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

NTP Technical Report on the Toxicity Studies of Myristicin (CASRN 607-91-0) Administered by Gavage to F344/NTac Rats and B6C3F1/N Mice

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Foreword

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

The Toxicity Study Report series began in 1991. The studies described in the Toxicity Study Report series are designed and conducted to characterize and evaluate the toxicologic potential of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in Toxicity Study Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection per se is not an indicator of a substance's toxic potential.

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

NTP Toxicity Study Reports are indexed in the National Center for Biotechnology Information (NCBI) Bookshelf databaseand are available free of charge electronically on the NTP website (http://ntp.niehs.nih.gov). Additional information regarding this study may be requested through Central Data Management (CDM) at cdm@niehs.nih.gov. Toxicity data are available through NTP's Chemical Effects in Biological Systems (CEBS) database: https://manticore.niehs.nih.gov/cebssearch.

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This report has been reformatted to meet new NTP publishing requirements;
its content has not changed.

About This Report

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Peer Review

The draft *NTP Technical Report on the Toxicity Studies of Myristicin (CASRN 607-91-0) Administered by Gavage to F344/NTac Rats and B6C3F1/N Mice* was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies were appropriate and ensured that this Toxicity Study Report presented the experimental results and conclusions fully and clearly.

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Abstract

Myristicin is derived from the tropical evergreen tree *Myristica fragrans*. It is a major constituent in essential oil extracted from either the seed, which is the source of the spice nutmeg, or the aril covering the seed, which is the source of the spice mace. Myristicin was nominated for study by the National Cancer Institute due to widespread human exposure from natural sources and extensive consumer exposure. Male and female F344/NTac rats and B6C3F1/N mice received myristicin (greater than 94% pure) in corn oil by gavage at doses of 0, 10, 30, 100, 300, or 600 mg/kg body weight 5 days per week for 13 weeks. Additional groups of 10 male and 10 female clinical pathology study rats were administered the same doses for 21 days. Genetic toxicology studies were conducted in *Salmonella typhimurium* and in rat and mouse peripheral blood erythrocytes.

All core study male rats survived to the end of the study. Three 600 mg/kg core study female rats died within 4 days of the start of the study. The mean body weights of 600 mg/kg males were significantly less than those of the vehicle controls. The livers of all 300 and 600 mg/kg rats surviving to the end of the study were enlarged at necropsy.

Small but significant increases in segmented neutrophil counts occurred in 300 and 600 mg/kg male rats at study termination and could be related to the liver necrosis or lesions in the glandular stomach observed microscopically at 600 mg/kg. Alanine aminotransferase and sorbitol dehydrogenase activities were significantly elevated in several dosed groups of males and females, but most consistently at 600 mg/kg, consistent with the observed liver necrosis. At week 14, cholesterol was significantly increased in the 100 mg/kg or greater males and in all dosed groups of females. Triglycerides were significantly increased in 300 and 600 mg/kg males and 600 mg/kg females at week 14. The reason for the alterations is not known, but they may be related to changes in lipid metabolism.

The absolute and relative liver weights of 100 mg/kg or greater male and female rats and the relative liver weight of 30 mg/kg males were significantly increased compared to those of the vehicle controls. The absolute and relative kidney weights of 100 mg/kg or greater males were significantly increased compared to the vehicle controls. The relative kidney weights of 300 and 600 mg/kg females were significantly greater than that of the vehicle control group.

Treatment-related lesions in the liver of rats included centrilobular hepatocyte hypertrophy, fatty change, and hepatocyte necrosis. Treatment-related lesions in the glandular stomach included epithelium atrophy and hyperplasia in male and female rats and necrosis in female rats. Treatment-related lesions in the submandibular salivary gland included secretory depletion in males and females. Treatment-related lesions in the kidney of male rats included renal tubule hyaline droplet accumulation and a slight increase in the severity of nephropathy.

Male rats in the 600 mg/kg group had significantly lower absolute left cauda and left epididymis weights and mean total number of sperm per cauda epididymis compared to those of the vehicle controls. Treatment-related lesions in the male rat reproductive system include germinal epithelium degeneration and elongated spermatid retention in seminiferous tubules of the testis and exfoliated germ cells in the duct lumina of the epididymis. Myristicin exposure via gavage exhibited the potential to be a reproductive toxicant in male F344/NTac rats.

All mice survived to the end of the study. The mean body weights of 300 and 600 mg/kg males and females were significantly less than those of the vehicle control groups. As in rats, the livers

of all 300 and 600 mg/kg male and female mice were enlarged at necropsy. A few treated mice had white or tan foci in the forestomach.

Leukocyte counts were significantly increased in 300 and 600 mg/kg male and 600 mg/kg female mice. Segmented neutrophil counts were significantly increased in 300 and 600 mg/kg males and females, the lymphocyte count was significantly increased in 600 mg/kg males, and the monocyte count was significantly increased in 600 mg/kg females. The increase in the leukon was consistent with the chronic inflammation observed in the stomach.

The absolute and relative liver weights of male mice administered 100 mg/kg or greater and all dosed groups of females were significantly greater than those of the vehicle control groups.

Treatment related lesions in the liver of mice included fatty change, centrilobular hepatocyte hypertrophy, hepatocyte necrosis, and oval cell hyperplasia. Treatment-related lesions in the nose of mice included epithelial atrophy, nerve atrophy, glands hyperplasia, and hyaline droplet accumulation of the olfactory epithelium and hyaline droplet accumulation and cytoplasmic vacuolization of the respiratory epithelium. The incidences of atrophy and hyperplasia in the epithelium of the glandular stomach were significantly increased in 600 mg/kg males and females compared to the vehicle controls. In the forestomach, the incidences of chronic and epithelial suppurative inflammation were significantly increased in 600 mg/kg males compared to vehicle controls.

Myristicin was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535, when tested with or without exogenous metabolic activation. In the 3-month in vivo studies, male and female rats had significant increases in micronucleated immature erythrocytes (polychromatic erythrocytes; PCEs) in the peripheral blood as well as significant increases in the percentage of circulating PCEs, suggesting that myristicin may have stimulated erythropoiesis in rats. No increases in micronucleated red blood cells were seen in peripheral blood of male or female mice from the 3-month study, but significant decreases in the percentage of PCEs in peripheral blood were seen in both sexes, suggesting toxicity to the bone marrow.

Under the conditions of the 3-month oral gavage studies, there were treatment-related lesions in male and female rats and mice. The major targets from myristicin administration in rats and mice included the liver and glandular stomach. In rats, additional targets included salivary glands in males and females, and the kidney, testis, and epididymis in males. Additional targets in the mice included the nose in males and females and the forestomach in males. The most sensitive measure of myristicin toxicity in male rats was a higher relative liver weight (lowest-observable-effect level (LOEL) = 30 mg/kg), and in female rats, the most sensitive measures were clinical chemistry findings (LOEL = 10 mg/kg) including increased cholesterol and alanine aminotransferase. In male mice, the most sensitive measures of myristicin toxicity included higher absolute and relative liver weights and a significantly increased incidence of fatty liver (LOEL = 100 m/kg). The most sensitive measure of myristicin toxicity in female mice was an increase in absolute and relative liver weight (LOEL = 10 mg/kg). No-observed-effect levels were 10 mg/kg for male rats and 30 mg/kg for male mice, but none was reached for female rats or mice.

Synonyms: 1-Allyl-5-methoxy-2,3-methylenedioxybenzene; 5-allyl-1-methoxy-2,3,-(methylenedioxy)benzene; 1-methoxy-2,3-methylenedioxy-5-allyl benzene; 4-methoxy-6-prop-2-enyl-1,3-benzodioxole

Summary of Findings Considered to Be Toxicologically Relevant in Rats and Mice Administered Myristicin by Gavage for Three Months

	Male F344/NTac Rats	Female F344/NTac Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Doses in corn oil	0, 10, 30, 100, 300, or 600 mg/kg	0, 10, 30, 100, 300, or 600 mg/kg	0, 10, 30, 100, 300, or 600 mg/kg	0, 10, 30, 100, 300, or 600 mg/kg
Survival rates	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 7/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10
Body weights	600 mg/kg group 20% less than the vehicle control group	Dosed groups similar to the vehicle control group	300 mg/kg group 12% less than the vehicle control group; 600 mg/kg group 24% less than the vehicle control group	300 mg/kg group 11% less than the vehicle control group; 600 mg/kg group 16% less than vehicle control group
Clinical findings	None	None	None	None
Organ weights	↑ Liver (absolute and relative[a]) ↑ Kidney (absolute and relative)	↑ Liver (absolute and relative) ↑ Kidney (relative)	↑ Liver (absolute and relative)	↑ Liver (absolute and relative)
Clinical pathology	↑ Neutrophil count ↑ Alanine aminotransferase activity ↑ Sorbitol dehydrogenase activity ↑ Cholesterol ↑ Triglycerides	↑ Alanine aminotransferase activity ↑ Sorbitol dehydrogenase activity ↑ Cholesterol ↑ Triglycerides	↑ Leukocyte counts ↑ Neutrophil counts ↑ Lymphocyte counts	↑ Leukocyte counts ↑ Neutrophil counts ↑ Monocyte counts
Reproductive toxicity	↓ Cauda epididymis wt. ↓ Epididymis wt. ↓ Sperm per cauda	Not determined	None	Not determined
Nonneoplastic effects	Liver: fatty change (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); centrilobular, hepatocyte, hypertrophy (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); hepatocyte, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 8/10) Glandular stomach: epithelium, atrophy (0/10, 0/10, 0/10, 0/10, 0/10,10/10); epithelium, hyperplasia (0/10,	Liver: fatty change (0/10, 0/10, 0/10, 0/10, 1/10, 10/10); centrilobular, hepatocyte, hypertrophy (0/10, 0/10, 0/10, 0/10, 10/10, 7/10); hepatocyte, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 6/10) Glandular stomach: epithelium, atrophy (0/10, 0/10, 0/10, 0/10, 5/10, 7/10); epithelium, hyperplasia (0/10,	Liver: fatty change (0/10, 0/10, 0/10, 8/10, 5/10, 10/10); centrilobular, hepatocyte, hypertrophy (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); hepatocyte, necrosis (0/10, 0/10, 0/10, 0/10, 1/10, 10/10); oval cell, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 10/10) Glandular stomach: epithelium, atrophy (0/10, 0/10, 0/10,	Liver: fatty change (0/10, 0/10, 0/10, 0/10, 0/10, 10/10); centrilobular, hepatocyte, hypertrophy (0/10, 0/10, 0/10, 10/10, 10/10, 10/10); hepatocyte, necrosis (0/10, 0/10, 0/10, 1/10, 10/10, 10/10); oval cell, hyperplasia (0/10, 0/10, 0/10, 0/10, 10/10, 10/10) Glandular stomach: epithelium, atrophy (0/10, 0/10, 0/10,

	Male F344/NTac Rats	Female F344/NTac Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
	0/10, 0/10, 0/10, 0/10,10/10) Salivary glands: submandibular gland, depletion secretory (0/10, 0/0, 0/0, 0/0, 0/10, 7/10) Kidney: renal tubule, accumulation, hyaline droplet (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); severity of nephropathy (1.0, 1.0, 1.0, 1.0, 1.0, 1.8) Testes: germinal epithelium, degeneration (5/10, 5/10, 3/10, 6/10, 8/10, 10/10); elongated spermatid, retention (5/10, 3/10, 0/10, 1/10, 6/10, 10/10) Epididymis: exfoliated germ cell (1/10, 3/10, 1/10, 2/10, 4/10, 10/10)	0/10, 0/10, 0/10, 0/10, 7/10); epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 2/10) Salivary glands: submandibular gland, depletion secretory (0/10, 0/0, 0/10, 0/10, 10/10, 7/10)	0/10, 0/10, 10/10); epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 10/10) Nose: olfactory epithelium, atrophy (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); nerve, atrophy (0/10, 0/10, 0/10, 0/10, 8/10, 10/10); glands, olfactory epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 7/10); olfactory epithelium, accumulation, hyaline droplet (0/10, 0/10, 0/10, 0/10, 2/10, 9/10); respiratory epithelium, accumulation, hyaline droplet (4/10, 5/10, 1/10, 5/10, 10/10, 10/10); respiratory epithelium, vacuolization, cytoplasmic (1/10, 1/10, 0/10, 0/10, 0/10, 6/10) Forestomach: inflammation, chronic (0/10, 0/10, 0/10, 0/10, 2/10, 4/10): epithelium, inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 0/10, 4/10)	0/10, 0/10, 10/10); epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 10/10) Nose: olfactory epithelium, atrophy (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); nerve, atrophy (0/10, 0/10, 0/10, 0/10, 9/10, 10/10); glands, olfactory epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 10/10); olfactory epithelium, accumulation, hyaline droplet (0/10, 1/10, 0/10, 3/10, 8/10, 10/10); respiratory epithelium, accumulation, hyaline droplet (5/10, 3/10, 1/10, 7/10, 10/10, 10/10); respiratory epithelium, vacuolization, cytoplasmic (0/10, 0/10, 0/10, 0/10, 2/10, 4/10)
Genetic toxicology				
Bacterial gene mutations:	Negative in TA97, TA98, TA100, and TA1535 with or without S9			
Micronucleated erythrocytes				
Rat peripheral blood in vivo:	Positive in males and females			
Mouse peripheral blood in vivo:	Negative in males and females			

[a]Relative to body weight.

Introduction

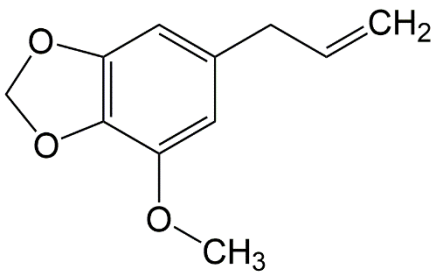

Figure 1. Myristicin (CASRN 607-91-0; Chemical Formula: $C_{11}H_{12}O_3$; Molecular Weight: 192.21)

Synonyms: 1-Allyl-5-methoxy-2,3-methylenedioxybenzene; 5-allyl-1-methoxy-2,3,-(methylenedioxy)benzene; 1-methoxy-2,3-methylenedioxy-5-allyl benzene; 4-methoxy-6-prop-2-enyl-1,3-benzodioxole.

Chemical and Physical Properties

The naturally occurring alkenylbenzene, myristicin, is a volatile, fragrant, and colorless oil. Myristicin has a specific gravity of 1.1416 g/mL at 20ºC, a melting point lower than −20ºC, a boiling point of 276.5ºC, and a vapor pressure of 0.00646 mmHg at 25ºC. Myristicin is soluble in benzene and ether, slightly soluble in ethanol, and insoluble in water.[1] As a member of the alkoxy-substituted allylbenzene family, myristicin shares many physical and chemical properties with the food additives apiole, safrole, elemicin, estragole, and methyleugenol.[2]

Production, Use, and Human Exposure

Exposure to myristicin is largely limited to ingestion of certain spices or plants and products incorporating their essential oils and oleoresins. Principally, myristicin is derived from the tropical evergreen tree *Myristica fragrans* Houtt. (Myristicaceae). Myristicin is a major constituent in essential oil extracted from either the seed (0.25% to 3.28%), which is the source of the spice nutmeg, or the aril covering the seed (0.25% to 5.92%), which is the source of the spice mace, of *M. fragrans*.[3-7] Myristicin is also in the oleoresin fraction of nutmeg and mace; one study reported 4.5% and another reported 8.1% myristicin in nutmeg oleoresin.[7; 8] Other notable components of nutmeg oil include elemicin, safrole, eugenol, isoeugenol, and methyleugenol.[9-11] Besides *M. fragrans*, the chemical myristicin is present in some plants of the Apiaceae family, including dill and parsley, as well as in celery, carrot, and parsnip.[4; 10; 12] Essential oils derived from these natural sources of myristicin are an important consideration when estimating myristicin exposure, the largest sources being nutmeg and mace.[2] The percentage of myristicin in dill herb aromatic fraction is 2.81% to 7.63% and in essential oil from parsley leaf, myristicin is as low as 1% and as high as 60%.[13; 14]

Nutmeg and mace are used widely as spices in a variety of cuisines. Essential oil extracted from nutmeg is used as a flavoring in various products, including soft drinks and cigarettes, sauces, baked goods, processed foods, and condiments, and as a fragrance in perfumes and cosmetics.[15-18] Exposure to myristicin is largely via ingestion, although inhalation and dermal exposure may be secondary routes, especially for those involved in the farming, transport, or processing of nutmeg. Import of nutmeg to the United States was nearly 4.5 million pounds each year in 2011, 2012, and 2013; an increase from 4 million pounds in 1971.[15; 19] Percentages of myristicin in

nutmeg and mace, and their essential oils, vary considerably depending upon the source and the processing technique.[20] Archer[21] found that myristicin comprises 1.14% to 2.54% of powdered nutmeg and 2.59% to 7.55% of powdered mace. Pure myristicin may be isolated from natural products, synthesized from pine oil, or derived synthetically from eugenol for research purposes.[22; 23] Absolute exposure of an individual to myristicin is difficult to estimate, due to the varying levels in essential oils and the large variation in spices and essential oils consumed. Based on the amount of essential oil from nutmeg and mace used in the United States food supply annually, the Food and Drug Administration[15] proposed an estimated exposure of 0.05 mg essential oil/kilogram body weight per day for adults. Based on intake of myristicin from the most frequent sources, such as condiments, nonalcoholic beverages, frozen dairy, processed vegetables, and baked goods, one study estimated average total intake of myristicin to be a few mg per person per day.[4] More recently, assuming the highest concentration of myristicin in essential oils, from nutmeg and other sources, the World Health Organization estimated intake of myristicin to be less than 1 mg/day.[2]

Regulatory Status

There are currently no FDA regulations regarding the levels of powders, oil, or oleoresins from nutmeg, mace, parsley, or dill or pure myristicin allowable in pharmaceutical or food products. Nutmeg, mace, dill, parsley, and the essential oils derived from these spices have been generally recognized as safe.[15; 24; 25] Similarly, there are no guidelines set forth by the Occupational Safety and Health Administration of acceptable levels in industrial settings. There are no guidelines or laws regarding the production or sale of synthetic myristicin or myristicin isolated from natural sources.

Absorption, Distribution, Metabolism, Excretion, and Toxicokinetics

Experimental Animals

There have been no comprehensive toxicokinetic (TK) studies or absorption, distribution, metabolism, and excretion (ADME) studies completed on myristicin to date; however, a few studies have reported in vitro and in vivo metabolism of myristicin in various species. Overall, these studies suggest that the myristicin was well absorbed following oral exposure, metabolized extensively, and excreted as CO_2 and in the urine of experimental animals.[26-29]

Male Swiss-Webster mice administered 5 µmol/kg radiolabeled myristicin by stomach tube were found to expire up to 73% as CO_2 after 48 hours, potentially formed from the hydroxylation of the methylene group of myristicin.[26; 27] Of the remaining radioactivity, 15% was recovered in urine, 3% in feces, 3% in intestine, and 1.5% in liver.

Myristicin was found to have several routes of metabolism, including hydroxylation, demethylenation, and methylation. In male Wistar rats administered myristicin (100 mg/kg) once by oral gavage,[29] the metabolites identified in urine included 1-hydroxy-1-(3′,4′-methylenedioxy-5′-methoxyphenyl)-prop-2-ene, 1-(3′,4′-dihydroxy-5′-methoxyphenyl)-prop-2-ene, 1-(3′,5′-dimethoxy-4′-hydroxyphenyl)-prop-2-ene, 1-(3′,4′-methylenedioxy-5′-hydroxyphenyl)-prop-2-ene, and 2,3-dihydroxy-1-(3′,4′-methylenedioxy-5′-methoxy phenyl)-propane (Figure 2). Isolation of these metabolites in Sprague Dawley rat urine after a single oral administration of

myristicin (100 mg/kg), and comparison before and after glucuronidase treatment, suggests that these metabolites are also excreted in their respective conjugated forms.[28] Oswald et al.[30] found that intraperitoneal injection of myristicin in rats and guinea pigs yielded 3-piperidyl-1-(3′-methoxy-4′,5′-methylenedioxyphenyl)-1-propanone and 3-pyrrolidinyl-1-(3′-methoxy-4′,5′-methylenedioxyphenyl)-1-propanone as the respective major urinary metabolites. This suggests that the many pathways of myristicin metabolism vary in prevalence depending upon species and route of exposure, and may indeed include amination of the allyl group.

Myristicin has the capacity to induce enzymes involved in xenobiotic metabolism. Male Sprague Dawley rats administered myristicin (10 to 500 µmol/kg) intraperitoneally exhibited a dose-dependent increase in microsomal cytochrome P450 (CYP) activity, including CYP1A1/1A2, CYP2B1/2B2, and CYP2E1.[31] In mouse cells, myristicin was found to induce CYP1A1.[32] Myristicin has also been shown to induce glutathione-S-transferase (GST) activity. Female A/J mice orally administered 30 mg myristicin over the course of 6 days exhibited increased levels of hepatic and intestinal GST activity.[33] A similar GST activity induction was observed in albino mice, which exhibited a preferential induction of GSTµ expression over GSTπ and GSTα.[34]

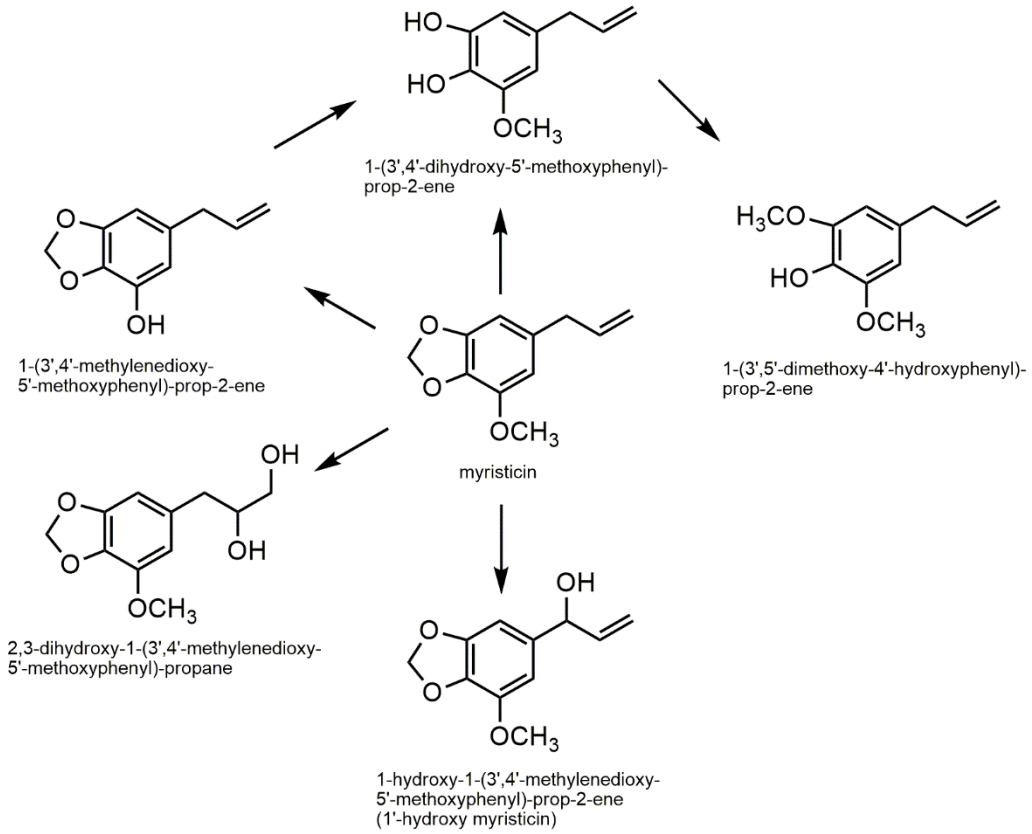

Figure 2. Urinary Metabolites of Myristicin in Rats

Incubation of myristicin in rat liver microsomes formed two major metabolites, 1′-hydroxymyristicin and 5-allyl-1-methoxy-2,3-dihydroxy-benzene,[28] which are the same metabolites identified in the Beyer et al.[29] study, 1-(3′,4′-dihydroxy-5′-methoxyphenyl)-prop-2-ene and 1-hydroxy-1-(3′,4′-methylenedioxy-5′-methoxyphenyl)-prop-2-ene. One study, using isolated perfused rat liver, found that the liver is also capable of biotransforming myristicin into

3-methoxy-4,5-methylenedioxyamphetamine, and proposed it as the likely mechanism for psychoactive activity of myristicin in instances of nutmeg overdose.[35] No other studies have identified amphetamine derivatives of myristicin, or any other safrole-related compound studied.

Humans

There have been no comprehensive studies in humans. However, one study examined metabolites present in the urine of a patient who had ingested five nutmeg seeds resulting in an overdose.[29] Of the metabolites detected, 2,3-dihydroxy-1-(3′,4′-methylenedioxy-5′-methoxyphenyl)-propane may have been derived from myristicin, 1-(3′,5′-dimethoxy-4′-hydroxyphenyl)-prop-2-ene may have been derived from myristicin or estragole, and 1-(3′,4′-methylenedioxy-5′-hydroxyphenyl)-prop-2-ene and 1-(3′,4′-dihydroxy-5′-methoxyphenyl)-prop-2-ene may have been formed from several nutmeg components. Biotransformation of myristicin in human liver microsomes to one of its major metabolites, 5-allyl-1-methoxy-2,3-dihydroxy-benzene, was found to be catalyzed by CYP3A4 and, to a lesser extent, CYP1A2.[36]

Toxicity

Because exposure to myristicin is limited to ingestion of plants and spices of which myristicin is one of many components, it is difficult to delineate the toxicologic responses specific to myristicin. Although myristicin is believed to be the active component of nutmeg oil, there is still some ambiguity as to whether its relative potency can fully explain nutmeg intoxication. Therefore, the reports summarized herein include studies on both nutmeg and myristicin.

