	X		X					X		9
Carcinoma																										1
Histiocytic sarcoma																										1
Lacrimal gland																			+							1

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

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-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 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	
Carcass ID Number	0 2 5	0 3 7	0 3 8	0 3 9	0 4 2	0 4 5	0 4 6	0 4 7	0 5 0	0 5 1	0 5 7	0 0 1	0 0 6	0 1 6	0 2 9	0 3 6	0 4 0	0 4 1	0 4 4	0 4 8	0 5 3	0 5 4	0 5 5	0 5 6	0 5 7	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Renal tubule, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant					X																					2

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

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-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 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	
Carcass ID Number	0 8 7	0 8 8	0 8 9	0 9 1	0 9 2	0 9 4	0 9 8	0 1 3	1 1 1	1 1 6	1 2 0	0 6 1	0 6 2	0 6 4	0 7 3	0 7 9	0 9 5	0 9 9	1 0 6	1 0 7	1 1 4	1 1 5	1 1 7	1 1 8	0 7 8	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma											X															5
Hepatocellular carcinoma, multiple																						X				3
Hepatocellular adenoma	X		X	X									X				X	X						X		11
Hepatocellular adenoma, multiple									X			X								X						3
Mesentery																										2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma				X																						1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma									X																	2
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	M	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
General Body System																										
None																										
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 2-Methylimidazole: 625 ppm

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-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 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	
Carcass ID Number	0 8 7	0 8 8	0 8 9	0 9 1	0 9 2	0 9 4	0 9 8	1 0 3	1 1 1	1 1 6	1 2 0	0 6 1	0 6 2	0 6 4	0 7 3	0 7 9	0 9 5	0 9 9	1 0 6	1 1 0 7	1 1 4	1 1 5	1 1 7	1 1 8	0 7 8	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Lymph node											+															3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	M	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	M	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrous histiocytoma																							X			1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteoma						X																				1
Skeletal muscle																										1
Hemangiosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X					X									X											7
Alveolar/bronchiolar carcinoma																										2
Alveolar/bronchiolar carcinoma, multiple																										1
Hepatocellular carcinoma, metastatic, liver																										3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma	X				X					X											X			X		6
Carcinoma						X			X																	3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant										X								X								3

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

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

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

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	13 36	13 39	14 00	14 01	14 02	14 45	15 07	16 00	16 08	18 00	12 03	12 29	13 00	13 04	13 07	14 43	14 66	14 92	15 55	16 03	16 65	17 00	17 74	17 77	17 78	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	42
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Polp adenomatous												X														1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Carcinoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma								X								X								X			8
Hepatocellular carcinoma, multiple			X						X										X			X				6
Hepatocellular adenoma			X						X				X				X								X	9
Hepatocellular adenoma, multiple										X								X								4
Histiocytic sarcoma																										1
Mesentery																			+							5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Blood vessel									+					+	+											3
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Prostate	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										1

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

Number of Days on Study	0 0 0 0 0 4 5 5 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 0 0 0 2 2 0 1 3 1 2 5 5 0 1 2 2 2 2 2 2 2 2 2 3 3 7 7 7 1 2 0 2 1 1 3 4 9 6 8 9 9 9 9 9 9 9 9 9 0 0
Carcass ID Number	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 5 7 5 7 2 6 3 5 7 6 3 5 5 2 2 2 3 4 4 4 5 6 7 2 2 3 8 0 3 6 6 5 2 1 3 1 9 3 2 1 4 6 8 4 7 8 4 1 2 2 2
Hematopoietic System	
Bone marrow	+ +
Lymph node, mandibular	+ + + + + + + + + + + + M + + + M + + + + + + + M
Lymph node, mesenteric	A + + A M + + + + + A + + + + + + + + + + + + + +
Spleen	+ + + + + + + + + + A + + + + + + + + + + + + +
Hemangiosarcoma	
Thymus	+ + + + + + + + M + + M + + + + + + + + + + + + +
Integumentary System	
Mammary gland	+ +
Skin	+ +
Subcutaneous tissue, schwannoma benign	X
Musculoskeletal System	
Bone	+ +
Nervous System	
Brain	+ +
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	X X
Alveolar/bronchiolar carcinoma	X X
Alveolar/bronchiolar carcinoma, multiple	X
Carcinoma, metastatic, kidney	X
Hepatocellular carcinoma, metastatic, liver	X X X X
Nose	+ +
Trachea	+ +
Special Senses System	
Eye	+ +
Harderian gland	+ + + + M + + + + + + + M + + + + + + + + + + + +
Adenoma	X X X
Carcinoma	X
Urinary System	
Kidney	+ +
Renal tubule, carcinoma	X
Urinary bladder	+ +
Systemic Lesions	
Multiple organs	+ +
Histiocytic sarcoma	X
Leukemia granulocytic	X

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-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 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	1 3 3 6	1 3 3 9	1 4 0 0	1 4 4 1	1 4 4 2	1 4 4 5	1 5 5 7	1 6 6 0	1 6 6 8	1 8 8 0	1 2 2 3	1 2 2 9	1 3 3 0	1 3 3 4	1 3 3 7	1 4 4 3	1 4 4 6	1 4 4 9	1 5 5 5	1 6 6 3	1 6 6 5	1 7 7 0	1 7 7 4	1 7 7 7	1 7 7 8	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	45
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																						X				1
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, schwannoma benign																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X			X										X			X								6
Alveolar/bronchiolar carcinoma																			X						X	4
Alveolar/bronchiolar carcinoma, multiple																										1
Carcinoma, metastatic, kidney																										1
Hepatocellular carcinoma, metastatic, liver									X														X			6
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																			X	X						5
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia granulocytic																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-Methylimidazole: 2,500 ppm

Number of Days on Study	0 0 0 0 5 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	0 0 0 2 3 5 5 9 9 0 2 2 2 2 2 2 2 2 2 2 2 2 3 3
	7 7 7 1 1 4 6 3 5 1 9 9 9 9 9 9 9 9 9 9 9 9 9 0
Carcass ID Number	1 2 2 2 1 2 2 2 2 2 1 1 1 1 1 2 2 2 2 2 2 2 2 1 1
	8 0 3 0 8 1 0 3 0 3 8 8 9 9 9 0 0 1 2 3 3 3 4 8 9
	2 4 1 9 9 7 3 5 1 0 3 6 2 7 8 5 7 0 7 2 7 8 0 8 6
Alimentary System	
Esophagus	+ +
Gallbladder	+ + + + + + + + + + + + M + + + + + + + + + + + +
Intestine large, colon	+ +
Intestine large, rectum	+ +
Intestine large, cecum	+ +
Intestine small, duodenum	+ +
Intestine small, jejunum	+ +
Intestine small, ileum	+ +
Liver	+ +
Hemangioma	
Hemangiosarcoma	
Hepatocellular carcinoma	X X
Hepatocellular carcinoma, multiple	X
Hepatocellular adenoma	X X X X X X X
Hepatocellular adenoma, multiple	X
Liposarcoma, metastatic, uncertain primary site	X
Osteosarcoma, metastatic, bone	X
Mesentery	+
Pancreas	+ +
Salivary glands	+ +
Stomach, forestomach	+ +
Squamous cell papilloma	X
Stomach, glandular	+ +
Tooth	+
Cardiovascular System	
Blood vessel	+ +
Heart	+ +
Liposarcoma, metastatic, uncertain primary site	X
Endocrine System	
Adrenal cortex	+ +
Capsule, adenoma	X
Adrenal medulla	+ +
Islets, pancreatic	+ +
Adenoma	X
Parathyroid gland	+ + + + + M M + + + + + + + + + + + + + + + + + +
Pituitary gland	+ +
Thyroid gland	+ +
Bilateral, follicular cell, adenoma	
Unilateral, follicular cell, adenoma	X X X
General Body System	
None	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-Methylimidazole: 2,500 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	732	
Carcass ID Number	20106	20212	20213	20220	20224	20226	20234	20239	21804	21901	21903	21905	21909	22002	22101	22115	22119	22212	22223	22235	22295	22333	22336	22348	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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
Hemangioma															X										1
Hemangiosarcoma																X			X						2
Hepatocellular carcinoma					X			X						X											5
Hepatocellular carcinoma, multiple																									1
Hepatocellular adenoma				X	X	X					X						X	X	X	X			X		15
Hepatocellular adenoma, multiple									X															X	3
Liposarcoma, metastatic, uncertain primary site																									1
Osteosarcoma, metastatic, bone																									1
Mesentery							+																		2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																									1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																									1
Cardiovascular System																									
Blood vessel																								+	3
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liposarcoma, metastatic, uncertain primary site																									1
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma																									1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																									1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, follicular cell, adenoma							X																		1
Unilateral, follicular cell, adenoma		X			X																	X			6
General Body System																									
None																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-Methylimidazole: 2,500 ppm

Number of Days on Study	0 0 0 0 5 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	0 0 0 2 3 5 5 9 9 0 2 2 2 2 2 2 2 2 2 2 2 2 3 3
	7 7 7 1 1 4 6 3 5 1 9 9 9 9 9 9 9 9 9 9 9 9 9 0
Carcass ID Number	1 2 2 2 1 2 2 2 2 2 1 1 1 1 1 2 2 2 2 2 2 2 2 1 1
	8 0 3 0 8 1 0 3 0 3 8 8 9 9 1 0 0 1 2 3 3 3 4 8 9
	2 4 1 9 9 7 3 5 1 0 3 6 2 7 8 5 7 0 7 2 7 8 0 8 6
Genital System	
Epididymis	+ +
Penis	+
Preputial gland	+ +
Prostate	+ +
Seminal vesicle	+ +
Testes	+ +
Interstitial cell, adenoma	
Hematopoietic System	
Bone marrow	+ +
Lymph node	+
Inguinal, osteosarcoma, metastatic, bone	X
Mediastinal, osteosarcoma, metastatic, bone	X
Lymph node, mandibular	+ +
Lymph node, mesenteric	+ + + M + + + M + + + + + + + + + + + + + + + + +
Spleen	+ +
Thymus	+ + + + I + + + + + + + + + + + M M + + + + + + +
Osteosarcoma, metastatic, bone	X
Integumentary System	
Mammary gland	+ +
Skin	+ +
Musculoskeletal System	
Bone	+ +
Liposarcoma, metastatic, uncertain primary site	X
Osteosarcoma	X
Skeletal muscle	+ +
Liposarcoma, metastatic, uncertain primary site	X
Nervous System	
Brain	+ +
Peripheral nerve	+
Spinal cord	+
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	X X X
Alveolar/bronchiolar adenoma, multiple	
Alveolar/bronchiolar carcinoma	X
Alveolar/bronchiolar carcinoma, multiple	X
Hemangiosarcoma, metastatic, liver	
Hepatocellular carcinoma, metastatic, liver	X
Liposarcoma, metastatic, uncertain primary site	X
Osteosarcoma, metastatic, bone	X
Nose	+ +
Trachea	+ +

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-Methylimidazole: 2,500 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	732	
Carcass ID Number	20106	21202	21303	22200	22304	22406	22643	23309	18404	19111	19913	19955	19992	20211	21159	21191	22201	22223	22235	22299	23313	23366	23228			Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma						X																				1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										1
Inguinal, osteosarcoma, metastatic, bone																										1
Mediastinal, osteosarcoma, metastatic, bone																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Osteosarcoma, metastatic, bone																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liposarcoma, metastatic, uncertain primary site																										1
Osteosarcoma																										1
Skeletal muscle																										2
Liposarcoma, metastatic, uncertain primary site																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X																							4
Alveolar/bronchiolar adenoma, multiple																		X								1
Alveolar/bronchiolar carcinoma					X			X				X														4
Alveolar/bronchiolar carcinoma, multiple																										1
Hemangiosarcoma, metastatic, liver																X										1
Hepatocellular carcinoma, metastatic, liver														X												2
Liposarcoma, metastatic, uncertain primary site																										1
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-Methylimidazole: 2,500 ppm

Number of Days on Study	0 0 0 0 5 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	0 0 0 2 3 5 5 9 9 0 2 2 2 2 2 2 2 2 2 2 2 2 3 3
	7 7 7 1 1 4 6 3 5 1 9 9 9 9 9 9 9 9 9 9 9 9 0 0
Carcass ID Number	1 2 2 2 1 2 2 2 2 2 1 1 1 1 1 2 2 2 2 2 2 2 2 1 1
	8 0 3 0 8 1 0 3 0 3 8 8 9 9 9 0 0 1 2 3 3 3 4 8 9
	2 4 1 9 9 7 3 5 1 0 3 6 2 7 8 5 7 0 7 2 7 8 0 8 6
Special Senses System	
Eye	+ +
Harderian gland	+ +
Adenoma	X
Carcinoma	
Urinary System	
Kidney	+ +
Liposarcoma, metastatic, uncertain primary site	X
Renal tubule, adenoma	
Urethra	+
Urinary bladder	+ +
Systemic Lesions	
Multiple organs	+ +
Lymphoma malignant	X

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 2-Methylimidazole: 2,500 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	20006	20212	20313	22020	22204	22406	23404	23909	18404	19901	19913	19935	19995	20202	21101	21155	21199	21221	22223	22323	22355	22429	23303	23336	23228	Total Tissues/ Tumors
Special Senses System																											
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma											X														X	3
Carcinoma	X																							X		2
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liposarcoma, metastatic, uncertain primary site																										1
Renal tubule, adenoma																			X							1
Urethra																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																										1

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Harderian Gland: Adenoma				
Overall rate[a]	9/50 (18%)	6/50 (12%)	5/50 (10%)	3/50 (6%)
Adjusted rate[b]	19.0%	12.4%	12.0%	6.7%
Terminal rate[c]	9/43 (21%)	6/46 (13%)	4/36 (11%)	2/40 (5%)
First incidence (days)	729 (T)	729 (T)	654	531
Poly-3 test[d]	P=0.060N	P=0.271N	P=0.271N	P=0.072N
Harderian Gland: Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	2.1%	6.2%	2.4%	4.5%
Terminal rate	0/43 (0%)	3/46 (7%)	0/36 (0%)	2/40 (5%)
First incidence (days)	657	729 (T)	623	729 (T)
Poly-3 test	P=0.485	P=0.312	P=0.730	P=0.475
Harderian Gland: Adenoma or Carcinoma				
Overall rate	10/50 (20%)	9/50 (18%)	6/50 (12%)	5/50 (10%)
Adjusted rate	21.0%	18.5%	14.3%	11.1%
Terminal rate	9/43 (21%)	9/46 (20%)	4/36 (11%)	4/40 (10%)
First incidence (days)	657	729 (T)	623	531
Poly-3 test	P=0.108N	P=0.482N	P=0.292N	P=0.157N
Liver: Hepatocellular Adenoma				
Overall rate	7/50 (14%)	14/50 (28%)	13/50 (26%)	18/50 (36%)
Adjusted rate	14.8%	28.8%	30.8%	40.3%
Terminal rate	7/43 (16%)	14/46 (30%)	10/36 (28%)	17/40 (43%)
First incidence (days)	729 (T)	729 (T)	611	656
Poly-3 test	P=0.006	P=0.077	P=0.058	P=0.005
Liver: Hepatocellular Carcinoma				
Overall rate	4/50 (8%)	8/50 (16%)	14/50 (28%)	6/50 (12%)
Adjusted rate	8.4%	16.3%	32.7%	13.4%
Terminal rate	3/43 (7%)	5/46 (11%)	9/36 (25%)	4/40 (10%)
First incidence (days)	611	657	531	656
Poly-3 test	P=0.261	P=0.190	P=0.003	P=0.330
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	10/50 (20%)	22/50 (44%)	22/50 (44%)	22/50 (44%)
Adjusted rate	20.9%	44.9%	50.9%	49.0%
Terminal rate	9/43 (21%)	19/46 (41%)	16/36 (44%)	20/40 (50%)
First incidence (days)	611	657	531	656
Poly-3 test	P=0.007	P=0.009	P=0.002	P=0.003
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	11/50 (22%)	7/50 (14%)	6/50 (12%)	5/50 (10%)
Adjusted rate	23.0%	14.4%	14.5%	11.3%
Terminal rate	9/43 (21%)	7/46 (15%)	6/36 (17%)	5/40 (13%)
First incidence (days)	657	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.103N	P=0.208N	P=0.229N	P=0.113N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	6/50 (12%)	3/50 (6%)	5/50 (10%)	5/50 (10%)
Adjusted rate	12.7%	6.2%	12.0%	11.3%
Terminal rate	6/43 (14%)	3/46 (7%)	4/36 (11%)	5/40 (13%)
First incidence (days)	729 (T)	729 (T)	654	729 (T)
Poly-3 test	P=0.521	P=0.231N	P=0.589N	P=0.545N

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	14/50 (28%)	9/50 (18%)	10/50 (20%)	9/50 (18%)
Adjusted rate	29.2%	18.5%	24.0%	20.3%
Terminal rate	12/43 (28%)	9/46 (20%)	9/36 (25%)	9/40 (23%)
First incidence (days)	657	729 (T)	654	729 (T)
Poly-3 test	P=0.264N	P=0.160N	P=0.376N	P=0.226N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	7/50 (14%)
Adjusted rate	0.0%	2.1%	0.0%	15.8%
Terminal rate	0/43 (0%)	0/46 (0%)	0/36 (0%)	7/40 (18%)
First incidence (days)	—[e]	657	—[f]	729 (T)
Poly-3 test	P<0.001	P=0.506	—	P=0.006
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.2%	2.1%	2.4%	6.8%
Terminal rate	1/43 (2%)	0/46 (0%)	1/36 (3%)	3/40 (8%)
First incidence (days)	668	726	729 (T)	729 (T)
Poly-3 test	P=0.294	P=0.493N	P=0.548N	P=0.468
All Organs: Malignant Lymphoma				
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	4.2%	6.2%	0.0%	2.3%
Terminal rate	2/43 (5%)	3/46 (7%)	0/36 (0%)	1/40 (3%)
First incidence (days)	729 (T)	729 (T)	—	729 (T)
Poly-3 test	P=0.266N	P=0.511	P=0.269N	P=0.523N
All Organs: Benign Neoplasms				
Overall rate	23/50 (46%)	25/50 (50%)	24/50 (48%)	30/50 (60%)
Adjusted rate	47.9%	51.2%	56.4%	66.0%
Terminal rate	20/43 (47%)	24/46 (52%)	20/36 (56%)	27/40 (68%)
First incidence (days)	657	657	611	531
Poly-3 test	P=0.037	P=0.451	P=0.273	P=0.057
All Organs: Malignant Neoplasms				
Overall rate	20/50 (40%)	16/50 (32%)	21/50 (42%)	14/50 (28%)
Adjusted rate	41.0%	32.7%	48.0%	31.1%
Terminal rate	15/43 (35%)	13/46 (28%)	14/36 (39%)	11/40 (28%)
First incidence (days)	611	657	512	656
Poly-3 test	P=0.291N	P=0.260N	P=0.322	P=0.218N
All Organs: Benign or Malignant Neoplasms				
Overall rate	32/50 (64%)	34/50 (68%)	34/50 (68%)	38/50 (76%)
Adjusted rate	65.6%	69.4%	77.0%	83.3%
Terminal rate	27/43 (63%)	31/46 (67%)	26/36 (72%)	34/40 (85%)
First incidence (days)	611	657	512	531
Poly-3 test	P=0.023	P=0.428	P=0.164	P=0.040

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, 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 C4a
Historical Incidence of Thyroid Gland (Follicular Cell) Neoplasms in Control Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Feed Studies			
trans-Cinnamaldehyde	0/50	0/50	0/50
Citral	1/50	0/50	1/50
p,p'-Dichlorodiphenyl sulfone	1/50	0/50	1/50
2-Methylimidazole	0/50	0/50	0/50
o-Nitrotoluene	1/59	0/59	1/59
p-Nitrotoluene	0/50	0/50	0/50
Overall Historical Incidence: Feed Studies			
Total (%)	3/309 (1.0%)	0/309 (0%)	3/309 (1.0%)
Mean ± standard deviation	1.0% ± 1.1%		1.0% ± 1.1%
Range	0%-2%		0%-2%
Overall Historical Incidence			
Total (%)	8/1,143 (0.7%)	2/1,143 (0.2%)	10/1,143 (0.9%)
Mean ± standard deviation	0.7% ± 0.9%	0.2% ± 0.6%	0.9% ± 1.0%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of March 3, 2003

TABLE C4b
Historical Incidence of Liver Neoplasms in Control Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence: Feed Studies			
trans-Cinnamaldehyde	5/50	6/50	10/50
Citral	10/50	4/50	13/50
p,p'-Dichlorodiphenyl sulfone	6/50	9/50	15/50
2-Methylimidazole	7/50	4/50	10/50
o-Nitrotoluene	18/60	12/60	27/60
p-Nitrotoluene	14/50	8/50	20/50
Overall Historical Incidence: Feed Studies			
Total (%)	60/310 (19.4%)	43/310 (13.9%)	95/310 (30.7%)
Mean ± standard deviation	19.0% ± 8.5%	13.7% ± 5.1%	30.2% ± 10.4%
Range	10%-30%	8%-20%	20%-45%
Overall Historical Incidence			
Total (%)	357/1,159 (30.8%)	247/1,159 (21.3%)	543/1,159 (46.9%)
Mean ± standard deviation	32.2% ± 10.5%	22.3% ± 8.7%	48.9% ± 14.5%
Range	12%-46%	8%-46%	20%-72%

[a] Data as of March 3, 2003

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
6-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	4	1	5	5
Natural deaths	3	3	9	5
Survivors				
Terminal sacrifice	43	46	36	40
Animals examined microscopically	60	60	60	60
6-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation			1 (10%)	
Infiltration cellular, mixed cell	2 (20%)	3 (30%)	6 (60%)	2 (20%)
Necrosis, focal		1 (10%)		
Hepatocyte, karyomegaly				5 (50%)
Hepatocyte, vacuolization cytoplasmic				4 (40%)
Mesentery				(1)
Fat, necrosis				1 (100%)
Cardiovascular System				
Heart	(10)	(10)	(10)	(10)
Valve, hypertrophy		1 (10%)		
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule	2 (20%)			1 (10%)
Hyperplasia, focal				1 (10%)
Hypertrophy, focal		1 (10%)		
Thyroid gland	(10)	(10)	(10)	(10)
Follicle, cyst				1 (10%)
Follicular cell, hypertrophy		5 (50%)	9 (90%)	10 (100%)
Genital System				
Preputial gland	(10)	(10)	(10)	(10)
Cyst				1 (10%)
Inflammation, chronic			1 (10%)	
Hematopoietic System				
Bone marrow	(10)	(10)	(10)	(10)
Hyperplasia	1 (10%)		2 (20%)	5 (50%)
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		5 (50%)	10 (100%)	10 (100%)
Pigmentation		1 (10%)	10 (100%)	10 (100%)
Lymphoid follicle, atrophy			5 (50%)	10 (100%)
Lymphoid follicle, hyperplasia	1 (10%)			
Thymus	(10)	(10)	(10)	(10)
Atrophy		1 (10%)		

[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 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
6-Month Interim Evaluation (continued)				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hemorrhage			1 (10%)	1 (10%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Cyst		1 (10%)		
Hydronephrosis	1 (10%)	1 (10%)		
Nephropathy	1 (10%)		1 (10%)	2 (20%)
Renal tubule, pigmentation			1 (10%)	10 (100%)
Urinary bladder	(10)	(10)	(10)	(10)
Transitional epithelium, hyperplasia	1 (10%)			
Systems Examined at 6 Months with No Neoplasms Observed				
General Body System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
2-Year Study				
Alimentary System				
Intestine large, cecum	(49)	(49)	(47)	(50)
Edema	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Intestine small, duodenum	(49)	(49)	(45)	(50)
Epithelium, hyperplasia				1 (2%)
Intestine small, jejunum	(50)	(49)	(46)	(50)
Hyperplasia, lymphoid		1 (2%)	1 (2%)	
Infiltration cellular, polymorphonuclear			1 (2%)	
Intestine small, ileum	(49)	(50)	(45)	(49)
Hyperplasia, lymphoid		1 (2%)		1 (2%)
Infiltration cellular, polymorphonuclear				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)		1 (2%)	
Basophilic focus	2 (4%)	3 (6%)	1 (2%)	3 (6%)
Clear cell focus	6 (12%)	9 (18%)	6 (12%)	8 (16%)
Cyst		1 (2%)		
Eosinophilic focus	3 (6%)	3 (6%)	2 (4%)	6 (12%)
Hematopoietic cell proliferation	1 (2%)		2 (4%)	2 (4%)
Hepatodiaphragmatic nodule				1 (2%)
Infarct	1 (2%)			
Infiltration cellular, mixed cell	5 (10%)	7 (14%)	7 (14%)	7 (14%)
Mixed cell focus	4 (8%)	3 (6%)	4 (8%)	4 (8%)
Necrosis, diffuse				1 (2%)
Necrosis, focal	2 (4%)	6 (12%)	2 (4%)	6 (12%)
Tension lipidosis		2 (4%)	1 (2%)	
Hepatocyte, cytoplasmic alteration			11 (22%)	37 (74%)
Hepatocyte, karyomegaly			10 (20%)	29 (58%)
Hepatocyte, vacuolization cytoplasmic		1 (2%)		1 (2%)
Kupffer cell, pigmentation		1 (2%)	1 (2%)	19 (38%)

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Mesentery	(6)	(2)	(5)	(2)
Angiectasis		1 (50%)		
Inflammation, chronic		1 (50%)		
Fat, necrosis	4 (67%)	1 (50%)	3 (60%)	2 (100%)
Pancreas	(50)	(50)	(49)	(50)
Atrophy	1 (2%)		1 (2%)	
Cyst	2 (4%)		3 (6%)	2 (4%)
Acinus, cytoplasmic alteration			2 (4%)	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	2 (4%)	2 (4%)		
Stomach, forestomach	(50)	(49)	(50)	(50)
Diverticulum		2 (4%)		
Inflammation, chronic active	1 (2%)	1 (2%)		
Ulcer		1 (2%)		1 (2%)
Epithelium, hyperplasia	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Stomach, glandular	(50)	(49)	(49)	(50)
Glands, dysplasia	1 (2%)			
Tooth				(1)
Inflammation, chronic				1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	1 (2%)			1 (2%)
Perivascular, inflammation, chronic	3 (6%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	2 (4%)	6 (12%)	8 (16%)	6 (12%)
Hyperplasia, focal	2 (4%)	4 (8%)	3 (6%)	1 (2%)
Hypertrophy, focal	13 (26%)	18 (36%)	10 (20%)	10 (20%)
Capsule, hyperplasia	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia			2 (4%)	
Islets, pancreatic	(50)	(50)	(49)	(50)
Hyperplasia		2 (4%)		1 (2%)
Parathyroid gland	(49)	(47)	(45)	(48)
Cyst	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Pituitary gland	(49)	(48)	(48)	(50)
Pars distalis, cyst	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Pars distalis, hyperplasia, focal		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Degeneration, cystic	10 (20%)	2 (4%)	3 (6%)	4 (8%)
Follicular cell, hyperplasia		2 (4%)	3 (6%)	33 (66%)
Follicular cell, hypertrophy	1 (2%)		6 (12%)	25 (50%)
General Body System				
None				