Experimental Animals

Perhaps due to the long history of nutmeg as a traditional home remedy, there have been several studies on the therapeutic and toxicologic properties both of nutmeg and of pure myristicin. Investigation into the hypolipidemic effect of nutmeg extract in rabbits revealed the ability to lower levels of total cholesterol in heart and liver (500 mg/kg over 60 days) with no toxicologically significant clinical effects.[37] In benzo[α]pyrene-induced tumor female A/J mouse model, myristicin exposure was associated with a 65% reduction in lung tumor formation and a 31% reduction in forestomach tumor formation after 18 weeks.[33] Myristicin was also shown to be hepatoprotective, attenuating the increased serum ALT and AST activities and DNA fragmentation in mouse livers following administration of lipopolysaccharide and D-galactosamine.[38] Myristicin has been found to have antibacterial, antifungal, and insecticidal activity, as well as to enhance the effectiveness of other insecticides.[12; 39; 40]

There are few studies reported that investigate the toxicity of nutmeg or myristicin in experimental animals. In a 42-day study in adult Wistar rats, oral administration of nutmeg spice (500 and 1,000 mg/kg) displayed degeneration and atrophy of the kidney.[41] However, in another study, male white rats (strain not specified) administered 10 mg/kg myristicin per day for 26 days exhibited no changes in body weight and no abnormalities of the liver or kidney.[42]

Acute administration of nutmeg essential oil (0.5 mL/cage) to mice by inhalation resulted in inhibition of locomotion, and disruption of rope-climbing and bar-pressing activities.[11; 43] Rats and guinea pigs dosed with myristicin (5 to 20 mg/kg intraperitoneal injection) were hyperactive for the first 30 minutes after exposure and then become sedated and nonresponsive for up to 2 hours.[30] Myristicin may promote anxiolytic behavior in male Sprague Dawley rats dosed with

midazolam.[44] A mouse tetrad study, characterizing the effect of nutmeg extract on locomotor activity, catalepsy, body temperature, and nociception compared to cannabinoids and amphetamines revealed that nutmeg-related responses depended upon the extraction method and route of exposure.[45] Oral administration of nutmeg extracts exhibited a stimulating effect on locomotor activity similar to amphetamine; whereas intraperitoneal administration of extracts led to either no effect or a depressive effect on locomotor activity and hypothermia similar to what is seen with cannabinoids.

Humans

Nutmeg has been used for centuries in traditional home remedies to treat illnesses ranging from rheumatism to various intestinal ailments including dysentery and cholera, kidney disorders, gastrointestinal disorders including distended stomach, diarrhea, and flatulence, or as a remedy for overeating, whooping cough, and for healing after childbirth.[5; 10; 46] As a home remedy, nutmeg was used as a sedative, and nutmeg butter was often used in soap due to its purported ability to reinvigorate the skin and heal boils.[46] Nutmeg was also believed to cure madness and relieve headaches, and it was speculated to be an abortifacient at high doses.[10; 46]

Toxic outcomes related to myristicin in humans are largely associated with acute overdoses of nutmeg, the symptoms of which are generally attributed to myristicin. Ingestion of excessive amounts of nutmeg (as little as one and a half seeds and up to 19 seeds) can result in a delirious stupor, with a combination of stimulation and drowsiness or weakness similar to opium and other narcotics.[10; 47; 48] Patients with nutmeg overdose present with facial flushing, tachycardia, hypertension, blurred vision, dry mouth, psychoactive hallucinations, and feelings of euphoria, anxiety, and fear; symptoms typically subside after 24 to 36 hours.[5; 49] Intoxication can be accidental, occurring when individuals would ingest too much nutmeg as a home remedy.[10]

In the modern era, overdoses are more likely to occur due to intentional ingestion of nutmeg as an inexpensive and legal alternative or addition to other abuse substances.[50] There have been several case reports of nutmeg overdoses in recent decades. A German poison information center in Erfurt reported seven cases of nutmeg poisoning from 1996 to 1998, ranging from suspended nutmeg powder (14 to 80 grams) to full nutmeg seeds (up to 19).[48] A retrospective review in an Illinois poison center revealed that, from 2001 to 2011, 32 cases of nutmeg ingestion were reported. Approximately 47% were intentional, and almost all the intentional exposures occurred in teenagers, often as a coexposure with drugs of abuse.[50] A similar retrospective review in California, from 1997 to 1998, reported 119 single-substance exposures to nutmeg, with 72% of them reported as intentional.[51] Nevertheless, to date, only two nutmeg-related deaths have been reported: one 8-year-old boy who ingested two nutmeg seeds (estimated 560 to 840 mg/kg of nutmeg) and one woman whose death was attributed to a drug-drug interaction between myristicin and flunitrazepam.[48; 52]

In the occupational exposure realm, a sampling of spice workers suggests that the processing of nutmeg does not generally elicit allergic responses, although one report of occupational asthma due to exposure to mace, which contains myristicin, has been recorded.[53; 54] Among patients tested for contact allergy to spices, 28% of the 32 patients who tested positive were allergic to nutmeg.[55] There is no information about whether these responses are due to myristicin, or other components of nutmeg.

Reproductive and Developmental Toxicity

Experimental Animals

In a report prepared for the FDA that evaluated the health aspects of nutmeg, mace, and their essential oils as food ingredients, results of available prenatal developmental toxicity studies were summarized. Oil of nutmeg was administered to CD-1 mice (up to 560 mg/kg), Wistar rats (up to 260 mg/kg), and hamsters (up to 600 mg/kg) through species-specific gestation periods (GD 6-15 for rats and mice, GD 6-10 for hamsters). No discernible changes in maternal or fetal survival, nor any malformations of the soft or skeletal tissues were observed in the experimental groups.[56] No studies directly addressing reproductive or developmental toxicity of myristicin in experimental animals have been published; however, there is some evidence of transplacental transfer of myristicin. One study identified alkenylbenzene-DNA adducts in fetal mouse liver after maternal exposure to myristicin by gastric intubation.[16]

Humans

There are no studies on the reproductive or developmental effects of either nutmeg or myristicin in humans. Although nutmeg was historically rumored to have abortifacient properties, these claims have not been substantiated in the recent scientific literature.[5]

Carcinogenicity

Experimental Animals

To date, there have been no 2-year exposure studies on myristicin to investigate carcinogenicity. Safrole, a structurally related chemical, is hepatocarcinogenic in mouse and rat and is reasonably anticipated to be a human carcinogen.[57] Methyleugenol was found by the National Toxicology Program (NTP) to be a hepatocarcinogen in male and female rats and is reasonably anticipated to be a human carcinogen.[58; 59] A panel of predictive models developed using alkenylbenzene toxicology data from subchronic studies suggested that when compared against known hepatocarcinogenic alkenylbenzenes, myristicin would likely be a weak carcinogen if studied at a dose level of 2 mmol/kg body weight per day for 2 years in male F344 rats.[60] Another study canvassed 23 alkenylbenzene derivatives for hepatocarcinogenic potential in mice. Myristicin was repeatedly administered to CD-1 mice over the first 22 days after birth (4.75 µmol).[61] After 13 and 18 months, myristicin-exposed animals exhibited a greater number of hepatomas, but the difference did not reach statistical significance compared to controls that received no treatment.

Humans

There are no studies investigating whether exposure to myristicin is associated with increased cancer incidence in humans.

Genetic Toxicity

Myristicin is metabolized to an electrophilic compound that forms DNA adducts. The 1′-carbon of the allyl side chain of alkenylbenzenes undergoes Phase I hydroxylation.[28] The resultant 1′-hydroxyl group is a target for Phase II conjugation by sulfotransferase; however, the O-sulfation product is unstable and a carbonium ion is generated when the sulfate group leaves.[62] This

carbonium ion forms a major and a minor DNA adduct at the N^2 position of guanine residues.[63; 64]

The formation of myristicin-DNA adducts in the livers of mice has been demonstrated in male B6C3F1 mice and female CD-1 mice exposed via intraperitoneal injection, female ICR mice exposed via oral gavage, and pregnant ICR mice and their fetuses when the dams were exposed via oral gavage.[16; 63; 65] Myristicin-DNA adducts were also detected in vitro in a human hepatoma (HepG2) cell line that exhibits metabolic activity.[64]

Although myristicin forms DNA adducts in mice in vivo and human cells in vitro, exposure to myristicin did not result in phosphorylation of H2AX, a marker of double-strand DNA breaks, in Chinese hamster ovary (CHO) cells, nor did it increase DNA migration, indicative of DNA damage, in CHO cells as measured in the comet assay when tested up to a concentration that reduced cell viability by 50%.[66] Deficiency for the DNA nucleotide excision repair protein, ERCC1, did not affect these results or increase the cytotoxic effects of myristicin.[66] However, an exogenous metabolic activation system was not used in these CHO cell experiments. In freshly-isolated hepatocytes from male Fischer 344 rats, in vitro exposure to myristicin produced a dose-dependent but modest level of unscheduled DNA synthesis, an indicator of DNA excision repair activity.[67]

DNA adduct formation and DNA damage were assessed using an avian egg model.[68] Turkey eggs were injected with myristicin once a day on days 22 through 24 of incubation and embryos were removed 3 hours after the last injection. Adduct levels and DNA damage were assessed in each developing liver. Myristicin-DNA adducts were formed in turkey embryos, and small but statistically significant increases in DNA damage were observed using the comet assay.

Study Rationale and Design

Myristicin was first nominated by the National Cancer Institute for metabolism, genetic toxicology, and carcinogenicity studies due to high potential for human exposure from natural sources and use as a flavoring in food substances. Additionally, myristicin bears structural similarity to safrole, estragole, and other members of the alkenylbenzene family. Based on these similarities, the current studies were conducted to better characterize the toxicity of myristicin in F344/NTac rats and B6C3F1/N mice over 3 months of exposure. To facilitate comparison with NTP studies of other alkenylbenzene flavorings, gavage was chosen as the route of exposure, and comparable doses were selected.

Materials and Methods

Procurement and Characterization

Myristicin

Myristicin was obtained from International Specialty Chemicals, Inc. (Tarrytown, NY), in one lot (PP/MYR/03-11-01) that was used in the 3-month studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle Chemistry Support Services (Columbus, OH) for the study laboratory at Battelle Columbus Operations (Columbus, OH) (Appendix F). Reports on the analyses performed in support of the myristicin studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a brownish orange to red, slightly sticky liquid was identified as myristicin using infrared and proton and carbon-13 nuclear magnetic resonance spectroscopy. The moisture content of lot PP/MYR/03-11-01 was determined by Galbraith Laboratories, Inc. (Knoxville, TN), using Karl Fischer titration. Elemental analyses of the bulk chemical for carbon and hydrogen were conducted by Prevalere Life Sciences, Inc. (Whitesboro, NY). The analytical chemistry laboratory determined the peroxide levels by titration and the purity of the test chemical using gas chromatography (GC) with flame ionization detection (FID) and high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. Reportable impurities were further characterized by the analytical chemistry laboratory using GC and HPLC with mass spectrometry (MS) detection.

Karl Fischer titration indicated ≤0.16% water. Elemental analyses for carbon were in agreement with the theoretical values, but hydrogen values were slightly low; titration indicated the presence of no peroxide. GC/FID by one system indicated one major peak with 96.5% of the total peak area and four reportable impurities with a combined area of 3.5% of the total peak area and individual relative areas of 0.2%, 0.4%, 1.2%, and 1.7%. GC/MS identified two of the four impurities as tributylamine and eugenol; standard addition using GC/FID by a second system estimated their concentrations to be 0.31% and 1.25%, respectively. HPLC/UV analysis indicated one major peak with 94.1% of the total peak area and three reportable impurities with individual relative areas of 1.5%, 1.9%, and 2.5%. HPLC/MS identified two of the three impurities as 5-hydroxy-eugenol (1.9%) and eugenol (1.5%). Standard addition using HPLC/UV estimated the concentration of eugenol to be 1.1%. The purity of lot PP/MYR/03-11-01 was estimated to be greater than 94%.

Stability studies of the bulk chemical were performed using GC/FID. These studies indicated that myristicin was stable as a bulk chemical for at least 14 days when stored in sealed amber glass bottles at temperatures up to 25°C. To ensure stability, the bulk chemical was stored at approximately 5°C, protected from light, in sealed glass bottles. Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 3-month studies with GC/FID, and no degradation of the bulk chemical was detected.

Corn Oil

Corn oil was obtained in two lots (098K0008 and 128K0040) from Sigma-Aldrich (St. Louis, MO) and was used as the vehicle in the 3-month studies. Periodic analyses of the corn oil

performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than the rejection level of 3 mEq/kg.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared four times by mixing myristicin with corn oil to give the required concentrations. The dose formulations were stored at approximately 25°C in amber glass bottles sealed with Teflon®-lined lids for no longer than 42 days.

The analytical chemistry laboratory performed a gavagability study of a 200 mg/mL formulation and stability studies of the 1 mg/mL dose formulation using GC/FID. Gavagability was confirmed for a 22 gauge or larger needle. Stability was confirmed for at least 42 days for dose formulations stored in amber glass bottles sealed with Teflon®-lined lids at ambient and refrigerated temperatures and for at least 3 hours under simulated animal room conditions.

During the 3-month studies, the dose formulations of myristicin were analyzed three times by the study laboratory using GC/FID; all 27 dose formulations were within 10% of the target concentrations (Table F-3). Animal room samples of these dose formulations were also analyzed; 14 of 15 for rats and 14 of 15 for mice were within 10% of the target concentrations.

Animal Source

Male and female F344/NTac rats were obtained from the commercial colony at Taconic BioSciences (formerly Taconic Farms, Inc.), Germantown, NY, and B6C3F1/N mice were obtained from the NTP colony maintained at Taconic BioSciences. The rationale for change of rat strain from F344/N to F344/NTac was a programmatic decision. For many years, NTP used the inbred F344/N rat for its toxicity and carcinogenicity studies. Over a period of time, the F344/N rat exhibited sporadic seizures and idiopathic chylothorax, and consistently high rates of mononuclear cell leukemia and testicular neoplasia. Because of these issues in the F344/N rat and NTP's desire to find a more fecund rat model that could be used in both reproductive and carcinogenesis studies for comparative purposes, a change in the rat model was explored. Following a workshop in 2005, the F344 rat from the Taconic commercial colony (F344/NTac) was used for a few NTP studies to allow NTP to evaluate different rat models. The F344/NTac rat was used in four subchronic and two chronic studies between 2005 and 2006.[69]

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by the AAALAC International. Studies were approved by the Battelle Columbus Operations Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Three-month Studies

On receipt, rats were 3 to 5 weeks old and mice were 4 to 5 weeks old. Animals were quarantined for 12 days; rats were 5 to 7 weeks old and mice were 6 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. The

health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix H). All test results were negative.

Groups of 10 male and 10 female core study rats and mice were administered myristicin in corn oil by gavage at doses of 0, 10, 30, 100, 300, or 600 mg/kg body weight 5 days per week for 13 weeks. Additional groups of 10 male and 10 female clinical pathology study rats were administered the same doses for 21 days. Vehicle control animals received the corn oil vehicle alone. Dosing volumes were 5 mL/kg for rats and 10 mL/kg for mice. Feed and water were available ad libitum. Rats and female mice were housed five per cage, and male mice were housed individually. Animals were observed twice daily. The animals were weighed on day 1, weekly thereafter, and at the end of the studies; clinical observations were recorded postdosing on day 1, weekly thereafter, and at the end of the studies for core study rats and mice. Details of the study design and animal maintenance are summarized in Table 1. Information on feed composition and contaminants is provided in Appendix G.

Animals were anesthetized with a carbon dioxide and oxygen mixture (CO_2:O_2 mixture at 70%:30%) and blood was collected from the retroorbital plexus of clinical pathology study rats on days 3 and 22 and from core study rats at study termination for hematology and clinical chemistry; blood was collected from the retroorbital sinus of mice for hematology analyses at study termination. The blood samples were collected into tubes containing EDTA for hematology determinations or serum separator tubes for clinical chemistry (Table 1). The following hematology parameters were determined using an Advia® 120 Hematology Analyzer (Bayer Diagnostics Division, Tarrytown, NY): hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials. A manual hematocrit was also performed. Peripheral blood smears were evaluated for any morphological abnormalities. Serum was analyzed for the following clinical chemistry parameters using a Cobas® c 501 chemistry analyzer (Roche Diagnostics Corporation, Indianapolis, IN): urea nitrogen, creatinine, glucose, total protein, albumin, cholesterol, triglyceride and bile acid concentrations, and alanine aminotransferase, alkaline phosphatase, creatine kinase, and sorbitol dehydrogenase activities.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats and mice in the 0, 100, 300, and 600 mg/kg groups. The parameters evaluated are listed in Table 1. For 16 consecutive days prior to scheduled terminal euthanasia, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained; however, due to technical issues with sample collection, an assessment of estrous cyclicity could not be made. Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrone's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-

buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin; however, the eyes were first fixed in Davidson's solution, and testes, vaginal tunics, and epididymides were first fixed in modified Davidson's solution. All tissues were trimmed, processed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Additional sections of the kidney from male rats were stained with the Mallory-Heidenhain stain for detection of protein. The study pathologist performed complete histopathologic examinations on rats and mice from the vehicle control and 600 mg/kg groups. The liver, glandular stomach, kidney (males only), testis, epididymis, and lung of rats; and the liver, stomach, testis, epididymis, and nose of mice were examined in all remaining dosed groups. The salivary gland of male and female rats was examined to a no-observed-effect level (300 mg/kg for males and 100 mg/kg for females). Table 1 lists the tissues and organs routinely examined.

Evaluation of the rat sperm parameters suggested an effect on spermatogenesis, but there was no microscopic correlation found during the routine pathology examination of the tissues from the 3-month studies. Therefore, a detailed qualitative histopathologic examination was conducted on H&E-stained slides of testes and epididymides from the rats and mice. Tubular stages of the spermatogenic cycle were taken into account in order to identify potential findings.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to an NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. A special pathology peer review was convened following the initial PWG to evaluate the testes and epididymides. Details of these review procedures have been described, in part, by Maronpot and Boorman[70] and Boorman et al.[71]

Table 1. Experimental Design and Materials and Methods in the Three-month Gavage Studies of Myristicin

Three-month Studies
Study Laboratory
Battelle Columbus Operations (Columbus, OH)
Strain and Species
F344/NTac rats B6C3F1/N mice
Animal Source
Taconic BioSciences (Germantown, NY)
Time Held Before Studies
Rats: 12 (males) or 13 (females) days Mice: 15 (males) or 14 (females) days
Average Age When Studies Began
Rats: 5 to 7 weeks Mice: 6 to 7 weeks
Date of First Dose
Rats: January 20 (males) or 21 (females), 2009 Mice: January 23 (males) or 22 (females), 2009
Duration of Dosing
5 days per week for 21 days (clinical pathology rats) or 13 weeks (core study rats and mice)
Date of Last Dose
Clinical pathology study rats: February 9 (males) or 10 (females), 2009 Core study rats: April 20 (males) or 21 (females), 2009 Mice: April 23 (males) or 22 (females), 2009
Necropsy Dates
Rats: April 21 (males) or 22 (females), 2009 Mice: April 24 (males) or 23 (females), 2009
Average Age at Necropsy
Rats: 18 to 20 weeks Mice: 19 to 20 weeks
Size of Study Groups
10 males and 10 females
Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.
Animals per Cage
Rats: 5
Mice: 1 (males) or 5 (females)
Method of Animal Identification
Tail tattoo

Three-month Studies

Diet

Irradiated NTP-2000 wafer feed (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed at least once weekly

Water

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

Cages

Polycarbonate (Lab Products, Inc., Seaford, DE), changed weekly (male mice) or twice weekly (rats and female mice)

Bedding

Irradiated Sani Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly (male mice) or twice weekly (rats and female mice)

Rack Filters

Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH) changed every 2 weeks

Racks

Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks

Animal Room Environment

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

Doses

0, 10, 30, 100, 300, or 600 mg/kg in corn oil; dosing volumes of 5 mL/kg (rats) or 10 mL/kg (mice)

Type and Frequency of Observation

Observed twice daily; animals were weighed on day 1, weekly thereafter, and at the end of the studies; clinical observations were recorded postdosing on day 1, weekly thereafter, and at the end of the studies for core study rats and mice.

Method of Euthanasia

100% Carbon dioxide

Necropsy

Necropsies were performed on all core study rats and mice. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.

Clinical Pathology

Blood was collected from the retroorbital plexus of clinical pathology study rats on days 3 and 22 and of core study rats at the end of the study for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of mice at the end of the study for hematology. Hematology: hematocrit (automated and manual); hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials
Clinical chemistry: urea nitrogen, creatinine, glucose, total protein, albumin, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids

Three-month Studies

Histopathology

Complete histopathology was performed on 0 and 600 mg/kg core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung (with mainstem bronchus), lymph nodes (mandibular and mesenteric), mammary gland (except male mice), nose, ovary, pancreas, pancreatic islets (mice), parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the kidney (males only), liver, lung, stomach (glandular), and testis (with epididymis) of rats; and the liver, nose, stomach (forestomach and glandular), and testis (with epididymis) of mice were examined in all remaining dosed groups. The salivary gland of male and female rats was examined to a no-observed-effect level.

Sperm Motility and Vaginal Cytology

At the end of the studies, spermatid and sperm samples were collected from male animals in the 0, 100, 300, and 600 mg/kg groups. The following parameters were evaluated: spermatid heads per gram testis and per testis, sperm motility, and sperm per gram cauda epididymis and per cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for 16 consecutive days prior to the end of the studies from females in the 0, 100, 300, and 600 mg/kg groups for vaginal cytology evaluations.

Statistical Methods

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test,[72] a procedure based on the overall proportion of affected animals, was used to determine significance.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and 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[73] and Williams.[74; 75] Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[76] (as modified by Williams[77]) and Dunn.[78] Jonckheere's test[79] 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[80] were examined by NTP personnel, and implausible values were eliminated from the analysis.

Quality Assurance Methods

The 3-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations.[81] In addition, as records from the 3-month studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent QA contractor. Separate audits covered completeness and accuracy of the pathology data, pathology

specimens, final pathology tables, and a draft of this NTP Toxicity Study 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 Toxicity Study Report.

Genetic Toxicology

Salmonella typhimurium Mutagenicity Test Protocol

Testing procedures were those reported by Zeiger et al.[82] Briefly, a commercially obtained sample of myristicin was sent to the laboratory under code. It was incubated with each of 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 at least five doses of myristicin. The high dose was limited by toxicity. All trials were repeated.

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, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

Rat and Mouse Peripheral Blood Micronucleus Test Protocol

At the termination of the 3-month studies, blood samples were collected from male and female rats and mice, placed in EDTA-coated tubes, fixed in ultracold methanol, and frozen at −80°C until analysis. Thawed blood samples were analyzed for frequency of micronucleated reticulocytes (polychromatic erythrocytes, PCEs) and mature erythrocytes (normochromatic erythrocytes, NCEs) using a flow cytometer[83]; both the mature erythrocyte population and the immature reticulocyte population can be analyzed separately by employing special cell surface markers to differentiate the two cell types. Because the very young reticulocyte population can be targeted using this technique, rat blood samples can be analyzed for damage in the bone marrow that occurred within the past 24 to 48 hours, before the rat spleen appreciably alters the percentage of micronucleated reticulocytes in circulation.[84] In mice, both the reticulocyte and mature erythrocyte populations can be evaluated for micronucleus frequency because the mouse spleen does not sequester and eliminate damaged erythrocytes. These achieve steady state in the peripheral blood of mice following 4 weeks of continuous exposure. Approximately 20,000 PCEs and 1×10^6 NCEs are analyzed per sample for frequency of micronucleated cells, and the percent PCEs is calculated as a measure of bone marrow toxicity resulting from chemical exposure.

Based on prior experience with the large number of cells scored using flow cytometric scoring techniques,[85; 86] it is reasonable to assume that the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the control group depend on whether the variances among the groups are equal. Levene's test at $\alpha = 0.05$ is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with dose and Williams' test is used to test for pairwise differences between each treatment group and the control group. In the case of unequal variances, Jonckheere's test is used to test for linear trend and Dunn's test is used for pairwise comparisons of each treatment group with the control group. Trend tests and pairwise comparisons with the controls are considered statistically significant for PCEs and NCEs when the one-sided P-value is less than 0.025, and for percent PCEs when the two-sided P-value is less than 0.05. In the micronucleus test, for each of the endpoints evaluated, a positive result is preferably based on the presence of both a significant trend as well as at least one significantly elevated dose group compared with the corresponding control group. The presence of either a significant trend or a single significant dose group generally results in an equivocal call. The absence of both a trend and a significant dose group results in a negative call. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed (in acute studies), and the magnitudes of those effects.

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 samples of a chemical were tested in the same assay, and different results were obtained among these samples and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the in vitro assays have another variable that must be considered in arriving at an overall test result. In vitro assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Study Report represent a scientific judgment of the overall evidence for activity of the chemical in an assay.

Results

Three-month Study in Rats

All core study male rats survived to the end of the study (Table 2). Three 600 mg/kg core study females died within 4 days of the start of the study; two of the females were found dead with no specific cause identified, and one female was euthanized due to its moribund condition, which included ataxia, abnormal breathing, and nasal/eye discharge. The final mean body weight and mean body weight gain of 600 mg/kg males were significantly less (20% and 30% less, respectively) than those of the vehicle controls (Table 2 and Figure 3). The final mean body weights and mean body weight gains of the remaining dosed groups of males and all dosed groups of females were similar (within 4%) to those of the vehicle control groups. Clinical findings included nasal and eye discharge observed in one 30 mg/kg male, one 300 mg/kg male, and three 600 mg/kg males on days 36, 22, and 15, respectively, and in one 10 mg/kg female on day 29. The livers of all 300 and 600 mg/kg rats surviving to the end of the study were enlarged at necropsy.

Table 2. Survival and Body Weights of Rats in the Three-month Gavage Study of Myristicin[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	110 ± 5	331 ± 6	222 ± 3	
10	10/10	110 ± 5	327 ± 5	217 ± 4	99
30	10/10	106 ± 5	334 ± 7	228 ± 4	101
100	10/10	107 ± 4	335 ± 7	227 ± 7	101
300	10/10	106 ± 5	317 ± 9	211 ± 6	96
600	10/10	109 ± 5	264 ± 6**	154 ± 4**	80
Female					
0	10/10	99 ± 3	179 ± 4	81 ± 3	
10	10/10	98 ± 2	182 ± 3	84 ± 3	102
30	10/10	95 ± 3	178 ± 4	83 ± 4	100
100	10/10	96 ± 2	179 ± 2	83 ± 2	100
300	10/10	95 ± 3	172 ± 2	77 ± 3	96
600	7/10[c]	96 ± 3	172 ± 7	72 ± 5	96

**Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test.
[a]Weights and weight changes are given as mean ± standard error.
[b]Number of animals surviving at 14 weeks/number initially in group.
[c]Week of deaths: 1.

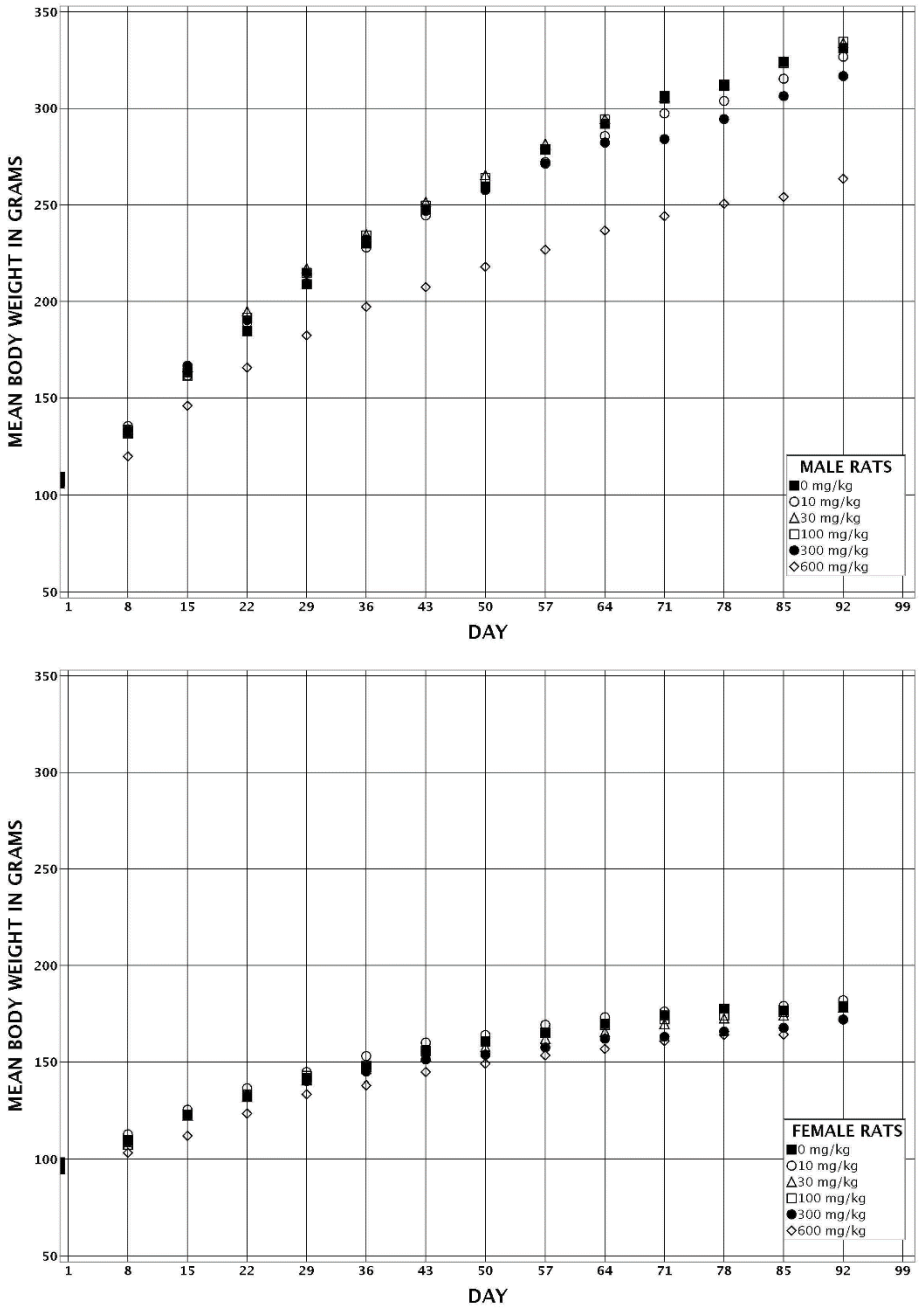

Figure 3. Growth Curves for Rats Administered Myristicin by Gavage for Three Months

On day 3, significant increases in the male rat erythron were observed, most consistently in the 600 mg/kg group; this was characterized by significantly increased erythrocyte count, hemoglobin concentration, and hematocrit values (Table 3 and Table B-1). These increases were consistent with hemoconcentration (i.e., dehydration) and supported by significantly increased blood urea nitrogen (BUN) concentration in 600 mg/kg males on day 3. These male erythron changes resolved by week 14. On day 22, the erythrocyte count, hemoglobin concentration, and hematocrit values were significantly decreased in female rats, generally in the 300 and 600 mg/kg groups. At week 14, similar significantly decreased incidences were observed in 300

and 600 mg/kg females, but the effect was minimal in comparison. The reason for the mild decrease in the erythron in females is uncertain.

Small but significant increases in segmented neutrophil counts occurred in 300 and 600 mg/kg males at study termination and could be related to the liver necrosis or lesions in the glandular stomach observed microscopically at 600 mg/kg (Table 3 and Table B-1). Several other significant hematological changes were observed in male and female rats; however, these changes were inconsistent or minimal and thus not considered toxicologically relevant.

Alanine aminotransferase and sorbitol dehydrogenase activities were significantly elevated in several dosed groups of males and females, but most consistently at 600 mg/kg (Table 3 and Table B-1). Alkaline phosphatase (ALP) was significantly decreased in males and females starting on day 22 (Table 3 and Table B-1). In male rats, affected dose groups varied over time with ALP consistently decreased throughout the study in 600 mg/kg males. In females, ALP was decreased in 30 mg/kg or greater groups on day 22 and in the 100 mg/kg or greater groups at week 14. Decreases in serum ALP activity are associated with decreases in food consumption, as the intestinal ALP isoenzyme contributes greatly to total serum ALP activity. While decreased body weight in 600 mg/kg males supports a decrease in food consumption, female body weights were relatively unchanged. Thus, the decreases in ALP may be related to alterations in hepatic enzyme metabolism.

Cholesterol and triglyceride concentrations were significantly elevated in male and female rats in various dose groups (Table 3 and Table B-1). In particular, at week 14, cholesterol was significantly increased in the 100 mg/kg or greater males and in all dosed female groups. Triglycerides were significantly increased in 300 and 600 mg/kg males and 600 mg/kg females at week 14. The reason for the alterations is not known, but may be related to changes in lipid metabolism.

BUN concentrations were significantly increased in 600 mg/kg males on day 3 and 100 mg/kg or greater males on day 22 (Table 3 and Table B-1). At week 14, creatinine, total protein, and albumin concentrations were significantly increased in 300 and 600 mg/kg males and BUN concentration was significantly increased in 600 mg/kg males; these elevations were minimal to mild in magnitude. This particular group of changes is consistent with a decrease in water intake. In females at week 14, total protein and albumin concentrations were significantly increased at 300 and 600 mg/kg; this was also consistent with decreased water intake. The renal nephropathy observed in males was generally minimal and not considered a cause for the mild increases in BUN and creatinine.

Table 3. Selected Clinical Pathology Data for Rats in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Male						
Hematology						
n						
Day 3	9	9	10	10	10	10
Day 22	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Hematocrit (auto) (%)						
Day 3	43.0 ± 0.6	43.9 ± 0.6	43.3 ± 0.5	43.0 ± 0.5	44.3 ± 0.5	45.4 ± 0.6**
Day 22	45.1 ± 0.3	43.9 ± 0.2	45.6 ± 0.5	46.2 ± 0.4	44.2 ± 0.4	42.4 ± 0.9*
Week 14	47.3 ± 0.5	47.3 ± 0.3	46.5 ± 0.4	46.9 ± 0.4	46.3 ± 0.4	46.4 ± 0.3
Manual hematocrit (%)						
Day 3	43.2 ± 0.4	44.6 ± 0.5	43.6 ± 0.4[b]	43.7 ± 0.4[b]	45.3 ± 0.4**[b]	46.3 ± 0.5**
Day 22	45.3 ± 0.5	45.1 ± 0.3	46.3 ± 0.4	46.4 ± 0.4	45.0 ± 0.5	43.3 ± 0.7
Week 14	47.6 ± 0.5	46.7 ± 0.4	46.8 ± 0.4	47.0 ± 0.4	46.3 ± 0.4	46.1 ± 0.3
Hemoglobin (g/dL)						
Day 3	13.5 ± 0.2	13.8 ± 0.1	13.6 ± 0.1	13.6 ± 0.2	14.1 ± 0.1**	14.7 ± 0.1**
Day 22	14.1 ± 0.1	13.8 ± 0.1	14.2 ± 0.1	14.3 ± 0.1	14.0 ± 0.1	13.4 ± 0.3
Week 14	15.0 ± 0.1	14.8 ± 0.1	14.7 ± 0.2	14.8 ± 0.1	14.6 ± 0.1	14.5 ± 0.1**
Erythrocytes (10^6/μL)						
Day 3	7.41 ± 0.15	7.49 ± 0.09	7.43 ± 0.10	7.39 ± 0.12	7.60 ± 0.09	7.90 ± 0.10**
Day 22	7.99 ± 0.09	7.78 ± 0.04	8.05 ± 0.10	8.16 ± 0.07	7.81 ± 0.07	7.70 ± 0.16
Week 14	9.25 ± 0.11	9.19 ± 0.07	9.11 ± 0.09	9.07 ± 0.07	9.00 ± 0.07	9.13 ± 0.06
Segmented neutrophils (10^3/μL)						
Day 3	1.46 ± 0.08	1.48 ± 0.07	1.60 ± 0.10	1.51 ± 0.07	1.51 ± 0.11	1.36 ± 0.09
Day 22	1.54 ± 0.08	1.52 ± 0.11	1.64 ± 0.11	1.72 ± 0.09	1.77 ± 0.11	1.62 ± 0.09
Week 14	1.53 ± 0.06	1.98 ± 0.20	1.85 ± 0.10	1.53 ± 0.07	1.96 ± 0.10*	1.90 ± 0.08*
Clinical Chemistry						
n						
Day 3	10	10	10	10	10	10
Day 22	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	12.9 ± 0.4[c]	13.5 ± 0.7	13.3 ± 0.3	13.8 ± 0.6[b]	14.2 ± 0.8	17.0 ± 0.8**[c]
Day 22	10.1 ± 0.4	10.1 ± 0.5	10.2 ± 0.5	13.1 ± 0.8*	12.0 ± 0.4*	12.3 ± 0.6*
Week 14	13.3 ± 0.6	13.1 ± 0.7	13.4 ± 0.4	14.1 ± 0.6	14.0 ± 0.4	15.8 ± 0.8*

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Creatinine (mg/dL)						
Day 3	0.61 ± 0.01[b]	0.61 ± 0.01	0.60 ± 0.00	0.60 ± 0.00[b]	0.61 ± 0.01	0.65 ± 0.03
Day 22	0.62 ± 0.01	0.60 ± 0.00	0.61 ± 0.01	0.61 ± 0.02	0.61 ± 0.01	0.62 ± 0.01
Week 14	0.70 ± 0.01	0.74 ± 0.04	0.71 ± 0.01	0.73 ± 0.02	0.75 ± 0.02*	0.76 ± 0.02*
Total protein (g/dL)						
Day 3	5.5 ± 0.1[c]	5.7 ± 0.1	5.5 ± 0.1	5.5 ± 0.1[b]	5.4 ± 0.1	5.4 ± 0.1[c]
Day 22	6.1 ± 0.1	5.8 ± 0.1*	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.4 ± 0.1
Week 14	7.2 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.7 ± 0.1**	8.0 ± 0.1**
Albumin (g/dL)						
Day 3	3.9 ± 0.1	4.0 ± 0.0	3.9 ± 0.0	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.0
Day 22	4.3 ± 0.0	4.1 ± 0.0	4.2 ± 0.1	4.2 ± 0.1	4.1 ± 0.0	4.4 ± 0.1
Week 14	4.6 ± 0.0	4.5 ± 0.0	4.6 ± 0.0	4.6 ± 0.0	4.8 ± 0.0*	5.0 ± 0.0**
Cholesterol (mg/dL)						
Day 3	88 ± 2	90 ± 2	92 ± 2	101 ± 3**	108 ± 4**	103 ± 3**[b]
Day 22	79 ± 1	73 ± 1	79 ± 1	81 ± 1	88 ± 2**	122 ± 4**
Week 14	79 ± 1	77 ± 2	83 ± 2	85 ± 1*	113 ± 2**	149 ± 3**
Triglycerides (mg/dL)						
Day 3	107 ± 8[b]	135 ± 28	110 ± 9	115 ± 4	127 ± 8	266 ± 34**[c]
Day 22	98 ± 8	101 ± 6	112 ± 8	80 ± 12	121 ± 10	179 ± 17**
Week 14	120 ± 9	113 ± 8	135 ± 10	128 ± 9	164 ± 9**	192 ± 20**
Alanine aminotransferase (IU/L)						
Day 3	64 ± 3	66 ± 4	64 ± 2	67 ± 2	72 ± 3*	78 ± 5*
Day 22	49 ± 1	51 ± 2	51 ± 1	55 ± 2*	56 ± 1**	62 ± 2**
Week 14	64 ± 2	61 ± 3	60 ± 3	58 ± 2	60 ± 2	84 ± 3*
Alkaline phosphatase (IU/L)						
Day 3	641 ± 29[d]	669 ± 24	623 ± 26[c]	616 ± 17[d]	661 ± 25[b]	537 ± 24[b]
Day 22	453 ± 10	435 ± 6	447 ± 12	399 ± 16*	377 ± 7**	330 ± 11**
Week 14	249 ± 9	265 ± 7	261 ± 5	249 ± 6	232 ± 6	223 ± 6*
Sorbitol dehydrogenase (IU/L)						
Day 3	10 ± 0[b]	11 ± 0	11 ± 0	10 ± 0[b]	12 ± 1**	10 ± 1[b]
Day 22	14 ± 1	13 ± 1	13 ± 0	13 ± 1	12 ± 1	15 ± 1
Week 14	16 ± 1	16 ± 1	14 ± 1	15 ± 1	15 ± 1	22 ± 1**

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Female						
Hematology						
n						
Day 3	10	9	10	9	10	9
Day 22	8	10	10	10	10	9
Week 14	10	9	10	10	10	7
Hematocrit (auto) (%)						
Day 3	45.4 ± 0.4	45.5 ± 0.5	45.2 ± 0.6	46.3 ± 0.9	45.0 ± 0.3	46.1 ± 0.8
Day 22	47.2 ± 0.7	46.1 ± 0.4	45.8 ± 0.5	46.2 ± 0.3	45.5 ± 0.5*	44.0 ± 0.4**
Week 14	45.7 ± 0.5	46.3 ± 0.2	45.3 ± 0.2	45.9 ± 0.4	44.2 ± 0.4**	44.6 ± 0.5*
Manual hematocrit (%)						
Day 3	45.2 ± 0.6[b]	45.3 ± 0.5	45.1 ± 0.5	45.4 ± 0.8[d]	45.1 ± 0.5	46.1 ± 0.7
Day 22	47.8 ± 0.4	46.4 ± 0.4	46.6 ± 0.4	46.3 ± 0.4*	46.4 ± 0.5*	44.3 ± 0.5**
Week 14	46.9 ± 0.4	47.3 ± 0.3	46.6 ± 0.2	47.1 ± 0.2	45.9 ± 0.3	46.3 ± 0.6
Hemoglobin (g/dL)						
Day 3	14.7 ± 0.1	14.7 ± 0.2	14.4 ± 0.2	14.9 ± 0.3	14.5 ± 0.1	15.2 ± 0.2
Day 22	15.4 ± 0.3	15.1 ± 0.1	14.9 ± 0.2	15.0 ± 0.1	14.8 ± 0.2*	14.1 ± 0.1**
Week 14	14.7 ± 0.1	14.9 ± 0.1	14.7 ± 0.0	14.6 ± 0.1	14.2 ± 0.1**	14.0 ± 0.2**
Erythrocytes (10^6/μL)						
Day 3	7.97 ± 0.08	7.99 ± 0.12	7.87 ± 0.08	8.08 ± 0.17	7.90 ± 0.08	8.18 ± 0.11
Day 22	8.62 ± 0.14	8.37 ± 0.11	8.29 ± 0.09*	8.38 ± 0.07*	8.17 ± 0.08**	8.10 ± 0.08**
Week 14	8.39 ± 0.10	8.51 ± 0.06	8.37 ± 0.04	8.42 ± 0.06	8.19 ± 0.06	8.55 ± 0.11
Clinical Chemistry						
n						
Day 3	10	10	10	10	10	10
Day 22	9	10	10	10	10	9
Week 14	10	10	10	10	10	7
Total protein (g/dL)						
Day 3	5.7 ± 0.1[b]	5.8 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.5 ± 0.0*	5.5 ± 0.1*
Day 22	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.6 ± 0.1**
Week 14	7.0 ± 0.1	7.3 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.7 ± 0.1**	7.9 ± 0.1**
Albumin (g/dL)						
Day 3	4.1 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.0 ± 0.1	4.0 ± 0.1
Day 22	4.5 ± 0.1	4.4 ± 0.0	4.5 ± 0.1	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.1
Week 14	4.8 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	5.0 ± 0.1**	5.1 ± 0.0**

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Cholesterol (mg/dL)						
Day 3	90 ± 2	90 ± 3	98 ± 2*	99 ± 2**	109 ± 3**	108 ± 4**
Day 22	88 ± 8	84 ± 4	86 ± 1	97 ± 2**	138 ± 2**	185 ± 4**
Week 14	77 ± 2	84 ± 3*	87 ± 1**	98 ± 2**	140 ± 3**	166 ± 6**
Triglycerides (mg/dL)						
Day 3	95 ± 5	85 ± 5	78 ± 3	86 ± 8	102 ± 8	209 ± 21**
Day 22	72 ± 8	67 ± 6	54 ± 5	56 ± 8	61 ± 5	94 ± 8
Week 14	50 ± 7	56 ± 4	53 ± 5	46 ± 4	67 ± 10	69 ± 5*
Alanine aminotransferase (IU/L)						
Day 3	52 ± 1	62 ± 3**	58 ± 2*	61 ± 2**	68 ± 2**	75 ± 3**
Day 22	43 ± 2	42 ± 2	41 ± 1	43 ± 1	49 ± 1**	57 ± 3**
Week 14	52 ± 2	56 ± 4	50 ± 2	47 ± 2	50 ± 2	73 ± 4*
Alkaline phosphatase (IU/L)						
Day 3	510 ± 10	555 ± 19	532 ± 13	541 ± 23	508 ± 12	488 ± 19
Day 22	374 ± 5	366 ± 6	345 ± 6**	352 ± 7*	331 ± 6**	333 ± 10**
Week 14	261 ± 6	267 ± 5	262 ± 6	223 ± 7**	193 ± 6**	246 ± 12*
Sorbitol dehydrogenase (IU/L)						
Day 3	12 ± 1	10 ± 1	12 ± 1	12 ± 1	12 ± 1	13 ± 2
Day 22	11 ± 1	11 ± 1	11 ± 0	15 ± 1*	18 ± 0**	16 ± 1**
Week 14	13 ± 1	14 ± 1	13 ± 1	15 ± 1	16 ± 1**	29 ± 2**

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b]n = 9.
[c]n = 8.
[d]n = 7.

The absolute and relative liver weights of 100 mg/kg or greater males and females and the relative liver weight of 30 mg/kg males were significantly increased compared to those of the vehicle controls (Table 4 and Table C-1). The absolute liver weights of 600 mg/kg males and females were 72% and 124% greater than those of the vehicle controls, respectively.

The mean absolute and relative kidney weights of 100 mg/kg or greater males were significantly increased compared to the vehicle controls (Table 4 and Table C-1). The absolute kidney weight was highest in 300 mg/kg males and was 15% greater than that of the vehicle controls. The relative kidney weights of 300 and 600 mg/kg females were significantly greater than that of the vehicle control group.

Other differences in organ weights include significantly increased relative heart weight in the 300 and 600 mg/kg males with significantly decreased absolute heart weight in 600 mg/kg males; significantly increased relative lung weight in 600 mg/kg males; and, significantly decreased absolute and relative thymus weight in 600 mg/kg males (Table C-1). These differences are of uncertain toxicologic significance given the decreased body weight and weight gain in the 600 mg/kg males and the lack of treatment-related, histologic lesions in these tissues.

Significant treatment-related, histologic lesions were observed in the liver, glandular stomach, and salivary gland of male and female rats and in the kidney, testis, and epididymis of male rats.

Liver: Treatment-related lesions in the liver included centrilobular hepatocyte hypertrophy, fatty change, and hepatocyte necrosis (Table 5, Table A-1, and Table A-2). The incidences of centrilobular hepatocyte hypertrophy were significantly increased in 300 and 600 mg/kg males and females compared to the vehicle controls. The incidences of fatty change in the liver were significantly increased in 300 and 600 mg/kg males and 600 mg/kg females, and the incidences of hepatocyte necrosis were significantly increased in 600 mg/kg male and females.

Microscopically, hepatocyte hypertrophy was mild to moderate in severity and characterized by the presence of enlarged centrilobular hepatocytes with eosinophilic granular cytoplasm (Figure 5 and Figure 6). At 300 mg/kg, hepatocyte hypertrophy was generally restricted to the centrilobular zone, but at 600 mg/kg, it often extended into the midzonal region or throughout the lobule. Increased liver weights and grossly observed liver enlargement corresponded microscopically with centrilobular hepatocyte hypertrophy and fatty change in 300 and 600 mg/kg male and female rats. Fatty change in the liver was minimal to mild in severity and characterized by numerous, variably sized (macrovesicular and microvesicular) discrete, clear vacuoles within the cytoplasm of scattered hepatocytes (Figure 5 and Figure 6).

Table 4. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	331 ± 6	327 ± 5	334 ± 7	335 ± 7	317 ± 9	264 ± 6**
R. Kidney						
Absolute	1.00 ± 0.03	0.97 ± 0.02	1.04 ± 0.03	1.09 ± 0.04*	1.15 ± 0.04**	1.09 ± 0.03**
Relative	3.00 ± 0.05	2.97 ± 0.06	3.12 ± 0.07	3.27 ± 0.09**	3.62 ± 0.05**	4.13 ± 0.07**
Liver						
Absolute	12.61 ± 0.26	12.44 ± 0.34	13.74 ± 0.35	14.42 ± 0.44**	18.97 ± 0.54**	21.71 ± 0.66**
Relative	38.07 ± 0.34	38.06 ± 0.73	41.20 ± 0.97*	43.04 ± 0.70**	59.91 ± 0.78**	82.39 ± 1.68**
Female						
n	10	10	10	10	10	7
Necropsy body wt	179 ± 4	182 ± 3	178 ± 4	179 ± 2	172 ± 2	172 ± 7
R. Kidney						
Absolute	0.64 ± 0.01	0.63 ± 0.02	0.62 ± 0.02	0.65 ± 0.01	0.66 ± 0.01	0.69 ± 0.02
Relative	3.55 ± 0.06	3.43 ± 0.04	3.51 ± 0.09	3.61 ± 0.06	3.83 ± 0.04**	4.03 ± 0.08**
Liver						
Absolute	6.55 ± 0.23	6.64 ± 0.18	6.81 ± 0.12	7.88 ± 0.18**	10.55 ± 0.29**	14.70 ± 0.50**
Relative	36.57 ± 0.97	36.47 ± 0.65	38.33 ± 1.04	44.12 ± 1.10**	61.34 ± 1.58**	85.76 ± 1.19**

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

Table 5. Incidences of Selected Nonneoplastic Lesions in Rats in the Three-month Gavage Study of Myristicin

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Male						
Liver[a]	10	10	10	10	10	10
Centrilobular, Hepatocyte, Hypertrophy[b]	0	0	0	0	10** (2.0)[c]	10** (3.0)
Fatty Change	0	0	0	0	10** (1.6)	10** (1.5)
Hepatocyte, Necrosis	0	0	0	0	0	8** (1.0)
Stomach, Glandular	10	10	10	10	10	10
Epithelium, Atrophy	0	0	0	0	0	10** (1.7)
Epithelium, Hyperplasia	0	0	0	0	0	10** (1.8)
Salivary Glands	10	0	0	0	10	10
Submandibular Gland, Depletion Secretory	0	–	–	–	0	7** (1.3)
Kidney	10	10	10	10	10	10
Renal Tubule, Accumulation, Hyaline Droplet	0	0	0	0	10** (1.0)	10** (1.9)
Nephropathy	10 (1.0)	10 (1.0)	9 (1.0)	10 (1.0)	10 (1.0)	10 (1.8)
Female						
Liver	10	10	10	10	10	10
Centrilobular, Hepatocyte, Hypertrophy	0	0	0	0	10** (2.0)	7** (3.0)
Fatty Change	0	0	0	0	1 (1.0)	10** (1.9)
Hepatocyte, Necrosis	0	0	0	0	0	6** (1.2)
Stomach, Glandular	10	10	10	10	10	10
Epithelium, Atrophy	0	0	0	0	5** (1.0)	7** (1.7)
Epithelium, Hyperplasia	0	0	0	0	0	7** (1.4)
Epithelium, Necrosis	0	0	0	0	0	2 (1.0)
Salivary Glands	10	0	0	10	10	10
Submandibular Gland, Depletion Secretory	0	–	–	0	10** (1.5)	7** (3.0)

**Significantly different ($P \leq 0.01$) from the vehicle control group by the Fisher exact test.
[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.