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
2-Year Study (continued)				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Granuloma sperm			2 (4%)	5 (10%)
Hyperplasia, lymphoid			2 (4%)	1 (2%)
Hypospermia			1 (2%)	
Inflammation, chronic active	1 (2%)	3 (6%)	7 (14%)	8 (16%)
Penis	(1)		(1)	(1)
Angiectasis			1 (100%)	1 (100%)
Preputial gland	(50)	(50)	(50)	(50)
Cyst	18 (36%)	23 (46%)	16 (32%)	21 (42%)
Inflammation, chronic	14 (28%)	10 (20%)	14 (28%)	7 (14%)
Prostate	(49)	(50)	(49)	(50)
Inflammation, chronic				2 (4%)
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, atrophy	1 (2%)	4 (8%)	8 (16%)	14 (28%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	10 (20%)	20 (40%)	42 (84%)
Lymph node	(2)	(3)		(1)
Bronchial, hyperplasia, lymphoid		1 (33%)		
Lymph node, mandibular	(48)	(46)	(45)	(50)
Atrophy	1 (2%)		2 (4%)	4 (8%)
Ectasia			1 (2%)	
Hyperplasia, lymphoid	10 (21%)	14 (30%)	9 (20%)	13 (26%)
Pigmentation	8 (17%)	6 (13%)	9 (20%)	6 (12%)
Lymph node, mesenteric	(47)	(47)	(46)	(48)
Angiectasis				1 (2%)
Atrophy	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Hemorrhage	4 (9%)	2 (4%)	2 (4%)	
Hyperplasia, lymphoid	7 (15%)	7 (15%)	15 (33%)	10 (21%)
Necrosis	1 (2%)			1 (2%)
Spleen	(50)	(50)	(49)	(50)
Angiectasis				1 (2%)
Atrophy		1 (2%)	4 (8%)	3 (6%)
Hematopoietic cell proliferation	10 (20%)	21 (42%)	38 (78%)	45 (90%)
Pigmentation	1 (2%)	16 (32%)	33 (67%)	43 (86%)
Lymphoid follicle, atrophy		4 (8%)	14 (29%)	30 (60%)
Lymphoid follicle, hyperplasia	5 (10%)	2 (4%)	2 (4%)	
Thymus	(47)	(46)	(46)	(47)
Atrophy	7 (15%)	5 (11%)	9 (20%)	8 (17%)
Cyst	1 (2%)		1 (2%)	1 (2%)
Hyperplasia, lymphoid		1 (2%)		
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Inflammation, chronic		1 (2%)		

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
2-Year Study (continued)				
Musculoskeletal System				
Skeletal muscle	(1)	(1)		(2)
Atrophy				1 (50%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression		1 (2%)		
Peripheral nerve	(1)			(1)
Atrophy				1 (100%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Edema	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Hemorrhage	4 (8%)	8 (16%)	4 (8%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)	2 (4%)	4 (8%)
Infiltration cellular, histiocyte	4 (8%)	4 (8%)	4 (8%)	2 (4%)
Inflammation, chronic			1 (2%)	2 (4%)
Metaplasia, osseous			2 (4%)	
Thrombosis	1 (2%)			1 (2%)
Alveolar epithelium, hyperplasia	5 (10%)	3 (6%)	1 (2%)	3 (6%)
Alveolar epithelium, hyperplasia, multifocal	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Foreign body	1 (2%)	3 (6%)	3 (6%)	2 (4%)
Inflammation, chronic	1 (2%)	4 (8%)	3 (6%)	2 (4%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract			1 (2%)	1 (2%)
Inflammation, chronic	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Cornea, hyperplasia	1 (2%)		1 (2%)	
Harderian gland	(50)	(50)	(48)	(50)
Hyperplasia, focal	1 (2%)		2 (4%)	
Inflammation, chronic	3 (6%)	4 (8%)		2 (4%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	13 (26%)	11 (22%)	12 (24%)	19 (38%)
Hydronephrosis	1 (2%)			
Hyperplasia, lymphoid	1 (2%)	3 (6%)	3 (6%)	3 (6%)
Infarct	6 (12%)	6 (12%)	6 (12%)	1 (2%)
Inflammation, suppurative				1 (2%)
Metaplasia, osseous	5 (10%)	3 (6%)	2 (4%)	3 (6%)
Nephropathy	34 (68%)	43 (86%)	32 (64%)	31 (62%)
Papilla, necrosis				1 (2%)
Renal tubule, hyperplasia	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Renal tubule, pigmentation	1 (2%)		2 (4%)	45 (90%)
Urethra				(1)
Angiectasis				1 (100%)
Inflammation, suppurative				1 (100%)
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, hyperplasia				1 (2%)

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

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
6-Month interim evaluation	10	10	10	10
Early deaths				
Other		1	1	
Moribund	2	3	4	4
Natural deaths	2	3	2	1
Survivors				
Died last week of study		1	1	
Terminal sacrifice	46	42	42	45
Animals examined microscopically	60	59	59	60
Systems Examined at 6 Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Intestine large, cecum	(50)	(48)	(48)	(50)
Intestine small, duodenum	(50)	(49)	(48)	(49)
Carcinoma				1 (2%)
Polyp adenomatous		1 (2%)		
Intestine small, jejunum	(50)	(46)	(48)	(49)
Serosa, histiocytic sarcoma				1 (2%)
Liver	(50)	(49)	(49)	(50)
Hepatocellular carcinoma	2 (4%)			
Hepatocellular adenoma	2 (4%)	3 (6%)	6 (12%)	9 (18%)
Hepatocellular adenoma, multiple	1 (2%)	1 (2%)		1 (2%)
Histiocytic sarcoma			1 (2%)	1 (2%)
Pancreas	(50)	(49)	(48)	(50)
Histiocytic sarcoma				1 (2%)
Salivary glands	(50)	(49)	(49)	(49)
Stomach, forestomach	(50)	(49)	(49)	(50)
Squamous cell papilloma	1 (2%)	1 (2%)		2 (4%)
Squamous cell papilloma, multiple	1 (2%)			
Stomach, glandular	(50)	(49)	(48)	(50)
Cardiovascular System				
Heart	(50)	(49)	(49)	(50)

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
2-Year Study (continued)				
Endocrine System				
Adrenal medulla	(50)	(49)	(49)	(50)
Pheochromocytoma malignant			1 (2%)	
Islets, pancreatic	(50)	(48)	(48)	(50)
Adenoma		1 (2%)		
Pituitary gland	(50)	(49)	(47)	(49)
Pars distalis, adenoma	1 (2%)	1 (2%)		
Thyroid gland	(49)	(48)	(48)	(50)
Unilateral, follicular cell, adenoma	1 (2%)			1 (2%)
General Body System				
None				
Genital System				
Ovary	(50)	(49)	(49)	(48)
Cystadenoma		1 (2%)		
Granulosa-theca tumor benign		1 (2%)		
Histiocytic sarcoma				1 (2%)
Luteoma			1 (2%)	1 (2%)
Uterus	(50)	(49)	(49)	(50)
Hemangiosarcoma	1 (2%)			
Histiocytic sarcoma				1 (2%)
Polyp stromal			2 (4%)	
Sarcoma stromal	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(49)	(48)	(50)
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma			1 (2%)	
Lymph node	(12)	(6)	(8)	(9)
Lymph node, mandibular	(48)	(48)	(46)	(48)
Lymph node, mesenteric	(48)	(48)	(47)	(48)
Histiocytic sarcoma				1 (2%)
Spleen	(50)	(49)	(49)	(50)
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma			1 (2%)	
Thymus	(50)	(48)	(48)	(50)
Integumentary System				
Mammary gland	(49)	(49)	(49)	(50)
Carcinoma	1 (2%)			2 (4%)
Skin	(50)	(49)	(49)	(50)
Basal cell carcinoma			1 (2%)	
Keratoacanthoma		1 (2%)		
Subcutaneous tissue, fibrosarcoma			3 (6%)	1 (2%)
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Subcutaneous tissue, hemangiosarcoma	1 (2%)	1 (2%)		

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
2-Year Study (continued)				
Musculoskeletal System				
Bone	(50)	(49)	(49)	(50)
Osteoma				1 (2%)
Osteosarcoma	1 (2%)			
Skeletal muscle		(1)	(1)	
Nervous System				
Brain	(50)	(49)	(49)	(50)
Cranial nerve, schwannoma malignant			1 (2%)	
Respiratory System				
Lung	(50)	(49)	(49)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	2 (4%)		1 (2%)
Alveolar/bronchiolar carcinoma		2 (4%)		
Histiocytic sarcoma			1 (2%)	
Special Senses System				
Harderian gland	(50)	(49)	(49)	(50)
Adenoma	3 (6%)	2 (4%)	6 (12%)	6 (12%)
Carcinoma	1 (2%)		1 (2%)	1 (2%)
Urinary System				
Kidney	(50)	(49)	(49)	(50)
Urinary bladder	(50)	(49)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(49)	(49)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Lymphoma malignant	6 (12%)	8 (16%)	6 (12%)	8 (16%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	23	20	23	29
Total primary neoplasms	28	28	30	36
Total animals with benign neoplasms	13	12	14	20
Total benign neoplasms	14	15	15	22
Total animals with malignant neoplasms	12	12	15	14
Total malignant neoplasms	14	13	15	14

[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 2-Methylimidazole: 0 ppm

Number of Days on Study	4 0 2	5 3 3	6 5 1	7 2 3	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3
Carcass ID Number	2 6 4 9	2 4 5 5	2 7 5 5	2 6 5 8	2 5 6 6	2 5 5 7	2 5 5 8	2 5 5 9	2 6 6 0	2 6 6 1	2 6 6 2	2 6 6 3	2 6 6 4	2 6 6 5	2 6 7 6	2 7 7 7	2 7 7 8	2 7 7 9	2 8 8 0	2 8 8 6	2 8 8 7	2 8 8 8	2 8 8 9	2 9 9 0	2 4 4 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma				X																					
Hepatocellular adenoma				X			X																		
Hepatocellular adenoma, multiple																									
Mesentery					+																				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma											X														
Squamous cell papilloma, multiple																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																								X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Unilateral, follicular cell, adenoma																	X								
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Sarcoma stromal										X															

+: 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 2-Methylimidazole: 0 ppm

Number of Days on Study	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 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 4 2	2 4 3	2 4 4	2 6 6	2 6 7	2 7 0	2 8 1	2 8 2	2 8 3	2 8 4	2 8 5	2 4 6	2 4 7	2 4 8	2 4 9	2 5 0	2 7 1	2 7 2	2 7 3	2 7 4	2 9 6	2 9 7	2 9 8	2 9 9	3 0 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																		X								2
Hepatocellular adenoma																										2
Hepatocellular adenoma, multiple																									X	1
Mesentery						+													+							3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Squamous cell papilloma, multiple						X																				1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Unilateral, follicular cell, adenoma																										1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																									X	1
Sarcoma stromal																										1

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

Number of Days on Study	4 0 2	5 3 3	6 5 1	7 2 3	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3
Carcass ID Number	2 6 6 9	2 4 4 5	2 7 5 5	2 6 6 8	2 5 6 6	2 5 5 7	2 5 5 8	2 5 5 9	2 6 6 0	2 6 6 1	2 6 6 2	2 6 6 3	2 6 6 4	2 6 6 5	2 6 7 6	2 7 7 7	2 7 7 8	2 7 7 9	2 8 8 0	2 8 8 6	2 8 8 7	2 8 8 8	2 8 8 9	2 9 9 0	2 4 4 1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph mode					+				+	+			+	+					+			+			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	M
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma										X															
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma		X																							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma	X																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									X
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X																
Carcinoma																									
Lacrimal gland																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant					X														X						

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

Number of Days on Study	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 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 4 2	2 4 3	2 4 4	2 6 6	2 6 7	2 7 0	2 8 1	2 8 2	2 8 3	2 8 4	2 8 5	2 4 6	2 4 7	2 4 8	2 4 9	2 5 0	2 7 1	2 7 2	2 7 3	2 7 4	2 9 6	2 9 7	2 9 8	2 9 9	3 0 0	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node							+							+			+			+	+					12
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma												X								X					X	4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X								X															3
Carcinoma							X																			1
Lacrimal gland						+																				1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant	X						X										X			X						6

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

Number of Days on Study	400	600	620	639	688	720	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	733	733
Carcass ID Number	32828	32255	32277	32399	34466	31060	30667	30078	30088	30099	31011	31123	31134	31144	31155	31188	32266	32299	33303	33366	33377	33388	34040	32212
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																X								
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma					X														X					
Hepatocellular adenoma, multiple															X									
Mesentery									+						+									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																		X						
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular Systems																								
Blood vessel																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X																					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																								
None																								
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																								
Granulosa-theca tumor benign																		X						
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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

Number of Days on Study	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 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	3 2 3	3 2 4	3 4 7	3 4 8	3 4 9	3 5 0	3 5 6	3 5 7	3 5 8	3 5 9	3 6 0	3 0 1	3 0 2	3 0 3	3 0 4	3 0 5	3 0 6	3 1 7	3 1 9	3 3 1	3 3 2	3 3 3	3 3 4	3 3 5	Total Tissues/ Tumors	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	47	
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48	
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	I	+	+	46	
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48	
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Polp adenomatous																									1	
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+		46	
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Hepatocellular adenoma																		X							3	
Hepatocellular adenoma, multiple																									1	
Mesentery					+																		+		4	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Squamous cell papilloma																									1	
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Cardiovascular System																										
Blood vessel												+						+							2	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Islets, pancreatic	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48	
Adenoma								X																	1	
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	46	
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Pars distalis, adenoma																									1	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	48	
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Cystadenoma														X											1	
Granulosa-theca tumor benign																									1	
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	

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

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

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

Number of Days on Study	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 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	3 2 3	3 2 4	3 4 7	3 4 8	3 4 9	3 5 0	3 5 6	3 5 7	3 5 8	3 5 9	3 6 0	3 0 1	3 0 2	3 0 3	3 0 4	3 0 5	3 1 6	3 1 7	3 1 9	3 3 1	3 3 2	3 3 3	3 3 4	3 3 5	Total Tissues/ Tumors
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																									1
Lymph node		+													+	+									6
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																									1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	48
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Keratoacanthoma																									1
Subcutaneous tissue, hemangiosarcoma																									1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skeletal muscle																									1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma																	X								2
Alveolar/bronchiolar carcinoma																									2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma								X											X						2
Lacrimal gland														+											1
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant			X			X						X			X	X						X			8

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

Number of Days on Study	1 2 8	5 5 5	6 2 3	6 5 8	6 9 3	7 2 0	7 2 9	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3
Carcass ID Number	3 7 5	3 8 0	4 0 2	4 1 1	4 1 4	3 6 7	4 1 2	3 8 1	3 8 2	3 8 3	3 8 4	3 8 5	4 0 6	4 0 7	4 0 8	4 0 9	4 1 0	4 1 3	4 1 5	4 1 6	4 1 7	4 1 8	4 1 9	4 2 0	3 9 6
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma									X											X					
Histiocytic sarcoma						X																			
Mesentery	+																								
Pancreas	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel																						+			
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant							X																		
Islets, pancreatic	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Luteoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal			X				X																		
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Lymph node		+		+							+					+	+					+			
Lymph node, mandibular	+	+	+	+	I	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	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	3 9 7	3 9 8	3 9 9	4 0 0	4 0 3	4 0 4	4 0 5	3 6 1	3 6 2	3 6 3	3 6 4	3 6 5	3 6 6	3 6 8	3 6 9	3 7 0	3 7 1	3 7 2	3 7 3	3 7 4	3 7 6	3 7 7	3 7 8	3 7 9	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma	X		X					X								X									6
Histiocytic sarcoma																									1
Mesentery											+			+			+	+	+						6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																									
Blood vessel																+									2
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant																									1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Luteoma						X																			1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp stromal																									2
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	48
Histiocytic sarcoma																									1
Lymph node				+								+													8
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																									1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

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

Number of Days on Study	1 2 8	5 5 5	6 2 3	6 5 8	6 9 3	7 2 0	7 2 9	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3
Carcass ID Number	3 7 5	3 8 0	4 0 2	4 1 1	4 1 4	3 6 7	4 1 2	3 8 1	3 8 2	3 8 3	3 8 4	3 8 5	4 0 6	4 0 7	4 0 8	4 0 9	4 1 0	4 1 3	4 1 5	4 1 6	4 1 7	4 1 8	4 1 9	4 2 0	3 9 6
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																										
Subcutaneous tissue, fibrosarcoma					X			X														X				
Subcutaneous tissue, fibrous histiocytoma																			X							
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																									
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranial nerve, schwannoma malignant																								X		
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Ear		+																								
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X									X									
Carcinoma			X																							
Lacrimal gland																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																				
Lymphoma malignant				X																						

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

Number of Days on Study	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	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	3 9 7	3 9 8	3 9 9	4 0 0	4 0 3	4 0 4	4 0 5	3 6 1	3 6 2	3 6 3	3 6 4	3 6 5	3 6 6	3 6 8	3 6 9	3 7 0	3 7 1	3 7 2	3 7 3	3 7 4	3 7 6	3 7 7	3 7 8	3 7 9	Total Tissues/ Tumors
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Basal cell carcinoma																				X					1
Subcutaneous tissue, fibrosarcoma																									3
Subcutaneous tissue, fibrous histiocytoma																									1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skeletal muscle																									1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cranial nerve, schwannoma malignant																									1
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																									1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																									
Ear																+									2
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma						X	X							X						X					6
Carcinoma																									1
Lacrimal gland																+									1
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																									1
Lymphoma malignant				X				X		X		X		X											6

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 2-Methylimidazole: 2,500 ppm

Number of Days on Study	5 1 7	6 0 4	6 6 4	7 2 0	7 2 7	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 2	7 3 2	7 3 2	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	4 3 5	4 6 5	4 6 6	4 7 0	4 2 9	4 3 6	4 3 7	4 3 8	4 3 9	4 4 0	4 4 1	4 6 2	4 6 3	4 6 4	4 6 7	4 6 8	4 6 9	4 2 6	4 2 7	4 2 8	4 3 0	4 4 1	4 4 2	4 4 3	4 4 4
Alimentary System																									
Esophagus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma		X																							
Intestine small, jejunum	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serosa, histiocytic sarcoma			X																						
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma								X					X					X					X		X
Hepatocellular adenoma, multiple			X																						
Histiocytic sarcoma			X																						
Mesentery	+		+	+											+										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Salivary glands	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma		X																							
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel											+														
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Unilateral, follicular cell, adenoma																	X								
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Histiocytic sarcoma			X																						
Luteoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 2-Methylimidazole: 2,500 ppm

Number of Days on Study	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	7 3 3	7 3 3	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	4 4 5	4 4 6	4 4 7	4 4 8	4 4 9	4 5 0	4 5 6	4 5 7	4 5 8	4 5 9	4 6 0	4 7 1	4 7 2	4 7 3	4 7 4	4 7 5	4 2 1	4 2 2	4 2 3	4 2 4	4 2 5	4 3 1	4 3 2	4 3 3	4 3 4	Total Tissues/ Tumors
Alimentary System																												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Serosa, histiocytic sarcoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma		X										X							X					X		9
Hepatocellular adenoma, multiple																										1
Histiocytic sarcoma																										1
Mesentery										+						+										6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																						X				2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																												
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																												
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Unilateral, follicular cell, adenoma																										1
General Body System																												
None																												
Genital System																												
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Luteoma																X										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 2-Methylimidazole: 2,500 ppm

Number of Days on Study	5 1 7	6 0 4	6 6 4	7 2 0	7 2 7	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 2	7 3 2	7 3 2	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	4 3 5	4 6 5	4 6 6	4 7 0	4 2 9	4 3 6	4 3 7	4 3 8	4 3 9	4 4 0	4 4 1	4 6 2	4 6 3	4 6 4	4 6 7	4 6 8	4 6 9	4 2 6	4 2 7	4 2 8	4 3 0	4 4 1	4 4 2	4 4 3	4 4 4
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+			+									+				+		+	+		
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma					X																		X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteoma			X																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+																								
Spinal cord	+																								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X									X									
Carcinoma																	X								
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Lymphoma malignant																X				X		X			

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 2-Methylimidazole: 2,500 ppm

Number of Days on Study	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	7 3 3	7 3 3	7 3 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	
Carcass ID Number	4 4 5	4 4 6	4 4 7	4 4 8	4 4 9	4 5 0	4 5 6	4 5 7	4 5 8	4 5 9	4 6 0	4 7 1	4 7 2	4 7 3	4 7 4	4 7 5	4 2 1	4 2 2	4 2 3	4 2 4	4 2 5	4 3 1	4 3 2	4 3 3	4 3 4	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node										+					+										+	9
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma	X			X			X		X																	6
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant			X							X					X					X				X		8

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Harderian Gland: Adenoma				
Overall rate[a]	3/50 (6%)	2/49 (4%)	6/49 (12%)	6/50 (12%)
Adjusted rate[b]	6.2%	4.3%	12.9%	12.3%
Terminal rate[c]	3/46 (7%)	2/43 (5%)	6/43 (14%)	6/45 (13%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test[d]	P=0.113	P=0.514N	P=0.226	P=0.247
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	2/49 (4%)	7/49 (14%)	7/50 (14%)
Adjusted rate	8.3%	4.3%	14.9%	14.4%
Terminal rate	4/46 (9%)	2/43 (5%)	6/43 (14%)	7/45 (16%)
First incidence (days)	729 (T)	729 (T)	623	729 (T)
Poly-3 test	P=0.107	P=0.351N	P=0.247	P=0.267
Liver: Hepatocellular Adenoma				
Overall rate	3/50 (6%)	4/49 (8%)	6/49 (12%)	10/50 (20%)
Adjusted rate	6.2%	8.5%	12.9%	20.5%
Terminal rate	2/46 (4%)	3/43 (7%)	6/43 (14%)	9/45 (20%)
First incidence (days)	723	688	729 (T)	664
Poly-3 test	P=0.015	P=0.485	P=0.226	P=0.037
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	4/50 (8%)	4/49 (8%)	6/49 (12%)	10/50 (20%)
Adjusted rate	8.3%	8.5%	12.9%	20.5%
Terminal rate	3/46 (7%)	3/43 (7%)	6/43 (14%)	9/45 (20%)
First incidence (days)	723	688	729 (T)	664
Poly-3 test	P=0.030	P=0.628	P=0.348	P=0.077
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	2/49 (4%)	0/49 (0%)	1/50 (2%)
Adjusted rate	8.3%	4.2%	0.0%	2.1%
Terminal rate	4/46 (9%)	1/43 (2%)	0/43 (0%)	1/45 (2%)
First incidence (days)	729 (T)	620	—[e]	729 (T)
Poly-3 test	P=0.085N	P=0.348N	P=0.065N	P=0.177N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	4/49 (8%)	0/49 (0%)	1/50 (2%)
Adjusted rate	8.3%	8.4%	0.0%	2.1%
Terminal rate	4/46 (9%)	2/43 (5%)	0/43 (0%)	1/45 (2%)
First incidence (days)	729 (T)	620	—	729 (T)
Poly-3 test	P=0.059N	P=0.635	P=0.065N	P=0.177N
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	0/50 (0%)	0/49 (0%)	3/49 (6%)	1/50 (2%)
Adjusted rate	0.0%	0.0%	6.4%	2.1%
Terminal rate	0/46 (0%)	0/43 (0%)	2/43 (5%)	0/45 (0%)
First incidence (days)	—	—[f]	693	720
Poly-3 test	P=0.254		P=0.113	P=0.502
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Fibrosarcoma				
Overall rate	0/50 (0%)	0/49 (0%)	4/49 (8%)	1/50 (2%)
Adjusted rate	0.0%	0.0%	8.6%	2.1%
Terminal rate	0/46 (0%)	0/43 (0%)	3/43 (7%)	0/45 (0%)
First incidence (days)	—	—	693	720
Poly-3 test	P=0.252	—	P=0.057	P=0.502

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
All Organs: Malignant Lymphoma				
Overall rate	6/50 (12%)	8/49 (16%)	6/49 (12%)	8/50 (16%)
Adjusted rate	12.4%	17.0%	12.8%	16.5%
Terminal rate	6/46 (13%)	7/43 (16%)	5/43 (12%)	8/45 (18%)
First incidence (days)	729 (T)	688	658	729 (T)
Poly-3 test	P=0.403	P=0.367	P=0.600	P=0.393
All Organs: Benign Neoplasms				
Overall rate	13/50 (26%)	12/49 (24%)	14/49 (29%)	20/50 (40%)
Adjusted rate	26.9%	25.3%	29.8%	40.6%
Terminal rate	12/46 (26%)	9/43 (21%)	13/43 (30%)	18/45 (40%)
First incidence (days)	723	620	623	604
Poly-3 test	P=0.058	P=0.521N	P=0.468	P=0.113
All Organs: Malignant Neoplasms				
Overall rate	12/50 (24%)	12/49 (24%)	15/49 (31%)	14/50 (28%)
Adjusted rate	24.1%	25.1%	31.6%	28.4%
Terminal rate	9/46 (20%)	8/43 (19%)	11/43 (26%)	10/45 (22%)
First incidence (days)	402	620	623	604
Poly-3 test	P=0.325	P=0.548	P=0.277	P=0.402
All Organs: Benign or Malignant Neoplasms				
Overall rate	23/50 (46%)	20/49 (41%)	23/49 (47%)	29/50 (58%)
Adjusted rate	46.3%	41.9%	48.5%	58.8%
Terminal rate	20/46 (44%)	16/43 (37%)	19/43 (44%)	25/45 (56%)
First incidence (days)	402	620	623	604
Poly-3 test	P=0.078	P=0.409N	P=0.494	P=0.148

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for 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 D4
Historical Incidence of Liver Neoplasms in Control Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence: Feed Studies			
trans-Cinnamaldehyde	4/50	1/50	4/50
Citral	5/50	1/50	6/50
p,p'-Dichlorodiphenyl sulfone	4/50	3/50	6/50
2-Methylimidazole	3/50	2/50	4/50
o-Nitrotoluene	7/60	2/60	9/60
p-Nitrotoluene	6/49	3/49	8/49
Overall Historical Incidence: Feed Studies			
Total (%)	29/309 (9.4%)	12/309 (3.9%)	37/309 (12.0%)
Mean ± standard deviation	9.3% ± 2.4%	3.9% ± 1.8%	11.9% ± 3.5%
Range	6%-12%	2%-6%	8%-16%
Overall Historical Incidence			
Total (%)	179/1,152 (15.5%)	87/1,152 (7.6%)	250/1,152 (21.7%)
Mean ± standard deviation	16.3% ± 6.6%	8.1% ± 4.2%	22.8% ± 9.4%
Range	8%-29%	3%-16%	8%-40%