Hepatocyte necrosis was generally minimal in severity and characterized by single or, less often, small clusters of hepatocytes with condensed, hypereosinophilic cytoplasm that had shrunken or karyorrhectic nuclei, sometimes associated with low numbers of neutrophils and mononuclear inflammatory cells. Additionally, a single early death 600 mg/kg female was diagnosed with mild necrosis in the liver, which differed from the diagnosis of hepatocyte necrosis in that it was characterized by multiple larger, discrete foci of coagulative necrosis.

Glandular Stomach: Treatment-related lesions in the glandular stomach included epithelium atrophy and hyperplasia in males and females and epithelium necrosis in females (Table 5, Table A-1, and Table A-2). The incidences of epithelium atrophy were significantly increased in 600 mg/kg males and in 300 and 600 mg/kg females compared to the vehicle controls. The incidences of epithelium hyperplasia were significantly increased in the 600 mg/kg males and females. Epithelium necrosis of the glandular stomach was observed in two 600 mg/kg females, which died on day 4 of the study. Although this increase was not statistically significant, the necrosis of the glandular stomach in these females is considered to be treatment related.

Microscopically, epithelium atrophy ranged from minimal to mild in severity and was characterized by decreased cellularity and height of the mucosa due to nearly complete or complete loss of chief and parietal cells with single cell death of both of these cell types (Figure 7 and Figure 8). In general, there was slight distortion of glands that remained and increased prominence of stroma between the glands. There were few granulocytes and mononuclear cells scattered within the lamina propria extending between and accumulating in gastric glands. Epithelium atrophy in 300 mg/kg females differed from that in 600 mg/kg males and females in that there was only a decrease in the number of chief cells and the remaining glandular epithelium appeared histologically normal. Epithelium atrophy was accompanied by minimal to mild epithelium hyperplasia within the overall atrophic, thinned mucosal epithelium. Epithelium hyperplasia was characterized by small, scattered foci with increased numbers of epithelial cells with basophilic cytoplasm, especially mucus neck cells, and increased numbers of mitotic figures (Figure 8). Epithelium necrosis of the glandular stomach was seen as single cell necrosis of chief cells and parietal cells with accumulation of desquamated epithelial cells in dilated gastric glands.

Salivary Gland: The incidences of secretory depletion in the submandibular gland were significantly increased in the 600 mg/kg males and 300 and 600 mg/kg females compared to the vehicle controls (Table 5, Table A-1, and Table A-2). Microscopically, this lesion was characterized by a decrease in the number of cytoplasmic zymogen granules within the epithelial cells of the granular convoluted ducts (Figure 9) as compared to the normal salivary gland (Figure 10). In males, the lesion was of minimal to mild severity and also encompassed disorganization of the epithelial cells of the granular convoluted ducts. In females, the lesion was of minimal to moderate severity, and the granular convoluted ducts were often severely shrunken due to lack of cytoplasm.

Kidney: Treatment-related lesions in the kidney of male rats included renal tubule hyaline droplet accumulation and a slight increase in the severity of nephropathy (Table 5 and Table A-1). The incidences of renal tubule hyaline droplet accumulation were significantly increased in the 300 and 600 mg/kg males compared to the vehicle controls. Hyaline droplet accumulation was present in all 300 and 600 mg/kg males and increased in severity from minimal at 300 mg/kg to mild at 600 mg/kg. Nephropathy, which was minimal in severity in vehicle control, 10, 30, 100, and 300 mg/kg males, increased to generally mild in severity in 600 mg/kg males.

Hyaline droplet accumulation was observed in the renal tubule epithelium of the proximal convoluted tubules. In contrast to the fine, round, cytoplasmic droplets characteristic of the vehicle control kidneys (Figure 11), the cytoplasmic droplets in the 300 and 600 mg/kg males were increased in number and size and were irregularly angular and more pleomorphic (Figure 12). There was also loss of the droplet-congested cells that are typical of the control

kidneys. Although evident in the H&E-stained sections, droplets in treated animals were more prominent upon staining with the Mallory-Heidenhain method or ultraviolet illumination, suggestive of accumulation of α_{2u}-globulin. Increases in kidney weights corresponded microscopically to the accumulation of hyaline droplets in the 300 and 600 mg/kg males. There was a slight increase in the severity (exacerbation) of nephropathy in the 600 mg/kg males, based upon an increase in the number of foci of regenerating tubules. Microscopically, nephropathy was consistent with the early stages of chronic progressive nephropathy typically observed in rats.

Male Reproductive System: Treatment-related histologic lesions in the male reproductive system included germinal epithelium degeneration and elongated spermatid retention in seminiferous tubules of the testis and exfoliated germ cells in the duct lumina of the epididymis (Table 6 and Table A-1). In the testis, the incidences of germinal epithelium degeneration and elongated spermatid retention in 600 mg/kg males were significantly increased compared to those of the vehicle control group; the severities of both lesions in this dose group were also greater than the severities found in the vehicle control group. In the epididymis, the incidence of exfoliated germ cell was significantly increased in 600 mg/kg males compared to that in the vehicle controls.

Microscopically, germinal epithelium degeneration was mild to moderate and characterized by a decrease and/or disorganization or vacuolization of the germinal epithelial layers (Figure 13 and Figure 14). Elongated spermatid retention in the testis was minimal to mild and characterized by the presence of mature elongated spermatids at the luminal surface or within the germinal epithelium of Stage IX and X seminiferous tubules (Figure 15). Exfoliated germ cells in the epididymis were characterized by minimal to mild increases in the numbers of rounded germ cells and cellular debris in the epididymal duct lumina, primarily in the tail of the epididymis, exceeding the numbers normally observed in the epididymis of vehicle control animals (Figure 16).

Male rats in the 600 mg/kg dose group had significantly decreased absolute left cauda and left epididymis weights and mean total number of sperm per cauda epididymis compared to those of the vehicle controls (approximately 22%, 16%, and 24% lower, respectively) (Table 6 and Table D-1). Myristicin exposure via gavage exhibited the potential to be a reproductive toxicant in male F344/NTac rats.

Other Findings: In the lung of females, significantly increased incidences of chronic active inflammation occurred at 30, 100, and 300 mg/kg (2/10, 6/10, 7/10, 7/10, 10/10, 5/10), and significantly increased incidences of alveolus infiltration cellular histiocyte occurred in 30 mg/kg or greater females (2/10, 5/10, 8/10, 8/10, 9/10, 7/10) (Table A-2). The lung lesions, which also were present in male rats, were characterized by focally extensive to multifocal infiltrates of lymphohistiocytic cells, resulting in alveolar septal wall thickening (i.e., interstitial pneumonia) accompanied by prominent perivascular "cuffing" of pulmonary veins by dense infiltrates of primarily lymphocytes. Histiocytic alveolus infiltration was characterized by the presence of intra-alveolar clusters of large, foamy macrophages. These lung changes are consistent with mild infection with *Pneumocystis carinii*,[87] which was not a recognized etiologic agent in rats at the time of the study. In general, the relationship of these changes to exposure is uncertain.

Table 6. Reproductive System Parameters of Male Rats in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
n	10	–	–	10	10	10
L. Testis Weight	1.4464 ± 0.0217	–	–	1.4943 ± 0.0226	1.5092 ± 0.0317	1.4106 ± 0.0220
L. Cauda Epididymis Weight	0.1365 ± 0.0033	–	–	0.1387 ± 0.0028	0.1292 ± 0.0045	0.1070 ± 0.0031**
L. Epididymis Weight	0.4090 ± 0.0071	–	–	0.4104 ± 0.0038	0.4008 ± 0.0096	0.3444 ± 0.0080**
Sperm (10^6/cauda epididymis)	89.37 ± 5.49	–	–	88.63 ± 4.42	79.11 ± 4.47	67.63 ± 3.18**
Epididymis[b]	10	10	10	10	10	10
Exfoliated Germ Cell[c]	1 (1.0)[d]	3 (1.3)	1 (1.0)	2 (1.0)	4 (2.3)	10▲▲ (1.5)
Testes	10	10	10	10	10	10
Germinal Epithelium, Degeneration	5 (1.4)	5 (2.2)	3 (1.3)	6 (1.3)	8 (1.6)	10▲ (2.3)
Elongated Spermatid, Retention	5 (1.0)	3 (1.0)	0	1 (1.0)	6 (1.0)	10▲ (1.4)

**Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' (tissue weights) or Shirley's (sperm measurements) test.
▲Significantly different from the vehicle control group ($P \leq 0.05$) by the Fisher exact test.
▲▲$P \leq 0.01$.
[a]Tissue weights and sperm data are presented as mean ± standard error. Tissue weights and sperm data were not evaluated for 10 and 30 mg/kg males.
[b]Number of animals with tissue 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.

Three-month Study in Mice

All mice survived to the end of the study (Table 7). The final mean body weights and mean body weight gains of 300 and 600 mg/kg males and females were significantly less than those of the vehicle control groups (Table 7 and Figure 4). The final mean body weights of 600 mg/kg males and females were 24% and 16% less, respectively, than those of vehicle controls. Clinical observations included thinness in one 300 mg/kg male and one 600 mg/kg female and ruffled fur in one 300 mg/kg male and one 600 mg/kg male. As seen in rats, the livers of all the 300 and 600 mg/kg male and female mice were also enlarged at scheduled termination. A few treated mice had white or tan foci in the forestomach.

Table 7. Survival and Body Weights of Mice in the Three-month Gavage Study of Myristicin[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	22.7 ± 0.4	38.8 ± 1.2	16.1 ± 1.0	
10	10/10	22.6 ± 0.3	38.1 ± 1.1	15.6 ± 0.9	98
30	10/10	22.6 ± 0.2	38.3 ± 0.8	15.7 ± 0.7	99
100	10/10	22.6 ± 0.2	37.7 ± 0.9	15.1 ± 0.8	97
300	10/10	22.8 ± 0.4	34.2 ± 1.1**	11.2 ± 0.8**	88
600	10/10	22.7 ± 0.3	29.3 ± 0.6**	6.6 ± 0.4**	76
Female					
0	10/10	17.9 ± 0.2	28.3 ± 0.8	10.4 ± 0.6	
10	10/10	18.0 ± 0.2	31.0 ± 0.8	12.9 ± 0.7	109
30	10/10	17.8 ± 0.2	29.1 ± 1.0	11.3 ± 0.9	103
100	10/10	18.3 ± 0.3	27.2 ± 0.6	8.9 ± 0.4	96
300	10/10	17.9 ± 0.4	25.3 ± 0.3**	7.4 ± 0.3**	89
600	10/10	17.8 ± 0.3	23.9 ± 0.4**	6.2 ± 0.4**	85

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

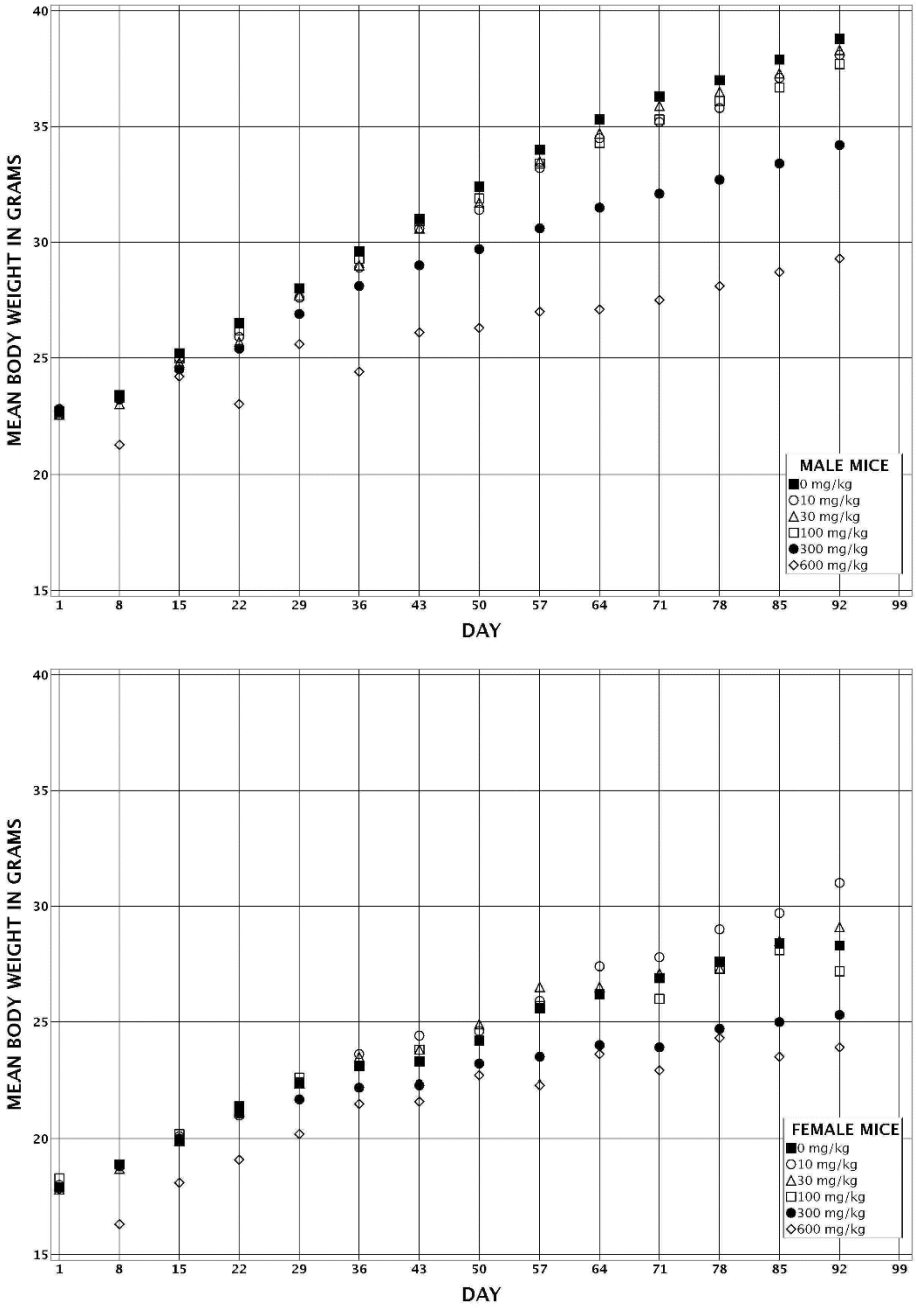

Figure 4. Growth Curves for Mice Administered Myristicin by Gavage for Three Months

The erythrocyte counts were significantly decreased in the 100 mg/kg and greater males, and the automated hematocrit value was significantly decreased in 600 mg/kg males (Table 8 and Table B-2). These erythron changes were mild and not observed in the females and, therefore, their toxicologic significance is uncertain. It is plausible that the mild decrease in the erythron was due to erythroid suppression secondary to the chronic inflammation observed in the stomach, which is supported by the changes in the leukon (increased leukocyte counts). Leukocyte counts were significantly increased in 300 and 600 mg/kg males and 600 mg/kg females. In addition, segmented neutrophil counts were significantly increased in 300 and

600 mg/kg males and females. Also, the lymphocyte count was significantly increased in 600 mg/kg males and the monocyte count was significantly increased in 600 mg/kg females. The increase in the leukon is consistent with the chronic inflammation observed in the stomach.

Table 8. Selected Hematology Data for Mice in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Male						
n	10	9	10	9	10	10
Hematocrit (auto) (%)	50.1 ± 0.6	50.0 ± 0.7	49.7 ± 0.5	48.2 ± 0.3	49.1 ± 0.6	48.0 ± 0.6*
Erythrocytes (10^6/µL)	10.72 ± 0.11	10.72 ± 0.18	10.70 ± 0.11	10.25 ± 0.06**	10.48 ± 0.14*	10.40 ± 0.15*
Leukocytes (10^3/µL)	5.68 ± 0.22	4.61 ± 0.26	5.87 ± 0.84	5.61 ± 0.26	7.36 ± 0.39*	8.86 ± 0.60**
Segmented neutrophils (10^3/µL)	0.80 ± 0.04	0.74 ± 0.10	0.85 ± 0.09	0.93 ± 0.07	1.24 ± 0.14**	2.12 ± 0.30**
Lymphocytes (10^3/µL)	4.66 ± 0.19	3.68 ± 0.17	4.83 ± 0.72	4.48 ± 0.21	5.80 ± 0.29	6.36 ± 0.36*
Monocytes (10^3/µL)	0.10 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.13 ± 0.02	0.18 ± 0.04
Female						
n	10	10	10	10	10	10
Leukocytes (10^3/µL)	5.16 ± 0.53	3.79 ± 0.12	4.16 ± 0.24	5.46 ± 0.27	6.12 ± 0.35	7.75 ± 0.76*
Segmented neutrophils (10^3/µL)	0.56 ± 0.08	0.52 ± 0.05	0.61 ± 0.08	0.72 ± 0.08	0.89 ± 0.08**	1.43 ± 0.23**
Lymphocytes (10^3/µL)	4.40 ± 0.47	3.19 ± 0.11	3.40 ± 0.19	4.52 ± 0.18	5.04 ± 0.32	5.97 ± 0.67
Monocytes (10^3/µL)	0.07 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.11 ± 0.03	0.09 ± 0.01	0.16 ± 0.02**

*Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test.
**$P \leq 0.01$.
[a]Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

The absolute and relative liver weights of males administered 100 mg/kg or greater and all dosed groups of females were significantly greater than those of the vehicle control groups (the absolute liver weights of 600 mg/kg males and females were 75% and 128% greater than those of the vehicle control groups, respectively) (Table 9 and Table C-2). Other changes in organ weights included significantly increased relative heart weight in 300 and 600 mg/kg males with significantly decreased absolute heart weight in 600 mg/kg males; significantly increased relative lung weight in 600 mg/kg males; and, significantly decreased absolute and relative thymus weight in 600 mg/kg males (Table C-2). These findings are of uncertain toxicologic significance given the decreased body weight and weight gain in the 600 mg/kg males and the lack of treatment-related lesions in these tissues. Significant histologic lesions were observed in the liver, nose, and stomach of male and female mice.

Table 9. Liver Weights and Liver Weight-to-Body Weight Ratios for Mice in the Three-month Gavage Study of Myristicin

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	38.8 ± 1.2	38.1 ± 1.1	38.3 ± 0.8	37.7 ± 0.9	34.2 ± 1.1**	29.3 ± 0.6**
Liver						
Absolute	1.54 ± 0.05	1.57 ± 0.05	1.58 ± 0.04	1.73 ± 0.04**	2.32 ± 0.06**	2.69 ± 0.06**
Relative	39.67 ± 0.46	41.18 ± 0.90	41.09 ± 0.47	45.97 ± 0.54**	67.94 ± 1.05**	91.64 ± 0.79**
Female						
Necropsy body wt	28.3 ± 0.8	31.0 ± 0.8	29.1 ± 1.0	27.2 ± 0.6	25.3 ± 0.3**	23.9 ± 0.4**
Liver						
Absolute	1.09 ± 0.02	1.29 ± 0.04**	1.21 ± 0.03**	1.27 ± 0.04**	1.76 ± 0.03**	2.49 ± 0.06**
Relative	38.77 ± 0.54	41.52 ± 0.74*	41.77 ± 0.56*	46.64 ± 0.97**	69.63 ± 1.22**	103.96 ± 1.23**

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

Liver: Treatment-related lesions in the liver included fatty change, centrilobular hepatocyte hypertrophy, and hepatocyte necrosis, as also seen in rats; additionally, oval cell hyperplasia, clear cell foci, and basophilic foci were observed in mice (Table 10, Table A-3, and Table A-4). The incidences of fatty change were significantly increased in 100 mg/kg or greater males and in 600 mg/kg females compared to those in the respective vehicle controls. The incidences of centrilobular hepatocyte hypertrophy were significantly increased in 300 and 600 mg/kg males and 100 mg/kg and greater females. The incidences of hepatocyte necrosis and oval cell hyperplasia were significantly increased in 600 mg/kg males and in 300 and 600 mg/kg females. A few incidences of clear cell foci were observed in 300 and 600 mg/kg females, and one female each in the 30 and 300 mg/kg groups had basophilic foci.

Microscopically, fatty change in mice was generally of minimal severity and was similar to that observed in rats (Figure 17 and Figure 18). Both numerous, small (microvesicular) intracytoplasmic vacuoles, generally in hypertrophied hepatocytes, and fewer, discrete, large (macrovesicular) vacuoles, generally in non-hypertrophied periportal hepatocytes, were present.

Hepatocyte hypertrophy was mild to moderate in severity, and in general, was morphologically similar to that observed in rats in both appearance and distribution. Affected hepatocytes were swollen with increased amounts of granular eosinophilic cytoplasm; often there was variation in nuclear size and increased numbers of mitotic figures (Figure 17 and Figure 18). In 300 and 600 mg/kg males and 100 mg/kg females, hypertrophied hepatocytes were mainly centrilobular or extended into the midzonal region, whereas in 300 and 600 mg/kg females, they occupied the entire lobule, resulting in obscured hepatic cords. Elevated liver weights and gross liver enlargement corresponded microscopically with centrilobular hepatocyte hypertrophy in 300 and

600 mg/kg male and female mice. Hepatocyte necrosis was seen as individual swollen or shrunken hepatocytes with hypereosinophilic cytoplasm and shrunken or fragmented nuclei, often accompanied by inflammatory cell infiltrates.

Oval cell hyperplasia was of minimal to mild severity and consisted of linear proliferative arrays, often along sinusoids, of small, oval, slightly basophilic cells with round to oval basophilic nuclei (Figure 18). Clear cell focus was seen as a discrete cluster of diffusely vacuolated hepatocytes clearly distinct from but not compressing the adjacent hepatic parenchyma. Basophilic focus was similar but composed of hepatocytes with basophilic cytoplasm.

Nose: Treatment-related lesions occurred in the nose of mice, unlike in rats, and included epithelial atrophy, nerve atrophy, glands hyperplasia, and hyaline droplet accumulation of the olfactory epithelium and hyaline droplet accumulation and cytoplasmic vacuolization of the respiratory epithelium (Table 10, Table A-3, and Table A-4). The incidences of olfactory epithelium atrophy and nerve atrophy in 300 and 600 mg/kg males and females were significantly greater than those in the vehicle controls. Both olfactory epithelium atrophy and nerve atrophy generally increased in severity from minimal at 300 mg/kg to mild at 600 mg/kg in both sexes (Table 10). The incidences of glands hyperplasia of the olfactory epithelium in 600 mg/kg males and females and hyaline droplet accumulation of the olfactory epithelium in 600 mg/kg males and 300 and 600 mg/kg females were significantly increased. In the respiratory epithelium, the incidences of hyaline droplet accumulation in 300 and 600 mg/kg males and females and cytoplasmic vacuolization in 600 mg/kg males and females were significantly increased.

Table 10. Incidences of Selected Nonneoplastic Lesions in Mice in the Three-month Gavage Study of Myristicin

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Male						
Liver[a]	10	10	10	10	10	10
Centrilobular, Hepatocyte, Hypertrophy[b]	0	0	0	0	10** (2.9)[c]	10** (3.0)
Fatty Change	0	0	0	8** (1.0)	5* (1.0)	10** (1.2)
Hepatocyte, Necrosis	0	0	0	0	1 (1.0)	10** (1.0)
Oval Cell, Hyperplasia	0	0	0	0	0	10** (1.0)
Nose	10	10	10	10	10	10
Olfactory Epithelium, Atrophy	0	0	0	0	10** (1.0)	10** (2.0)
Nerve, Atrophy	0	0	0	0	8** (1.0)	10** (2.0)
Glands, Olfactory Epithelium, Hyperplasia	0	0	0	0	0	7** (1.0)
Olfactory Epithelium, Accumulation, Hyaline Droplet	0	0	0	0	2 (1.0)	9** (1.3)
Respiratory Epithelium, Accumulation, Hyaline Droplet	4 (1.0)	5 (1.4)	1 (1.0)	5 (1.0)	10** (1.2)	10** (2.1)
Respiratory Epithelium, Vacuolization, Cytoplasmic	1 (1.0)	1 (1.0)	0	0	0	6* (1.3)
Stomach, Glandular	10	10	10	10	10	10
Epithelium, Atrophy	0	0	0	0	0	10** (1.7)
Epithelium, Hyperplasia	0	0	0	0	0	10** (2.0)
Stomach, Forestomach	10	10	10	10	10	10
Inflammation, Chronic	0	0	0	0	2 (1.0)	4* (3.5)
Ulcer	0	0	0	0	0	2 (2.5)
Epithelium, Hyperkeratosis	0	0	0	0	2 (1.0)	3 (3.7)
Epithelium, Hyperplasia	0	0	0	0	1 (2.0)	3 (4.0)
Epithelium, Inflammation, Suppurative	0	0	0	0	0	4* (3.5)
Female						
Liver	10	10	10	10	10	10
Centrilobular, Hepatocyte, Hypertrophy	0	0	0	10** (2.0)	10** (3.0)	10** (3.0)
Fatty Change	0	0	0	0	0	10** (1.0)
Hepatocyte, Necrosis	0	0	0	1 (1.0)	10** (1.0)	10** (1.0)
Oval Cell, Hyperplasia	0	0	0	0	10** (1.0)	10** (2.4)

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Clear Cell Focus	0	0	0	0	3	2
Basophilic Focus	0	0	1	0	1	0
Nose	10	10	10	10	10	10
Olfactory Epithelium, Atrophy	0	0	0	0	10** (1.0)	10** (2.0)
Nerve, Atrophy	0	0	0	0	9** (1.2)	10** (2.0)
Glands, Olfactory Epithelium Hyperplasia	0	0	0	0	0	10** (1.0)
Olfactory Epithelium, Accumulation, Hyaline Droplet	0	1 (1.0)	0	3 (1.0)	8** (1.0)	10** (1.9)
Respiratory Epithelium, Accumulation, Hyaline Droplet	5 (1.2)	3 (1.7)	1 (1.0)	7 (1.4)	10* (2.5)	10* (2.7)
Respiratory Epithelium, Vacuolization, Cytoplasmic	0	0	0	0	2 (2.0)	4* (2.0)
Stomach, Glandular	10	10	10	10	10	10
Epithelium, Atrophy	0	0	0	0	0	10** (1.2)
Epithelium, Hyperplasia	0	0	0	0	0	10** (1.6)
Stomach, Forestomach	10	10	10	10	10	10
Inflammation, Chronic	0	0	2 (3.0)	1 (3.0)	1 (2.0)	3 (3.7)
Ulcer	0	0	0	1 (1.0)	0	2 (3.0)
Epithelium, Hyperkeratosis	0	0	2 (3.0)	1 (4.0)	1 (2.0)	3 (4.0)
Epithelium, Hyperplasia	0	0	2 (2.0)	1 (4.0)	1 (2.0)	3 (3.7)
Epithelium, Inflammation, Suppurative	0	0	2 (2.0)	1 (3.0)	0	3 (3.7)

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

Microscopically, olfactory epithelium atrophy was minimal to mild in severity and characterized by decreased cellularity and height of the olfactory epithelium, most commonly along the dorsomedial aspect of the Level II and III sections of the nose and sometimes scattered along turbinates in the Level III sections (Figure 19 and Figure 20). At the junction of the atrophied epithelium with the more normal adjacent olfactory epithelium, the epithelium was disorganized and lined by numerous shrunken epithelial cells with eosinophilic, granular cytoplasm and irregularly shaped, condensed nuclei. Olfactory epithelium atrophy was accompanied by atrophy of the olfactory nerves, characterized by shrinkage of the nerve axons in the lamina propria subjacent to the atrophied olfactory epithelium (Figure 21 and Figure 22). Hyperplasia of the glands of the olfactory epithelium was minimal in severity and characterized by increased prominence and cellularity of Bowman's glands in the lamina propria underlying the atrophic olfactory epithelium; in some animals, the glandular epithelial cells were also hypertrophied (Figure 21 and Figure 22).

Hyaline droplet accumulation occurred in both the olfactory and respiratory epithelia, and was characterized by accumulation of brightly eosinophilic, globular, homogeneous material that expanded the cytoplasm (Figure 23 and Figure 24).

Respiratory epithelium cytoplasmic vacuolization was minimal to mild in severity and characterized by the presence of expansive clear spaces in the apical cytoplasm of respiratory epithelium along the nasal septum in the Level II sections of the nose in treated mice that had concomitant hyaline droplet accumulation (Figure 25).

Stomach (Glandular and Forestomach): The incidences of atrophy and hyperplasia in the epithelium of the glandular stomach were significantly increased in 600 mg/kg males and females compared to the vehicle controls (Table 10, Table A-3, and Table A-4). Epithelium atrophy and epithelium hyperplasia in the glandular stomach of mice were morphologically similar to that observed in rats. Rarely, the replicative, hyperplastic population of epithelial cells formed dilated or irregularly formed glands with accumulations of desquamated cells. Low numbers of neutrophils and mononuclear cells were present within the lamina propria and extending between the gastric glands.

In the forestomach, low incidences of lesions occurred in 300 and 600 mg/kg males and in 30 mg/kg or greater females and included epithelial hyperplasia and hyperkeratosis, ulcers, chronic inflammation, and epithelial suppurative inflammation (Table 10, Table A-3, and Table A-4). In general, these lesions co-occurred in the same animals. The incidences of chronic inflammation and epithelial suppurative inflammation in 600 mg/kg males were significantly increased compared to those of the vehicle controls. However, the incidences of these forestomach lesions were not statistically significant in females. Severities for these forestomach lesions were highest in the 600 mg/kg groups of males and females. These lesions likely corresponded to the white or tan foci observed grossly in the forestomachs of a few treated mice. Similar lesions were not observed in the forestomachs of rats. Chronic inflammation was seen microscopically as mixed inflammatory cell infiltrates of mainly mononuclear cells in the lamina propria and submucosa, and chronic inflammation was in some cases associated with ulceration. Epithelial suppurative inflammation was characterized by multifocal pustule-like accumulations of neutrophils within the superficial epithelium of the forestomach and was also sometimes associated with ulceration.

Male Reproductive System: Male mice in the 600 mg/kg dose group had significantly lower absolute left cauda, left epididymis, and left testis weights (approximately 21%, 18%, and 7% lower, respectively) compared to those of the vehicle controls; however, the high dose mice weighed 24% less than the vehicle control mice, so these organ weight changes are considered secondary to body weight (Table 11 and Table D-2). Reproductive organ weight changes were not accompanied by any significant histopathologic lesions (Table A-3). There were also no changes in spermatid counts/mg of cauda, spermatid heads/testis or per mg testis, or sperm motility in the exposed groups as compared to the vehicle control group (Table D-2).

Table 11. Selected Reproductive Tissue Weights for Male Mice in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	100 mg/kg	300 mg/kg	600 mg/kg
n	10	10	10	10
L. Cauda Epididymis Weight	0.0132 ± 0.0006	0.0138 ± 0.0007	0.0138 ± 0.0007	0.0104 ± 0.0006**
L. Epididymis Weight	0.0409 ± 0.0012	0.0414 ± 0.0007	0.0401 ± 0.0009	0.0337 ± 0.0010**
L. Testis Weight	0.1118 ± 0.0016	0.1110 ± 0.0018	0.1147 ± 0.0014	0.1045 ± 0.0010**

**Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' or Dunnett's test.
[a]Reproductive tissue weights are presented as mean ± standard error. Reproductive tissues were not weighed for 10 or 30 mg/kg males.

Genetic Toxicology

Myristicin (3.3 to 833 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535, when tested with or without exogenous metabolic activation provided by Aroclor 1254-induced rat or hamster liver S9 and cofactors (Table E-1). The highest testable dose in the absence of S9 was 217 µg/plate; with S9, doses up to 833 µg/plate were able to be tested.

In rats, the reticulocyte population is the only red blood cell population that can be accurately assessed for micronucleus frequency in peripheral blood due to efficient splenic scavenging of damaged erythrocytes soon after they emerge from the bone marrow. In both sexes of rats in the 3-month study, the frequencies of micronucleated reticulocytes (polychromatic erythrocytes; PCEs) were significantly increased at 600 mg/kg (Table E-2). The effect at this dose resulted in statistically significant trend tests for both sexes, and results were judged to be positive for both male and female rats. In addition, and in contrast with what was seen in the mice, myristicin appeared to stimulate erythropoiesis in male and female rats, with significant, dose-dependent increases in the percent PCEs observed in both sexes. It should be noted that stimulation of erythropoiesis may, in some instances, result in elevated levels of micronucleated red blood cells due to an increase in mitotic errors associated with rapid cell division, although in in vivo micronucleus studies conducted by the National Toxicology Program, no consistent association between increases in percent PCEs and increases in micronuclei has been observed.

No increases in the frequency of micronucleated erythrocytes (either immature or mature) were seen in the peripheral blood of male or female mice in the 3-month study (Table E-3). Significant, dose-dependent decreases in the percentage of PCEs in peripheral blood seen in both sexes of mice suggest that the bone marrow was a target for myristicin-induced cytotoxicity.

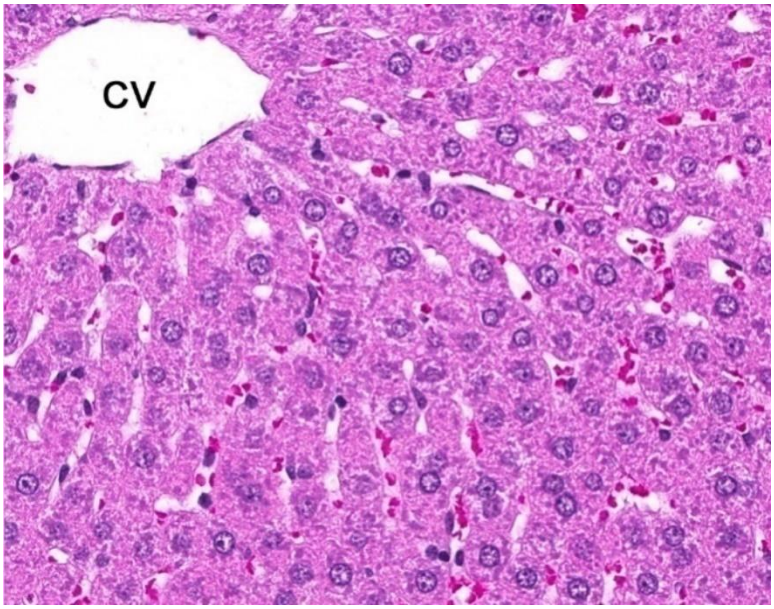

Figure 5. Normal Liver in a Vehicle Control Female F344/NTac Rat at Three Months in the Gavage Study of Myristicin (H&E)

Note the uniform size of hepatocytes. CV = central vein.

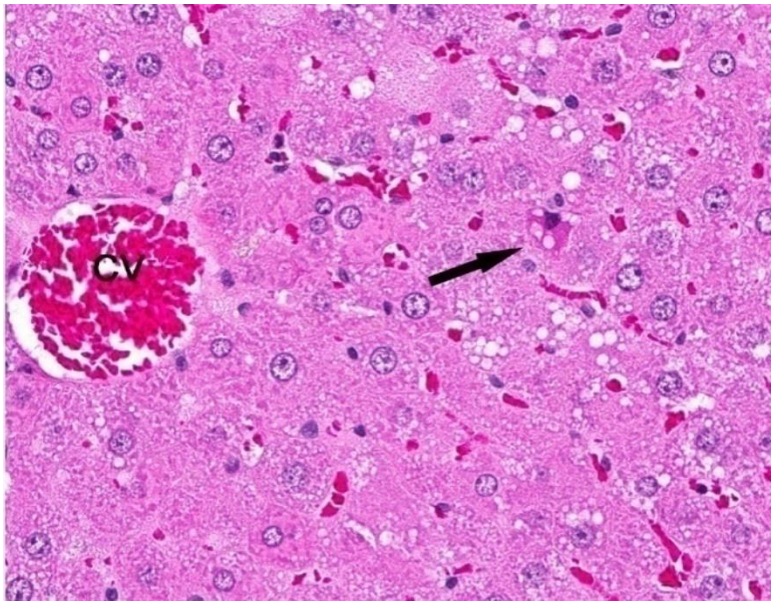

Figure 6. Centrilobular Hepatocyte Hypertrophy and Fatty Change in the Liver of a Female F344/NTac Rat Administered 600 Mg/Kg Myristicin by Gavage for Three Months (H&E)

Note that hepatocytes centered around central veins (CV) are enlarged with lightly eosinophilic, granular cytoplasm, with some containing vacuoles consistent with lipid accumulation. Occasional hepatocytes are necrotic (arrow). Compare to the normal liver in Figure 5 at the same magnification.

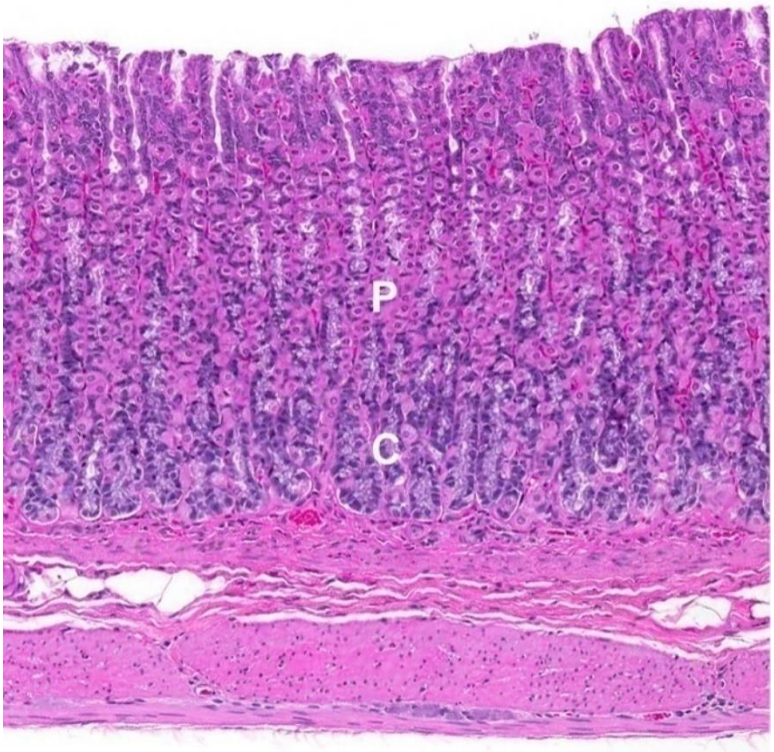

Figure 7. Normal Glandular Stomach in a Vehicle Control Female F344/NTac Rat at Three Months in the Gavage Study of Myristicin (H&E)

Note the orderly progression of the epithelial cells in the gastric glands with midzonal, eosinophilic parietal (P) cells and basal, dark staining chief cells (C).

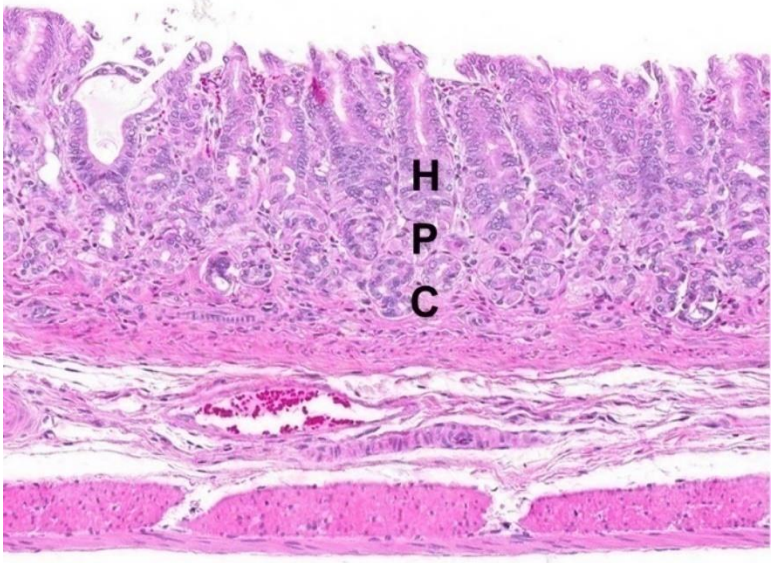

Figure 8. Epithelium Atrophy and Epithelium Hyperplasia in the Glandular Stomach of a Female F344/NTac Rat Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

Epithelium atrophy is demonstrated by an overall decrease in mucosal height due to loss of many of the parietal (P) and chief cells (C), and the glands have lost the orderly progression in cell types. There is also hyperplasia of some other glandular epithelial cells, especially mucous neck cells (H). Compare to the normal glandular stomach in Figure 7 at the same magnification.

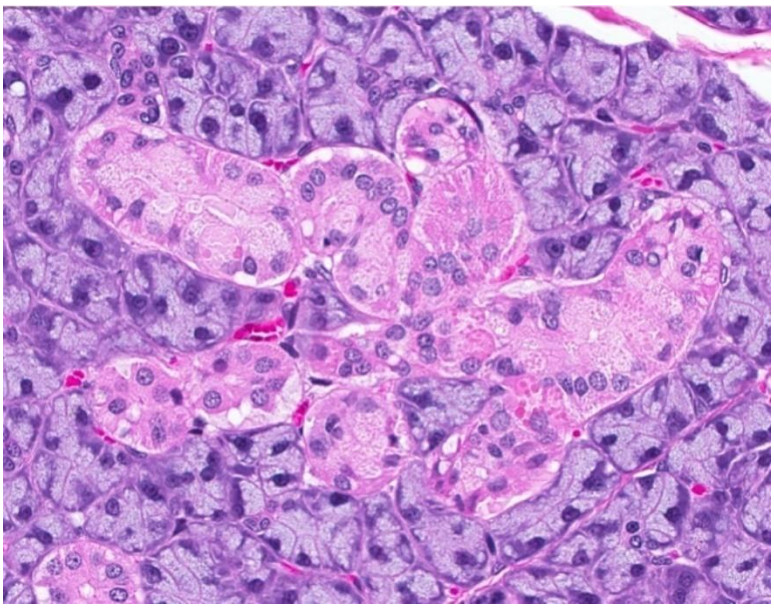

Figure 9. Secretory Depletion in the Submandibular Salivary Gland of a Male F344/NTac Rat Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

Note the decreased number of eosinophilic, cytoplasmic granules in granular convoluted ducts when compared to the normal submandibular salivary gland in Figure 10.

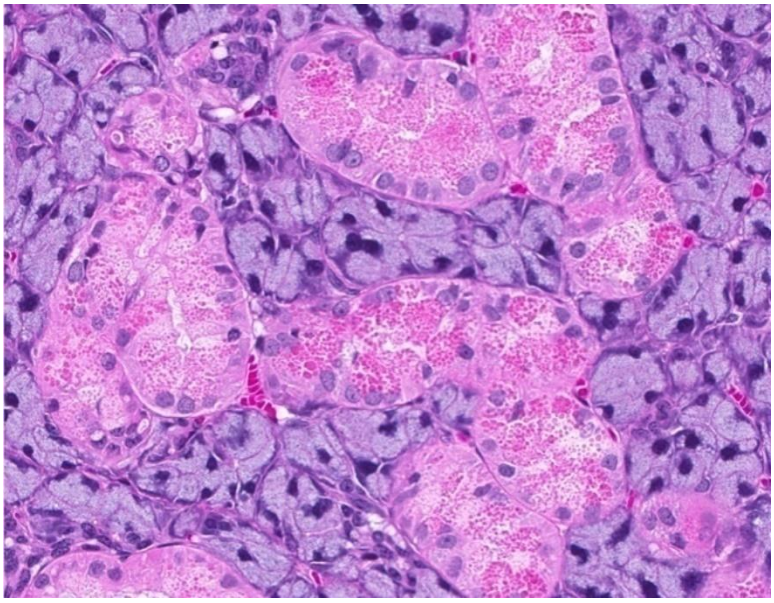

Figure 10. Normal Submandibular Salivary Gland in a Vehicle Control Male F344/NTac Rat at Three Months in the Gavage Study of Myristicin (H&E)

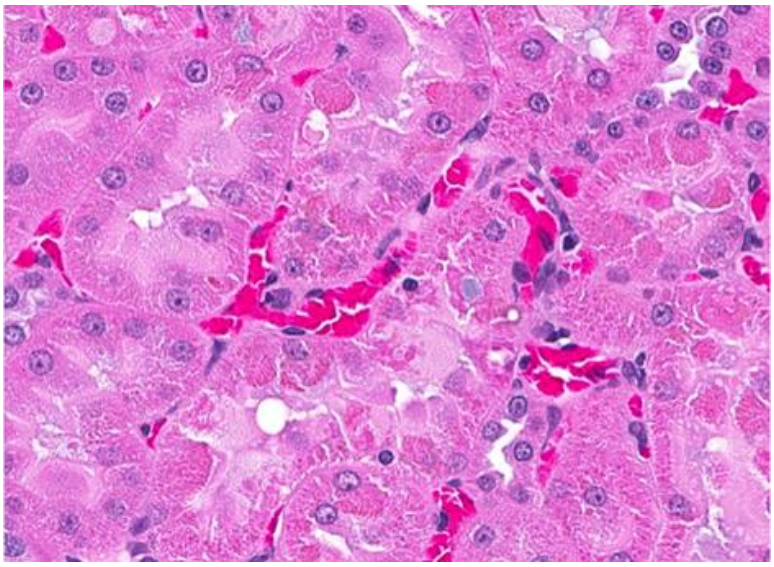

Figure 11. Normal Kidney in a Vehicle Control Male F344/NTac Rat at Three Months in the Gavage Study of Myristicin Showing the Normal Background Level of Uniformly Fine, Eosinophilic (Hyaline) Droplets within the Cytoplasm of Renal Tubule Epithelial Cells (H&E)

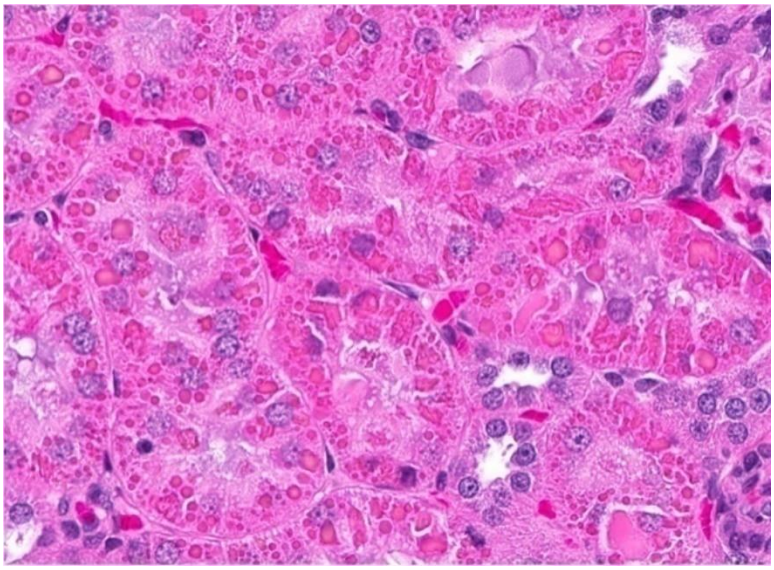

Figure 12. Hyaline Droplet Accumulation in the Kidney of a Male F344/NTac Rat Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

Compared to the normal kidney in Figure 11, there are larger, variably-sized, round to irregularly angular eosinophilic (hyaline) droplets within the cytoplasm of several renal tubule epithelial cells.

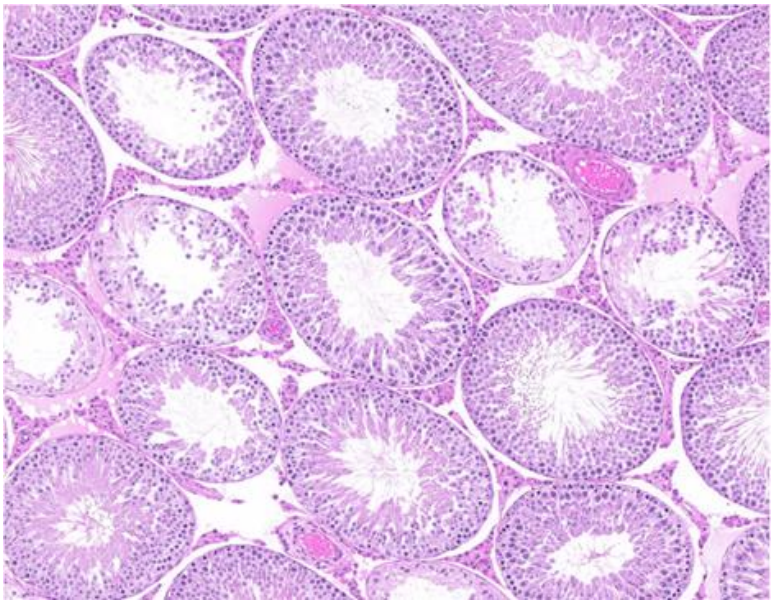

Figure 13. Germinal Epithelium Degeneration in Several Seminiferous Tubules of the Testis of a Male F344/NTac Rat Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

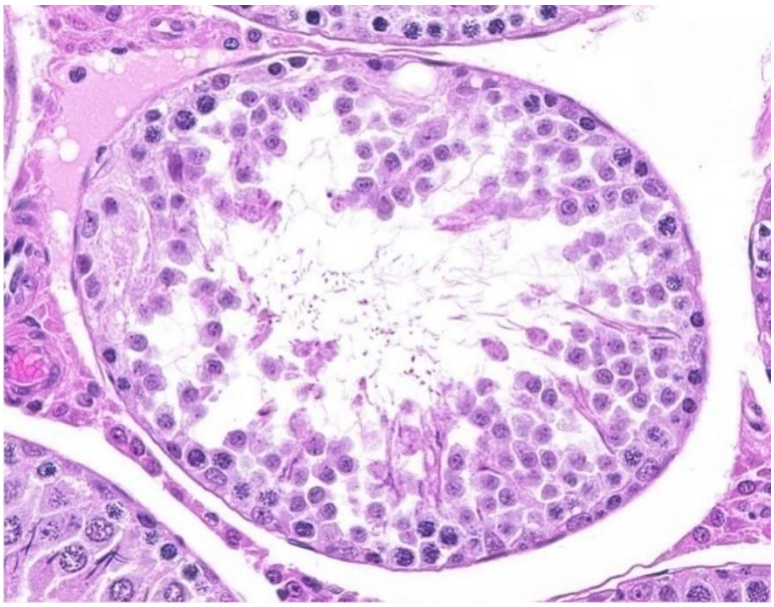

Figure 14. Higher Magnification of Germinal Epithelium Degeneration in the Testis of a Male F344/NTac Rat Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

An early-stage seminiferous tubule displays disorganization of the germinal epithelium, depletion of round and elongating spermatids, and vacuolation.