[a] Data as of March 3, 2003

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
6-Month interim evaluation	10	10	10	10
Early deaths				
Other		1	1	
Moribund	2	3	4	4
Natural deaths	2	3	2	1
Survivors				
Died last week of study		1	1	
Terminal sacrifice	46	42	42	45
Animals examined microscopically	60	59	59	60
6-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	1 (10%)			
Infiltration cellular, mixed cell	6 (60%)	7 (70%)	3 (30%)	5 (50%)
Necrosis, focal		1 (10%)		
Hepatocyte, vacuolization cytoplasmic		1 (10%)	2 (20%)	4 (40%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule	2 (20%)			1 (10%)
Thyroid gland	(8)	(10)	(10)	(10)
Follicular cell, hypertrophy		8 (80%)	10 (100%)	10 (100%)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Cyst	1 (10%)	1 (10%)		
Uterus	(10)	(10)	(10)	(10)
Hyperplasia, cystic	3 (30%)	1 (10%)	4 (40%)	1 (10%)
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(10)
Pigmentation	2 (20%)	1 (10%)		2 (20%)
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	2 (20%)	5 (50%)	6 (60%)	10 (100%)
Pigmentation	1 (10%)	3 (30%)	7 (70%)	10 (100%)
Lymphoid follicle, atrophy				4 (40%)
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Inflammation, chronic	1 (10%)			
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hyperplasia, lymphoid				1 (10%)

[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 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
6-Month Interim Evaluation (continued)				
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Nephropathy	1 (10%)	1 (10%)		
Systems Examined at 6 Months with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				
2-Year Study				
Alimentary System				
Intestine large, cecum	(50)	(48)	(48)	(50)
Edema	2 (4%)		2 (4%)	2 (4%)
Intestine small, jejunum	(50)	(46)	(48)	(49)
Hyperplasia, lymphoid		1 (2%)		1 (2%)
Peyer's patch, infiltration cellular, polymorphonuclear				1 (2%)
Intestine small, ileum	(47)	(49)	(47)	(48)
Hyperplasia, lymphoid	1 (2%)			
Peyer's patch, infiltration cellular, polymorphonuclear				1 (2%)
Liver	(50)	(49)	(49)	(50)
Angiectasis	1 (2%)			
Basophilic focus		4 (8%)	1 (2%)	1 (2%)
Clear cell focus	1 (2%)		1 (2%)	4 (8%)
Eosinophilic focus			1 (2%)	
Hematopoietic cell proliferation	3 (6%)	3 (6%)	5 (10%)	3 (6%)
Hemorrhage		1 (2%)		
Hepatodiaphragmatic nodule			1 (2%)	2 (4%)
Hyperplasia, lymphoid	3 (6%)	3 (6%)		6 (12%)
Infarct		1 (2%)		
Infiltration cellular, mixed cell	13 (26%)	5 (10%)	12 (24%)	10 (20%)
Mixed cell focus	4 (8%)	1 (2%)	3 (6%)	
Necrosis, focal	5 (10%)	2 (4%)	3 (6%)	4 (8%)
Tension lipidosis			2 (4%)	
Bile duct, hyperplasia		1 (2%)		
Hepatocyte, vacuolization cytoplasmic			1 (2%)	
Kupffer cell, pigmentation		2 (4%)		
Mesentery	(3)	(4)	(6)	(6)
Inflammation, chronic	1 (33%)	1 (25%)		
Fat, angiectasis			1 (17%)	1 (17%)
Fat, necrosis	2 (67%)	3 (75%)	6 (100%)	6 (100%)
Pancreas	(50)	(49)	(48)	(50)
Atrophy	1 (2%)			
Cyst	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Acinus, cytoplasmic alteration			2 (4%)	1 (2%)
Salivary glands	(50)	(49)	(49)	(49)
Atrophy	2 (4%)			1 (2%)
Hyperplasia, lymphoid		5 (10%)	3 (6%)	2 (4%)

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Stomach, forestomach	(50)	(49)	(49)	(50)
Diverticulum	1 (2%)			5 (10%)
Inflammation, chronic active	1 (2%)	1 (2%)	2 (4%)	
Epithelium, hyperplasia	1 (2%)		4 (8%)	1 (2%)
Cardiovascular System				
Heart	(50)	(49)	(49)	(50)
Cardiomyopathy				2 (4%)
Thrombosis				1 (2%)
Myocardium, necrosis			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(49)	(49)	(50)
Accessory adrenal cortical nodule	7 (14%)	5 (10%)	4 (8%)	6 (12%)
Hyperplasia, focal				2 (4%)
Capsule, hyperplasia	3 (6%)			
Adrenal medulla	(50)	(49)	(49)	(50)
Hyperplasia			1 (2%)	
Islets, pancreatic	(50)	(48)	(48)	(50)
Hyperplasia	1 (2%)	1 (2%)		1 (2%)
Parathyroid gland	(48)	(46)	(45)	(48)
Cyst		1 (2%)	1 (2%)	1 (2%)
Pituitary gland	(50)	(49)	(47)	(49)
Pars distalis, angiectasis		3 (6%)	1 (2%)	1 (2%)
Pars distalis, cyst	1 (2%)	3 (6%)	1 (2%)	
Pars distalis, hyperplasia, focal	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Thyroid gland	(49)	(48)	(48)	(50)
Degeneration, cystic	15 (31%)	14 (29%)	12 (25%)	9 (18%)
Follicle, cyst			1 (2%)	1 (2%)
Follicular cell, hyperplasia	1 (2%)	1 (2%)	1 (2%)	9 (18%)
Follicular cell, hypertrophy	6 (12%)	3 (6%)	23 (48%)	46 (92%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(48)	(48)	(50)
Inflammation, chronic				1 (2%)
Ovary	(50)	(49)	(49)	(48)
Angiectasis	12 (24%)	7 (14%)	8 (16%)	8 (17%)
Cyst	10 (20%)	11 (22%)	15 (31%)	10 (21%)
Inflammation, chronic	3 (6%)		2 (4%)	
Thrombosis				1 (2%)
Corpus luteum, hyperplasia				1 (2%)
Uterus	(50)	(49)	(49)	(50)
Angiectasis	3 (6%)	2 (4%)	2 (4%)	6 (12%)
Inflammation, chronic	6 (12%)	2 (4%)	9 (18%)	4 (8%)
Metaplasia, squamous	5 (10%)		5 (10%)	1 (2%)
Cervix, cyst epithelial inclusion		1 (2%)		
Endometrium, fibrosis				1 (2%)
Endometrium, hyperplasia, cystic	48 (96%)	47 (96%)	46 (94%)	45 (90%)

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
2-Year Study (continued)				
Hematopoietic System				
Bone marrow	(50)	(49)	(48)	(50)
Hyperplasia	10 (20%)	5 (10%)	13 (27%)	16 (32%)
Lymph node	(12)	(6)	(8)	(9)
Bronchial, hyperplasia, lymphoid	1 (8%)			2 (22%)
Iliac, ectasia			1 (13%)	
Iliac, hematopoietic cell proliferation	2 (17%)	1 (17%)	1 (13%)	
Iliac, hyperplasia, lymphoid	5 (42%)	3 (50%)	4 (50%)	2 (22%)
Iliac, pigmentation		1 (17%)		
Inguinal, hyperplasia, lymphoid			1 (13%)	
Lumbar, hyperplasia, lymphoid	1 (8%)	1 (17%)	2 (25%)	2 (22%)
Lumbar, pigmentation		1 (17%)		
Mediastinal, hyperplasia, lymphoid	1 (8%)	1 (17%)		
Pancreatic, hyperplasia, lymphoid			1 (13%)	
Renal, hematopoietic cell proliferation	1 (8%)		1 (13%)	
Renal, hyperplasia, lymphoid	3 (25%)	1 (17%)	2 (25%)	
Lymph node, mandibular	(48)	(48)	(46)	(48)
Atrophy			2 (4%)	
Hematopoietic cell proliferation	3 (6%)			
Hemorrhage				1 (2%)
Hyperplasia, lymphoid	11 (23%)	12 (25%)	10 (22%)	15 (31%)
Hyperplasia, plasma cell	2 (4%)	1 (2%)	4 (9%)	
Pigmentation	24 (50%)	20 (42%)	21 (46%)	21 (44%)
Lymph node, mesenteric	(48)	(48)	(47)	(48)
Ectasia				1 (2%)
Hematopoietic cell proliferation	5 (10%)	3 (6%)		3 (6%)
Hemorrhage		1 (2%)		1 (2%)
Hyperplasia, lymphoid	3 (6%)	7 (15%)	4 (9%)	6 (13%)
Hyperplasia, plasma cell		1 (2%)		
Spleen	(50)	(49)	(49)	(50)
Angiectasis			1 (2%)	
Atrophy		1 (2%)	1 (2%)	
Congestion	2 (4%)			
Hematopoietic cell proliferation	15 (30%)	20 (41%)	24 (49%)	39 (78%)
Hyperplasia, plasma cell		1 (2%)		
Pigmentation		4 (8%)	11 (22%)	34 (68%)
Lymphoid follicle, atrophy	1 (2%)	3 (6%)	5 (10%)	4 (8%)
Lymphoid follicle, hyperplasia	12 (24%)	14 (29%)	13 (27%)	16 (32%)
Thymus	(50)	(48)	(48)	(50)
Atrophy	5 (10%)	2 (4%)	7 (15%)	3 (6%)
Hyperplasia, lymphoid				4 (8%)
Integumentary System				
Mammary gland	(49)	(49)	(49)	(50)
Hyperplasia		2 (4%)		2 (4%)
Skin	(50)	(49)	(49)	(50)
Edema			1 (2%)	
Musculoskeletal System				
Bone	(50)	(49)	(49)	(50)
Hyperostosis	9 (18%)	13 (27%)	10 (20%)	11 (22%)
Skeletal muscle		(1)	(1)	
Necrosis			1 (100%)	

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

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
2-Year Study (continued)				
Nervous System				
Brain	(50)	(49)	(49)	(50)
Hemorrhage		1 (2%)	1 (2%)	
Inflammation, chronic active		1 (2%)		
Vacuolization cytoplasmic				1 (2%)
Respiratory System				
Lung	(50)	(49)	(49)	(50)
Edema	2 (4%)			1 (2%)
Foreign body	2 (4%)	2 (4%)	2 (4%)	
Hemorrhage	4 (8%)	3 (6%)	1 (2%)	4 (8%)
Hyperplasia, lymphoid	9 (18%)	6 (12%)	3 (6%)	7 (14%)
Infiltration cellular, histiocyte	2 (4%)	2 (4%)	3 (6%)	
Thrombosis		1 (2%)		1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Nose	(50)	(49)	(49)	(50)
Foreign body		1 (2%)		
Special Senses System				
Eye	(50)	(49)	(49)	(50)
Atrophy				1 (2%)
Cataract		1 (2%)	2 (4%)	
Inflammation, chronic	1 (2%)		2 (4%)	
Harderian gland	(50)	(49)	(49)	(50)
Hyperplasia	1 (2%)			
Hyperplasia, focal		3 (6%)	2 (4%)	1 (2%)
Urinary System				
Kidney	(50)	(49)	(49)	(50)
Cyst	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid	8 (16%)	16 (33%)	8 (16%)	4 (8%)
Infarct			2 (4%)	2 (4%)
Inflammation, chronic				1 (2%)
Metaplasia, osseous		1 (2%)		2 (4%)
Nephropathy	27 (54%)	17 (35%)	21 (43%)	19 (38%)
Papilla, necrosis				1 (2%)
Renal tubule, accumulation, hyaline droplet		1 (2%)		1 (2%)
Renal tubule, dilatation			1 (2%)	
Renal tubule, necrosis			1 (2%)	1 (2%)
Renal tubule, pigmentation		2 (4%)		
Urinary bladder	(50)	(49)	(49)	(50)
Hyperplasia, lymphoid	16 (32%)	16 (33%)	6 (12%)	11 (22%)
Inflammation, chronic	1 (2%)	1 (2%)		
Transitional epithelium, hyperplasia		1 (2%)		

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1988). 2-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 2-methylimidazole. The high dose was limited to 10,000 µg/plate. Trials initially conducted with 10% S9 were repeated with 30% S9.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

RAT AND MOUSE BONE MARROW MICRONUCLEUS TEST PROTOCOL

Preliminary range-finding studies were performed; the high dose was limited by toxicity. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Groups of five male F344/N rats or five male B6C3F$_1$ mice were injected intraperitoneally three times at 24-hour intervals with 2-methylimidazole dissolved in phosphate-buffered saline. Solvent control animals were injected with phosphate-buffered saline only. The positive control rats and mice received injections of cyclophosphamide. The animals were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained with acridine orange; 2,000 polychromatic erythrocytes (PCEs) were scored per animal for the frequency of micronucleated cells. Two hundred erythrocytes were counted to establish the percentage of PCEs.

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 dose group is less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 14-week toxicity study of 2-methylimidazole (NTP, 2004), peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were later stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per exposure group. The frequency of micronucleated cells among NCEs was analyzed by the same software package and methods used to analyze PCEs in the bone marrow micronucleus tests. One thousand erythrocytes were examined to determine the percentage of PCEs; the data were analyzed by a one-way analysis of variance to determine the significance of stimulation.

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

2-Methylimidazole (100 to 10,000 µg/plate) was negative in the *S. typhimurium* gene mutation assay when tested in strains TA97, TA98, TA100, and TA1535, with and without S9 metabolic activation enzymes (Table E1). 2-Methylimidazole was also tested in three *in vivo* assays for induction of chromosomal damage as measured by micronucleated erythrocyte frequency, and the results were mixed (Tables E2, E3, and E4). 2-Methylimidazole, administered to male mice by intraperitoneal injection three times at 24-hour intervals, produced small increases in the frequency of micronucleated PCEs in bone marrow, but these increases were not significant and the results of the assay were concluded to be negative. Results of a three-injection bone marrow micronucleus test in male rats were also negative. In contrast to the results obtained in these two short-term studies, results of the 14-week 2-methylimidazole study in mice showed significant exposure-related increases in the frequencies of micronucleated NCEs in peripheral blood samples of males and females. The increases in the frequencies of micronuclei noted in female mice were greater than those observed in male mice (the three highest doses tested in females induced micronucleated frequencies that were significantly elevated above the control frequency), but the overall magnitudes of the responses in males and females were similar. An exposure concentration-related increase in the percentage of micronucleated PCEs in peripheral blood was seen in male and female mice in the 14-week study. It is possible that an increase in the rate of hematopoiesis, evidenced by the increase in percentage of PCEs, may have contributed to an enhancement of the micronucleus frequencies in mice treated with 2-methylimidazole for 14 weeks.

TABLE E1
Mutagenicity of 2-Methylimidazole in *Salmonella typhimurium*[a]

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

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

TABLE E2
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with 2-Methylimidazole by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	PCEs (%)
Phosphate-buffered saline[c]		5	1.7 ± 0.3	47.8
2-Methylimidazole	25	5	1.3 ± 0.4	46.6
	50	5	1.2 ± 0.3	44.8
	100	5	0.8 ± 0.2	46.9
	200	4	1.3 ± 0.4	30.0
	400	0	Lethal	
			P=0.813[d]	
Cyclophosphamide[e]	7.5	5	22.3 ± 1.6	34.0

[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.
Mean ± standard error; differences of 2-methylimidazole groups versus the solvent control group are not significant by pairwise comparison (P≤0.006) (ILS, 1990)
[c] Solvent control
[d] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at P≤0.025 (ILS, 1990); 400 mg/kg group excluded from statistical analysis due to 100% mortality
[e] Positive control

TABLE E3
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with 2-Methylimidazole by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	PCEs (%)
Phosphate-buffered saline[d]		5	1.2 ± 0.3		51.2
2-Methylimidazole	200	5	2.5 ± 0.4	0.0174	45.8
	300	5	1.9 ± 0.3	0.0949	44.6
	400	5	2.2 ± 0.4	0.0420	45.8
	500	1	3.5		44.0
			P=0.068[e]		
Cyclophosphamide[f]	25	5	23.8 ± 0.7		42.7

[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.
Mean ± standard error
[c] Pairwise comparison with the solvent control group; significant at P≤0.008 (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); 500 mg/kg group excluded from statistical analysis due to poor survival (minimum of three animals required for a valid data point)
[f] Positive control

TABLE E4
Frequency of Micronuclei in Mouse Peripheral Blood Normochromatic Erythrocytes Following Treatment with 2-Methylimidazole in Feed for 14 Weeks[a]

Compound	Dose (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs (%)
Male					
NIH-07 Feed[d]		5	2.5 ± 0.3		8.7
2-Methylimidazole	625	5	2.6 ± 0.3	0.4442	8.7
	1,250	5	3.0 ± 0.4	0.2498	12.6
	2,500	5	3.8 ± 0.5	0.0505	19.3
	5,000	5	4.0 ± 0.6	0.0312	27.3
	10,000	5	4.6 ± 0.6	0.0063	31.2
			P=0.001[e]		P=0.000[f]
Female					
NIH-07 Feed		5	1.7 ± 0.3		7.2
2-Methylimidazole	625	5	1.8 ± 0.4	0.4329	7.5
	1,250	5	2.2 ± 0.3	0.2114	8.5
	2,500	5	3.6 ± 0.3	0.0045	13.2
	5,000	5	3.7 ± 0.3	0.0032	16.7
	10,000	5	4.9 ± 0.4	0.0000	17.4
			P≤0.001[e]		P=0.000[f]

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by MacGregor *et al*. (1990).
[b] NCE=normochromatic erythrocyte.
[c] Mean ± standard error
[d] Pairwise comparison with the untreated control group; significant at P≤0.005 (ILS, 1990)
[e] Untreated control
[f] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025 (ILS, 1990)
One-way analysis of variance; significant at P≤0.05

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
Male				
Hematology				
Month 6				
n	9	10	10	10
Automated hematocrit (%)	46.4 ± 0.6	46.8 ± 0.5	45.4 ± 0.4	44.0 ± 0.4**
Manual hematocrit (%)	45.5 ± 0.6	45.7 ± 0.4	44.7 ± 0.4	43.3 ± 0.5*
Hemoglobin (g/dL)	15.6 ± 0.2	15.7 ± 0.1	15.2 ± 0.1	14.9 ± 0.1**
Erythrocytes (10^6/μL)	8.96 ± 0.10	9.01 ± 0.09	8.71 ± 0.11	8.52 ± 0.07**
Reticulocytes (10^6/μL)	2.64 ± 0.09	2.83 ± 0.06	3.08 ± 0.17*	2.88 ± 0.04
Mean cell volume (fL)	51.8 ± 0.2	51.9 ± 0.2	52.2 ± 0.6	51.6 ± 0.1
Mean cell hemoglobin (pg)	17.4 ± 0.1	17.4 ± 0.1	17.4 ± 0.1	17.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.6 ± 0.2	33.5 ± 0.2	33.4 ± 0.3	33.8 ± 0.1
Platelets (10^3/μL)	576.3 ± 8.1	594.9 ± 9.8	638.4 ± 16.2**	655.9 ± 9.8**
Leukocytes (10^3/μL)	8.02 ± 0.38	8.46 ± 0.23	9.00 ± 0.27	8.85 ± 0.27
Segmented neutrophils (10^3/μL)	1.60 ± 0.07	1.55 ± 0.06	1.63 ± 0.05	1.52 ± 0.06
Lymphocytes (10^3/μL)	5.97 ± 0.34	6.50 ± 0.21	6.89 ± 0.29	6.91 ± 0.27
Monocytes (10^3/μL)	0.15 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
Basophils (10^3/μL)	0.011 ± 0.003	0.009 ± 0.002	0.015 ± 0.004	0.010 ± 0.001
Eosinophils (10^3/μL)	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.06 ± 0.00
Large unstained cells (10^3/μL)	0.211 ± 0.027	0.182 ± 0.004	0.221 ± 0.021	0.205 ± 0.011
Clinical Chemistry				
n	10	10	10	10
Thyroid-stimulating hormone (TSH) (ng/mL)				
Day 8	3.01 ± 0.15	3.82 ± 0.32*	8.85 ± 0.89**	19.60 ± 0.61**
Week 14	2.84 ± 0.37[b]	2.95 ± 0.31	2.25 ± 0.32	3.22 ± 0.27
Month 6	2.27 ± 0.49	1.71 ± 0.18	1.85 ± 0.23	2.50 ± 0.36
Triiodothyronine (T_3) (ng/dL)				
Day 8	120.40 ± 7.03	130.30 ± 7.20	102.40 ± 5.10	53.90 ± 2.46**
Week 14	102.60 ± 5.93	105.90 ± 8.08	100.40 ± 4.91	88.50 ± 6.06
Month 6	94.70 ± 4.54	95.44 ± 6.73[b]	95.11 ± 2.09[b]	87.20 ± 4.79
Thyroxine (T_4) (μg/dL)				
Day 8	5.73 ± 0.33	5.98 ± 0.33	5.23 ± 0.21	1.07 ± 0.10**
Week 14	5.13 ± 0.19	4.55 ± 0.25	4.78 ± 0.15	4.89 ± 0.19
Month 6	3.78 ± 0.20	3.93 ± 0.30	3.96 ± 0.16	3.80 ± 0.27

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	1,000 ppm	2,500 ppm	5,000 ppm
Female				
Hematology				
Month 6				
n	10	9	10	10
Automated hematocrit (%)	44.7 ± 0.5	44.2 ± 0.4	42.4 ± 1.1	41.5 ± 0.6**
Manual hematocrit (%)	44.2 ± 0.4	43.3 ± 0.4	41.3 ± 1.1**	41.1 ± 0.4**
Hemoglobin (g/dL)	15.3 ± 0.1	15.1 ± 0.1	14.4 ± 0.4*	14.0 ± 0.2**
Erythrocytes (10^6/µL)	8.13 ± 0.07	8.14 ± 0.08	7.89 ± 0.19	7.92 ± 0.11
Reticulocytes (10^6/µL)	2.51 ± 0.08	2.37 ± 0.09	2.57 ± 0.10	2.70 ± 0.07
Mean cell volume (fL)	55.0 ± 0.2	54.3 ± 0.2*	53.7 ± 0.2**	52.4 ± 0.2**
Mean cell hemoglobin (pg)	18.8 ± 0.1	18.6 ± 0.1*	18.2 ± 0.1**	17.7 ± 0.0**
Mean cell hemoglobin concentration (g/dL)	34.2 ± 0.2	34.2 ± 0.2	33.9 ± 0.2	33.8 ± 0.1
Platelets (10^3/µL)	599.7 ± 17.4	609.3 ± 9.2	611.9 ± 15.1	661.1 ± 11.7**
Leukocytes (10^3/µL)	6.03 ± 0.27	7.70 ± 0.33**	9.58 ± 0.81**	10.46 ± 0.48**
Segmented neutrophils (10^3/µL)	1.09 ± 0.04	1.33 ± 0.05**	1.55 ± 0.11**	1.58 ± 0.08**
Lymphocytes (10^3/µL)	4.65 ± 0.23	6.00 ± 0.28**	7.54 ± 0.68**	8.43 ± 0.46**
Monocytes (10^3/µL)	0.10 ± 0.01	0.14 ± 0.01**	0.17 ± 0.02**	0.15 ± 0.01**
Basophils (10^3/µL)	0.008 ± 0.002	0.010 ± 0.002	0.014 ± 0.003	0.014 ± 0.004
Eosinophils (10^3/µL)	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Large unstained cells (10^3/µL)	0.132 ± 0.009	0.173 ± 0.016	0.246 ± 0.032**	0.241 ± 0.023**
Clinical Chemistry				
n	10	10	10	10
Thyroid-stimulating hormone (TSH) (ng/mL)				
Day 8	2.00 ± 0.08	4.00 ± 0.32**	16.73 ± 0.80**	17.36 ± 0.62**
Week 14	1.61 ± 0.18[c]	1.98 ± 0.23[b]	2.65 ± 0.44	7.42 ± 1.19**
Month 6	1.31 ± 0.19[b]	1.37 ± 0.11	1.92 ± 0.19*	3.75 ± 0.42**
Triiodothyronine (T_3) (ng/dL)				
Day 8	112.80 ± 4.06	98.30 ± 3.77*	60.20 ± 3.65**	41.40 ± 0.95**
Week 14	113.60 ± 3.68	113.80 ± 6.04	108.40 ± 5.20	97.10 ± 3.57
Month 6	108.50 ± 2.40	102.80 ± 4.54	95.10 ± 7.02*	80.90 ± 2.42**
Thyroxine (T_4) (µg/dL)				
Day 8	4.07 ± 0.24	3.32 ± 0.34	1.29 ± 0.14**	0.57 ± 0.05**
Week 14	3.98 ± 0.30	3.78 ± 0.30	3.97 ± 0.25	3.16 ± 0.14
Month 6	3.55 ± 0.24	3.25 ± 0.33	3.34 ± 0.28	2.71 ± 0.16

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=8

TABLE F2
Hematology and Clinical Chemistry Data for Mice in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
Hematology				
Month 6				
n	10	10	9	10
Automated hematocrit (%)	52.1 ± 0.7	50.9 ± 0.7	48.4 ± 0.7**	42.5 ± 1.0**
Manual hematocrit (%)	52.1 ± 0.6	51.4 ± 0.6	48.8 ± 0.6**[b]	42.9 ± 1.0**
Hemoglobin (g/dL)	17.6 ± 0.2	17.4 ± 0.2	16.4 ± 0.2**	13.6 ± 0.3**
Erythrocytes (10^6/μL)	10.79 ± 0.18	10.41 ± 0.12	9.50 ± 0.16**	7.76 ± 0.22**
Reticulocytes (10^6/μL)	4.15 ± 0.09	5.71 ± 0.54**	7.01 ± 0.74**	13.56 ± 1.82**
Mean cell volume (fL)	48.3 ± 0.5	48.9 ± 0.2	51.0 ± 0.4**	54.9 ± 0.5**
Mean cell hemoglobin (pg)	16.3 ± 0.1	16.7 ± 0.1*	17.3 ± 0.1**	17.5 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.2	34.2 ± 0.2	33.8 ± 0.2	31.9 ± 0.3**
Platelets (10^3/μL)	836.7 ± 67.1	967.6 ± 67.4	940.8 ± 45.9	919.4 ± 73.1
Leukocytes (10^3/μL)	6.32 ± 0.40	6.59 ± 0.68	5.93 ± 0.62	6.02 ± 0.62
Segmented neutrophils (10^3/μL)	0.85 ± 0.18	0.81 ± 0.09	0.74 ± 0.08	0.70 ± 0.08
Lymphocytes (10^3/μL)	5.29 ± 0.33	5.55 ± 0.57	5.01 ± 0.56	5.16 ± 0.54
Monocytes (10^3/μL)	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
Basophils (10^3/μL)	0.002 ± 0.001	0.004 ± 0.002	0.004 ± 0.002	0.005 ± 0.002
Eosinophils (10^3/μL)	0.07 ± 0.01	0.09 ± 0.02	0.07 ± 0.02	0.06 ± 0.01
Large unstained cells (10^3/μL)	0.059 ± 0.011	0.062 ± 0.011	0.047 ± 0.007	0.048 ± 0.007
Clinical Chemistry				
Day 8	8	9	10	7
Week 14	8	9	8	9
Month 6	10	10	9	10
Thyroid-stimulating hormone (TSH) (ng/mL)				
Day 8	224.14 ± 14.23[c]	192.43 ± 5.21[c]	219.63 ± 28.28[d]	236.29 ± 16.66
Week 14	232.83 ± 24.22[e]	208.71 ± 13.42[c]	209.17 ± 8.73[e]	273.56 ± 21.01
Triiodothyronine (T_3) (ng/dL)				
Day 8	74.50 ± 7.37	95.13 ± 9.72[d]	92.86 ± 5.76[c]	90.83 ± 10.49[e]
Week 14	169.38 ± 7.04	153.00 ± 8.01	173.29 ± 7.41[c]	161.33 ± 8.29
Month 6	99.90 ± 6.50	104.60 ± 6.87	105.44 ± 5.92	106.90 ± 7.38
Thyroxine (T_4) (μg/dL)				
Day 8	5.25 ± 0.38	5.71 ± 0.39	6.51 ± 0.95	6.30 ± 0.83
Week 14	5.09 ± 0.35[c]	4.63 ± 0.23	5.44 ± 0.36	5.95 ± 0.21*
Month 6	5.72 ± 0.31	5.10 ± 0.17	5.35 ± 0.19[f]	6.82 ± 0.20*