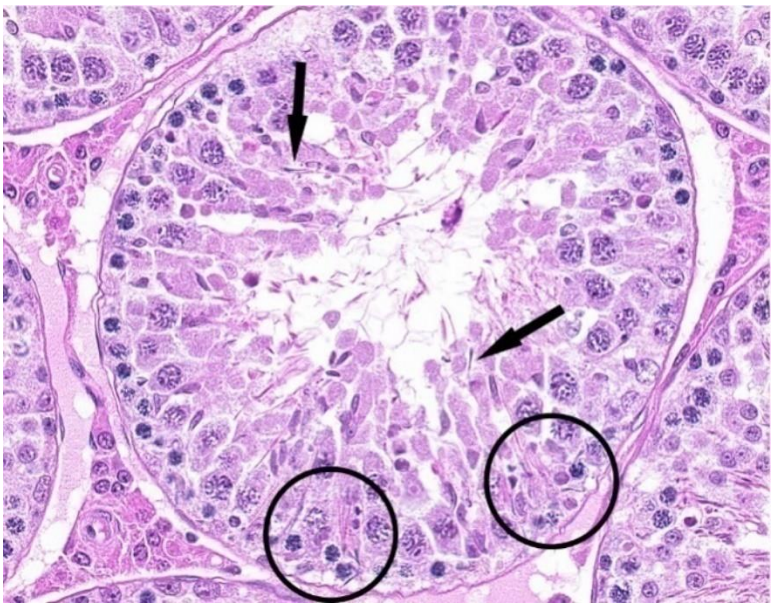

Figure 15. Spermatid Retention in a Late-Stage Seminiferous Tubule in the Testis of a Male F344/NTac Rat Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

Several mature, elongated step 19 spermatids are retained within the germinal epithelium near the luminal surface (arrows) as well as within the basal cytoplasm (circles).

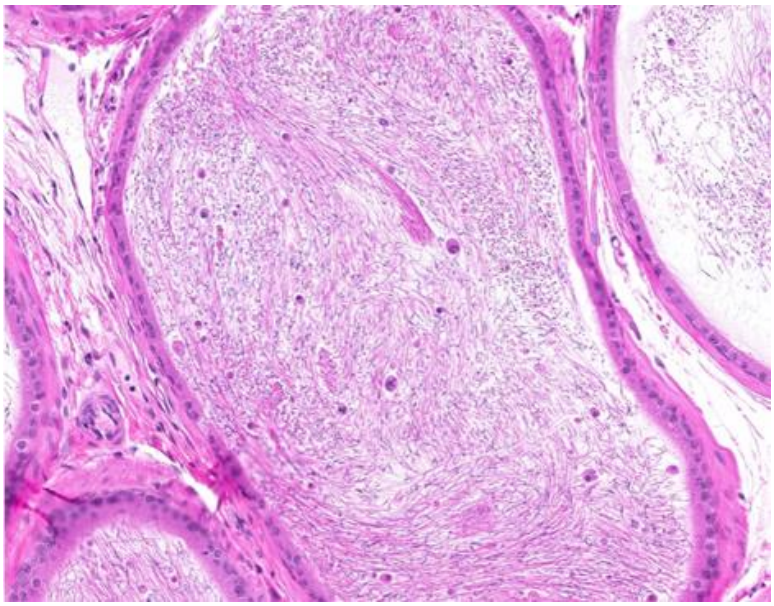

Figure 16. Exfoliated Germ Cells in the Duct Lumina of the Epididymis of a Male F344/NTac Rat Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

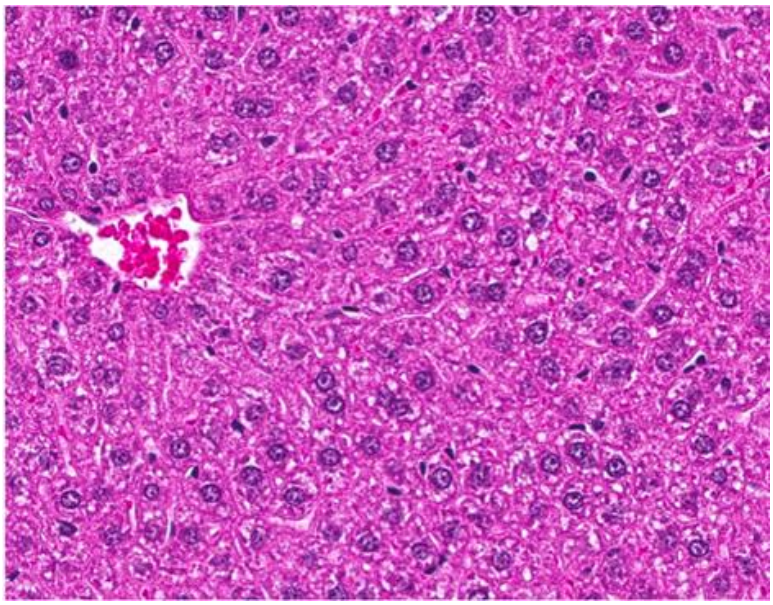

Figure 17. Normal Liver in a Vehicle Control Female B6C3F1/N Mouse at Three Months in the Gavage Study of Myristicin (H&E)

Note the uniform size of hepatocytes and distinct sinusoids.

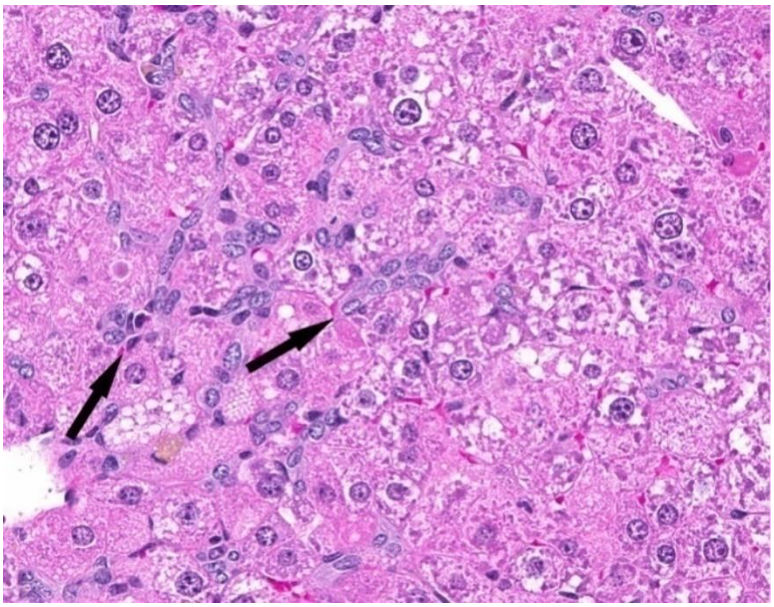

Figure 18. Centrilobular Hepatocyte Hypertrophy, Fatty Change, and Oval Cell Hyperplasia in the Liver of a Female B6C3F1/N Mouse Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

Hepatocytes are enlarged with lightly eosinophilic, granular cytoplasm, with some containing vacuoles consistent with lipid accumulation. Oval cell hyperplasia is seen as proliferative arrays of small, oval, basophilic cells along sinusoids (black arrows). Occasional hepatocytes are necrotic (white arrow). Compare to the normal liver in Figure 17 at the same magnification.

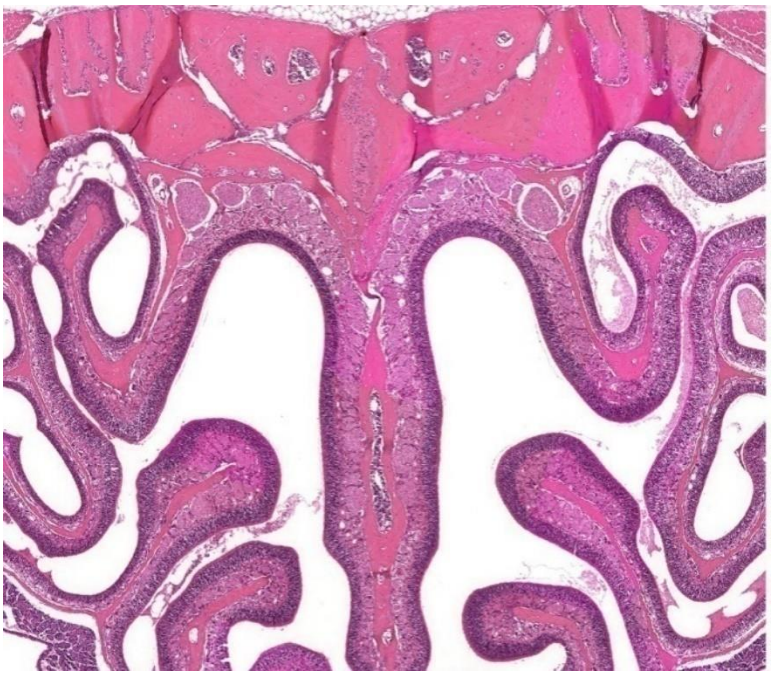

Figure 19. Low Magnification of a Normal Level III Section of the Nose in a Vehicle Control Female B6C3F1/N Mouse at Three Months in the Gavage Study of Myristicin (H&E)

The thickness of the olfactory epithelium is uniform.

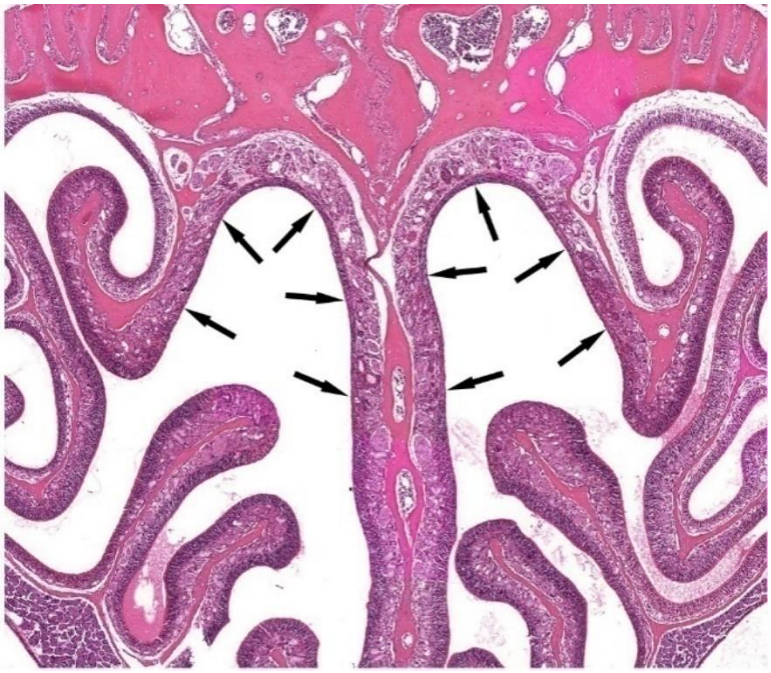

Figure 20. Low Magnification of a Level III Section of the Nose in a Female B6C3F1/N Mouse Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

The olfactory epithelium is atrophied and thinned along the dorsomedial aspect (arrows). Compare to the normal Level III section of the nose in Figure 19 at the same magnification.

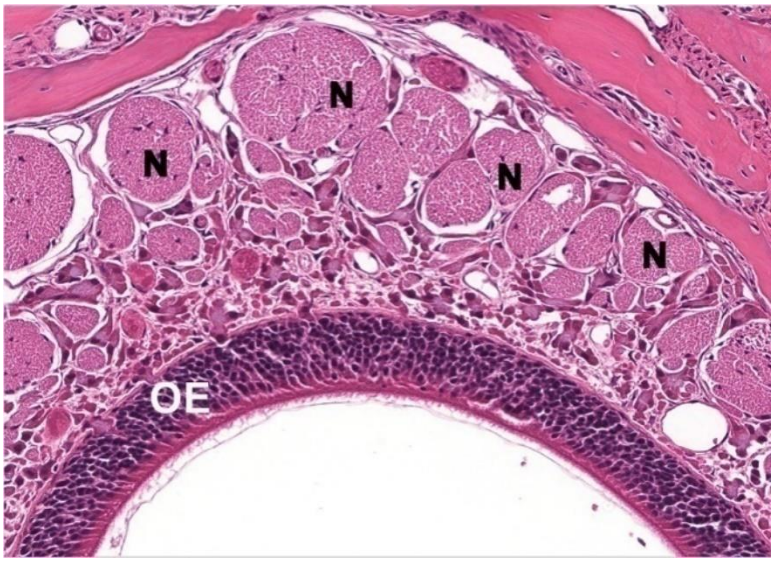

Figure 21. Normal Olfactory Epithelium in a Vehicle Control Female B6C3F1/N Mouse at Three Months in the Gavage Study of Myristicin (H&E)

Higher magnification of the dorsal meatus of the nasal cavity from the Level III section shown in Figure 19. The olfactory epithelium (OE) is thick, and nerve bundles (N) are prominent in the underlying lamina propria.

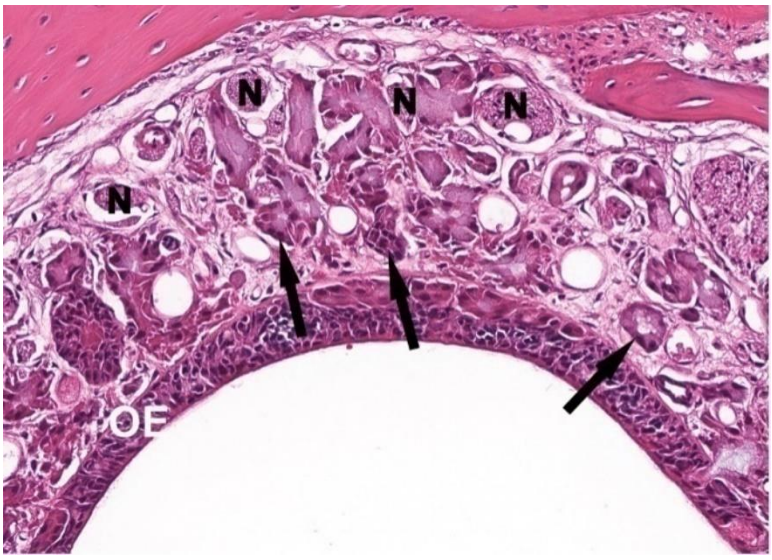

Figure 22. Olfactory Epithelium Atrophy, Nerve Atrophy, and Glands Hyperplasia of the Olfactory Epithelium in a Female B6C3F1/N Mouse Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

Higher magnification of the dorsal meatus of the nasal cavity from the Level III section shown in Figure 20. Compared to Figure 21, the olfactory epithelium (OE) is thinned and less cellular, and the olfactory nerves (N) in the lamina propria are decreased in size and number. In contrast, the Bowman's glands (arrows) in the lamina propria are increased in size and number in the treated mouse compared to the vehicle control mouse in Figure 21.

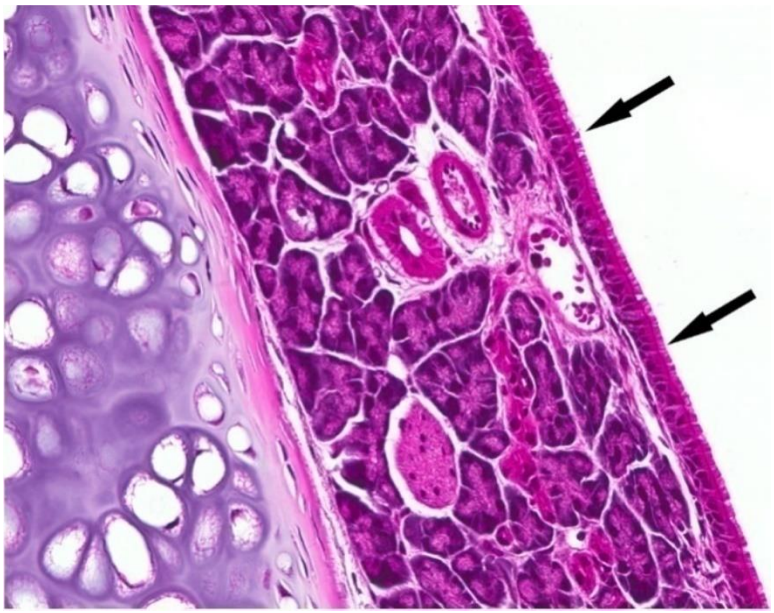

Figure 23. Normal Respiratory Epithelium (arrows) Along the Nasal Septum in a Level II Section of the Nose from a Vehicle Control Female B6C3F1/N Mouse at Three Months in the Gavage Study of Myristicin (H&E)

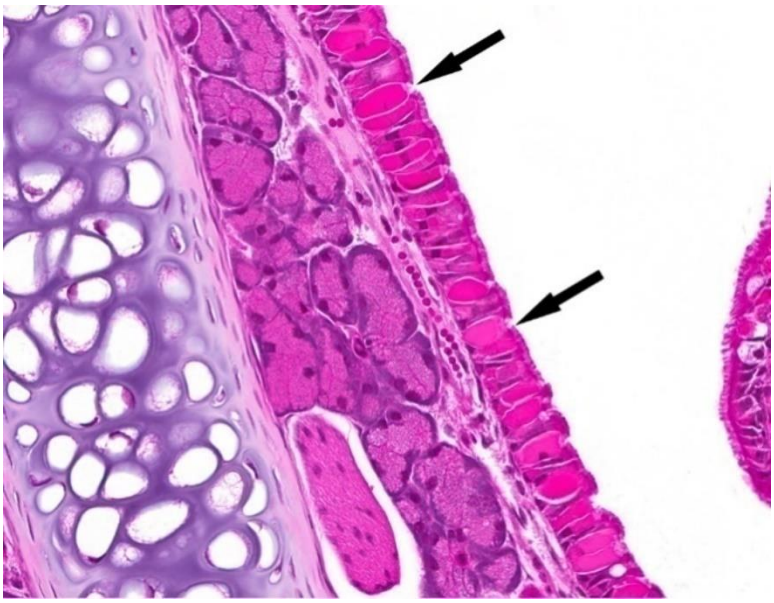

Figure 24. Hyaline Droplet Accumulation in the Respiratory Epithelium (Arrows) Along the Nasal Septum in a Level II Section of the Nose from a Male B6C3F1/N Mouse Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

Brightly eosinophilic, homogeneous material expands the cytoplasm of respiratory epithelial cells. Compare to the normal respiratory epithelium in Figure 23.

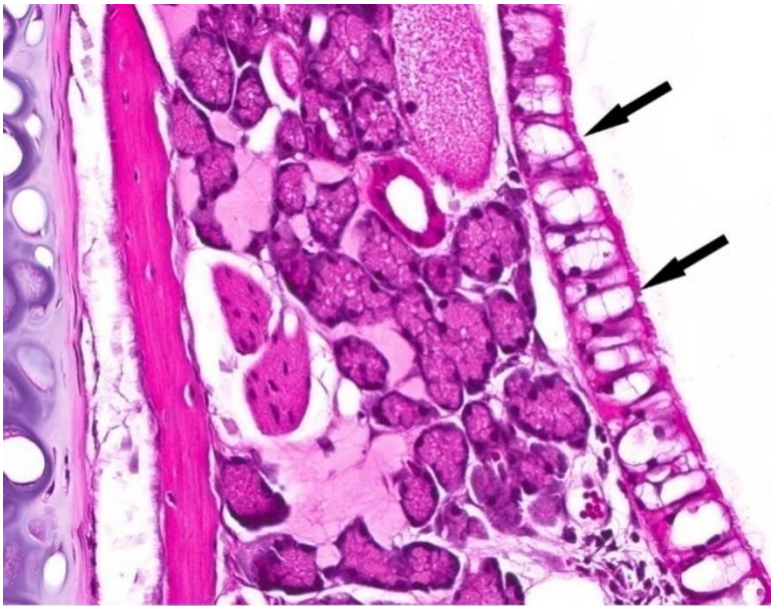

Figure 25. Cytoplasmic Vacuolization in the Respiratory Epithelium (Arrows) Along the Nasal Septum in a Level II Section of the Nose from a Female B6C3F1/N Mouse Administered 600 mg/kg Myristicin by Gavage for Three Months (H&E)

Clear spaces expand the cytoplasm of respiratory epithelial cells. Compare to the normal respiratory epithelium in Figure 23.

Discussion

Myristicin is a naturally occurring compound, with potentially widespread human exposure from ingestion of the plant products from which it is derived and food and consumer products containing myristicin as a flavoring agent.[16; 17] Myristicin exposure is primarily through ingestion of the spice nutmeg or its oils and oleoresins, but myristicin is also present in the spice mace and other plants such as parsley and dill.[7; 8; 12-14] Human exposure to myristicin can also occur through the ingestion of nutmeg for medicinal or recreational drug purposes. There are well-documented reports of the acute toxicity of nutmeg.[50-52] Daily intake of myristicin by ingestion from all sources is estimated to not exceed 1 mg.[2] Myristicin production or distribution is not regulated; moreover, natural and synthetic sources of myristicin are all generally recognized as safe.[15]

Because of its widespread use and structural similarity to other alkenylbenzene flavoring agents such as eugenol, methyleugenol, isoeugenol, estragole, and safrole, some of which have exhibited carcinogenic activity in rodents,[57; 58; 88-90] NTP characterized the toxicological effects of myristicin administration in 3-month studies in male and female F344/NTac rats and B6C3F1/N mice. NTP has conducted subchronic studies of these chemicals and 2-year carcinogenicity bioassays of eugenol, methyleugenol, and a similar compound, isoeugenol. Eugenol exhibited no evidence of carcinogenic activity under the conditions of the studies, whereas methyleugenol was considered to demonstrate clear evidence of carcinogenic activity in male and female rats and mice.[58; 88] Isoeugenol was found to have equivocal evidence of carcinogenic activity in male rats and female mice, no evidence of carcinogenic activity in female rats, and clear evidence of carcinogenic activity in male mice.[89] In the 3-month subchronic study, estragole induced hepatocellular adenomas and cholangiocarcinomas in male rats.[90]

NTP conducted comparative toxicogenomic and subchronic toxicity studies to develop predictive models of carcinogenicity in chemicals structurally related to safrole.[60] The alkenylbenzene family of compounds was used to validate computational models developed to predict the hepatocarcinogenic potential of chemicals. The models correctly distinguished between chemicals that had been shown to be liver carcinogens (safrole, estragole) in rats and those that had not (eugenol and isoeugenol). Additional validation studies of these models were used to predict the carcinogenic potential of myristicin and isosafrole, which had not been previously assessed in a carcinogenicity bioassay. The studies predicted that based on hepatic gene expression changes after 3 months, myristicin would be weakly carcinogenic in the liver of rats administered 2 mmol/kg per day for 2 years (almost 400 mg/kg).[60] Some toxicity markers observed in that study were used in the selection of a maximum dose of 600 mg/kg for the current studies.

In the current 3-month studies of myristicin, all male and female rats and mice survived to the end of the studies, except for three 600 mg/kg female rats that died within 4 days of the start of the study. The three 600 mg/kg rats that died early had lymphoid necrosis of the cortex of the thymus; two also had necrosis of the mucosal epithelium of the glandular stomach. Final mean body weights and mean body weight gains of 600 mg/kg male rats and 300 and 600 mg/kg male and female mice were significantly less than those of the respective vehicle control groups. Primary target organs identified for rats administered myristicin included liver, glandular

stomach, and salivary gland for males and females, and testis, epididymis, and kidney in males. Primary target organs identified for mice included liver, glandular stomach, and nose of males and females, and the forestomach of males.