TABLE F2
Hematology and Clinical Chemistry Data for Mice in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Female				
Month 6				
Hematology				
n	10	9	10	10
Automated hematocrit (%)	50.6 ± 0.5	50.3 ± 0.8	49.3 ± 0.6	47.2 ± 0.7**
Manual hematocrit (%)	51.1 ± 0.5	51.2 ± 0.8[b]	49.7 ± 0.5	48.1 ± 0.5**
Hemoglobin (g/dL)	17.4 ± 0.2	17.3 ± 0.2	16.9 ± 0.2*	16.1 ± 0.2**
Erythrocytes (10^6/μL)	10.49 ± 0.10	10.40 ± 0.15	9.84 ± 0.11**	9.15 ± 0.14**
Reticulocytes (10^6/μL)	3.94 ± 0.57	5.08 ± 1.47	4.20 ± 0.30	6.53 ± 0.61**
Mean cell volume (fL)	48.3 ± 0.2	48.4 ± 0.2	50.1 ± 0.2**	51.6 ± 0.3**
Mean cell hemoglobin (pg)	16.6 ± 0.1	16.7 ± 0.1	17.1 ± 0.1**	17.6 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	34.4 ± 0.2	34.4 ± 0.1	34.2 ± 0.1	34.0 ± 0.3
Platelets (10^3/μL)	828.7 ± 53.9	759.6 ± 79.7	893.5 ± 55.4	997.1 ± 74.3
Leukocytes (10^3/μL)	5.39 ± 0.38	5.40 ± 0.67	5.24 ± 0.57	6.07 ± 0.50
Segmented neutrophils (10^3/μL)	0.73 ± 0.07	0.97 ± 0.33	0.72 ± 0.09	0.78 ± 0.06
Lymphocytes (10^3/μL)	4.47 ± 0.33	4.28 ± 0.43	4.35 ± 0.49	5.09 ± 0.43
Monocytes (10^3/μL)	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
Basophils (10^3/μL)	0.004 ± 0.002	0.007 ± 0.002	0.004 ± 0.002	0.006 ± 0.002
Eosinophils (10^3/μL)	0.10 ± 0.02	0.06 ± 0.02	0.09 ± 0.02	0.10 ± 0.02
Large unstained cells (10^3/μL)	0.040 ± 0.006	0.036 ± 0.006	0.039 ± 0.006	0.047 ± 0.007
Clinical Chemistry				
Day 8	10	9	7	9
Week 14	8	9	9	8
Month 6	10	10	10	10
Thyroid-stimulating hormone (TSH) (ng/mL)				
Day 8	73.50 ± 11.73[e]	67.50 ± 14.92	76.86 ± 15.22	82.20 ± 17.30[f]
Week 14	131.00 ± 8.18[e]	126.29 ± 14.86[c]	110.63 ± 11.29[d]	106.71 ± 9.16[c]
Triiodothyronine (T_3) (ng/dL)				
Day 8	62.20 ± 7.91[f]	63.86 ± 4.65[c]	65.57 ± 3.68	65.17 ± 5.43[e]
Week 14	164.00 ± 11.49[c]	150.25 ± 15.33[d]	154.14 ± 7.36[c]	153.38 ± 10.16
Month 6	72.60 ± 3.84	77.88 ± 6.31[d]	85.70 ± 5.85	82.90 ± 2.04
Thyroxine (T_4) (μg/dL)				
Day 8	4.76 ± 0.23	5.00 ± 0.15	5.43 ± 0.22	4.79 ± 0.16
Week 14	6.51 ± 0.37[d]	5.47 ± 0.51	5.60 ± 0.24	5.21 ± 0.27*[e]
Month 6	6.02 ± 0.37	5.79 ± 0.24	5.36 ± 0.26	4.90 ± 0.23*[g]

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=10
[c] n=7
[d] n=8
[e] n=6
[f] n=5
[g] n=9

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

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at 8 Days in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
n	10	10	10	10
Male				
Necropsy body wt	146 ± 8	149 ± 6	148 ± 7	142 ± 6
Liver				
Absolute	6.427 ± 0.314	6.756 ± 0.230	6.947 ± 0.270	7.083 ± 0.226
Relative	44.1 ± 0.8	45.5 ± 0.4	47.3 ± 0.9**	50.2 ± 0.8**
Pituitary gland				
Absolute	0.0066 ± 0.0003	0.0067 ± 0.0004	0.0070 ± 0.0003	0.0076 ± 0.0005
Relative	0.046 ± 0.002	0.045 ± 0.002	0.048 ± 0.003	0.054 ± 0.004
Spleen				
Absolute	0.403 ± 0.020	0.405 ± 0.012	0.394 ± 0.018	0.312 ± 0.015**
Relative	2.764 ± 0.056	2.737 ± 0.062	2.671 ± 0.035	2.202 ± 0.055**
Thyroid gland				
Absolute	0.011 ± 0.000	0.012 ± 0.001	0.021 ± 0.002**	0.023 ± 0.002**
Relative	0.075 ± 0.006	0.079 ± 0.008	0.146 ± 0.015**	0.160 ± 0.012**
	0 ppm	**1,000 ppm**	**2,500 ppm**	**5,000 ppm**
Female				
Necropsy body wt	122 ± 3	113 ± 4	119 ± 3	109 ± 3*
Liver				
Absolute	4.952 ± 0.129	4.813 ± 0.133	5.421 ± 0.154	4.940 ± 0.127
Relative	40.8 ± 0.9	42.9 ± 0.9	45.7 ± 0.9**	45.3 ± 0.9**
Pituitary gland				
Absolute	0.0084 ± 0.0003	0.0075 ± 0.0002*	0.0080 ± 0.0002*	0.0065 ± 0.0002**
Relative	0.070 ± 0.003	0.067 ± 0.002	0.068 ± 0.003	0.060 ± 0.002*
Spleen				
Absolute	0.322 ± 0.013	0.303 ± 0.013	0.267 ± 0.010**	0.227 ± 0.009**
Relative	2.639 ± 0.063	2.710 ± 0.138	2.244 ± 0.042**	2.075 ± 0.053**
Thyroid gland				
Absolute	0.011 ± 0.001	0.014 ± 0.001*	0.022 ± 0.001**	0.019 ± 0.001**
Relative	0.090 ± 0.008	0.129 ± 0.011**	0.187 ± 0.010**	0.174 ± 0.009**

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

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at 14 Weeks in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
n	10	10	10	10
Male				
Necropsy body wt	338 ± 11	334 ± 4	325 ± 9	298 ± 8**
Liver				
Absolute	12.649 ± 0.598	13.223 ± 0.334	12.499 ± 0.450	11.778 ± 0.389
Relative	37.4 ± 1.1	39.6 ± 0.9	38.5 ± 1.0	39.6 ± 1.0
Pituitary gland				
Absolute	0.0094 ± 0.0006	0.0098 ± 0.0009[b]	0.0101 ± 0.0004	0.0095 ± 0.0004
Relative	0.027 ± 0.001	0.029 ± 0.003[b]	0.031 ± 0.001	0.032 ± 0.001
Spleen				
Absolute	0.696 ± 0.020	0.682 ± 0.019	0.681 ± 0.021	0.604 ± 0.014**
Relative	2.067 ± 0.041	2.038 ± 0.041	2.099 ± 0.055	2.031 ± 0.030
Thyroid gland				
Absolute	0.015 ± 0.001	0.015 ± 0.001	0.020 ± 0.001**	0.025 ± 0.001**
Relative	0.044 ± 0.002	0.046 ± 0.002	0.062 ± 0.003**	0.083 ± 0.003**
	0 ppm	**1,000 ppm**	**2,500 ppm**	**5,000 ppm**
Female				
Necropsy body wt	187 ± 4	190 ± 3	180 ± 3	166 ± 3**
Liver				
Absolute	5.860 ± 0.147	5.745 ± 0.102	5.950 ± 0.100	5.584 ± 0.090
Relative	31.5 ± 0.7	30.3 ± 0.5	33.2 ± 0.8	33.8 ± 0.4*
Pituitary gland				
Absolute	0.0139 ± 0.0005	0.0139 ± 0.0007	0.0123 ± 0.0008	0.0084 ± 0.0003**
Relative	0.075 ± 0.003	0.074 ± 0.004	0.069 ± 0.005	0.051 ± 0.002**
Spleen				
Absolute	0.450 ± 0.027	0.407 ± 0.005	0.413 ± 0.010	0.371 ± 0.011**
Relative	2.419 ± 0.150	2.150 ± 0.038	2.306 ± 0.077	2.249 ± 0.084
Thyroid gland				
Absolute	0.014 ± 0.001	0.014 ± 0.000	0.019 ± 0.001**	0.028 ± 0.001**
Relative	0.075 ± 0.004	0.072 ± 0.003	0.107 ± 0.004**	0.167 ± 0.006**

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

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at 6 Months in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
n	10	10	10	10
Male				
Necropsy body wt	406 ± 8	389 ± 2	395 ± 9	361 ± 5**
Liver				
Absolute	12.676 ± 0.235	12.459 ± 0.214	12.453 ± 0.375	11.856 ± 0.262
Relative	31.3 ± 0.4	32.0 ± 0.5	31.5 ± 0.4	32.8 ± 0.3*
Pituitary gland				
Absolute	0.0105 ± 0.0003	0.0100 ± 0.0004	0.0105 ± 0.0004	0.0094 ± 0.0003
Relative	0.026 ± 0.001	0.026 ± 0.001	0.027 ± 0.001	0.026 ± 0.001
Spleen				
Absolute	0.767 ± 0.016	0.731 ± 0.013	0.751 ± 0.019	0.667 ± 0.016**
Relative	1.891 ± 0.023	1.879 ± 0.036	1.902 ± 0.027	1.846 ± 0.025
Thyroid gland				
Absolute	0.021 ± 0.001	0.019 ± 0.001	0.024 ± 0.001	0.029 ± 0.001**
Relative	0.052 ± 0.002	0.048 ± 0.003	0.060 ± 0.002	0.079 ± 0.004**
	0 ppm	**1,000 ppm**	**2,500 ppm**	**5,000 ppm**
Female				
Necropsy body wt	204 ± 2	206 ± 2	202 ± 4	183 ± 5**
Liver				
Absolute	5.591 ± 0.085	6.077 ± 0.132*	6.015 ± 0.088	5.520 ± 0.200
Relative	27.4 ± 0.5	29.5 ± 0.5**	29.8 ± 0.5**	30.1 ± 0.5**
Pituitary gland				
Absolute	0.0156 ± 0.0008	0.0158 ± 0.0006	0.0162 ± 0.0005	0.0110 ± 0.0006**
Relative	0.077 ± 0.004	0.077 ± 0.003	0.080 ± 0.003	0.060 ± 0.002**
Spleen				
Absolute	0.416 ± 0.009	0.435 ± 0.015	0.458 ± 0.018	0.399 ± 0.009
Relative	2.037 ± 0.037	2.110 ± 0.061	2.262 ± 0.072*	2.184 ± 0.036
Thyroid gland				
Absolute	0.014 ± 0.001	0.016 ± 0.001	0.019 ± 0.001**	0.027 ± 0.001**
Relative	0.068 ± 0.006	0.077 ± 0.003	0.096 ± 0.005**	0.150 ± 0.007**

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

TABLE G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at 8 Days in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
n	10	10	10	10
Male				
Necropsy body wt	21.8 ± 0.3	21.5 ± 0.7	20.1 ± 1.1	21.2 ± 0.9
Liver				
Absolute	1.090 ± 0.023	1.066 ± 0.053	1.021 ± 0.088	1.125 ± 0.076
Relative	49.9 ± 0.6	49.4 ± 1.7	49.9 ± 2.4	52.4 ± 2.3
Pituitary gland				
Absolute	0.0016 ± 0.0002	0.0014 ± 0.0001	0.0016 ± 0.0001	0.0015 ± 0.0001[b]
Relative	0.072 ± 0.008	0.063 ± 0.005	0.082 ± 0.006	0.073 ± 0.005[b]
Spleen				
Absolute	0.059 ± 0.003	0.059 ± 0.007	0.047 ± 0.005	0.056 ± 0.005
Relative	2.701 ± 0.133	2.701 ± 0.296	2.266 ± 0.204	2.613 ± 0.192
Thyroid gland				
Absolute	0.003 ± 0.000	0.002 ± 0.000**	0.003 ± 0.000	0.003 ± 0.000[b]
Relative	0.147 ± 0.010	0.105 ± 0.008*	0.144 ± 0.012	0.127 ± 0.009[b]
Female				
Necropsy body wt	17.1 ± 0.3	16.7 ± 0.3	17.3 ± 0.2	17.2 ± 0.3
Liver				
Absolute	0.721 ± 0.028	0.704 ± 0.021	0.841 ± 0.021**	0.821 ± 0.026**
Relative	42.2 ± 1.1	42.2 ± 0.8	48.6 ± 1.0**	47.8 ± 1.4**
Pituitary gland				
Absolute	0.0018 ± 0.0001	0.0014 ± 0.0001	0.0018 ± 0.0002	0.0017 ± 0.0002
Relative	0.106 ± 0.006	0.083 ± 0.006	0.101 ± 0.010	0.096 ± 0.013
Spleen				
Absolute	0.065 ± 0.004	0.050 ± 0.003*	0.065 ± 0.004	0.056 ± 0.002
Relative	3.812 ± 0.249	2.982 ± 0.163*	3.752 ± 0.232	3.265 ± 0.133
Thyroid gland				
Absolute	0.003 ± 0.000	0.002 ± 0.000	0.002 ± 0.001	0.003 ± 0.000
Relative	0.159 ± 0.023	0.133 ± 0.014	0.140 ± 0.032	0.153 ± 0.016

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

TABLE G5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at 14 Weeks in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
Male				
n	10	10	9	10
Necropsy body wt	32.9 ± 1.0	31.2 ± 0.6	31.5 ± 0.8	31.2 ± 0.5
Liver				
Absolute	1.398 ± 0.046	1.362 ± 0.035	1.558 ± 0.056*	1.635 ± 0.032**
Relative	42.5 ± 0.5	43.7 ± 1.2	49.4 ± 0.8**	52.5 ± 0.6**
Pituitary gland				
Absolute	0.0016 ± 0.0001[b]	0.0012 ± 0.0002[b]	0.0009 ± 0.0002*	0.0016 ± 0.0001
Relative	0.050 ± 0.004[b]	0.039 ± 0.006[b]	0.029 ± 0.006*	0.050 ± 0.003
Spleen				
Absolute	0.063 ± 0.003	0.068 ± 0.002	0.102 ± 0.006**	0.193 ± 0.012**
Relative	1.933 ± 0.115	2.183 ± 0.083	3.240 ± 0.168**	6.167 ± 0.349**
Thyroid gland				
Absolute	0.002 ± 0.000	0.002 ± 0.000[c]	0.002 ± 0.000	0.004 ± 0.000**[b]
Relative	0.066 ± 0.006	0.058 ± 0.008[c]	0.062 ± 0.009	0.131 ± 0.009**[b]
Female				
n	10	10	10	10
Necropsy body wt	27.0 ± 0.7	26.8 ± 0.6	25.7 ± 0.7	25.7 ± 0.7
Liver				
Absolute	1.075 ± 0.026	1.111 ± 0.025	1.122 ± 0.043	1.152 ± 0.045
Relative	39.9 ± 0.4	41.6 ± 0.8	43.6 ± 0.7**	44.8 ± 1.1**
Pituitary gland				
Absolute	0.0023 ± 0.0002	0.0025 ± 0.0001	0.0023 ± 0.0001	0.0019 ± 0.0002
Relative	0.085 ± 0.007	0.095 ± 0.006	0.091 ± 0.005	0.074 ± 0.007
Spleen				
Absolute	0.081 ± 0.005	0.075 ± 0.002	0.088 ± 0.006	0.108 ± 0.007**
Relative	3.005 ± 0.180	2.816 ± 0.089	3.431 ± 0.220	4.209 ± 0.256**
Thyroid gland				
Absolute	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.004 ± 0.000**
Relative	0.096 ± 0.011	0.115 ± 0.006	0.099 ± 0.010	0.171 ± 0.012**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9
[c] n=8

TABLE G6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at 6 Months in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
n	10	10	10	10
Male				
Necropsy body wt	40.5 ± 0.7	40.3 ± 1.2	36.7 ± 1.3*	34.5 ± 0.7**
Liver				
Absolute	1.539 ± 0.025	1.633 ± 0.071	1.627 ± 0.077	1.686 ± 0.043
Relative	38.1 ± 0.6	40.4 ± 0.8	44.3 ± 1.0**	48.9 ± 0.9**
Pituitary gland				
Absolute	0.0017 ± 0.0001	0.0019 ± 0.0001	0.0018 ± 0.0001	0.0019 ± 0.0001
Relative	0.042 ± 0.002	0.046 ± 0.002	0.051 ± 0.003**	0.056 ± 0.002**
Spleen				
Absolute	0.071 ± 0.003	0.078 ± 0.003	0.115 ± 0.005**	0.234 ± 0.013**
Relative	1.761 ± 0.092	1.940 ± 0.066	3.165 ± 0.170**	6.776 ± 0.346**
Thyroid gland				
Absolute	0.004 ± 0.000	0.004 ± 0.000	0.005 ± 0.000	0.006 ± 0.001**
Relative	0.091 ± 0.007	0.098 ± 0.007	0.130 ± 0.004*	0.170 ± 0.019**
Female				
Necropsy body wt	33.5 ± 1.2	30.7 ± 0.7	29.6 ± 0.7**	30.9 ± 0.7
Liver				
Absolute	1.222 ± 0.054	1.196 ± 0.041	1.268 ± 0.037	1.425 ± 0.035**
Relative	36.4 ± 0.6	39.0 ± 1.0	43.0 ± 1.1**	46.3 ± 1.1**
Pituitary gland				
Absolute	0.0027 ± 0.0001	0.0027 ± 0.0001	0.0028 ± 0.0001	0.0027 ± 0.0001
Relative	0.081 ± 0.003	0.088 ± 0.004	0.095 ± 0.003*	0.087 ± 0.003
Spleen				
Absolute	0.096 ± 0.006	0.091 ± 0.004	0.096 ± 0.004	0.142 ± 0.008**
Relative	2.861 ± 0.147	2.981 ± 0.141	3.261 ± 0.137	4.634 ± 0.312**
Thyroid gland				
Absolute	0.003 ± 0.000	0.004 ± 0.000	0.004 ± 0.000*	0.006 ± 0.000**
Relative	0.102 ± 0.006	0.131 ± 0.010*	0.151 ± 0.008**	0.183 ± 0.013**

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

APPENDIX H
LIVER ENZYME RESULTS

TABLE H1
Liver Enzyme Activities of Rats in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	300 ppm	1,000 ppm	3,000 ppm
n	10	10	10	10
Male				
Cytochrome P450 (nmol/mg protein)				
Day 8	0.828 ± 0.041	0.828 ± 0.031	0.855 ± 0.055	0.810 ± 0.031
Week 14	0.523 ± 0.045	0.290 ± 0.037**	0.400 ± 0.032	0.353 ± 0.044
Month 6	0.712 ± 0.012	0.665 ± 0.017*	0.642 ± 0.026*	0.628 ± 0.024**
Cytochrome P450 (nmol/g liver)				
Day 8	11.9 ± 0.7	12.0 ± 0.4	11.5 ± 1.1	9.7 ± 0.7
Week 14	5.7 ± 0.7	2.9 ± 0.5**	4.1 ± 0.5	3.7 ± 0.6
Month 6	7.5 ± 0.2	6.8 ± 0.2	7.0 ± 0.5	6.4 ± 0.4
UDP-Glucuronosyltransferase (nmol/mg protein per minute)				
Day 8	12.5 ± 0.8	19.3 ± 1.0**	25.3 ± 1.3**	18.9 ± 2.7**
Week 14	8.0 ± 0.5	10.0 ± 0.6*	12.6 ± 0.6**	17.7 ± 0.8**
Month 6	8.5 ± 0.2	10.3 ± 0.4**	10.9 ± 0.4**	18.3 ± 0.6**
UDP-Glucuronosyltransferase (nmol/g liver per minute)				
Day 8	182 ± 18	283 ± 19*	335 ± 25**	215 ± 25
Week 14	84 ± 7	96 ± 8	123 ± 8**	181 ± 6**
Month 6	89 ± 3	106 ± 5*	118 ± 5**	184 ± 7**
	0 ppm	**1,000 ppm**	**2,500 ppm**	**5,000 ppm**
Female				
Cytochrome P450 (nmol/mg protein)				
Day 8	0.687 ± 0.023	0.665 ± 0.010	0.768 ± 0.030	0.709 ± 0.025
Week 14	0.401 ± 0.021	0.409 ± 0.016	0.438 ± 0.019	0.408 ± 0.013
Month 6	0.584 ± 0.017	0.546 ± 0.017	0.522 ± 0.021	0.514 ± 0.016*
Cytochrome P450 (nmol/g liver)				
Day 8	11.6 ± 0.4	11.0 ± 0.3	10.5 ± 0.7	10.1 ± 0.5
Week 14	5.5 ± 0.4	5.6 ± 0.3	6.5 ± 0.4	5.9 ± 0.3
Month 6	8.7 ± 0.6	7.4 ± 0.3	6.7 ± 0.4*	7.2 ± 0.3
UDP-Glucuronosyltransferase (nmol/mg protein per minute)				
Day 8	10.7 ± 0.6	13.2 ± 1.0*	21.4 ± 1.3**	23.3 ± 1.7**
Week 14	7.0 ± 0.6	11.6 ± 0.6**	15.6 ± 0.5**	27.7 ± 1.5**
Month 6	9.5 ± 0.4	10.9 ± 0.4*	16.1 ± 0.6**	23.9 ± 0.6**
UDP-Glucuronosyltransferase (nmol/g liver per minute)				
Day 8	179 ± 10	217 ± 17*	291 ± 20**	330 ± 24**
Week 14	96 ± 9	158 ± 10**	231 ± 16**	393 ± 11**
Month 6	139 ± 5	148 ± 7	206 ± 8**	336 ± 12**

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error

TABLE H2
Liver Enzyme Activities of Mice in the 2-Year Feed Study of 2-Methylimidazole[a]

	0 ppm	625 ppm	1,250 ppm	2,500 ppm
n	10	10	10	10
Male				
Cytochrome P450 (nmol/mg protein)				
Day 8	0.635 ± 0.017	0.664 ± 0.047	0.672 ± 0.045	0.679 ± 0.046
Week 14	0.602 ± 0.030	0.591 ± 0.020	0.626 ± 0.021[b]	0.635 ± 0.009
Month 6	0.534 ± 0.021	0.553 ± 0.023	0.531 ± 0.018	0.508 ± 0.019
Cytochrome P450 (nmol/g liver)				
Day 8	11.9 ± 0.5	10.6 ± 0.7	11.1 ± 0.6	10.0 ± 0.4*
Week 14	13.3 ± 0.7	13.3 ± 0.5	12.1 ± 1.2[b]	11.8 ± 0.3
Month 6	10.0 ± 0.8	9.8 ± 0.7	9.8 ± 0.6	9.3 ± 0.8
UDP-Glucuronosyltransferase (nmol/mg protein per minute)				
Day 8	27.5 ± 1.5	30.7 ± 1.9	28.3 ± 1.6	32.2 ± 1.7
Week 14	19.8 ± 0.4	21.6 ± 1.0	21.8 ± 0.9[b]	23.3 ± 0.7**
Month 6	17.4 ± 1.0	18.7 ± 0.8	18.6 ± 0.6	16.0 ± 0.9
UDP-Glucuronosyltransferase (nmol/g liver per minute)				
Day 8	514 ± 31	486 ± 21	460 ± 12	477 ± 26
Week 14	438 ± 12	485 ± 23	419 ± 36[b]	432 ± 7
Month 6	325 ± 29	330 ± 25	354 ± 19	294 ± 32
Female				
Cytochrome P450 (nmol/mg protein)				
Day 8	0.664 ± 0.046	0.633 ± 0.036	0.562 ± 0.025	0.495 ± 0.019**
Week 14	0.444 ± 0.019	0.389 ± 0.024	0.377 ± 0.032	0.327 ± 0.025**
Month 6	0.336 ± 0.023	0.346 ± 0.020	0.375 ± 0.025	0.325 ± 0.024
Cytochrome P450 (nmol/g liver)				
Day 8	10.4 ± 0.3	9.9 ± 0.4	8.7 ± 0.3**	7.8 ± 0.5**
Week 14	9.9 ± 0.5	7.4 ± 0.5**	6.8 ± 0.5**	6.1 ± 0.2**
Month 6	5.9 ± 0.5	6.4 ± 0.6	7.1 ± 0.4	6.4 ± 0.5
UDP-Glucuronosyltransferase (nmol/mg protein per minute)				
Day 8	20.6 ± 1.5	26.1 ± 1.7	24.3 ± 2.0	26 ± 1.1
Week 14	14.9 ± 0.7	14.5 ± 1.4	14.7 ± 1.5	14.2 ± 0.6
Month 6	10.6 ± 0.9	11.7 ± 0.8	12.9 ± 1.3	11.6 ± 0.7
UDP-Glucuronosyltransferase (nmol/g liver per minute)				
Day 8	321 ± 16	402 ± 19*	370 ± 25	398 ± 28*
Week 14	336 ± 21	277 ± 30	265 ± 20	278 ± 23
Month 6	183 ± 18	217 ± 26	242 ± 21	229 ± 16

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

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of 2-Methylimidazole

2-Methylimidazole was obtained from Aldrich Chemical Company (Milwaukee, WI) in two lots (08222CN and 04209TQ). The two lots were combined at the analytical chemistry laboratory, Battelle Columbus (Columbus, OH), and assigned lot number 081497. Lot 081497 was used during the 2-year studies. Identity, purity, and stability analyses on lot 081497 were conducted by the analytical chemistry laboratory and the study laboratory. Reports on analyses performed in support of the 2-methylimidazole studies are on file at the National Institute of Environmental Health Sciences.

Lot 081497, a dry white powder, was identified as 2-methylimidazole by the analytical chemistry laboratory and the study laboratory using infrared and proton nuclear magnetic resonance (NMR) spectroscopy, by the analytical chemistry laboratory using carbon-13 NMR, and by Galbraith Laboratories, Inc. (Knoxville, TN) using elemental analyses and melting point determination. Infrared and NMR spectra were consistent with literature spectra (Aldrich, 1974, 1981, 1992; Sigma, 1986). The infrared, proton NMR, and carbon-13 NMR spectra are presented in Figures I1, I2, and I3. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for 2-methylimidazole. The determined melting point range agreed with information provided by the manufacturer, agreed with one literature range (MSDS, 1995), but was slightly above another literature range (Sax's, 1996).

The purity of lot 081497 was determined by the analytical chemistry laboratory using capillary gas chromatography (GC) and high-performance liquid chromatography (HPLC) by system A. Gas chromatography was performed with a gas chromatograph (Hewlett Packard, Palo Alto, CA) using a flame ionization detector with a helium carrier gas flow rate of 1.2 mL per minute. A Stabilwax column (30 m × 0.25 mm, 0.25-µm film thickness; Restek, Bellefonte, PA) was used with an oven temperature program of 100° C for 2 minutes, then 15° C per minute to 235° C, then a 5 minute hold. The moisture content was determined by Galbraith Laboratories using Karl Fischer titration.

A) Hewlett Packard instrument; Prodigy C8 column (250 × 4.6 mm, 5-µm pore size; Phenomenex, Torrance, CA); using an isocratic mobile phase of 50% 3.75 mM sodium dodecyl sulfate and 37.5 mM sodium dihydrogen phosphate monobasic, and 50% methanol at a flow rate of 0.8 mL per minute, using ultraviolet detection at 221 nm

B) Hewlett-Packard instrument; Phenomenex Primesphere 5C-18-HC column (150 × 3.2 mm, 5-µm pore size; Phenomenex); with a mobile phase of 70% 7.5 mM sodium dodecyl sulfate and 75 mM sodium phosphate monobasic, and 30% methanol at a flow rate of 1.0 mL per minute, and ultraviolet detection at 215 nm

For lot 081497, GC at the analytical chemistry laboratory and the study laboratory indicated a purity of 100% with one major peak and no impurities. HPLC at the analytical chemistry laboratory indicated a purity of 100%. Karl Fischer titration indicated a moisture content of less than 0.16%. The overall purity of lot 081497 was determined to be greater than 99.5%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC. Samples stored under minimal headspace in sealed glass containers, protected from light, were stable for at least 2 weeks at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in glass bottles. Stability was monitored during the 2-year studies by the study laboratory using capillary 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 2-methylimidazole with feed (Table I1). A premix was prepared by hand and then blended with additional feed in a Patterson-Kelly V-shell blender for 30 minutes with the intensifier bar on for the initial 15 minutes. Formulations were stored in double thickness plastic bags placed inside heavy-duty opaque plastic bags and sealed inside plastic containers, protected from light at approximately 5° C for up to 35 days.

Homogeneity studies of the 300 and 5,000 ppm dose formulations were performed by the study laboratory by HPLC using System B. Stability studies of the 100 ppm dose formulation were performed by the analytical chemistry laboratory by HPLC using a system similar to system A. Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored in sealed plastic containers, protected from light at approximately 5° C and 25° C, and for at least 7 days when exposed to light and air.

Analyses of the dose formulations of 2-methylimidazole were conducted by the study laboratory every 8 to 12 weeks (Table I2) by HPLC using system B. Of the dose formulations analyzed for rats, 208 of 223 (93%) had concentrations that were within specifications. Of the 15 formulations that did not meet specifications, three were used and not remixed. Of those three, none were more than 13% from the target concentration. The concentrations of animal room samples for rats ranged from 89% to 104% of the target concentrations. Of the dose formulations analyzed for mice, 89 of 95 (94%) were within specifications. Of the four formulations not within specifications, one was used and not remixed. Animal room samples ranged from 90% to 101% of the target concentrations.

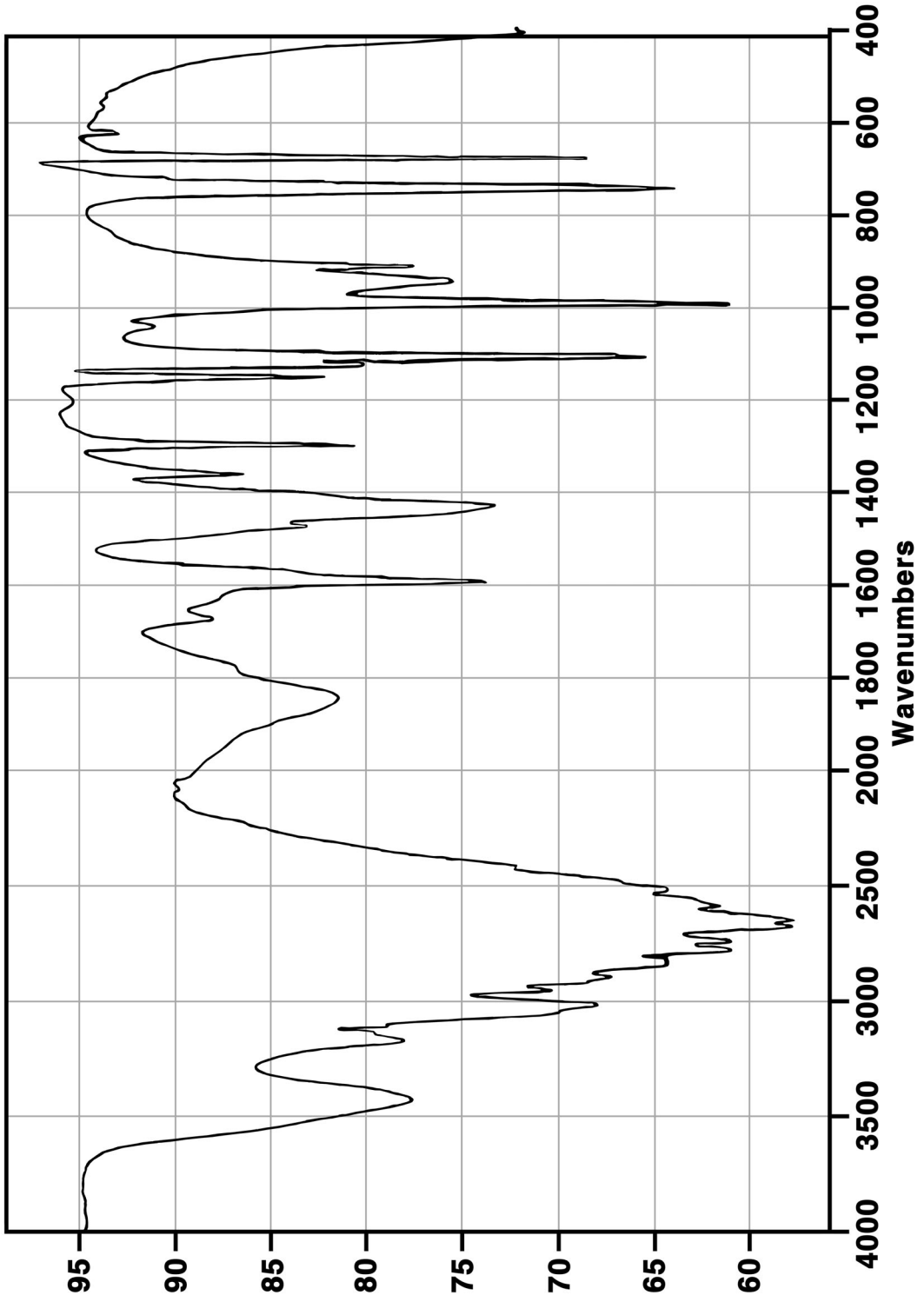

FIGURE I1
Infrared Absorption Spectrum of 2-Methylimidazole

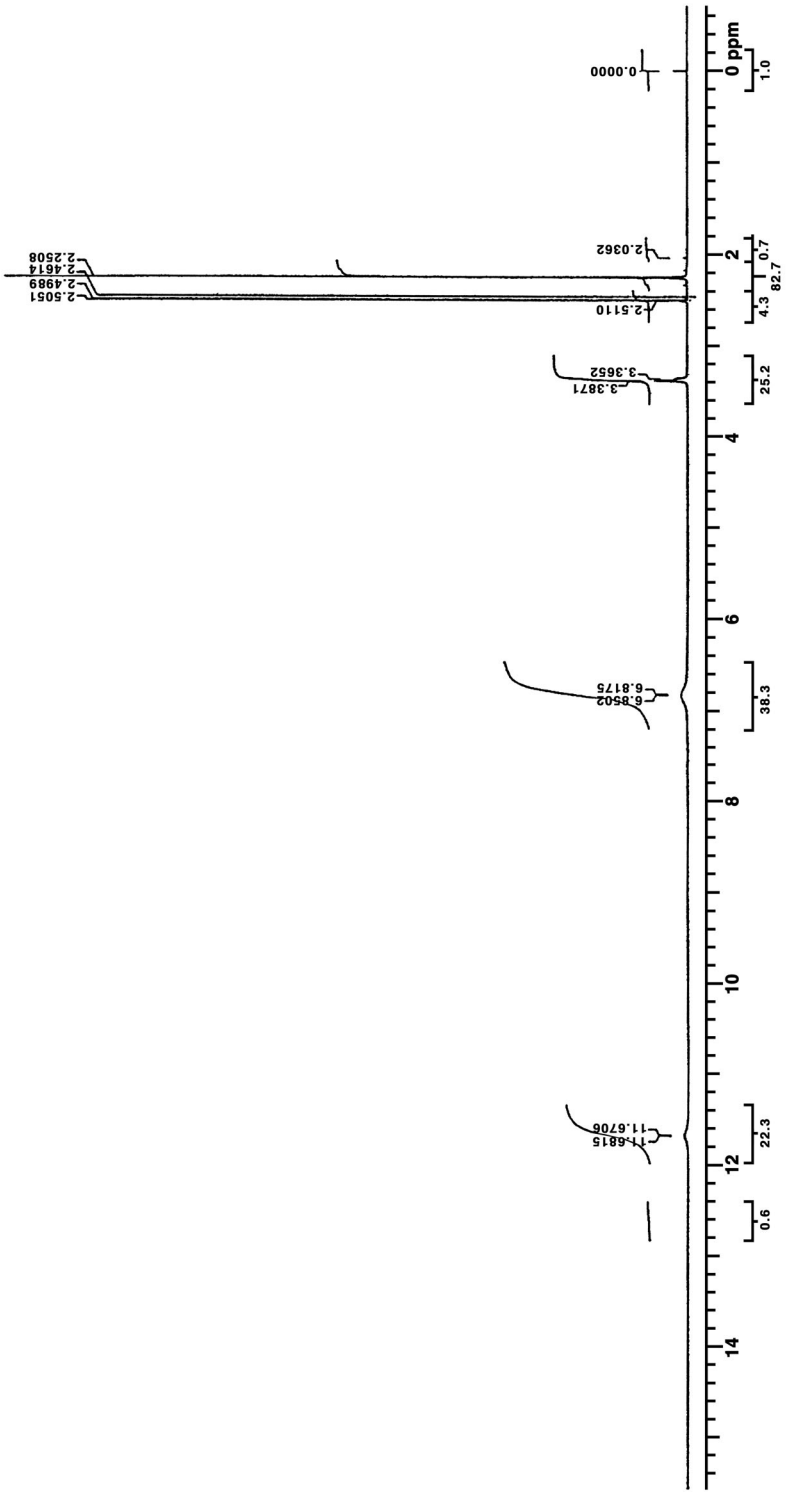

FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of 2-Methylimidazole

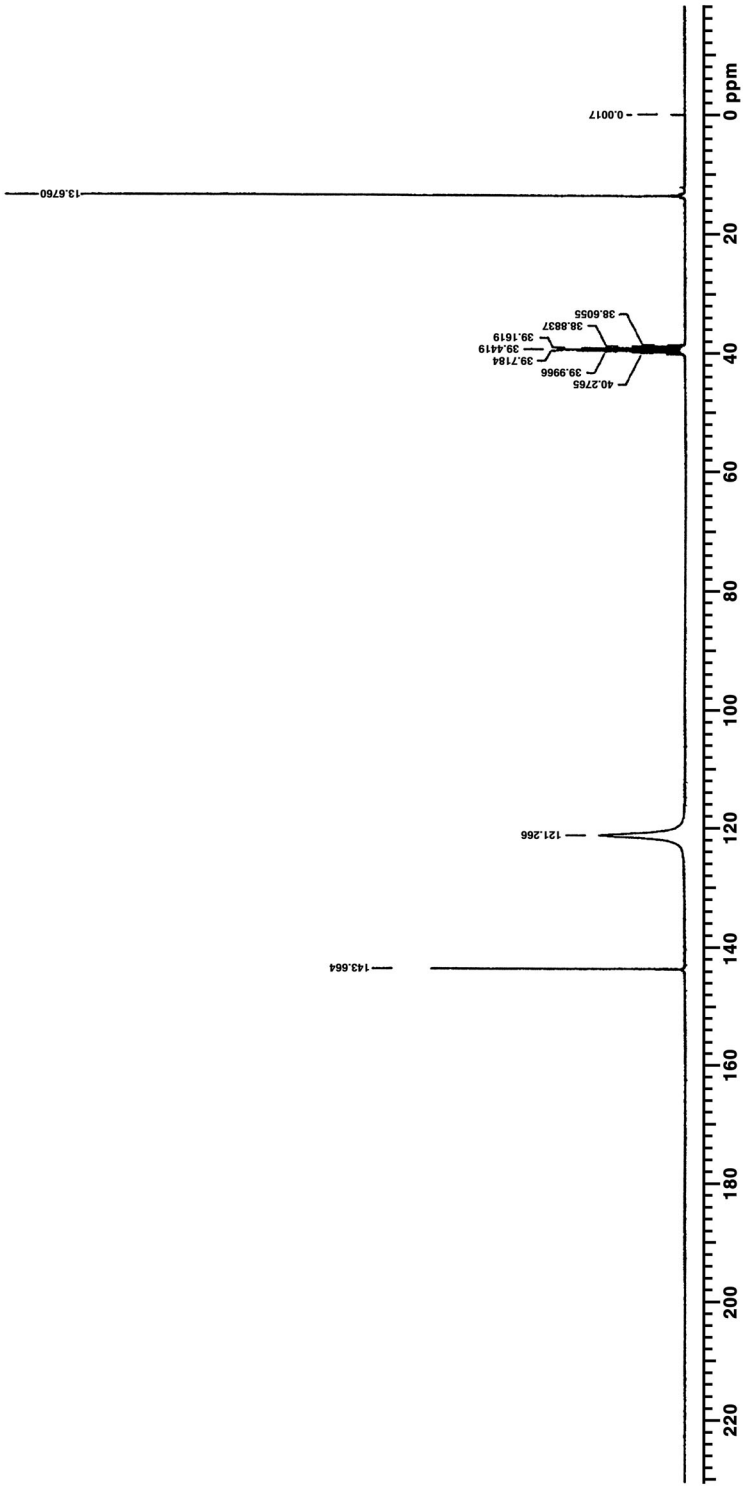

FIGURE I3
^{13}C-Nuclear Magnetic Resonance Spectrum of 2-Methylimidazole

TABLE I1
Preparation and Storage of Dose Formulations in the 2-Year Feed Studies of 2-Methylimidazole

Preparation
A premix of feed and 2-methylimidazole was prepared then layered into the remaining feed and blended in a Patterson-Kelly V-shell blender for 30 minutes with the intensifier bar on for the first 15 minutes. The dose formulations were prepared every 2 weeks.

Chemical Lot Number
081497

Maximum Storage Time
35 days

Storage Conditions
Stored in double-thickness plastic bags placed inside heavy-duty opaque plastic bags and sealed inside plastic containers, protected form light, at approximately 5° C

Study Laboratory
Southern Research Institute (Birmingham, AL)

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration[a] (mg/g)	Difference from Target (%)
Rats				
July 8 and 9, 1998	July 8-10, 1998	0.300	0.296	–1
		0.300	0.296	–1
		0.300	0.300	0
		1.00	0.997	0
		1.00	1.02	+2
		1.00	1.02	+2
		1.00	1.01	+1
		2.50	2.39	–4
		2.50	2.49	0
		3.00	2.89	–4
		3.00	2.99	0
		3.00	2.98	–1
		5.00	4.87	–3
		5.00	5.10	+2
July 8, 1998	July 31, 1998[b]	0.300	0.290	–3
		1.00	0.960	–4
		2.50	2.38	–5
		3.00	2.93	–2
		5.00	4.81	–4
July 22 and 23, 1998	July 23-25 and 29-30, 1998	0.300	0.305	+2
		0.300	0.328	+9
		0.300	0.319	+6
		0.300	0.314[c]	+5
		1.00	0.981	–2
		1.00	1.07	0
		1.00	1.02	+2
		1.00	1.04[c]	+4
		1.00	1.02	+2
		1.00	1.09	+9
		1.00	1.09	+9
		2.50	2.52	+1
		2.50	2.59	+4
		2.50	2.41[c]	–4
		2.50	2.66	+6
		3.00	2.97	–1
		3.00	3.04	+1
		3.00	3.13	+4
		3.00	3.19[c]	+6
		5.00	5.07	+1
		5.00	4.93	–1
		5.00	5.08	+2
August 3, 1998	August 5-6, 1998	0.300	0.300[d]	0
		1.00	1.00[d]	0
		2.50	2.59[d]	+3
		3.00	3.05[d]	+2

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
October 13-14, 1998	October 14-16, 1998	0.300	0.283	–6
		0.300	0.269[e]	–10
		0.300	0.299	0
		0.300	0.336[e]	+12
		1.00	0.996	0
		1.00	0.998	0
		1.00	1.03	+3
		1.00	0.971	–3
		1.00	1.01	+1
		1.00	0.996	0
		1.00	1.04	+4
		2.50	2.49[c]	0
		2.50	2.35	–6
		2.50	2.49	0
		2.50	2.49	0
		2.50	2.51	0
		3.00	3.15	+5
		3.00	3.05	+2
		3.00	2.87	–4
		3.00	3.01	0
		5.00	4.99[c]	0
		5.00	4.78	–4
		5.00	5.02	0
		5.00	4.88	–2
		5.00	4.98	0
October 19, 1998	October 20, 1998	0.300	0.283[d]	–6
		0.300	0.321[d]	+7
		2.50	2.57[d]	+3
		5.00	4.65[d]	–7
October 27-29, 1998	November 3-5, 1998	0.300	0.290	–3
		0.300	0.290	–4
		0.300	0.283	–6
		0.300	0.296	–1
		1.00	0.977	–2
		1.00	0.950	–5
		1.00	0.950	–5
		1.00	0.919	–8
		1.00	0.950	–5
		2.50	2.50	0
		2.50	2.44	–2
		2.50	2.48	–1
		2.50	2.46	–2
		2.50	2.42	–3
		3.00	2.99	0
		3.00	2.96	–1
		3.00	2.99	0
		3.00	2.85	–5

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
October 27-29, 1998	November 3-5, 1998	5.00	4.92	–2
		5.00	4.81	–4
		5.00	4.89	–2
		5.00	4.71	–6
		5.00	4.63	–7
January 6 and 8, 1999	January 7-9, 1999	0.300	0.280	–7
		0.300	0.279	–7
		0.300	0.281	–6
		1.00	1.03	+3
		1.00	0.977	–2
		1.00	0.996	0
		1.00	0.965	–4
		1.00	1.03	+3
		1.00	1.03	+3
		2.50	2.50	0
		2.50	2.48	–1
		2.50	2.45	–2
		2.50	2.45	–2
		2.50	2.51	+1
		3.00	2.94	–2
		3.00	2.82	–6
		3.00	2.88	–4
		5.00	5.06	+1
		5.00	5.15	+3
		5.00	4.92	–2
March 17 and 18, 1999	March 18-20 and 22, 1999	0.300	0.291	–3
		0.300	0.274	–9
		0.300	0.285	–5
		1.00	0.990	–1
		1.00	0.983	–2
		1.00	0.997	0
		1.00	0.989	–1
		1.00	0.993	–1
		2.50	2.53	+1
		2.50	2.52	+1
		2.50	2.55	+2
		2.50	2.55	+2
		3.00	2.98	–1
		3.00	3.07	+3
		3.00	3.11	+4
		5.00	4.77	–5
		5.00	4.97	–1
March 17, 1999	April 6-8, 1999[b]	0.300	0.300	0
		1.00	0.967	–3
		2.50	2.40	–4
		3.00	3.20	+7
		5.00	4.99	0

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
May 25 and 26, 1999	May 27-29, 1999	0.300	0.255[e]	–15
		0.300	0.294	–2
		0.300	0.268[e]	–11
		1.00	1.04[c]	+4
		1.00	1.03[c]	+3
		1.00	1.00	0
		1.00	1.02	+2
		1.00	1.04	+4
		2.50	2.51	0
		2.50	2.48	–1
		2.50	2.55	+2
		2.50	2.46	–2
		3.00	2.95	–2
		3.00	2.91	–3
		3.00	3.12	+4
		5.00	4.79	–4
		5.00	4.88	–2
June 2, 1999	June 2-3, 1999	0.300	0.296[d]	–1
		0.300	0.313[d]	+4
		1.00	0.992[d]	–1
		1.00	0.936[d]	–6
August 4 and 6, 1999	August 5-7, 1999	0.300	0.288	–4
		0.300	0.277	–8
		0.300	0.288	–4
		1.00	0.950	–5
		1.00	1.01	+1
		1.00	0.910	–9
		1.00	1.05	+5
		1.00	1.06	+6
		2.50	2.53	+1
		2.50	2.55	+2
		2.50	2.46	–2
		2.50	2.50	0
		3.00	3.10	+3
		3.00	3.08	+3
		3.00	2.99	0
		5.00	5.02	0
		5.00	5.01	0

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
October 13 and 15, 1999	October 14-16 and 18, 1999	0.300	0.291	–3
		0.300	0.296	–1
		0.300	0.297	–1
		1.00	0.981	–2
		1.00	1.01	+1
		1.00	0.950	–5
		1.00	1.03	+3
		1.00	1.03	+3
		2.50	2.44	–3
		2.50	2.57	+3
		2.50	2.43	–3
		2.50	2.48	–1
		3.00	3.00	0
		3.00	3.01	0
		3.00	2.93	–2
		5.00	4.97	–1
		5.00	5.11	+2
October 13, 1999	November 10-11, 1999[b]	0.300	0.287	–4
		1.00	0.942	–6
		2.50	2.42	–3
		3.00	2.85	–5
		5.00	4.99	0
December 21 and 22, 1999	December 27-28, 1999	0.300	0.292	–3
		0.300	0.284	–5
		0.300	0.278	–8
		1.00	1.01	+1
		1.00	1.01	+1
		1.00	0.991	–1
		1.00	1.01	+1
		1.00	1.01	+1
		2.50	2.50	0
		2.50	2.41	–4
		2.50	2.49	0
		2.50	2.61	+5
		3.00	2.94	–2
		3.00	3.03	+1
		3.00	2.96	–1
		5.00	5.07	+1
		5.00	5.21	+4

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
March 1 and 3, 2000	March 6-7, 2000	0.300	0.278	–7
		0.300	0.298	–1
		0.300	0.298	–1
		1.00	1.06	+6
		1.00	0.940	–6
		1.00	0.951	–5
		1.00	0.942	–6
		1.00	0.949	–5
		2.50	2.46	–1
		2.50	2.35	–6
		2.50	2.38	–5
		2.50	2.43	–3
		3.00	2.88	–4
		3.00	2.84	–6
		3.00	2.83	–6
		5.00	4.72	–6
		5.00	4.64	–7
May 10 and 12, 2000	May 15-16, 2000	0.300	0.288	–4
		0.300	0.281	–6
		0.300	0.261	–13[f]
		1.00	1.06	+6
		1.00	1.09	+9
		1.00	1.05	+5
		1.00	1.03	+3
		1.00	0.985	–2
		2.50	2.64	+6
		2.50	2.75	+10
		2.50	2.70	+8
		2.50	2.69	+7
		3.00	3.32	+11[f]
		3.00	3.33	+11[f]
		3.00	3.29	+10
		5.00	5.39	+8
		5.00	5.37	+7
May 10 and 12, 2000	June 6-7, 2000[b]	0.300	0.271	+4
		1.00	0.971	–11
		2.50	2.41	–11
		3.00	2.94	–11
		5.00	4.87	–9

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration[a] (mg/g)	Difference from Target (%)
Mice				
July 22 and 23, 1998	July 23-25 and 29-30, 1998	0.625	0.654	+5
		0.625	0.660	+6
		1.25	1.24	–1
		1.25	1.26	+1
		2.50	2.52	+1
		2.50	2.59	+4
		2.50	2.41[c]	–4
		2.50	2.66	+6
August 3, 1998	August 5-6, 1998	2.50	2.59[d]	+3
August 4, 1998	August 26-28, 1998[b]	0.625	0.609	–3
		1.25	1.24	–1
		2.50	2.34	–6
October 13 and 14, 1998	October 14-16, 1998	0.625	0.654	+5
		0.625	0.618	–1
		0.625	0.564	–10
		1.25	1.29	+3
		1.25	1.27[c]	+2
		1.25	1.26	+1
		2.50	2.49[c]	0
		2.50	2.35	–6
		2.50	2.49	0
		2.50	2.49	0
		2.50	2.51	0
October 19, 1998	October 20, 1998	1.25	1.28[d]	+2
		2.50	2.57[d]	+3
October 27 and 28, 1998	November 3-5, 1998	0.625	0.640[c]	+2
		0.625	0.625	0
		0.625	0.596	–5
		1.25	1.20	–4
		1.25	1.23	–1
		1.25	1.21	–3
		2.50	2.50	0
		2.50	2.44	–2
		2.50	2.48	–1
		2.50	2.46	–2
		2.50	2.42	–3
January 6 and 8, 1999	January 7-9 and 11, 1999	0.625	0.623	0
		0.625	0.575	–8
		1.25	1.26	+1
		1.25	1.29	+3
		2.50	2.50	0
		2.50	2.48	–1
		2.50	2.45	–2
		2.50	2.45	–2
		2.50	2.51	+1

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice (continued)				
March 17, 1999	March 18-20 and 22, 1999	0.625	0.578[c]	–7
		0.625	0.573	–8
		1.25	1.28[c]	+2
		1.25	1.24	–1
		2.50	2.53	+1
		2.50	2.52	+1
		2.50	2.55	+2
		2.50	2.55	+2
March 24, 1999	March 24, 1999	0.625	0.615[d]	–2
		1.25	1.21[d]	–3
March 24, 1999	April 6-8, 1999[b]	0.625	0.634	+1
		1.25	1.33	+6
March 17, 1999		2.50	2.54	+2
May 25 and 26, 1999	May 27-29, 1999	0.625	0.602	–4
		0.625	0.632	+1
		1.25	1.28	+3
		1.25	1.29	+3
		2.50	2.51	0
		2.50	2.48	–1
		2.50	2.55	+2
		2.50	2.46	–2
August 4, 1999	August 5-7 and 9, 1999	0.625	0.644	+3
		0.625	0.604	–3
		1.25	1.32	+5
		1.25	1.20	–4
		2.50	2.53	+1
		2.50	2.55	+2
		2.50	2.46	–2
		2.50	2.50	0
October 13 and 15, 1999	October 14-16 and 18, 1999	0.625	0.639	+2
		0.625	0.603	–4
		1.25	1.23	–2
		1.25	1.15	–8
		2.50	2.44	–3
		2.50	2.57	+3
		2.50	2.43	–3
		2.50	2.48	–1
October 13 and 15, 1999	November 8, 1999[b]	0.625	0.586	–6
		1.25	1.20	–4
		2.50	2.41	–4