In the 3-month myristicin studies, the liver was a target organ of toxicity in both rats and mice. Rats and mice exhibited hepatocellular hypertrophy, fatty change, and hepatocellular necrosis. In the 3-month study of estragole,[90] hepatocellular hypertrophy was seen in both rats and mice, with mice also exhibiting fatty change and liver necrosis. In myristicin, these findings were largely limited to the two highest doses (300 and 600 mg/kg), although mice were more sensitive to some endpoints down to the 100 mg/kg dose. Enlarged livers observed in all 300 and 600 mg/kg male and female rats and mice at necropsy corresponded with increased absolute and relative liver weights and the microscopic lesion of centrilobular hepatocellular hypertrophy. Increased liver weights (absolute and relative) also occurred in the 100 mg/kg groups of male and female rats and mice, and centrilobular hepatocellular hypertrophy also occurred in 100 mg/kg female mice. The increases in liver weights and incidences of hepatocellular hypertrophy, which was centrilobular in distribution in lower dose groups and throughout the lobule in higher dose groups, may have been due in part to the induction of cytochrome P450 enzyme systems. Myristicin has been shown to induce the P450 enzymes CYP1A1/2, CYP2B1/2, and CYP2E1.[31] Alkenylbenzenes in general have been shown to be potent inducers of these enzyme systems.[91; 92] Fatty change observed microscopically in hepatocytes in rats and mice and elevations in rat serum triglycerides and cholesterol concentrations suggest potential derangements in lipid metabolism due to the administration of myristicin. Hepatocellular injury caused by myristicin administration was evidenced by the microscopic lesion of hepatocellular necrosis in 600 mg/kg male and female rats and mice and 300 mg/kg female mice, along with increases in serum alanine aminotransferase and sorbitol dehydrogenase activities in several dosed groups of rats.

In addition to the liver findings seen in rats, mice administered myristicin also exhibited the additional hepatic lesions of oval cell hyperplasia, clear cell foci, and basophilic foci. These potentially preneoplastic hepatic lesions have also occurred in previous studies conducted at NTP on the structurally related compounds methyleugenol, isoeugenol, and estragole.[58; 89; 90] Clear cell foci occurred at low incidences in the liver of female mice in the higher myristicin dose groups (300 and 600 mg/kg). Clear cell foci were noted in male mice dosed at 75 and 150 mg/kg in the 2-year isoeugenol bioassay,[89] along with basophilic foci, hepatocellular adenoma, and hepatocellular carcinoma. Another liver lesion of note in mice was oval cell hyperplasia, which occurred in all 300 mg/kg females and all 600 mg/kg males and females in the current study. Oval cell hyperplasia does not occur spontaneously but often occurs following exposure to chemicals that cause hepatotoxicity. Oval cell hyperplasia was a prominent lesion noted in male and female rats and mice in the 3-month estragole study at doses as low as 37.5 mg/kg in rats.[90] Lesions in the estragole study included bile duct hyperplasia; basophilic, mixed cell, eosinophilic, and clear cell foci; cholangiofibrosis; two cholangiocarcinomas; and one hepatocellular adenoma in rats and hepatocyte hypertrophy, necrosis, and fatty change in mice.[90] The development of hepatocellular adenoma and cholangiocarcinoma seen in male rats administered estragole is an unusual occurrence in short-term studies such as a 3-month subchronic study. Overall, the similarities of liver lesions across the subchronic and chronic alkenylbenzene studies may suggest similarly triggered carcinogenic pathways. In a toxicogenomic study, myristicin had a weaker hepatocarcinogenic response than estragole and

methyleugenol but a stronger response than isoeugenol and eugenol.[60] The 3-month and 2-year eugenol studies did not exhibit major histopathologic findings or evidence of carcinogenicity.[88]

In the current 3-month studies of myristicin, the stomach was also a target of toxicity in rats and mice. High-dose male and female rats and mice had atrophy of the glandular stomach mucosa, which was characterized as diffuse mucosal thinning due to extensive loss and ongoing single-cell death of chief and parietal cells, accompanied by infiltration of low numbers of neutrophils and mononuclear cells. The multifocal epithelial hyperplasia also observed in the glandular stomach of rats and mice possibly represents a regenerative response in the face of the overall, diffuse atrophy. In the 3-month study of estragole in male and female rats, the lesions that occurred in the glandular stomach were morphologically similar to those in the current studies of myristicin, including diffuse mucosal atrophy with single-cell necrosis of parietal and chief cells, dilation at the base of glands, and infiltration of neutrophils.[90] Male and female rats administered methyleugenol for 3 months also exhibited similar glandular stomach mucosal atrophy and inflammation.[58] In the subsequent 2-year study, methyleugenol induced rare neuroendocrine cell hyperplasia and benign and malignant neuroendocrine tumors in the glandular stomach of both male and female rats.[58; 93]

Females appear to be especially sensitive to glandular stomach toxicity from exposure to alkenylbenzenes. In addition to the gastric mucosal atrophy seen as decreased numbers of chief and parietal cells in 600 mg/kg male and female rats and mice in the current studies of myristicin, the 300 mg/kg female rats also had increased incidences of gastric mucosal atrophy, which was characterized by decreased numbers of primarily chief cells. Additionally, of the three early-death female rats in the current study, two had necrosis of the glandular stomach epithelium. This epithelial necrosis, primarily of chief and parietal cells, in two early-death female rats may represent an early manifestation of the glandular stomach epithelial atrophy, with loss and death of chief and parietal cells, seen at study termination in 600 mg/kg male and female rats and 300 mg/kg female rats. Another example of potential increased sensitivity in females with a related compound is the increased incidences of stomach lesions in female mice, but not in male mice, in the 3-month study of estragole.[90] Additionally, in the 2-year study of methyleugenol in rats, neoplastic and nonneoplastic lesions of the glandular stomach were more prevalent or more severe in female rats than in male rats.[58]

In addition to the glandular stomach, the forestomach was also a target of myristicin administration in mice, but not in rats, although the increases in the incidences of forestomach lesions as compared to the vehicle control groups were generally not statistically significant. There were low incidences of chronic inflammation in the forestomach as well as suppurative inflammation, hyperplasia, and hyperkeratosis in the forestomach epithelium of 300 and 600 mg/kg male mice and 30 mg/kg or greater female mice. These lesions tended to co-occur in the same animal and presumably were related to the low observed incidences of ulcers. In the 3-month study of the related compound estragole, 600 mg/kg female mice had significant increases in the incidences of squamous hyperplasia, mineralization, and ulcer in the forestomach.[90] In the 2-year study of isoeugenol, the incidences of forestomach squamous hyperplasia and inflammation in male and female mice and of forestomach ulcer in male mice were significantly increased in animals exposed to 300 mg/kg.[89] In the 2-year study of the related compound methyleugenol, a positive trend occurred in the incidences of squamous cell papilloma or carcinoma (combined) in the forestomach of female rats.[58]

The nose was also a target of myristicin administration in mice, but not in rats. Nasal lesions associated with myristicin treatment included epithelium atrophy, nerve atrophy, glandular hyperplasia, and hyaline droplet accumulation, all within the olfactory epithelium, as well as hyaline droplet accumulation and cytoplasmic vacuolization within the respiratory epithelium. These lesions occurred in the highest dose groups of both male and female mice. The accumulation of hyaline material in the olfactory and respiratory epithelium can occur spontaneously in aging rodents or can increase with toxicant exposure. The significance or composition of this eosinophilic, hyaline material in the nasal epithelium is not definitively known; however, it is reported in mice to contain Ym1/2 chitinase proteins.[45] In the estragole 3-month study, male and female rats and mice exhibited a dose-related similar lesion of olfactory epithelium degeneration.[90] Similarly, male and female rats and mice dosed with isoeugenol had olfactory epithelium atrophy, metaplasia, and degeneration in the 3-month and 2-year studies.[89] The significance of the nasal lesions in mice in the current study and in studies of related compounds is uncertain, but the toxicity may be related to activation by cytochrome P450 or other enzymes, which are generally expressed at higher levels in the olfactory epithelium than the respiratory epithelium of the nose.

The salivary glands were a target of myristicin administration in rats, but not in mice. There were increased incidences of secretory depletion of the submandibular salivary glands in 600 mg/kg male and female rats and 300 mg/kg female rats. A similar lesion occurred in the 3-month (100 mg/kg or greater males and 30 mg/kg or greater females) and 2-year rat studies (all dosed groups) of methyleugenol; these lesions did not occur in dosed groups of mice.[58] A morphologically similar lesion also occurred in all rats administered 75 mg/kg or greater in a 3-month gavage study of the structurally related compound estragole.[90] The biologic significance of this salivary gland lesion is uncertain.

In the current studies, myristicin exhibited the potential to be a reproductive toxicant in male rats but not in mice. Rats in the high-dose group had increased incidences of germinal epithelium degeneration and elongated spermatid retention in seminiferous tubules of the testis and exfoliated germ cells in the duct lumina of the epididymis. High-dose male rats, but not mice, had a lower mean total number of sperm per cauda. Male rats and mice in the highest dose group also had decreased left cauda and left epididymal weights, but in mice these occurred in the presence of substantially lower body weights. Of the other alkenylbenzene compounds studied by NTP, estragole and methyleugenol also induced testicular and epididymal lesions. Male rats administered estragole 300 or 600 mg/kg for 3 months displayed germinal epithelium degeneration of the testis, bilateral hypospermia of the epididymis, and lower absolute and relative testis weights.[90] In the methyleugenol 3-month study, rats had testicular germinal epithelium degeneration and seminiferous tubule dilation at a dose of 1,000 mg/kg.[58] In the cases of estragole, methyleugenol, and now myristicin, rats were the more sensitive species to male reproductive lesions.

In the current studies, myristicin administration resulted in renal injury in male rats. Absolute and relative kidney weights were increased in a dose-dependent manner in male rats. In male rats, myristicin administration was associated with increases in the incidences and severities of hyaline droplet accumulation in tubular epithelial cells. There was also evidence of slight exacerbation of chronic progressive nephropathy (which is a common spontaneous change in the kidneys of male F344/N rats), as evidenced by the slight increase in severity in the 600 mg/kg group. Chronic progressive nephropathy was characterized by randomly distributed multifocal

clusters of regenerating tubules within the parenchyma of the kidney. The presence of hyaline droplet accumulation in this study is potentially suggestive of α_{2u}-globulin nephropathy, which occurs in male but not female F344/N rats and has been linked to the development of renal tubule neoplasms.[94] However, other changes typically observed in cases of α_{2u}-globulin nephropathy (e.g., tubular degeneration, necrosis, linear papillary mineralization) were lacking, suggesting that this may be a mild response.

The genotoxicity of myristicin and other alkenylbenzenes studied by NTP (estragole, safrole, eugenol, isoeugenol, and methyleugenol) is influenced by Phase I and Phase II metabolic pathways that produce electrophilic intermediates capable of forming DNA adducts. Phase I enzymes may convert the carbon-carbon double bond of the allyl side chain to a highly reactive epoxide. However, this epoxide is rapidly detoxified and does not appear to be a significant source of DNA adduct formation in vivo.[95] Alternatively, Phase I enzymes may hydroxylate the 1′-carbon of the allyl side chain of myristicin and other alkenylbenzenes (e.g., safrole, estragole, and methyleugenol). This 1′-hydroxyl group is then a target for Phase II sulfonation. However, this sulfonated group is unstable and a DNA-reactive carbonium ion is generated at the 1′-carbon when the sulfur group leaves. The metabolism of isoeugenol does not lead to generation of the reactive carbonium ion, as the allyl side chain undergoes hydroxylation at the 3′-carbon, targeting isoeugenol for elimination by glucuronidation. Isoeugenol and eugenol can also be eliminated via glucuronidation of a phenolic hydroxyl group. Genotoxicity studies in rodents, turkey fetal liver, and human HepG2 cells have shown that myristicin, safrole, estragole, and methyleugenol form DNA adducts in liver tissue and in HepG2 cells, whereas liver adducts were not detected with eugenol or isoeugenol, consistent with what is known about the different propensities for the formation of electrophilic intermediates of these chemicals and their elimination.[16; 63-65; 68; 96; 97] Notably, safrole, estragole, and methyleugenol have been shown to enhance the mutation frequency and change the mutation spectrum in DNA obtained from *gpt*-delta transgenic rat liver tissue,[98-100] whereas eugenol was not mutagenic in the liver tissue of lacZ transgenic mice (Muta™Mouse).[101] To date, the in vivo mutagenic potential of myristicin and isoeugenol has not been assessed using approaches such as transgenic rodent models. Although myristicin and other alkenylbenzenes studied by NTP were negative in bacterial mutagenicity tests with and without exogenous metabolic activation,[58; 88-90] the negative results may be a consequence of the limited metabolic activation provided by the S9 mix. Consistent with this notion, positive results were obtained when electrophilic metabolic intermediates of alkenylbenzenes were tested in bacterial mutagenicity assays with S9 mix.[102] Furthermore, use of a TA100 strain engineered to express human sulfotransferase, a Phase II enzyme, promoted the activation of methyleugenol to a mutagenic form.[103] Therefore, the standard bacterial mutagenicity tests conducted by NTP may be inadequate to predict the in vivo mutagenic potential of these alkenylbenzenes. Other researchers have also found a lack of mutagenicity for safrole, estragole, methyleugenol, eugenol, and isoeugenol using standard *Salmonella typhimurium* strains and *Escherichia coli* WP2 with S9 mix.[104]

NTP has tested myristicin, estragole, methyleugenol, eugenol, and isoeugenol in the in vivo rodent micronucleus assay. Methyleugenol and estragole did not induce micronuclei in erythrocytes of mice exposed by gavage in 3-month studies, and micronuclei were not induced in erythrocytes of mice given three daily intraperitoneal injections of eugenol.[58; 88; 90] A weak response in the micronucleus test was seen with isoeugenol in female mice, and results in male mice were negative; the small increase in micronucleated erythrocytes seen in female mice,

which was within the historical control range, was judged to be of limited biological significance.[89] Myristicin was negative for induction of micronuclei in mice. In rats, myristicin induced small but statistically significant increases in micronuclei that were within or just at the limit of the laboratory historical control ranges (mean ± two standard deviations). Finally, safrole, not tested for micronucleus induction by NTP, was reported to be negative in a rat micronucleus test.[97] Thus, some alkenylbenzenes studied by NTP, including myristicin, have been reported to induce DNA adducts and mutations in vivo, but most do not appear to induce chromosomal changes in erythrocytes of rats or mice.

Taken together, studies performed by NTP provide limited evidence for the genotoxicity of myristicin; however, studies in the literature indicate that myristicin, similar to safrole, estragole, and methyleugenol, forms DNA adducts in vivo and in human HepG2 cells in vitro.[16; 63-65; 68; 97] The consequence of these adducts is unknown, as myristicin has not been tested for mutation induction in vivo.

Under the conditions of the 3-month oral gavage studies, there were treatment-related lesions in male and female rats and mice. The major targets from myristicin administration in rats and mice included the liver and glandular stomach. In rats, additional targets included salivary glands in males and females, and the kidney, testis, and epididymis in males. Additional targets in the mice included the nose in males and females and the forestomach in males. The most sensitive measure of myristicin toxicity in male rats was a higher relative liver weight [lowest-observable-effect level (LOEL) = 30 mg/kg], and in female rats, the most sensitive measures were clinical chemistry findings (LOEL = 10 mg/kg) including increased cholesterol and alanine aminotransferase. In male mice, the most sensitive measures of myristicin toxicity included higher absolute and relative liver weights and a significantly increased incidence of fatty liver (LOEL = 100 m/kg). The most sensitive measure of myristicin toxicity in female mice was an increase in absolute and relative liver weight (LOEL = 10 mg/kg). No-observed-effect levels were 10 mg/kg for male rats and 30 mg/kg for male mice, but none was reached for female rats or mice.

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Appendix A. Summary of Lesions in Rats and Mice

Tables

Table A-1. Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Male Rats in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal euthanasia	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic	–	–	–	–	–	1 (10%)
Intestine large, cecum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, colon	(10)	(0)	(0)	(0)	(0)	(10)
Parasite metazoan	–	–	–	–	–	1 (10%)
Intestine large, rectum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, duodenum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, ileum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, jejunum	(10)	(0)	(0)	(0)	(0)	(10)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Fatty change	–	–	–	–	10 (100%)	10 (100%)
Hepatodiaphragmatic nodule	1 (10%)	1 (10%)	1 (10%)	3 (30%)	–	1 (10%)
Mixed cell focus	–	–	–	–	–	1 (10%)
Centrilobular, hepatocyte, hypertrophy	–	–	–	–	10 (100%)	10 (100%)
Hepatocyte, necrosis	–	–	–	–	–	8 (80%)
Pancreas	(10)	(0)	(0)	(0)	(0)	(10)
Salivary glands	(10)	(0)	(0)	(0)	(10)	(10)
Submandibular gland, depletion secretory	–	–	–	–	–	7 (70%)
Stomach, forestomach	(10)	(0)	(0)	(0)	(0)	(10)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, atrophy	–	–	–	–	–	10 (100%)
Epithelium, hyperplasia	–	–	–	–	–	10 (100%)
Cardiovascular System						
Blood vessel	(10)	(0)	(0)	(0)	(0)	(10)
Heart	(10)	(0)	(0)	(0)	(0)	(10)
Cardiomyopathy	10 (100%)	–	–	–	–	4 (40%)

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(0)	(0)	(10)
Adrenal medulla	(10)	(0)	(0)	(0)	(0)	(10)
Parathyroid gland	(7)	(0)	(0)	(0)	(0)	(10)
Pituitary gland	(10)	(0)	(0)	(0)	(0)	(10)
Thyroid gland	(10)	(0)	(0)	(0)	(0)	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Epididymis	(10)	(10)	(10)	(10)	(10)	(10)
Exfoliated germ cell	1 (10%)	3 (30%)	1 (10%)	2 (20%)	4 (40%)	10 (100%)
Preputial gland	(10)	(0)	(0)	(0)	(0)	(10)
Prostate	(10)	(0)	(0)	(0)	(0)	(10)
Seminal vesicle	(10)	(0)	(0)	(0)	(0)	(10)
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Elongated spermatid, retention	5 (50%)	3 (30%)	–	1 (10%)	6 (60%)	10 (100%)
Germinal epithelium, degeneration	5 (50%)	5 (50%)	3 (30%)	6 (60%)	8 (80%)	10 (100%)
Hematopoietic System						
Bone marrow	(10)	(0)	(0)	(0)	(0)	(10)
Lymph node, mandibular	(10)	(0)	(0)	(0)	(0)	(10)
Lymph node, mesenteric	(10)	(0)	(0)	(0)	(0)	(10)
Spleen	(10)	(0)	(0)	(0)	(0)	(10)
Thymus	(10)	(0)	(0)	(0)	(0)	(10)
Integumentary System						
Mammary gland	(10)	(0)	(0)	(0)	(0)	(10)
Skin	(10)	(1)	(0)	(0)	(0)	(10)
Squamous cell papilloma	–	1 (100%)	–	–	–	–
Musculoskeletal System						
Bone	(10)	(0)	(0)	(0)	(0)	(10)
Nervous System						
Brain	(10)	(0)	(0)	(0)	(0)	(10)
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	9 (90%)	10 (100%)	10 (100%)	10 (100%)	9 (90%)	9 (90%)
Metaplasia, osseous	1 (10%)	–	1 (10%)	–	–	1 (10%)

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Mineralization	1 (10%)	–	–	–	–	–
Alveolar epithelium, hyperplasia	–	1 (10%)	–	–	–	–
Alveolus, infiltration cellular, histiocyte	9 (90%)	6 (60%)	8 (80%)	9 (90%)	10 (100%)	8 (80%)
Pleura, inflammation, chronic	–	–	–	–	–	1 (10%)
Nose	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic active	–	–	–	–	–	1 (10%)
Olfactory epithelium, accumulation, hyaline droplet	2 (20%)	–	–	–	–	2 (20%)
Olfactory epithelium, degeneration	1 (10%)	–	–	–	–	–
Olfactory epithelium, metaplasia, squamous	1 (10%)	–	–	–	–	–
Respiratory epithelium, accumulation, hyaline droplet	1 (10%)	–	–	–	–	2 (20%)
Trachea	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic	–	–	–	–	–	1 (10%)
Special Senses System						
Eye	(10)	(0)	(0)	(0)	(0)	(10)
Harderian gland	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic	1 (10%)	–	–	–	–	–
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Mineralization	9 (90%)	7 (70%)	7 (70%)	9 (90%)	10 (100%)	10 (100%)
Nephropathy	10 (100%)	10 (100%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Renal tubule, accumulation, hyaline droplet	–	–	–	–	10 (100%)	10 (100%)
Renal tubule, dilatation	–	–	–	–	–	1 (10%)
Urinary bladder	(10)	(0)	(0)	(0)	(0)	(10)

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

Table A-2. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths	–	–	–	–	–	–
Moribund	–	–	–	–	–	1
Natural deaths	–	–	–	–	–	2
Survivors	–	–	–	–	–	–
Terminal euthanasia	10	10	10	10	10	7
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, cecum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, colon	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, rectum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, duodenum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, ileum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, jejunum	(10)	(0)	(0)	(0)	(0)	(8)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Fatty change	–	–	–	–	1 (10%)	10 (100%)
Hepatodiaphragmatic nodule	1 (10%)	3 (30%)	1 (10%)	2 (20%)	1 (10%)	1 (10%)
Inflammation, granulomatous	6 (60%)	6 (60%)	8 (80%)	5 (50%)	10 (100%)	–
Necrosis	–	–	–	–	–	1 (10%)
Centrilobular, hepatocyte, hypertrophy	–	–	–	–	10 (100%)	7 (70%)
Hepatocyte, necrosis	–	–	–	–	–	6 (60%)
Pancreas	(10)	(0)	(0)	(0)	(0)	(10)
Salivary glands	(10)	(0)	(0)	(10)	(10)	(10)
Submandibular gland, depletion secretory	–	–	–	–	10 (100%)	7 (70%)
Stomach, forestomach	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, acute	–	–	–	–	–	1 (10%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, atrophy	–	–	–	–	5 (50%)	7 (70%)
Epithelium, hyperplasia	–	–	–	–	–	7 (70%)
Epithelium, necrosis	–	–	–	–	–	2 (20%)

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Cardiovascular System						
Blood vessel	(10)	(0)	(0)	(0)	(0)	(10)
Heart	(10)	(0)	(0)	(0)	(0)	(10)
Cardiomyopathy	6 (60%)	–	–	–	–	4 (40%)
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(0)	(0)	(10)
Adrenal medulla	(10)	(0)	(0)	(0)	(0)	(10)
Parathyroid gland	(10)	(0)	(0)	(0)	(0)	(8)
Pituitary gland	(10)	(0)	(0)	(0)	(0)	(10)
Thyroid gland	(10)	(0)	(0)	(0)	(0)	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Clitoral gland	(10)	(0)	(0)	(0)	(0)	(10)
Ovary	(10)	(1)	(2)	(1)	(1)	(10)
Cyst	–	1 (100%)	2 (100%)	1 (100%)	1 (100%)	–
Uterus	(10)	(0)	(0)	(0)	(0)	(10)
Hematopoietic System						
Bone marrow	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, granulomatous	2 (20%)	–	–	–	–	–
Lymph node, mandibular	(10)	(0)	(0)	(0)	(0)	(10)
Lymph node, mesenteric	(10)	(0)	(0)	(0)	(0)	(10)
Spleen	(10)	(0)	(0)	(0)	(0)	(10)
Thymus	(10)	(0)	(0)	(0)	(0)	(10)
Cortex, depletion lymphoid	–	–	–	–	–	1 (10%)
Cortex, necrosis, lymphoid	–	–	–	–	–	3 (30%)
Integumentary System						
Mammary gland	(10)	(0)	(0)	(0)	(0)	(10)
Skin	(10)	(0)	(0)	(0)	(0)	(10)
Musculoskeletal System						
Bone	(10)	(0)	(0)	(0)	(0)	(10)
Nervous System						
Brain	(10)	(0)	(0)	(0)	(0)	(10)

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Foreign body	–	–	1 (10%)	–	–	–
Inflammation, chronic active	2 (20%)	6 (60%)	7 (70%)	7 (70%)	10 (100%)	5 (50%)
Mineralization	1 (10%)	–	–	–	–	–
Alveolus, infiltration cellular, histiocyte	2 (20%)	5 (50%)	8 (80%)	8 (80%)	9 (90%)	7 (70%)
Nose	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation	1 (10%)	–	–	–	–	–
Inflammation, chronic	1 (10%)	–	–	–	–	–
Olfactory epithelium, accumulation, hyaline droplet	6 (60%)	–	–	–	–	2 (20%)
Respiratory epithelium, accumulation, hyaline droplet	3 (30%)	–	–	–	–	2 (20%)
Trachea	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation	1 (10%)	–	–	–	–	–
Special Senses System						
Eye	(10)	(1)	(0)	(0)	(0)	(10)
Hemorrhage	–	1 (100%)	–	–	–	–
Anterior chamber, exudate	–	–	–	–	–	1 (10%)
Cornea, inflammation, acute	–	–	–	–	–	1 (10%)
Posterior chamber, exudate	–	–	–	–	–	1 (10%)
Retina, degeneration	–	–	–	–	–	1 (10%)
Harderian gland	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic	1 (10%)	–	–	–	–	–
Acinus, metaplasia	–	–	–	–	–	1 (10%)
Urinary System						
Kidney	(10)	(0)	(0)	(0)	(0)	(10)
Mineralization	8 (80%)	–	–	–	–	8 (80%)
Nephropathy	3 (30%)	–	–	–	–	2 (20%)
Renal tubule, dilatation	1 (10%)	–	–	–	–	–
Urinary bladder	(10)	(0)	(0)	(0)	(0)	(10)