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 2-Methylimidazole

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice (continued)				
December 21and 22, 1999	December 27-28, 1999	0.625	0.633	+1
		0.625	0.647	+3
		1.25	1.29	+3
		1.25	1.27	+1
		2.50	2.50	0
		2.50	2.41	–4
		2.50	2.49	0
		2.50	2.61	+5
March 1 and 3, 2000	March 6-7, 2000	0.625	0.587	–6
		0.625	0.603	–4
		1.25	1.23	–2
		1.25	1.27	+2
		2.50	2.46	–1
		2.50	2.35	–6
		2.50	2.38	–5
		2.50	2.43	–3
May 10, 2000	May 15-16, 2000	0.625	0.648	+4
		0.625	0.626	0
		1.25	1.34	+8
		1.25	1.31	+5
		2.50	2.64	+6
		2.50	2.75	+10
		2.50	2.70	+8
		2.50	2.69	+7
May 10 and 12, 2000	June 6-7, 2000[b]	0.625	0.627	0
		1.25	1.23	–2
		2.50	2.41	–4

[a] Results of duplicate analyses except for 0.3 mg/g formulations (triplicate analyses)
[b] Animal room samples
[c] Sample was remixed; ratio of the low and high concentration was less than 0.9
[d] Results of remix
[e] Sample was remixed; concentration was not within 10% of target concentration
[f] Sample was not remixed

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

TABLE J1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm		300 ppm			1,000 ppm			3,000 ppm		
Week	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)
4	18.7	213	18.5	207	27	18.1	209	87	15.8	193	246
8	18.0	284	16.9	280	18	18.0	280	64	16.2	257	189
12	16.6	320	16.8	315	16	16.1	315	51	15.2	289	158
16	16.9	348	16.9	344	15	17.0	341	50	15.6	318	147
20	16.6	367	16.5	360	14	16.6	360	46	15.1	334	136
24	16.6	378	16.0	371	13	15.8	369	43	15.5	347	134
28	15.5	386	15.9	382	12	15.7	377	42	14.1	362	117
32	16.5	401	16.6	395	13	16.3	392	42	16.7	377	133
36	16.6	415	16.7	405	12	15.8	403	39	16.3	388	126
40	16.8	422	16.9	419	12	16.8	414	41	15.7	400	118
44	16.2	429	17.0	423	12	16.9	421	40	16.3	406	121
48	16.3	436	15.7	428	11	15.9	426	37	15.4	411	112
52	15.7	437	16.2	435	11	16.1	432	37	15.3	414	111
56	16.6	444	16.8	437	11	16.2	432	37	16.1	417	116
60	16.9	446	17.3	438	12	16.8	435	39	16.4	420	117
64	16.3	444	16.2	440	11	16.2	433	37	15.2	413	110
72	15.8	447	16.4	439	11	15.6	437	36	14.9	422	106
76	15.9	443	15.7	443	11	15.3	435	35	14.6	420	104
80	15.7	440	15.9	433	11	15.6	430	36	15.3	410	112
84	15.0	436	15.1	431	11	15.2	425	36	15.1	407	111
88	14.3	431	15.3	429	11	15.2	422	36	14.8	400	111
92	13.4	422	14.1	425	10	14.2	416	34	14.1	400	105
96	14.7	421	14.8	420	11	15.0	412	36	14.8	397	112
100	14.9	419	16.3	421	12	14.2	408	35	14.1	390	108
104	15.1	415	15.6	414	11	15.2	407	37	13.1	375	104
Mean for weeks											
1-13	17.8	272	17.4	267	20	17.4	268	67	15.7	246	197
14-52	16.4	402	16.4	396	13	16.3	393	42	15.6	376	125
53-104	15.4	434	15.8	431	11	15.4	424	36	14.9	406	110

[a] Grams of feed consumed per animal per day
[b] Milligrams of 2-methylimidazole consumed per kilogram body weight per day

TABLE J2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of 2-Methylimidazole

	0 ppm		1,000 ppm			2,500 ppm			5,000 ppm		
Week	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)
4	13.2	148	13.2	148	89	11.5	142	202	10.1	133	381
8	11.4	173	11.3	175	65	11.1	168	165	9.6	154	310
12	10.8	184	11.1	184	61	10.4	177	147	9.1	160	286
16	10.3	193	10.5	191	55	9.8	184	134	8.2	166	245
20	10.2	198	10.0	197	51	9.5	191	124	8.3	171	242
24	10.1	204	10.4	203	51	9.3	197	119	8.2	178	229
28	9.8	210	10.2	208	49	9.8	202	121	8.4	185	226
32	10.5	217	10.6	215	49	9.9	208	119	9.2	193	237
36	10.3	224	10.7	220	49	10.1	214	118	9.1	197	232
40	10.6	231	10.6	228	47	10.1	221	115	9.0	204	220
44	11.1	238	11.0	235	47	10.7	225	119	9.2	206	223
48	10.7	242	11.0	240	46	10.5	230	114	9.3	211	221
52	10.8	246	11.9	247	48	10.9	235	116	9.1	211	215
56	11.9	257	11.3	257	44	10.8	240	112	9.6	217	221
60	11.9	268	12.2	266	46	11.6	249	117	9.8	220	224
64	11.5	275	11.6	273	42	11.2	254	111	9.5	223	214
72	12.3	291	11.8	288	41	10.8	264	102	8.6	228	189
76	11.8	297	11.6	291	40	11.6	269	108	9.5	230	207
80	12.1	297	12.0	293	41	11.8	273	109	9.8	230	214
84	11.2	300	11.3	295	38	11.3	275	102	10.0	232	214
88	11.1	304	11.8	299	39	11.4	279	102	9.3	234	199
92	11.0	312	10.8	304	35	10.4	283	92	9.0	232	194
96	12.3	314	12.7	306	41	12.0	285	105	10.0	233	215
100	12.1	319	12.0	308	39	11.3	285	99	9.7	231	210
104	12.0	321	12.0	309	39	12.0	283	106	10.0	228	219
Mean for weeks											
1-13	11.8	168	11.9	169	71	11.0	162	171	9.6	149	325
14-52	10.4	220	10.7	219	49	10.1	211	120	8.8	192	229
53-104	11.8	296	11.8	291	41	11.4	270	105	9.6	228	210

[a] Grams of feed consumed per animal per day
[b] Milligrams of 2-methylimidazole consumed per kilogram body weight per day

TABLE J3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of 2-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)
4	4.5	23.1	4.8	23.0	129	4.7	23.0	255	4.8	23.2	515
8	3.9	26.6	4.1	26.1	98	3.8	24.9	192	4.0	25.2	400
12	4.2	31.4	4.6	30.2	96	4.4	29.7	184	4.4	29.0	376
16	4.4	33.4	4.3	32.9	81	4.3	32.0	168	4.2	31.2	338
20	4.5	35.9	4.7	34.4	85	4.5	33.3	168	4.3	32.9	325
24	4.4	38.0	4.5	36.7	77	4.5	35.1	159	4.5	34.1	328
28	4.4	40.2	4.3	39.1	69	4.5	37.9	150	4.4	37.2	298
32	4.7	41.9	4.6	40.7	71	4.5	39.7	143	4.6	38.3	297
36	4.3	43.5	4.6	42.4	68	4.8	41.4	145	4.6	40.0	288
40	4.3	44.5	4.4	43.7	63	4.4	42.6	129	4.5	40.8	273
44	4.8	44.5	4.8	43.4	69	4.7	42.4	139	4.8	40.4	297
48	4.8	44.7	4.6	44.2	66	4.6	43.0	134	4.8	40.7	294
52	4.8	44.8	4.8	44.8	67	4.7	43.8	135	4.6	41.1	281
56	5.3	45.5	5.3	44.3	74	5.2	43.8	148	5.0	40.8	308
60	5.0	45.2	4.9	44.5	69	4.9	43.8	141	4.9	39.9	310
64	5.1	45.9	5.1	45.0	71	5.0	43.7	145	4.9	40.1	306
68	5.2	46.2	5.1	45.4	70	5.2	44.4	146	5.1	40.9	312
72	5.1	46.8	4.9	46.1	67	5.0	44.9	139	4.8	41.3	290
76	4.8	46.4	4.8	45.6	66	4.7	44.0	134	4.6	41.0	279
80	4.3	46.6	4.3	45.5	59	4.4	43.8	124	4.1	41.0	251
84	5.0	45.7	5.1	45.0	71	5.3	43.1	152	4.7	40.1	295
88	4.7	45.7	4.6	45.5	64	4.7	42.9	138	4.6	40.3	285
92	4.7	44.9	5.0	45.1	69	5.0	42.2	149	4.6	40.1	285
96	5.3	44.7	5.2	44.6	73	5.3	42.9	153	5.2	39.1	334
100	5.3	45.2	5.0	44.0	72	5.1	42.2	152	4.9	39.2	312
104	4.9	42.5	5.1	42.2	76	4.8	40.8	148	4.8	37.3	322
Mean for weeks											
1-13	4.2	27.0	4.5	26.4	108	4.3	25.9	210	4.4	25.8	431
14-52	4.5	41.1	4.6	40.2	72	4.6	39.1	147	4.5	37.7	302
53-104	5.0	45.5	5.0	44.8	69	5.0	43.3	144	4.8	40.1	299

[a] Grams of feed consumed per animal per day
[b] Milligrams of 2-methylimidazole consumed per kilogram body weight per day

TABLE J4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of 2-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)
4	2.7	20.3	3.3	20.1	102	2.9	20.0	180	2.9	20.2	364
8	3.6	23.1	3.7	23.6	98	3.4	23.2	182	3.6	22.9	389
12	3.4	25.0	3.5	24.5	89	3.5	24.2	181	3.5	24.1	365
16	3.3	25.8	3.3	25.9	81	3.5	25.4	170	3.4	24.7	345
20	3.1	27.7	3.4	27.6	77	3.2	26.8	151	3.3	26.7	308
24	3.6	28.7	3.4	29.2	74	3.6	28.6	159	3.7	27.9	336
28	3.7	30.5	4.1	31.6	81	3.9	30.7	157	4.0	29.7	333
32	3.6	31.0	3.7	31.9	72	3.6	31.4	143	3.6	29.6	306
36	3.8	30.3	3.7	32.3	73	4.0	32.0	157	3.7	29.8	314
40	3.8	32.9	4.0	34.0	73	3.8	34.6	136	3.8	32.1	298
44	4.0	32.6	4.5	33.4	84	4.3	33.5	159	4.4	31.2	352
48	3.6	32.4	3.7	33.4	70	3.8	33.7	141	3.9	31.7	308
52	4.0	32.5	3.9	34.7	70	3.8	34.6	137	3.5	32.2	275
56	4.3	33.6	4.5	34.5	82	4.5	34.1	164	4.4	31.5	350
60	4.1	34.2	4.2	35.1	75	4.2	34.5	151	4.0	31.1	321
64	4.0	34.3	4.0	35.3	71	4.0	34.7	144	3.9	32.0	306
68	4.5	34.8	4.6	35.5	81	4.6	35.2	164	4.4	32.5	339
72	4.2	35.0	4.3	36.9	72	4.4	35.4	157	4.8	31.8	373
76	3.2	34.7	3.4	35.7	59	3.3	35.3	117	3.2	31.3	257
80	4.1	37.0	4.1	37.5	69	4.0	36.6	135	4.0	33.0	300
84	4.4	38.8	4.8	36.5	82	4.9	38.1	159	4.6	34.3	338
88	4.2	38.5	4.2	38.4	68	4.3	38.7	137	4.1	33.8	302
92	3.8	38.2	3.8	38.0	63	3.9	37.9	128	3.8	33.5	281
96	4.3	38.2	4.4	38.2	72	4.2	38.0	138	4.1	34.4	297
100	4.8	38.2	4.2	38.8	68	4.2	38.6	137	4.1	34.1	303
104	4.5	37.8	5.1	38.2	84	4.6	38.0	153	4.5	33.6	336
Mean for weeks											
1-13	3.3	22.8	3.5	22.8	96	3.3	22.5	181	3.3	22.4	373
14-52	3.6	30.5	3.8	31.4	75	3.7	31.1	151	3.7	29.6	317
53-104	4.2	36.4	4.3	36.8	73	4.2	36.5	145	4.1	32.8	316

[a] Grams of feed consumed per animal per day
[b] Milligrams of 2-methylimidazole consumed per kilogram body weight per day

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

TABLE K1
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 K2
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 K3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	13.8 ± 0.46	13.0 – 15.4	25
Crude fat (% by weight)	8.1 ± 0.31	7.4 – 8.6	25
Crude fiber (% by weight)	9.0 ± 0.47	8.2 – 9.8	25
Ash (% by weight)	5.1 ± 0.28	4.7 – 6.0	25
Amino Acids (% of total diet)			
Arginine	0.731 ± 0.050	0.670 – 0.800	8
Cystine	0.224 ± 0.012	0.210 – 0.240	8
Glycine	0.684 ± 0.041	0.620 – 0.740	8
Histidine	0.333 ± 0.018	0.310 – 0.350	8
Isoleucine	0.524 ± 0.046	0.430 – 0.590	8
Leucine	1.061 ± 0.061	0.960 – 1.130	8
Lysine	0.708 ± 0.056	0.620 – 0.790	8
Methionine	0.401 ± 0.035	0.350 – 0.460	8
Phenylalanine	0.598 ± 0.036	0.540 – 0.640	8
Threonine	0.501 ± 0.051	0.430 – 0.590	8
Tryptophan	0.126 ± 0.014	0.110 – 0.150	8
Tyrosine	0.390 ± 0.056	0.280 – 0.460	8
Valine	0.640 ± 0.049	0.550 – 0.690	8
Essential Fatty Acids (% of total diet)			
Linoleic	3.97 ± 0.284	3.59 – 4.54	8
Linolenic	0.30 ± 0.042	0.21 – 0.35	8
Vitamins			
Vitamin A (IU/kg)	5,643 ± 1,504	2,960 – 8,710	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	82.2 ± 14.08	62.2 – 107.0	8
Thiamine (ppm)[b]	9.7 ± 3.4	6.3 – 23.0	25
Riboflavin (ppm)	5.6 ± 1.12	4.20 – 7.70	8
Niacin (ppm)	74.3 ± 5.94	66.4 – 85.8	8
Pantothenic acid (ppm)	22.5 ± 3.96	17.4 – 29.1	8
Pyridoxine (ppm)[b]	9.04 ± 2.37	6.4 – 12.4	8
Folic acid (ppm)	1.64 ± 0.38	1.26 – 2.32	8
Biotin (ppm)	0.333 ± 0.15	0.225 – 0.704	8
Vitamin B_{12} (ppb)	68.7 ± 63.0	18.3 – 174.0	8
Choline (ppm)[b]	3,155 ± 325	2,700 – 3,790	8
Minerals			
Calcium (%)	1.031 ± 0.079	0.933 – 1.240	25
Phosphorus (%)	0.579 ± 0.041	0.527 – 0.737	25
Potassium (%)	0.659 ± 0.022	0.627 – 0.691	8
Chloride (%)	0.357 ± 0.027	0.300 – 0.392	8
Sodium (%)	0.189 ± 0.019	0.160 – 0.212	8
Magnesium (%)	0.199 ± 0.009	0.185 – 0.213	8
Sulfur (%)	0.178 ± 0.021	0.153 – 0.209	8
Iron (ppm)	160 ± 14.7	135 – 177	8
Manganese (ppm)	50.3 ± 4.82	42.1 – 56.0	8
Zinc (ppm)	50.7 ± 6.59	43.3 – 61.1	8
Copper (ppm)	6.29 ± 0.828	5.08 – 7.59	8
Iodine (ppm)	0.461 ± 0.187	0.233 – 0.843	8
Chromium (ppm)	0.542 ± 0.128	0.330 – 0.707	7
Cobalt (ppm)	0.23 ± 0.049	0.20 – 0.30	7

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

TABLE K4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.15 ± 0.075	0.10 – 0.38	25
Cadmium (ppm)	0.04 ± 0.007	0.04 – 0.07	25
Lead (ppm)	0.09 ± 0.061	0.05 – 0.29	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.20 ± 0.028	0.15 – 0.26	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	11.2 ± 3.25	9.04 – 22.9	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	13 ± 14	10 – 80	25
Coliform (MPN/g)	0.14 ± 0.7	0.0 – 3.6	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.9 ± 1.45	2.3 – 7.7	25
N-Nitrosodimethylamine (ppb)[e]	2.1 ± 0.69	1.0 – 3.5	25
N-Nitrosopyrrolidine (ppb)[e]	2.8 ± 1.1	1.0 – 5.5	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.147 ± 0.138	0.020 – 0.536	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.168 ± 0.139	0.020 – 0.432	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

[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 L
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 randomly selected rats and mice during the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to MA Bioservices/BioReliance (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	6 months and study termination
Mycoplasma pulmonis	6 months and 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
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	6 months and study termination
M. pulmonis	6 months and 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

Method and Test	Time of Analysis
MICE (continued)	
Immunofluorescence Assay	
EDIM	Study termination
GDVII	Study termination
LCM	Study termination
MCMV (mouse cytomegalovirus)	6 months and study termination
M. arthritidis	Study termination
Parvovirus	6, 12, and 18 months, study termination

RESULTS

All test results were negative.

APPENDIX M
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

The single-dose intravenous injection and gavage toxicokinetic studies of 2-methylimidazole were designed to estimate toxicokinetic parameters for the elimination of 2-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 gavage dose of 25, 50, or 100 mg/kg. Postdose plasma samples were analyzed for 2-methylimidazole, and the results were used to calculate toxicokinetic parameters.

MATERIALS AND METHODS

2-Methylimidazole (lot 081497) was obtained from Aldrich Chemical Company (Milwaukee, WI) and stored at room temperature in the original bottles. The material was analyzed for purity and identity; the results and analytical systems are described in Appendix I.

On the day of the dosing, male and female F344/N rats were approximately 14 to 15 weeks old and ranged in weight from 283.8 to 330.4 and 151.2 to 191.8 grams, respectively; male and female B6C3F$_1$ mice were approximately 11 weeks old and ranged in weight from 18.6 to 32.9 and 11.5 to 27.1 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 a CO_2/O_2 mixture, and blood samples were collected from the retroorbital sinus (rats) or cardiac puncture (mice) and placed into individual tubes containing EDTA as an anticoagulant. Rats were bled twice (minimum of 55 minutes between bleedings), mice were bled once, and three rats and three mice were bled at each timepoint. Rats were bled at 5, 10, 15, 30, 60, 120, 240, 360, 720, and 1,440 (100 mg/kg groups only) minutes after dosing. Mice were bled at 5, 10, 15, 30, 45 (25 and 50 mg/kg groups only), 60, 90, 180, 360, and 720 (100 mg/kg groups only) minutes after dosing. Samples of approximately 1 mL (rats) and the maximum volume obtainable for mice (approximately 0.5 to 1 mL) were gently rocked by hand to ensure adequate mixing with the anticoagulant and 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 approximately 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 5 minutes at 1,700 rpm. The organic layer was transferred to automated liquid sampler vials for analysis. Gas chromatography was then performed on a (HP-6890; Hewlett-Packard, Palo Alto, CA) equipped with a nitrogen phosphorous detector and a Carbowax-Amine column (Supelco, Bellefonte, PA), which was either 30 m × 0.53 mm ID and 1-µm film thickness or 30 m × 0.32 mm ID and 0.23-µm film thickness. The column oven was programmed from 150° C for 2 minutes to 235° C for 5 minutes with 5° C per minute ramp rate. 2-Methylimidazole was quantitated using least-square linear regression of a calibration curve generated from spiked plasma of untreated F344/N rats.

The analytical method for determining 2-methylimidazole in plasma samples was validated and determined to be linear within a range of 0.1 to 20 µg/mL in F344/N rat plasma. The limit of quantitation was 0.1 µg/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

2-Methylimidazole plasma concentration versus time data were evaluated using the nonlinear least-squares estimation program WinNONLIN® version 2.1 (rats) or 1.5A (mice), 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, 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 (area 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

2-Methylimidazole was rapidly absorbed and distributed when administered by gavage in an aqueous formulation to male and female F344/N rats and male and female $B6C3F_1$ mice (Figures M1 and M2), and thus absorption and distribution cannot be differentiated in this toxicokinetic study. Therefore, the early timepoints probably represent a combination of the two processes. The administration of 2-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 much larger than elimination rate constants and were similar in males and females of each species (data not shown). The absorption half-life values ranged from 10 to 18 minutes in rats and 2 to 4 minutes in mice and were generally linear with dose (Table M1). Elimination half-life values ranged from 61 to 96 minutes in rats and from 15 to 20 minutes in mice and were generally increased in the 100 mg/kg groups. Differences in clearance across the treatment groups were not all statistically significant, but clearance was decreased in all 100 mg/kg groups. 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. 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.

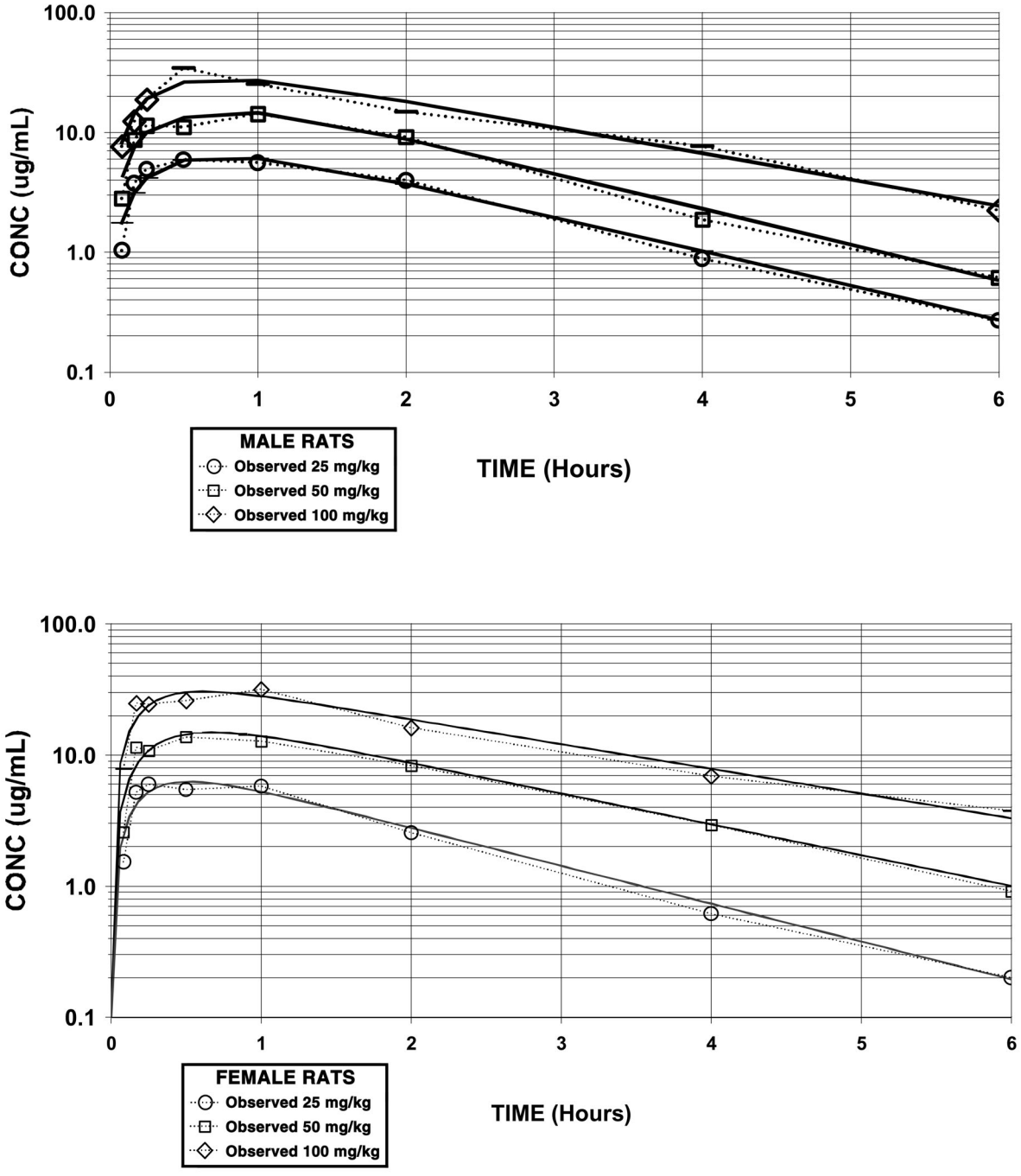

FIGURE M1
Plasma Concentrations of 2-Methylimidazole in Male and Female F344/N Rats after a Single Gavage Dose of 25, 50, or 100 mg/kg 2-Methylimidazole
Solid lines represent the predicted best-fit curves (WinNONLIN®) plotted through the observed data points.

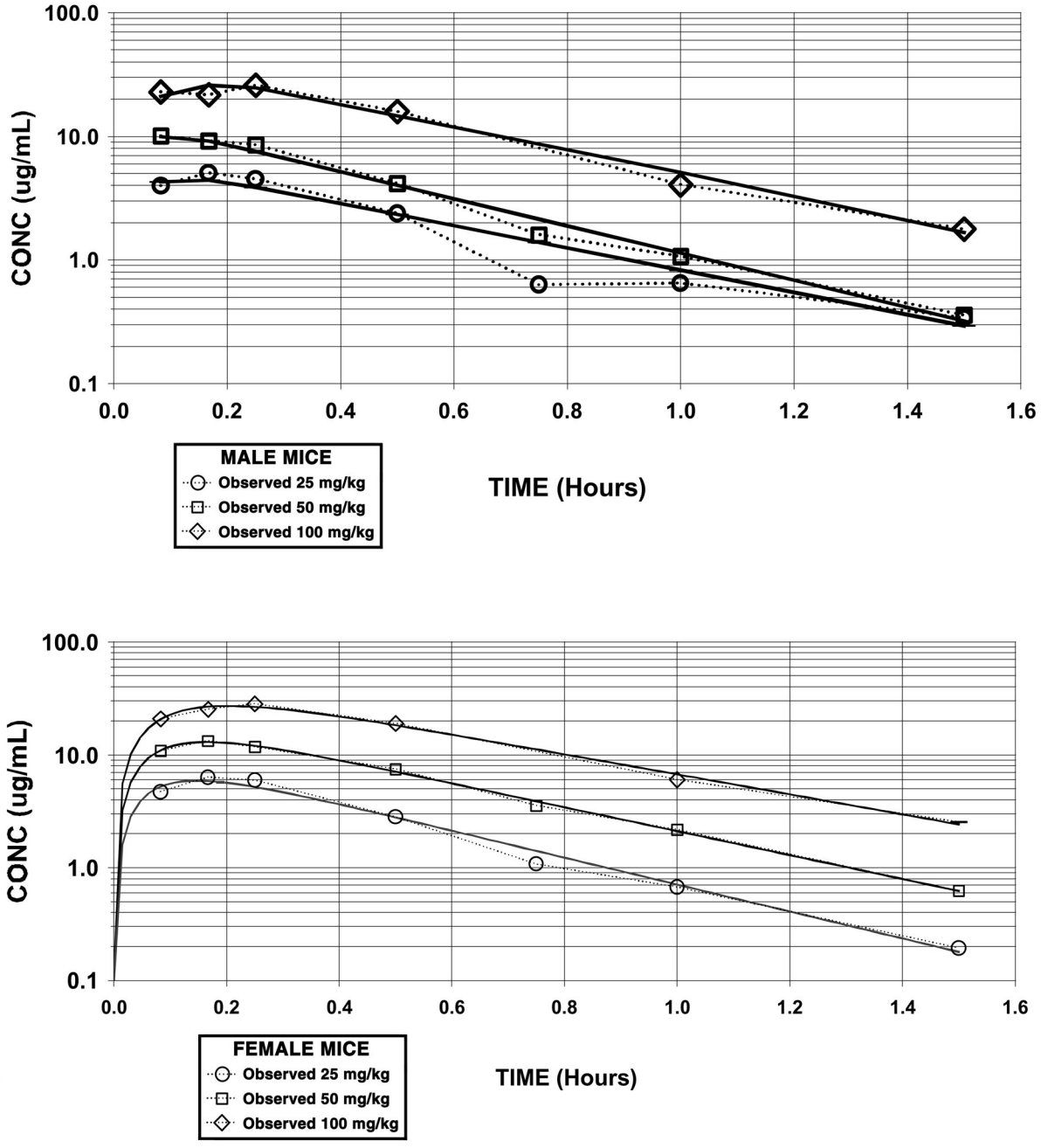

Figure M2
Plasma Concentrations of 2-Methylimidazole in Male and Female B6C3F$_1$ Mice after a Single Gavage Dose of 25, 50, or 100 mg/kg 2-Methylimidazole
Solid lines represent the predicted best-fit curves (WinNONLIN®) plotted through the observed data points.

TABLE M1
Toxicokinetic Parameter Estimates for the Elimination of 2-Methylimidazole from the Plasma of F344/N Rats and B6C3F$_1$ Mice after a Single Gavage Dose of 2-Methylimidazole[a]

Parameter	25 mg/kg		50 mg/kg		100 mg/kg	
	Male	Female	Male	Female	Male	Female
Rats						
C_{max} (µg/mL)	6.5 ± 0.6	6.2 ± 0.5	13.6 ± 1.3	14.7 ± 1.8	27.7 ± 2.5	31.4 ± 3.0
C_{max}/dose	0.260 ± 0.024	0.248 ± 0.020	0.272 ± 0.026	0.294 ± 0.036	0.277 ± 0.025	0.314 ± 0.030
AUC [(µg • hour)/mL]	16 ± 1	13 ± 1	34 ± 3	41 ± 5	83 ± 7	92 ± 7
AUC/dose	0.64 ± 0.04▲	0.52 ± 0.04▼■	0.68 ± 0.06	0.82 ± 0.1▼	0.83 ± 0.07▲	0.92 ± 0.07■
Absorption half-life (hour)	0.263 ± 0.065	0.183 ± 0.039	0.243 ± 0.061	0.230 ± 0.075	0.244 ± 0.058	0.172 ± 0.043
Elimination half-life (hour)	1.10 ± 0.12▲	1.03 ± 0.07▼	1.13 ± 0.11	1.35 ± 0.19	1.45 ± 0.16▲	1.54 ± 0.17▼
Clearance (L/hour)	4.97 ± 0.45	10.6 ± 0.8	4.73 ± 0.45	6.80 ± 0.73	3.83 ± 0.29	6.12 ± 0.51
Mice						
C_{max} (µg/mL)	4.35 ± 0.87	5.89 ± 0.53	10.0 ± 1.2	13.0 ± 0.4	25.4 ± 2.2	27.1 ± 1.2
C_{max}/dose	0.174 ± 0.035▼	0.236 ± 0.021▲	0.200 ± 0.024■	0.260 ± 0.008	0.254 ± 0.022▼■	0.271 ± 0.012▲
AUC [(µg • hour)/mL]	2.84 ± 0.35	3.21 ± 0.21	5.09 ± 0.32	7.93 ± 0.17	16.7 ± 1.1	20.0 ± 0.7
AUC/dose	0.114 ± 0.014▲	0.128 ± 0.008▼	0.102 ± 0.006■	0.159 ± 0.003▼	0.167 ± 0.011▲■	0.200 ± 0.070
Absorption half-life (hour)	0.035 ± 0.033	0.051 ± 0.016	0.027 ± 0.016	0.057 ± 0.006	0.054 ± 0.019	0.071 ± 0.012
Elimination half-life (hour)	0.334 ± 0.054	0.252 ± 0.018▲▼	0.274 ± 0.016	0.284 ± 0.008▲■	0.318 ± 0.028	0.339 ± 0.020▼■
Clearance (L/hour)	8.80 ± 2.86	7.79 ± 4.76	9.82 ± 3.12	6.30 ± 5.88	5.98 ± 0.91	5.00 ± 1.43

[a] On each row, values (mean ± standard error) for the same sex with the same symbol do not have overlapping means and errors (P≤0.05).
C_{max}=maximum mean plasma concentration; AUC=area under the plasma concentration versus time curve from 0 to infinity

APPENDIX N
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION

A physiologically based pharmacokinetic (PBPK) model representing the uptake, distribution, and metabolism of 2-methylimidazole in rats and mice was developed to describe the processes involved in 2-methylimidazole toxicokinetics. Model development was based on published data for 2-methylimidazole disposition in male F344 rats (Sanders *et al.*, 1998) and single-dose toxicokinetic data for male and female rats and mice from the current studies (Appendix M). Most of the model parameters were obtained from estimates in the literature. However, the model has 11 parameters that did not have literature estimates. These parameters were estimated with the model and the current toxicokinetic data using maximum likelihood techniques. Once the unknown parameters were estimated, the model was used to test for differences between species and sexes. There were tests for general differences as well as tests for differences attributed to a particular process such as metabolism or urinary excretion.

MODEL DEVELOPMENT

The toxicokinetic data in Appendix M include plasma concentrations of 2-methylimidazole for up to 24 hours following a single gavage dose to male and female rats and mice; although the single-dose intravenous injection data are not presented in Appendix M, they are used in this model. Published data for the disposition of 2-methylimidazole in male F344 rats include tissue concentrations of 2-methylimidazole for 24 hours after intravenous administration of 5 mg/kg in the following tissues: blood, liver, kidney, muscle, skin, adipose, brain, and thyroid gland. To enable full use of these data, the PBPK model presented here has separate compartments representing liver, kidney, muscle, skin, adipose, brain, gastrointestinal tract, thyroid gland, and other aggregated tissues (Figure N1). The data of Sanders *et al.* (1998) were used to evaluate the model against data not used to estimate any of the unknown model parameters. The model also includes separate compartments for arterial and venous blood. The representation of the kidney includes tubule lumen and glomerular filtration of arterial blood. Urinary excretion is modeled as a saturable process with Michaelis-Menten kinetics. A separate lumenal space is included for the gastrointestinal tract in order to model uptake and excretion of 2-methylimidazole more realistically. Metabolism of 2-methylimidazole is assumed to follow Michaelis-Menten kinetics and takes place in the liver. Each tissue compartment is represented as diffusion-limited with separate tissue and capillary spaces. The permeability of a tissue is related to the fraction of the permeant in the blood that is taken up during a single pass (Kohn, 1997). This fraction is referred to as the extraction ratio. The relationship between permeability (π), the extraction ratio (ε), and blood flow to the tissue (Q) is shown in the equation:

$$\pi = \frac{\varepsilon}{1-\varepsilon} Q$$

Sanders *et al.* (1998) concluded that 2-methylimidazole is rapidly distributed from blood to most tissues, but more slowly to the adipose tissue. Therefore, the model has a different extraction ratio for the adipose tissue than for the other tissues (equivalently, the relative permeability π/Q is similar for all tissues except adipose).

All model equations were encoded in MATLAB® (The Math Works, Inc., Natick, MA) using Simulink® code. Model equations are listed at the end of this section. Physiological parameter estimates for tissue volumes, tissue blood flows, lumenal spaces, cardiac outputs, and capillary volumes in rats and mice were obtained from the

literature (Table N1; Brown *et al.*, 1997). Cardiac output was derived from allometric relationships with body weight. Mean body weights from the toxicokinetic studies were used in fitting the model. The model was evaluated against the Sanders *et al*. (1998) data using the average weight of male F344 rats in that study (250 g).

The 2-methylimidazole tissue to blood partition coefficients (Table N2) for adipose, liver, muscle, kidney, skin, and brain were derived from the octanol:water partition coefficient for 2-methylimidazole (Poulin and Krishnan, 1995). Estimates for the 11 fitted parameters are shown in Table N3. Estimates were obtained by maximizing the log-likelihood function (Casella and Berger, 1990) using the toxicokinetic plasma data. Some of the data were below the limit of quantitation (LOQ), and for these data the cumulative distribution function was used in the likelihood rather than the probability density function (Koo *et al.*, 2002). This method allows all the data to be used without making assumptions about the below-LOQ values for fitting purposes. Tests for species or sex differences were done with likelihood ratio tests.

Definitions of Abbreviations

A = Amount (mg)
V = Volume of tissue or blood (L)
P = Partition coefficient
C = Concentration (mg/L)
Q = Blood flow rate (L/hour)
cap = Capillary blood
filt = Kidney blood to kidney tubule filtration rate (hour^{-1})
GI = Gastrointestinal
metab = Metabolism
IV = Intravenous
art = Arterial blood
ven = Venous blood
k_{tubule} = Permeability of the kidney tubule (hour^{-1})
$k_{excretion}$ = Urinary excretion rate (hour^{-1})
k_{bile} = Bile secretion rate (hour^{-1})
k_{feces} = Fecal elimination rate (hour^{-1})
$k_{absorption}$ = Absorption rate from the gastrointestinal lumen (hour^{-1})

Model Equations

For the muscle, brain, thyroid gland, adipose, skin, and aggregated other tissues, the equations for the amount of 2-methylimidazole in the tissue and capillary space are as follows:

$$\frac{dA_{tissue}}{dt} = \pi \frac{A_{capillary}}{V_{capillary}} - \pi \frac{A_{tissue}}{V_{tissue} \cdot P_{tissue}}$$

$$\frac{dA_{capillary}}{dt} = Q_{tissue} C_{art} - Q_{tissue} C_{tissuecap} - \pi \frac{A_{capillary}}{V_{capillary}} + \pi \frac{A_{tissue}}{V_{tissue} \cdot P_{tissue}}$$

The kidney equations are similar to those for the other tissues, but include an equation for the tubule space:

$$\frac{dA_{kidneytissue}}{dt} = \pi \frac{A_{capillary}}{V_{capillary}} - \pi \frac{A_{kidneytissue}}{V_{tissue} \cdot P_{tissue}} - k_{tubule} \frac{A_{kidneytissue}}{V_{tissue}} + k_{tubule} \frac{A_{tubule}}{V_{tubule}}$$

$$\frac{dA_{kidneycapillary}}{dt} = Q_{kidney} C_{art} - Q_{kidney} C_{fatcap} - \pi \frac{A_{kidneycapillary}}{V_{kidneycapillary}} + \pi \frac{A_{kidney}}{V_{kidney} \cdot P_{kidney}} - filt \frac{A_{kidneycapillary}}{V_{kidneycapillary}}$$

$$\frac{dA_{tubule}}{dt} = k_{tubule} \frac{A_{kidneytissue}}{V_{tissue}} - k_{tubule} \frac{A_{tubule}}{V_{tubule}} + filt \frac{A_{kidneycapillary}}{V_{kidneycapillary}} - k_{excretion} \frac{A_{tubule}}{V_{tubule}}$$

The liver equations are similar to those for the other tissues but include equations for capillary flow from the gastrointestinal tract, metabolism, and bile secretion:

$$\frac{dA_{livercapillary}}{dt} = Q_{liver} C_{art} + Q_{GI} C_{GI} - Q_{liver} C_{livercapillary} - \pi \frac{A_{livercapillary}}{V_{livercapillary}} + \pi \frac{A_{liver}}{V_{liver} \cdot P_{liver}}$$

$$\frac{dA_{livertissue}}{dt} = \pi \frac{A_{livercapillary}}{V_{livercapillary}} - \pi \frac{A_{livertissue}}{V_{liver} \cdot P_{liver}} - metab - k_{bile} \frac{A_{livertissue}}{V_{liver} \cdot P_{liver}}$$

$$metab = \frac{V_{max} C_{liver}}{K_m + C_{liver}} \text{ where } C_{liver} = \frac{A_{livertissue}}{V_{liver} \cdot P_{liver}}$$

The gastrointestinal equations are the same as those for the other tissues with the addition of a lumen equation. 2-Methylimidazole absorbed from the lumen goes to the gastrointestinal capillary space:

$$\frac{dA_{lumen}}{dt} = dose_{oral} - k_{feces} \frac{A_{lumen}}{V_{lumen}} + k_{bile} \frac{A_{livertissue}}{V_{liver} \cdot P_{liver}} - k_{absorption} \frac{A_{lumen}}{V_{lumen}}$$

The blood equations include the intravenous dose going to the venous blood:

$$\frac{dA_{art}}{dt} = C_{ven}Q_{total} - C_{art}Q_{total}$$

$$\frac{dA_{ven}}{dt} = \sum C_{tissuecap}Q_{tissue} - C_{ven}Q_{total} + dose_{IV}$$

RESULTS

The first test was for species differences between rats and mice. The full model had 22 parameters (11 for rats and 11 for mice) while the reduced model had 11 parameters. There were statistically significant differences between species. The second and third tests were for differences between males and females within each species. Again, the full model had 22 parameters and the reduced model had 11. There were statistically significant differences between males and females. The final two tests checked to see if the differences between males and females could be explained by a difference in only one process. For the fourth test, the only parameters that were different between males and females were the two metabolism parameters. In both rats and mice, there were statistically significant differences between males and females. Similarly, for the fifth test, the only parameters that were significantly different between males and females were the two excretion parameters. The overall conclusion is that there are statistically significant differences between rats and mice and between males and females within each species. A difference in urinary clearance can account for the statistically significant differences between genders and species. The unique parameters for each group with different metabolism are shown in Table N3. The PBPK model predictions were plotted along with the data for each group (Figures N2 to N5).

DISCUSSION AND CONCLUSIONS

The PBPK model fits the gavage data for male and female rats and mice, but overpredicts plasma concentrations following intravenous administration of 2-methylimidazole (Figures N2 to N5). The overprediction is magnified on the semilogarithmic plots, but it is real. Sanders *et al.* (1998) reported that the route of exposure had little effect on the disposition of 2-methylimidazole but the elimination of 2-methylimidazole-derived radioactivity was more rapid following intravenous administration. There is minimal metabolism in any of the groups with the exception of the male rats. Urinary clearance is the primary route of elimination of the parent compound. The rate of urinary elimination is higher in rats than in mice. The extraction ratios are similar for the species and clearly lower in adipose tissue. Sanders *et al.* (1998) concluded that only a small amount of 2-methylimidazole reaches the adipose tissue. The extraction ratios resulted from fitting NTP toxicokinetic data that excluded adipose tissue concentration. However, the parameters do support the conclusion that very little 2-methylimidazole reaches the adipose tissue.

Data from Sanders *et al.* (1998) were compared to the PBPK model-based fit for male rats (Figure N6) and reflected total radioactivity while the model predictions are strictly tissue concentrations of the parent compound. The model and data match very well for venous blood, skin, brain, thyroid gland, and muscle tissue. The experimental data for adipose tissue had a high value only at the 2-hour time point, and the adipose model prediction peaks soon after 2 hours. In the model, the relatively high adipose partition coefficient and low extraction ratio suggest that any 2-methylimidazole that reaches the adipose will be slow to return to the plasma. Kidney and liver are the two tissues where the model and data appear to differ the most. The kidney predictions

are probably low because in the model there is no storage of urine and elimination is instantaneous. The liver predictions miss the high peak shown in the data, possibly because the liver is the site of metabolism and the data include parent and metabolites while the predictions do not. A comparison here is difficult without more knowledge of the metabolites and metabolic pathways. The overall fits to the tissue data are good considering that the model parameters were estimated from a different data set having only plasma concentration data.

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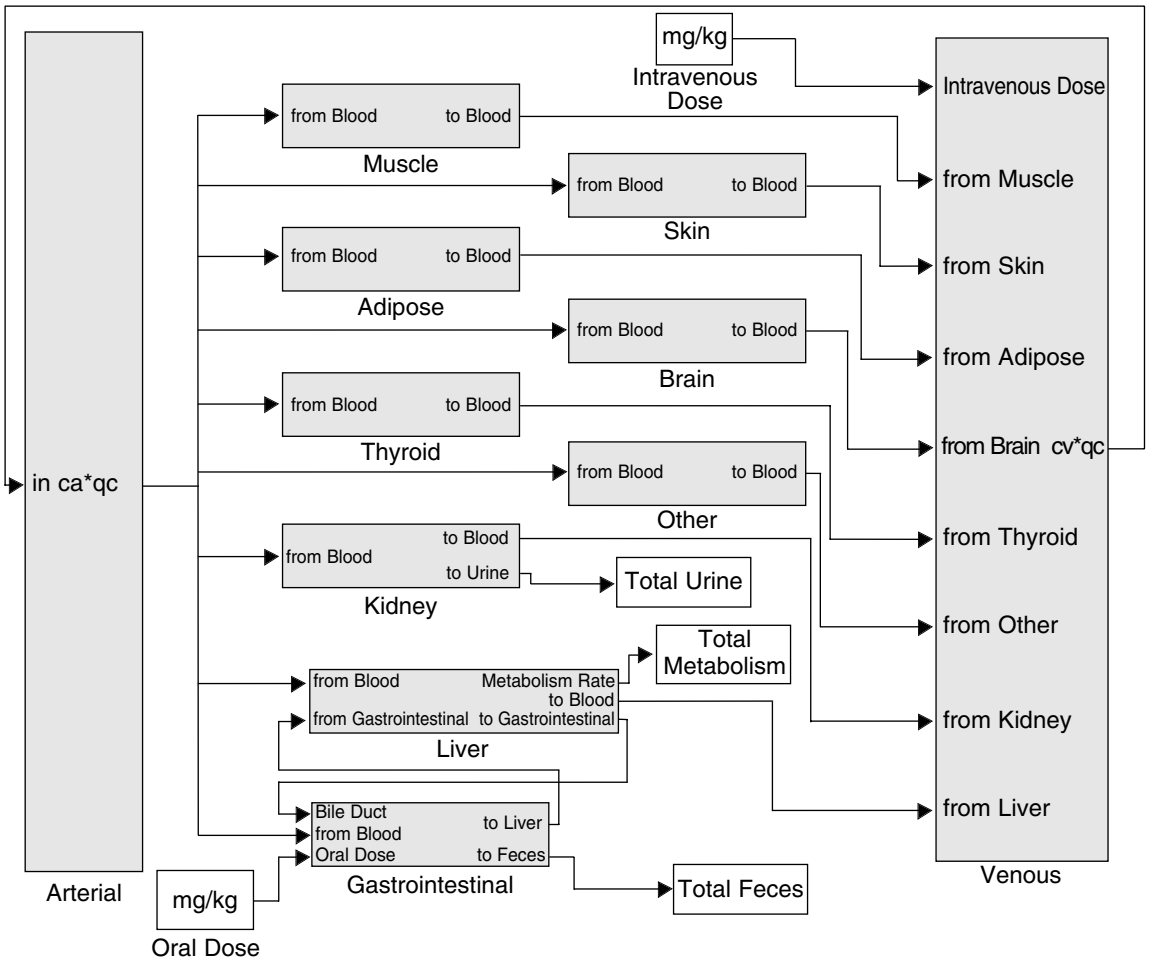

FIGURE N1
Physiologically Based Pharmacokinetic Model for Rats and Mice
Exposed to 2-Methylimidazole by Single-Dose Intravenous Injection or Oral Gavage

TABLE N1
Physiological Parameters of Rats and Mice for the Physiologically Based Pharmacokinetic Model of 2-Methylimidazole[a]

Tissue	Tissue Space (%)	Capillary Space (%)	Lumenal Space (%)	Blood Flow (%)
Rats				
Adipose	7.0	2.0		7.0
Liver	3.4	21.0		4.3
Muscle	40.0	4.0		27.8
Kidney	0.7	16.0	2.0	14.1
Skin	19.0	2.0		5.8
Thyroid gland	5e-4	18.0		0.5
Gastrointestinal	2.7	5.6	5.6	14.0
Brain	0.6	3.0		2.0
Other	24.0	4.0		24.5
Venous blood	1.3			
Arterial blood	0.4			
Mice				
Adipose	8.0	3.0		5.0
Liver	5.5	31.0		2.2
Muscle	38.4	4.0		15.9
Kidney	1.7	24.0	2.0	9.1
Skin	16.5	3.0		5.8
Thyroid gland	5e-4	18.0		0.5
Gastrointestinal	4.2	2.6	5.6	14.0
Brain	1.7	3.0		3.3
Other	22.059	4.0		44.2
Venous blood	1.0			
Arterial blood	0.3			

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

TABLE N2
Partition Coefficients for 2-Methylimidazole for the Physiologically Based Pharmacokinetic Model of 2-Methylimidazole

Tissue	Partition Coefficient[a]
Adipose	4.24
Liver	1.05
Muscle	0.94
Kidney	1.23
Skin	1.50
Thyroid gland	1.05[b]
Gastrointestinal	0.94[c]
Brain	1.28
Other	0.94[d]

[a] All coefficients expressed as tissue:blood ratios. Coefficients for adipose, liver, muscle, kidney, skin, and brain were derived from the octanol:water partition coefficient for 2-methylimidazole (Poulin and Krishnan, 1995).
[b] Used the estimate for liver.
[c] Used the estimate for muscle (Kohn and Melnick, 1999)
[d] Used the estimate for muscle (Poulin and Krishnan, 1995)

TABLE N3
Parameter Estimates for Rats and Mice from the Physiologically Based Pharmacokinetic Model of 2-Methylimidazole

Parameter	Rats		Mice	
	Male	Female	Male	Female
Extraction ratio	0.26		0.19	
Adipose extraction ratio	0.05		0.09	
Perm tubule ($hour^{-1}$)	1.021		0.65	
V_{max} (mg/L/hour)	6.48		2.05	
K_m (mg/L)	14.23		4.42	
Glomerular filtration ($hour^{-1}$)	0.01		0.09	
V_{max} (mg/L/hour)	1.20	0.14	0.09	0.03
K_m (mg/L)	11.60	2.16	4.65	7.38
Bile secretion ($hour^{-1}$)	0.61		0.28	
Absorption gastrointestinal ($hour^{-1}$)	0.73		0.69	
Fecal elimination ($hour^{-1}$)	0.63		1.32	

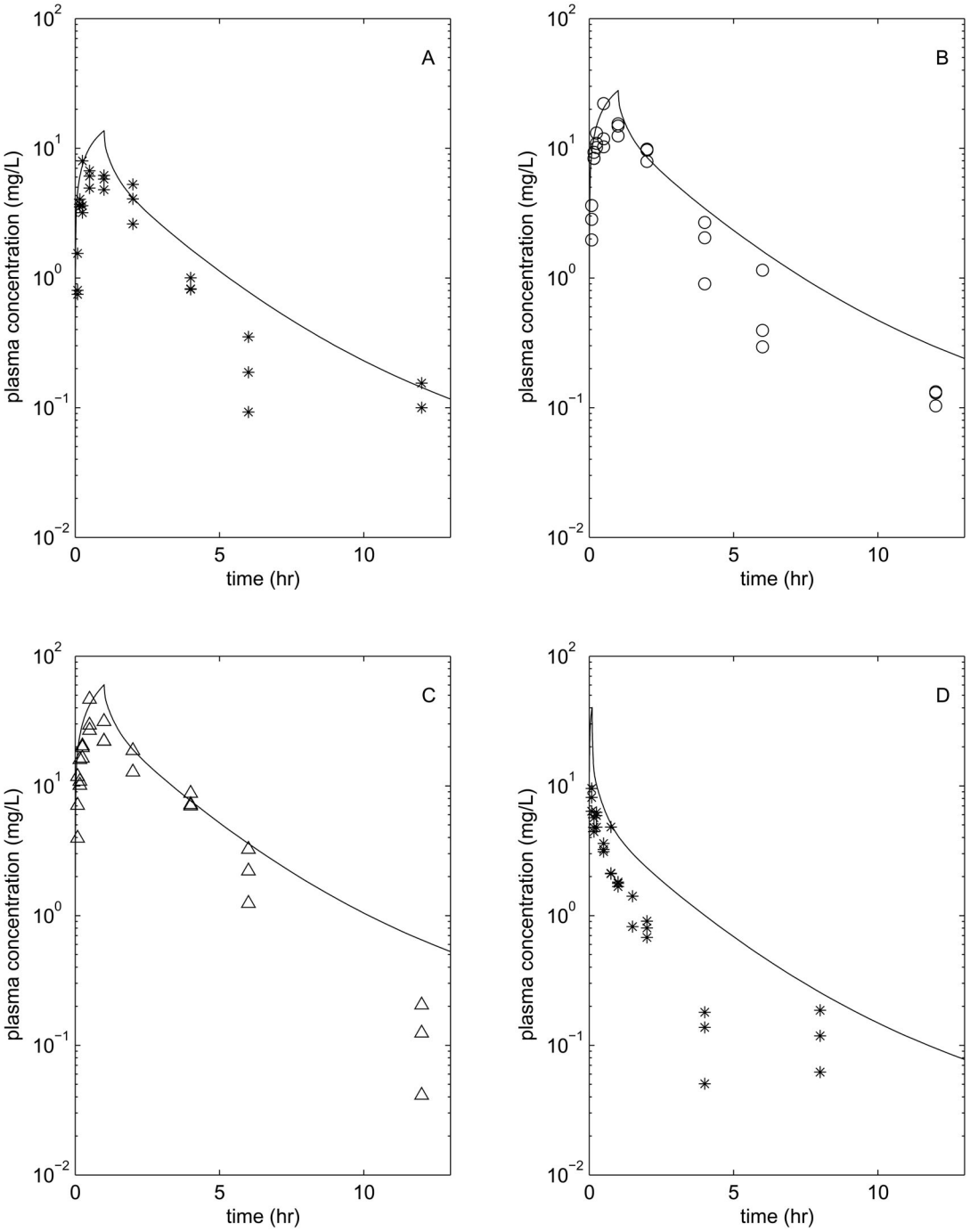

Figure N2
Plasma Concentrations of 2-Methylimidazole in Male Rats in the Single-Dose Intravenous Injection and Gavage Toxicokinetic Studies of 2-Methylimidazole
Male rat data and PBPK model prediction at 25 (A), 50 (B), and 100 (C) mg/kg by gavage and 10 mg/kg (D) intravenously

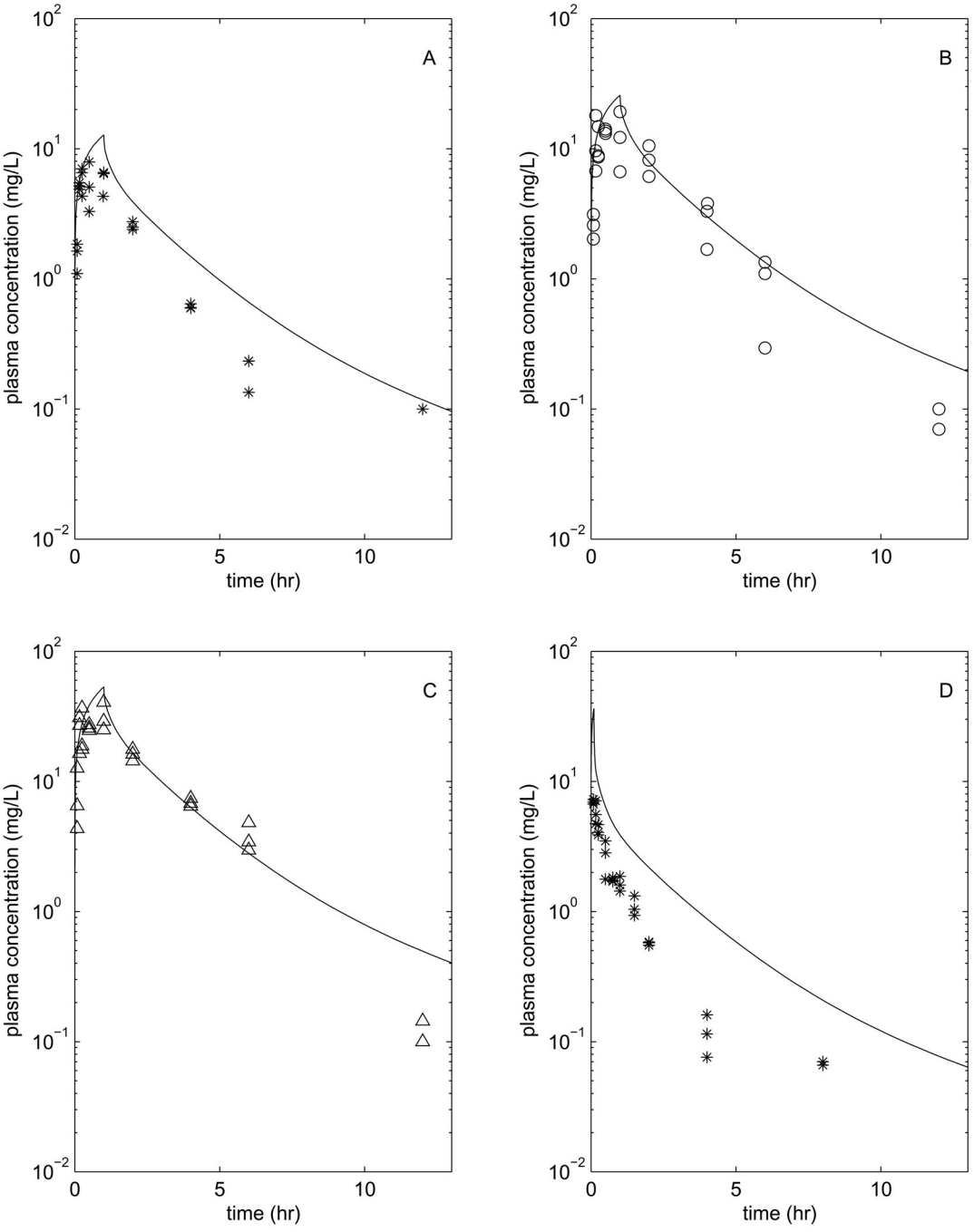

Figure N3
Plasma Concentrations of 2-Methylimidazole in Female Rats in the Single-Dose Intravenous Injection and Gavage Toxicokinetic Studies of 2-Methylimidazole
Female rat data and PBPK model prediction at 25 (A), 50 (B), and 100 (C) mg/kg by gavage and 10 mg/kg (D) intravenously

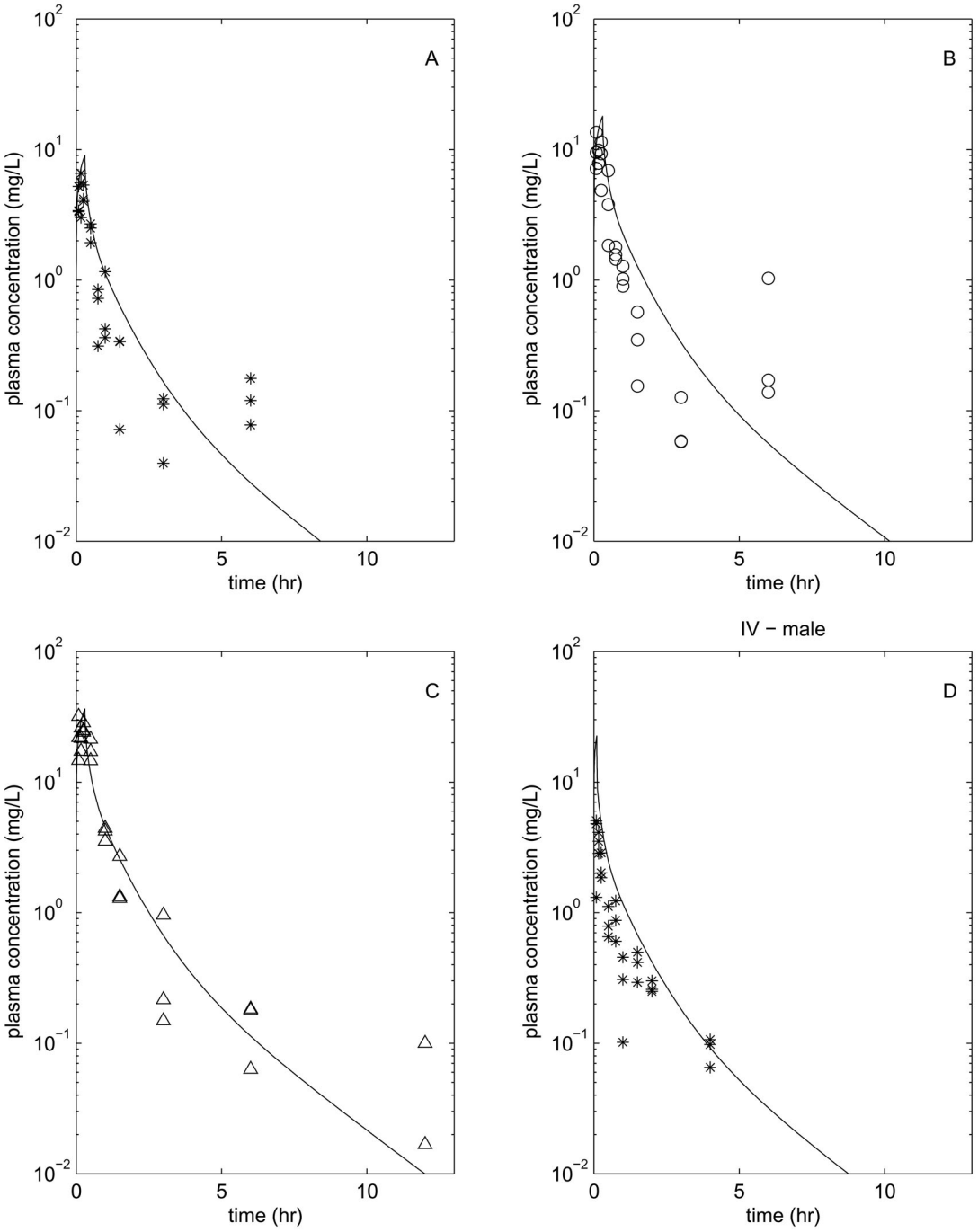

Figure N4
Plasma Concentrations of 2-Methylimidazole in Male Mice in the Single-Dose Intravenous Injection and Gavage Toxicokinetic Studies of 2-Methylimidazole
Male mouse data and PBPK model prediction at 25 (A), 50 (B), and 100 (C) mg/kg by gavage and 10 mg/kg (D) intravenously

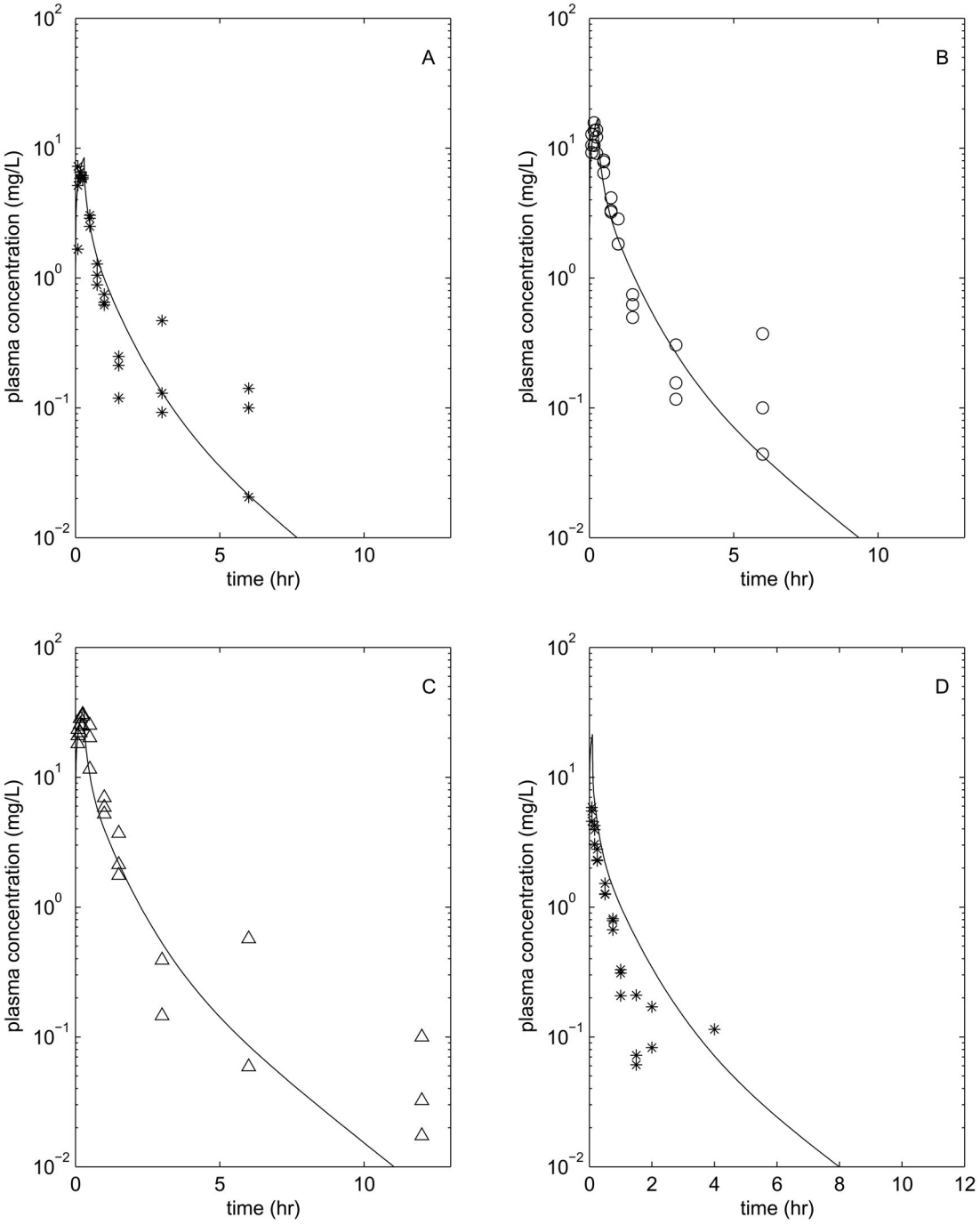

Figure N5
Plasma Concentrations of 2-Methylimidazole in Female Mice in the Single-Dose Intravenous Injection and Gavage Toxicokinetic Studies of 2-Methylimidazole
Female mouse data and PBPK model prediction at 25 (A), 50 (B), and 100 (C) mg/kg by gavage and 10 mg/kg (D) intravenously

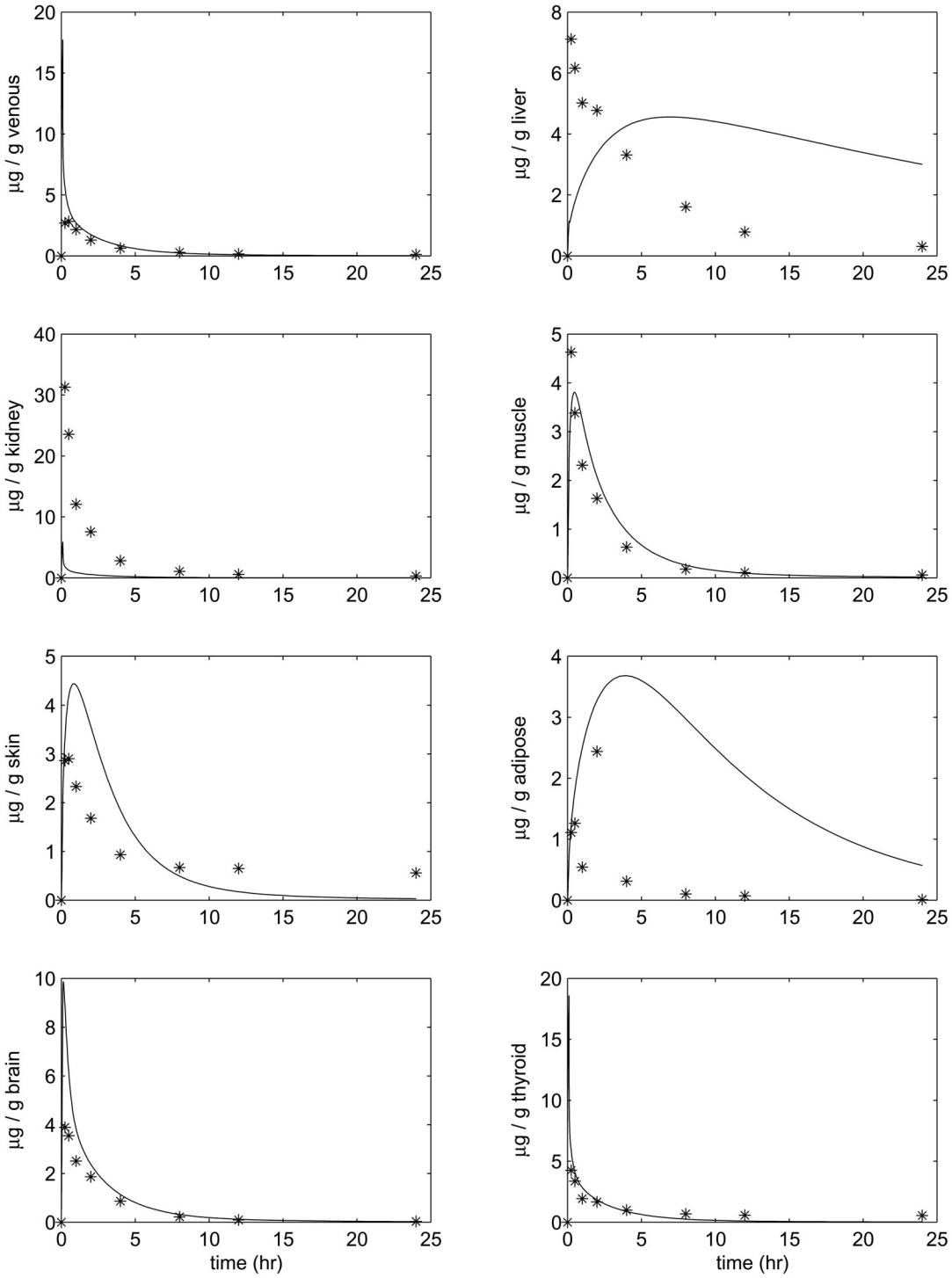

Figure N6
Tissue Concentrations of 2-Methylimidazole (µg/g tissue) after a Single Intravenous Injection of 5 mg 2-Methylimidazole/kg: Model versus Data
Data points (∗) are for total radioactivity (Sanders *et al.*, 1998); solid line represents the PBPK model-based estimate of tissue concentrations of parent compound only.

National Toxicology Program Technical Reports

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Chemical	TR No.
Acetaminophen	394
Acetonitrile	447
Acrylonitrile	506
Agar	230
Allyl Glycidyl Ether	376
Allyl Isothiocyanate	234
Allyl Isovalerate	253
1-Amino-2,4-Dibromoanthraquinone	383
2-Amino-4-Nitrophenol	339
2-Amino-5-Nitrophenol	334
11-Aminoundecanoic Acid	216
dl-Amphetamine Sulfate	387
Ampicillin Trihydrate	318
Asbestos, Amosite (Hamsters)	249
Asbestos, Amosite (Rats)	279
Asbestos, Chrysotile (Hamsters)	246
Asbestos, Chrysotile (Rats)	295
Asbestos, Crocidolite	280
Asbestos, Tremolite	277
L-Ascorbic Acid	247
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Barium Chloride Dihydrate	432
Benzaldehyde	378
Benzene	289
Benzethonium Chloride	438
Benzofuran	370
Benzyl Acetate (Gavage)	250
Benzyl Acetate (Feed)	431
Benzyl Alcohol	343
o-Benzyl-*p*-Chlorophenol (Gavage)	424
o-Benzyl-*p*-Chlorophenol (Mouse Skin)	444
2-Biphenylamine Hydrochloride	233
2,2-Bis(Bromomethyl)-1,3-Propanediol	452
Bis(2-Chloro-1-Methylethyl) Ether	239
Bisphenol A	215
Boric Acid	324
Bromodichloromethane	321
Bromoethane	363
1,3-Butadiene	288
1,3-Butadiene	434
t-Butyl Alcohol	436
Butyl Benzyl Phthalate	213
Butyl Benzyl Phthalate	458
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Chloral Hydrate	503
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Chlorinated Paraffins: C_{12}, 60% Chlorine	308
Chlorinated Trisodium Phosphate	294
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p-Chloroaniline Hydrochloride	351
Chlorobenzene	261
Chlorodibromomethane	282
Chloroethane	346
2-Chloroethanol	275
3-Chloro-2-Methylpropene	300
Chloroprene	467
1-Chloro-2-Propanol	477
Chlorpheniramine Maleate	317
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C.I. Acid Orange 10	211
C.I. Acid Red 14	220
C.I. Acid Red 114	405
C.I. Basic Red 9 Monohydrochloride	285
C.I. Direct Blue 15	397
C.I. Direct Blue 218	430
C.I. Disperse Blue 1	299
C.I. Disperse Yellow 3	222
C.I. Pigment Red 3	407
C.I. Pigment Red 23	411
C.I. Solvent Yellow 14	226
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Cobalt Sulfate Heptahydrate	471
Coconut Oil Acid Diethanolamine Condensate	479
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CS2	377
Cytembena	207
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D&C Yellow No. 11	463
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Diallyl Phthalate (Mice)	242
Diallyl Phthalate (Rats)	284
4,4′-Diamino-2,2′-Stilbenedisulfonic Acid, Disodium Salt	412
2,4-Diaminophenol Dihydrochloride	401
1,2-Dibromo-3-Chloropropane	206
1,2-Dibromoethane	210
2,3-Dibromo-1-Propanol	400
1,2-Dichlorobenzene (*o*-Dichlorobenzene)	255
1,4-Dichlorobenzene (*p*-Dichlorobenzene)	319
p,p′-Dichlorodiphenyl sulfone	501
2,4-Dichlorophenol	353
2,6-Dichloro-*p*-Phenylenediamine	219
1,2-Dichloropropane	263
1,3-Dichloropropene (Telone II)	269
Dichlorvos	342
Dietary Restriction	460
Diethanolamine	478
Di(2-Ethylhexyl) Adipate	212
Di(2-Ethylhexyl) Phthalate	217
Diethyl Phthalate	429
Diglycidyl Resorcinol Ether	257
3,4-Dihydrocoumarin	423
1,2-Dihydro-2,2,4-Trimethylquinoline (Monomer)	456
Dimethoxane	354
3,3′-Dimethoxybenzidine Dihydrochloride	372
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3,3′-Dimethylbenzidine Dihydrochloride	390
Dimethyl Hydrogen Phosphite	287
Dimethyl Methylphosphonate	323
Dimethyl Morpholinophosphoramidate	298
Dimethylvinyl Chloride	316
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Emodin	493
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Epinephrine Hydrochloride	380

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Ethyl Acrylate	259
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Ethylene Glycol Monobutyl Ether	484
Ethylene Oxide	326
Ethylene Thiourea	388
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Geranyl Acetate	252
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Gum Arabic	227
HC Blue 1	271
HC Blue 2	293
HC Red 3	281
HC Yellow 4	419
Hexachlorocyclopentadiene	437
Hexachloroethane	361
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4-Hexylresorcinol	330
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8-Hydroxyquinoline	276
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Iodinated Glycerol	340
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α-Methylbenzyl Alcohol	369
Methyl Bromide	385
Methyl Carbamate	328
Methyldopa Sesquihydrate	348
Methylene Chloride	306
4,4′-Methylenedianiline Dihydrochloride	248
Methyleugenol	491
2-Methylimidazole	516
Methyl Methacrylate	314
N-Methylolacrylamide	352
Methylphenidate Hydrochloride	439
Mirex	313
Molybdenum Trioxide	462
Monochloroacetic Acid	396
Monuron	266
Nalidixic Acid	368
Naphthalene (Mice)	410
Naphthalene (Rats)	500
Nickel (II) Oxide	451
Nickel Sulfate Hexahydrate	454
Nickel Subsulfide	453
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o-Nitroanisole	416
p-Nitrobenzoic Acid	442
Nitrofurantoin	341
Nitrofurazone	337
Nitromethane	461
p-Nitrophenol	417
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Penicillin VK	336
Pentachloroanisole	414
Pentachloroethane	232
Pentachloronitrobenzene	325
Pentachlorophenol, Purified	483
Pentachlorophenol, Technical Grade	349
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Phenylbutazone	367
Phenylephrine Hydrochloride	322
N-Phenyl-2-Naphthylamine	333
o-Phenylphenol	301
Polybrominated Biphenyl Mixture (Firemaster FF-1) (Gavage)	244
Polybrominated Biphenyl Mixture (Firemaster FF-1) (Feed)	398
Polysorbate 80 (Glycol)	415
Polyvinyl Alcohol	474
Primidone	476
Probenecid	395
Promethazine Hydrochloride	425
Propylene	272
Propylene Glycol Mono-*t*-butyl Ether	515
1,2-Propylene Oxide	267
Propyl Gallate	240
Pyridine	470
Quercetin	409
Riddelliine	508
Resorcinol	403
Rhodamine 6G	364
Rotenone	320
Roxarsone	345
Salicylazosulfapyridine	457
Scopolamine Hydrobromide Trihydrate	445
Sodium Azide	389
Sodium Fluoride	393
Sodium Nitrite	495
Sodium Xylenesulfonate	464
Stannous Chloride	231
Stoddard Solvent IIC	519
Succinic Anhydride	373
Talc	421
Tara Gum	224
2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (Dermal)	201
2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (Gavage)	209
1,1,1,2-Tetrachloroethane	237
Tetrachloroethylene	311
Tetracycline Hydrochloride	344
Tetrafluoroethylene	450
1-Trans-Delta^9-Tetrahydrocannabinol	446
Tetrahydrofuran	475
Tetrakis(Hydroxymethyl)Phosphonium Sulfate	296
Tetrakis(Hydroxymethyl)Phosphonium Chloride	296
Tetranitromethane	386

Chemical	TR No.	Chemical	TR No.
Theophylline	473	Tris(2-Chloroethyl) Phosphate	391
4,4-Thiobis(6-*t*-Butyl-*m*-Cresol)	435	Tris(2-Ethylhexyl) Phosphate	274
Titanocene Dichloride	399	Turmeric Oleoresin (Curcumin)	427
Toluene	371	Urethane, Ethanol, and Urethane/Ethanol	510
2,4- & 2,6-Toluene Diisocyanate	251	Vanadium Pentoxide	507
Triamterene	420	4-Vinylcyclohexene	303
Tribromomethane	350	4-Vinyl-1-Cyclohexene Diepoxide	362
Trichloroethylene	243	Vinylidene Chloride	228
Trichloroethylene	273	Vinyl Toluene	375
1,2,3-Trichloropropane	384	Xylenes (Mixed)	327
Tricresyl Phosphate	433	2,6-Xylidine	278
Triethanolamine	449	Zearalenone	235
Triethanolamine	518	Ziram	238

NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2-Methylimidazole in F344/N Rats and B6C3F$_1$ Mice (TR 516)

Erratum[1]:

p. 7 Abstract 1st paragraph
It has also been ~~identified as a by-product in foods and has been~~ detected in ~~mainstream and sidestream~~ tobacco smoke.

p. 15-16 Intro 4th and 5th paragraphs
Ammoniation of carbohydrate-containing material, including hay, to increase nonprotein nitrogen content is a common practice on farms (Ray *et al.*, 1984). 2-Methylimidazole, formed by interaction of ammonia with reducing sugars, has been identified as a toxic by-product in ammoniated hay forage for livestock animals (Ray *et al.*, 1984), and it has been identified in milk, plasma, and urine from cows and sheep given ammoniated feed (~~Morgan and Edwards, 1986; Perdok and Leng, 1987; Sivertsen *et al.*, 1997;~~ Muller *et al.*, 1998a; 1998b). ~~2-Methylimidazole has been identified as an undesirable by-product in fermented foods and is found 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). During cooking, 2-methylimidazole may be formed when ammonium hydroxide, glycine, and monosodium glutamate are present (Wong and Bernhard, 1988). Humans may also be exposed to low concentrations of 2-methylimidazole in mainstream and sidestream cigarette smoke (Moree-Testa *et al.*, 1984; Sakuma *et al.*,1984).~~

Insert: Results of model experiments where glucose was heated in the presence of various nitrogen sources commonly present in foods suggest that 2-methylimidazole can be formed during cooking (Wong and Bernhard, 1988).

Insert: 2-Methylimidazole has also been detected in cigarette smoke (Moree-Testa *et al.*, 1984).

~~A joint committee of the Food and Agriculture Organization of the United Nations recommended in 1971 that daily intake of caramel should be restricted to no more than 100 mg caramel/kg of body weight (Chappel and Howell, 1992). Citing a cancer risk, legislation enacted in August of 1976 restricted the use of caramel coloring in food and beverages in Denmark (Chappel and Howell, 1992).~~ The National Occupational Exposure Survey conducted from 1980 to 1983 estimated that 7,000 workers in 22 occupations at 318 facilities in 11 industries were potentially exposed to 2-methylimidazole annually in the United States (NIOSH, 1990). No standard or guideline has been set in the United States for allowable occupational exposure or environmental concentration of 2-methylimidazole ~~(Chappel and Howell, 1992)~~.

p. 67 (References)

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~~Huang, Y., Zhang, S., and Yang, R. (1983). Survey of communal industrial production of caramel by the ammonium process. *Tiaowei Fushipin Keji* **3**, 11-12 (Abstr.).~~

[1] Key: underline denotes added text and strikethrough denotes deleted text

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