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

Table A-3. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal euthanasia	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(0)	(0)	(0)	(0)	(10)
Gallbladder	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, cecum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, colon	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, rectum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, duodenum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, ileum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, jejunum	(10)	(0)	(0)	(0)	(0)	(10)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Fatty change	–	–	–	8 (80%)	5 (50%)	10 (100%)
Centrilobular, hepatocyte, hypertrophy	–	–	–	–	10 (100%)	10 (100%)
Hepatocyte, necrosis	–	–	–	–	1 (10%)	10 (100%)
Hepatocyte, necrosis, focal	1 (10%)	–	–	–	–	–
Oval cell, hyperplasia	–	–	–	–	–	10 (100%)
Pancreas	(10)	(0)	(0)	(0)	(0)	(10)
Salivary glands	(10)	(0)	(0)	(0)	(0)	(10)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic	–	–	–	–	2 (20%)	4 (40%)
Ulcer	–	–	–	–	–	2 (20%)
Epithelium, hyperkeratosis	–	–	–	–	2 (20%)	3 (30%)
Epithelium, hyperplasia	–	–	–	–	1 (10%)	3 (30%)
Epithelium, inflammation, suppurative	–	–	–	–	–	4 (40%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, atrophy	–	–	–	–	–	10 (100%)
Epithelium, hyperplasia	–	–	–	–	–	10 (100%)
Epithelium, necrosis	–	–	–	–	1 (10%)	–

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Cardiovascular System						
Blood vessel	(10)	(0)	(0)	(0)	(0)	(10)
Heart	(10)	(0)	(0)	(0)	(0)	(10)
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(0)	(0)	(10)
Subcapsular, hyperplasia	2 (20%)	–	–	–	–	1 (10%)
Adrenal medulla	(10)	(0)	(0)	(0)	(0)	(10)
Islets, pancreatic	(10)	(0)	(0)	(0)	(0)	(10)
Parathyroid gland	(9)	(0)	(0)	(0)	(0)	(10)
Pituitary gland	(10)	(0)	(0)	(0)	(0)	(10)
Thyroid gland	(10)	(0)	(0)	(0)	(0)	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Epididymis	(10)	(10)	(10)	(10)	(10)	(10)
Preputial gland	(10)	(0)	(0)	(0)	(0)	(10)
Prostate	(10)	(0)	(0)	(0)	(0)	(10)
Seminal vesicle	(10)	(0)	(0)	(0)	(0)	(10)
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic System						
Bone marrow	(10)	(0)	(0)	(0)	(0)	(10)
Lymph node, mandibular	(10)	(0)	(0)	(0)	(0)	(10)
Lymph node, mesenteric	(10)	(0)	(0)	(0)	(0)	(10)
Spleen	(10)	(0)	(0)	(0)	(0)	(10)
Thymus	(10)	(0)	(0)	(0)	(0)	(10)
Integumentary System						
Skin	(10)	(0)	(0)	(0)	(0)	(10)
Musculoskeletal System						
Bone	(10)	(0)	(0)	(0)	(0)	(10)
Nervous System						
Brain	(10)	(0)	(0)	(0)	(0)	(10)
Respiratory System						
Lung	(10)	(0)	(0)	(0)	(1)	(10)
Serosa, inflammation, granulomatous	–	–	–	–	1 (100%)	–

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Glands, olfactory epithelium, hyperplasia	–	–	–	–	–	7 (70%)
Nerve, atrophy	–	–	–	–	8 (80%)	10 (100%)
Olfactory epithelium, accumulation, hyaline droplet	–	–	–	–	2 (20%)	9 (90%)
Olfactory epithelium, atrophy	–	–	–	–	10 (100%)	10 (100%)
Respiratory epithelium, accumulation, hyaline droplet	4 (40%)	5 (50%)	1 (10%)	5 (50%)	10 (100%)	10 (100%)
Respiratory epithelium, vacuolization cytoplasmic	1 (10%)	1 (10%)	–	–	–	6 (60%)
Trachea	(10)	(0)	(0)	(0)	(0)	(10)
Special Senses System						
Eye	(10)	(0)	(0)	(0)	(0)	(10)
Retina, atrophy	1 (10%)	–	–	–	–	–
Harderian gland	(10)	(0)	(0)	(0)	(0)	(10)
Urinary System						
Kidney	(10)	(0)	(0)	(0)	(0)	(10)
Inflammation, chronic	1 (10%)	–	–	–	–	–
Nephropathy	1 (10%)	–	–	–	–	1 (10%)
Urinary bladder	(10)	(0)	(0)	(0)	(0)	(10)

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

Table A-4. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal euthanasia	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(0)	(0)	(0)	(0)	(10)
Gallbladder	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, cecum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, colon	(10)	(0)	(0)	(0)	(0)	(10)
Intestine large, rectum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, duodenum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, ileum	(10)	(0)	(0)	(0)	(0)	(10)
Intestine small, jejunum	(9)	(0)	(0)	(0)	(0)	(10)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Basophilic focus	–	–	1 (10%)	–	1 (10%)	–
Clear cell focus	–	–	–	–	3 (30%)	2 (20%)
Fatty change	–	–	–	–	–	10 (100%)
Mineralization, focal	–	–	1 (10%)	–	–	–
Necrosis	–	–	–	–	1 (10%)	–
Centrilobular, hepatocyte, hypertrophy	–	–	–	10 (100%)	10 (100%)	10 (100%)
Hepatocyte, necrosis	–	–	–	1 (10%)	10 (100%)	10 (100%)
Oval cell, hyperplasia	–	–	–	–	10 (100%)	10 (100%)
Pancreas	(10)	(0)	(0)	(0)	(0)	(10)
Salivary glands	(10)	(0)	(0)	(0)	(0)	(10)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic	–	–	2 (20%)	1 (10%)	1 (10%)	3 (30%)
Ulcer	–	–	–	1 (10%)	–	2 (20%)
Epithelium, hyperkeratosis	–	–	2 (20%)	1 (10%)	1 (10%)	3 (30%)
Epithelium, hyperplasia	–	–	2 (20%)	1 (10%)	1 (10%)	3 (30%)
Epithelium, inflammation, suppurative	–	–	2 (20%)	1 (10%)	–	3 (30%)

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, atrophy	–	–	–	–	–	10 (100%)
Epithelium, hyperplasia	–	–	–	–	–	10 (100%)
Epithelium, necrosis	–	–	–	–	1 (10%)	–
Cardiovascular System						
Blood vessel	(10)	(0)	(0)	(0)	(0)	(10)
Heart	(10)	(0)	(0)	(0)	(0)	(10)
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(0)	(0)	(10)
Subcapsular, hyperplasia	6 (60%)	–	–	–	–	5 (50%)
Adrenal medulla	(10)	(0)	(0)	(0)	(0)	(10)
Islets, pancreatic	(10)	(0)	(0)	(0)	(0)	(10)
Parathyroid gland	(9)	(0)	(0)	(0)	(0)	(9)
Pituitary gland	(10)	(0)	(0)	(0)	(0)	(10)
Thyroid gland	(10)	(0)	(0)	(0)	(0)	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Clitoral gland	(10)	(0)	(0)	(0)	(0)	(10)
Ovary	(10)	(0)	(0)	(0)	(0)	(10)
Uterus	(10)	(0)	(0)	(0)	(0)	(10)
Hematopoietic System						
Bone marrow	(10)	(0)	(0)	(0)	(0)	(10)
Lymph node, mandibular	(10)	(0)	(0)	(0)	(0)	(10)
Lymph node, mesenteric	(10)	(0)	(0)	(0)	(0)	(10)
Spleen	(10)	(0)	(0)	(0)	(0)	(10)
Thymus	(10)	(0)	(0)	(0)	(0)	(10)
Integumentary System						
Mammary gland	(10)	(0)	(0)	(0)	(0)	(10)
Skin	(10)	(0)	(0)	(0)	(0)	(10)
Musculoskeletal System						
Bone	(10)	(0)	(0)	(0)	(0)	(10)
Nervous System						
Brain	(10)	(0)	(0)	(0)	(0)	(10)

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Respiratory System						
Lung	(10)	(0)	(0)	(0)	(0)	(10)
Arteriole, inflammation, chronic	1 (10%)	–	–	–	–	–
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Glands, olfactory epithelium, hyperplasia	–	–	–	–	–	10 (100%)
Nerve, atrophy	–	–	–	–	9 (90%)	10 (100%)
Olfactory epithelium, accumulation, hyaline droplet	–	1 (10%)		3 (30%)	8 (80%)	10 (100%)
Olfactory epithelium, atrophy	–	–	–	–	10 (100%)	10 (100%)
Respiratory epithelium, accumulation, hyaline droplet	5 (50%)	3 (30%)	1 (10%)	7 (70%)	10 (100%)	10 (100%)
Respiratory epithelium, vacuolization cytoplasmic	–	–	–	–	2 (20%)	4 (40%)
Trachea	(10)	(0)	(0)	(0)	(0)	(10)
Special Senses System						
Eye	(10)	(0)	(0)	(0)	(0)	(10)
Harderian gland	(10)	(0)	(0)	(0)	(0)	(10)
Urinary System						
Kidney	(10)	(0)	(0)	(0)	(0)	(10)
Urinary bladder	(10)	(0)	(0)	(0)	(0)	(10)

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

Appendix B. Clinical Pathology Results

Tables

Table B-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Male						
Hematology						
n						
Day 3	9	9	10	10	10	10
Day 22	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Hematocrit (auto) (%)						
Day 3	43.0 ± 0.6	43.9 ± 0.6	43.3 ± 0.5	43.0 ± 0.5	44.3 ± 0.5	45.4 ± 0.6**
Day 22	45.1 ± 0.3	43.9 ± 0.2	45.6 ± 0.5	46.2 ± 0.4	44.2 ± 0.4	42.4 ± 0.9*
Week 14	47.3 ± 0.5	47.3 ± 0.3	46.5 ± 0.4	46.9 ± 0.4	46.3 ± 0.4	46.4 ± 0.3
Manual hematocrit (%)						
Day 3	43.2 ± 0.4	44.6 ± 0.5	43.6 ± 0.4[b]	43.7 ± 0.4[b]	45.3 ± 0.4**[b]	46.3 ± 0.5**
Day 22	45.3 ± 0.5	45.1 ± 0.3	46.3 ± 0.4	46.4 ± 0.4	45.0 ± 0.5	43.3 ± 0.7
Week 14	47.6 ± 0.5	46.7 ± 0.4	46.8 ± 0.4	47.0 ± 0.4	46.3 ± 0.4	46.1 ± 0.3
Hemoglobin (g/dL)						
Day 3	13.5 ± 0.2	13.8 ± 0.1	13.6 ± 0.1	13.6 ± 0.2	14.1 ± 0.1**	14.7 ± 0.1**
Day 22	14.1 ± 0.1	13.8 ± 0.1	14.2 ± 0.1	14.3 ± 0.1	14.0 ± 0.1	13.4 ± 0.3
Week 14	15.0 ± 0.1	14.8 ± 0.1	14.7 ± 0.2	14.8 ± 0.1	14.6 ± 0.1	14.5 ± 0.1**
Erythrocytes (10^6/μL)						
Day 3	7.41 ± 0.15	7.49 ± 0.09	7.43 ± 0.10	7.39 ± 0.12	7.60 ± 0.09	7.90 ± 0.10**
Day 22	7.99 ± 0.09	7.78 ± 0.04	8.05 ± 0.10	8.16 ± 0.07	7.81 ± 0.07	7.70 ± 0.16
Week 14	9.25 ± 0.11	9.19 ± 0.07	9.11 ± 0.09	9.07 ± 0.07	9.00 ± 0.07	9.13 ± 0.06
Reticulocytes (10^3/μL)						
Day 3	424 ± 31	405 ± 25	450 ± 31	414 ± 22	412 ± 27	344 ± 14
Day 22	275 ± 8	269 ± 8	287 ± 7	285 ± 6	289 ± 7	319 ± 29
Week 14	186 ± 6	193 ± 8	208 ± 5*	203 ± 5*	226 ± 6**	254 ± 6**
Mean cell volume (fL)						
Day 3	58.2 ± 0.5	58.6 ± 0.3	58.3 ± 0.4	58.3 ± 0.4	58.3 ± 0.5	57.5 ± 0.3
Day 22	56.5 ± 0.4	56.4 ± 0.2	56.6 ± 0.3	56.7 ± 0.2	56.6 ± 0.3	55.1 ± 0.2**
Week 14	51.1 ± 0.1	51.5 ± 0.2	51.0 ± 0.1	51.7 ± 0.1*	51.5 ± 0.2	50.8 ± 0.2

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Mean cell hemoglobin (pg)						
Day 3	18.3 ± 0.1	18.5 ± 0.1	18.4 ± 0.1	18.5 ± 0.1	18.6 ± 0.1	18.6 ± 0.1
Day 22	17.6 ± 0.1	17.7 ± 0.1	17.7 ± 0.1	17.6 ± 0.1	18.0 ± 0.1	17.4 ± 0.1
Week 14	16.2 ± 0.1	16.1 ± 0.0	16.2 ± 0.1	16.3 ± 0.0	16.2 ± 0.1	15.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	31.4 ± 0.2	31.6 ± 0.1	31.5 ± 0.2	31.7 ± 0.2	31.9 ± 0.2	32.4 ± 0.2**
Day 22	31.2 ± 0.1	31.4 ± 0.1	31.3 ± 0.1	31.0 ± 0.1	31.7 ± 0.1*	31.5 ± 0.2
Week 14	31.7 ± 0.2	31.3 ± 0.1	31.7 ± 0.2	31.5 ± 0.1	31.5 ± 0.2	31.1 ± 0.1
Platelets (10^3/μL)						
Day 3	1,078 ± 27	1,025 ± 36	1,073 ± 32	1,030 ± 23	944 ± 25**	1,021 ± 40
Day 22	817 ± 17	823 ± 21	784 ± 34	779 ± 15	829 ± 28	935 ± 28*
Week 14	626 ± 14	633 ± 22	670 ± 12	656 ± 18	701 ± 20**	785 ± 31**
Leukocytes (10^3/μL)						
Day 3	8.27 ± 0.42	8.87 ± 0.25	8.60 ± 0.20	8.67 ± 0.32	8.01 ± 0.47	7.34 ± 0.74
Day 22	7.81 ± 0.34	8.49 ± 0.30	8.76 ± 0.44	7.83 ± 0.38	8.92 ± 0.23	8.16 ± 0.23
Week 14	9.53 ± 0.50	8.96 ± 0.48	9.93 ± 0.23	9.29 ± 0.28	10.65 ± 0.51	9.31 ± 0.30
Segmented neutrophils (10^3/μL)						
Day 3	1.46 ± 0.08	1.48 ± 0.07	1.60 ± 0.10	1.51 ± 0.07	1.51 ± 0.11	1.36 ± 0.09
Day 22	1.54 ± 0.08	1.52 ± 0.11	1.64 ± 0.11	1.72 ± 0.09	1.77 ± 0.11	1.62 ± 0.09
Week 14	1.53 ± 0.06	1.98 ± 0.20	1.85 ± 0.10	1.53 ± 0.07	1.96 ± 0.10*	1.90 ± 0.08*
Lymphocytes (10^3/μL)						
Day 3	6.44 ± 0.37	6.96 ± 0.20	6.60 ± 0.18	6.79 ± 0.29	6.16 ± 0.37	5.69 ± 0.66
Day 22	5.96 ± 0.30	6.62 ± 0.30	6.78 ± 0.39	5.81 ± 0.29	6.77 ± 0.23	6.19 ± 0.22
Week 14	7.66 ± 0.45	6.64 ± 0.43	7.74 ± 0.19	7.48 ± 0.24	8.35 ± 0.45	7.04 ± 0.25
Monocytes (10^3/μL)						
Day 3	0.30 ± 0.02	0.33 ± 0.02	0.33 ± 0.02	0.29 ± 0.02	0.28 ± 0.03	0.24 ± 0.03
Day 22	0.20 ± 0.03	0.24 ± 0.01	0.25 ± 0.03	0.20 ± 0.03	0.27 ± 0.02	0.29 ± 0.02*
Week 14	0.23 ± 0.01	0.22 ± 0.02	0.23 ± 0.01	0.19 ± 0.01	0.26 ± 0.02	0.30 ± 0.03
Basophils (10^3/μL)						
Day 3	0.017 ± 0.002	0.022 ± 0.006	0.018 ± 0.002	0.018 ± 0.002	0.018 ± 0.002	0.013 ± 0.003
Day 22	0.044 ± 0.009	0.045 ± 0.007	0.038 ± 0.007	0.024 ± 0.003	0.038 ± 0.007	0.026 ± 0.002
Week 14	0.031 ± 0.003	0.025 ± 0.003	0.030 ± 0.003	0.026 ± 0.003	0.026 ± 0.004	0.026 ± 0.002

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Eosinophils ($10^3/\mu L$)						
Day 3	0.05 ± 0.00	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.04 ± 0.00*
Day 22	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.02	0.07 ± 0.02	0.03 ± 0.00**
Week 14	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.06 ± 0.01**	0.04 ± 0.00**
Clinical Chemistry						
n						
Day 3	10	10	10	10	10	10
Day 22	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	12.9 ± 0.4[c]	13.5 ± 0.7	13.3 ± 0.3	13.8 ± 0.6[b]	14.2 ± 0.8	17.0 ± 0.8**[c]
Day 22	10.1 ± 0.4	10.1 ± 0.5	10.2 ± 0.5	13.1 ± 0.8*	12.0 ± 0.4*	12.3 ± 0.6*
Week 14	13.3 ± 0.6	13.1 ± 0.7	13.4 ± 0.4	14.1 ± 0.6	14.0 ± 0.4	15.8 ± 0.8*
Creatinine (mg/dL)						
Day 3	0.61 ± 0.01[b]	0.61 ± 0.01	0.60 ± 0.00	0.60 ± 0.00[b]	0.61 ± 0.01	0.65 ± 0.03
Day 22	0.62 ± 0.01	0.60 ± 0.00	0.61 ± 0.01	0.61 ± 0.02	0.61 ± 0.01	0.62 ± 0.01
Week 14	0.70 ± 0.01	0.74 ± 0.04	0.71 ± 0.01	0.73 ± 0.02	0.75 ± 0.02*	0.76 ± 0.02*
Glucose (mg/dL)						
Day 3	140 ± 2[c]	141 ± 3	137 ± 2	136 ± 1	135 ± 3	128 ± 6[c]
Day 22	139 ± 2	139 ± 3	140 ± 2	136 ± 3	137 ± 3	140 ± 2
Week 14	133 ± 2	133 ± 2	130 ± 2	133 ± 2	131 ± 2	137 ± 8
Total protein (g/dL)						
Day 3	5.5 ± 0.1[c]	5.7 ± 0.1	5.5 ± 0.1	5.5 ± 0.1[b]	5.4 ± 0.1	5.4 ± 0.1[c]
Day 22	6.1 ± 0.1	5.8 ± 0.1*	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.4 ± 0.1
Week 14	7.2 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.7 ± 0.1**	8.0 ± 0.1**
Albumin (g/dL)						
Day 3	3.9 ± 0.1	4.0 ± 0.0	3.9 ± 0.0	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.0
Day 22	4.3 ± 0.0	4.1 ± 0.0	4.2 ± 0.1	4.2 ± 0.1	4.1 ± 0.0	4.4 ± 0.1
Week 14	4.6 ± 0.0	4.5 ± 0.0	4.6 ± 0.0	4.6 ± 0.0	4.8 ± 0.0*	5.0 ± 0.0**
Cholesterol (mg/dL)						
Day 3	88 ± 2	90 ± 2	92 ± 2	101 ± 3**	108 ± 4**	103 ± 3**[b]
Day 22	79 ± 1	73 ± 1	79 ± 1	81 ± 1	88 ± 2**	122 ± 4**
Week 14	79 ± 1	77 ± 2	83 ± 2	85 ± 1*	113 ± 2**	149 ± 3**

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Triglycerides (mg/dL)						
Day 3	107 ± 8[b]	135 ± 28	110 ± 9	115 ± 4	127 ± 8	266 ± 34**[c]
Day 22	98 ± 8	101 ± 6	112 ± 8	80 ± 12	121 ± 10	179 ± 17**
Week 14	120 ± 9	113 ± 8	135 ± 10	128 ± 9	164 ± 9**	192 ± 20**
Alanine aminotransferase (IU/L)						
Day 3	64 ± 3	66 ± 4	64 ± 2	67 ± 2	72 ± 3*	78 ± 5*
Day 22	49 ± 1	51 ± 2	51 ± 1	55 ± 2*	56 ± 1**	62 ± 2**
Week 14	64 ± 2	61 ± 3	60 ± 3	58 ± 2	60 ± 2	84 ± 3*
Alkaline phosphatase (IU/L)						
Day 3	641 ± 29[d]	669 ± 24	623 ± 26[c]	616 ± 17[d]	661 ± 25[b]	537 ± 24[b]
Day 22	453 ± 10	435 ± 6	447 ± 12	399 ± 16*	377 ± 7**	330 ± 11**
Week 14	249 ± 9	265 ± 7	261 ± 5	249 ± 6	232 ± 6	223 ± 6*
Creatine kinase (IU/L)						
Day 3	742 ± 186[b]	388 ± 43	526 ± 80	553 ± 108	536 ± 117	647 ± 118[d]
Day 22	335 ± 71	411 ± 112[b]	479 ± 135	318 ± 44[b]	324 ± 82	310 ± 39[c]
Week 14	476 ± 64[b]	346 ± 38	535 ± 51	555 ± 108	469 ± 49	468 ± 43
Sorbitol dehydrogenase (IU/L)						
Day 3	10 ± 0[b]	11 ± 0	11 ± 0	10 ± 0[b]	12 ± 1**	10 ± 1[b]
Day 22	14 ± 1	13 ± 1	13 ± 0	13 ± 1	12 ± 1	15 ± 1
Week 14	16 ± 1	16 ± 1	14 ± 1	15 ± 1	15 ± 1	22 ± 1**
Bile acids (μmol/L)						
Day 3	15.4 ± 2.8[b]	20.1 ± 2.8	15.0 ± 2.6	15.4 ± 2.4[b]	18.1 ± 2.4	19.2 ± 2.7[b]
Day 22	12.2 ± 1.9	19.3 ± 1.3	13.5 ± 1.8	10.9 ± 1.2	6.9 ± 0.9*	7.1 ± 1.0*
Week 14	8.2 ± 1.4	10.7 ± 1.3	10.2 ± 1.0	9.8 ± 1.1	6.7 ± 0.5	6.5 ± 1.2
Female						
Hematology						
n						
Day 3	10	9	10	9	10	9
Day 22	8	10	10	10	10	9
Week 14	10	9	10	10	10	7
Hematocrit (auto) (%)						
Day 3	45.4 ± 0.4	45.5 ± 0.5	45.2 ± 0.6	46.3 ± 0.9	45.0 ± 0.3	46.1 ± 0.8
Day 22	47.2 ± 0.7	46.1 ± 0.4	45.8 ± 0.5	46.2 ± 0.3	45.5 ± 0.5*	44.0 ± 0.4**
Week 14	45.7 ± 0.5	46.3 ± 0.2	45.3 ± 0.2	45.9 ± 0.4	44.2 ± 0.4**	44.6 ± 0.5*

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Manual hematocrit (%)						
Day 3	45.2 ± 0.6[b]	45.3 ± 0.5	45.1 ± 0.5	45.4 ± 0.8[d]	45.1 ± 0.5	46.1 ± 0.7
Day 22	47.8 ± 0.4	46.4 ± 0.4	46.6 ± 0.4	46.3 ± 0.4*	46.4 ± 0.5*	44.3 ± 0.5**
Week 14	46.9 ± 0.4	47.3 ± 0.3	46.6 ± 0.2	47.1 ± 0.2	45.9 ± 0.3	46.3 ± 0.6
Hemoglobin (g/dL)						
Day 3	14.7 ± 0.1	14.7 ± 0.2	14.4 ± 0.2	14.9 ± 0.3	14.5 ± 0.1	15.2 ± 0.2
Day 22	15.4 ± 0.3	15.1 ± 0.1	14.9 ± 0.2	15.0 ± 0.1	14.8 ± 0.2*	14.1 ± 0.1**
Week 14	14.7 ± 0.1	14.9 ± 0.1	14.7 ± 0.0	14.6 ± 0.1	14.2 ± 0.1**	14.0 ± 0.2**
Erythrocytes (10^6/μL)						
Day 3	7.97 ± 0.08	7.99 ± 0.12	7.87 ± 0.08	8.08 ± 0.17	7.90 ± 0.08	8.18 ± 0.11
Day 22	8.62 ± 0.14	8.37 ± 0.11	8.29 ± 0.09*	8.38 ± 0.07*	8.17 ± 0.08**	8.10 ± 0.08**
Week 14	8.39 ± 0.10	8.51 ± 0.06	8.37 ± 0.04	8.42 ± 0.06	8.19 ± 0.06	8.55 ± 0.11
Reticulocytes (10^3/μL)						
Day 3	260 ± 16	293 ± 24	323 ± 20	291 ± 32	284 ± 23	237 ± 16
Day 22	167 ± 9	167 ± 3	159 ± 8	186 ± 7	177 ± 3	240 ± 12**
Week 14	192 ± 6	197 ± 11	202 ± 9	217 ± 14	197 ± 6	213 ± 9
Mean cell volume (fL)						
Day 3	56.9 ± 0.4	57.0 ± 0.5	57.4 ± 0.4	57.3 ± 0.6	57.0 ± 0.5	56.3 ± 0.5
Day 22	54.8 ± 0.2	55.1 ± 0.4	55.3 ± 0.2	55.2 ± 0.3	55.7 ± 0.3	54.3 ± 0.3
Week 14	54.4 ± 0.2	54.4 ± 0.2	54.2 ± 0.1	54.5 ± 0.1	54.1 ± 0.2	52.1 ± 0.2**
Mean cell hemoglobin (pg)						
Day 3	18.4 ± 0.1	18.3 ± 0.1	18.3 ± 0.1	18.4 ± 0.1	18.4 ± 0.1	18.6 ± 0.1
Day 22	17.9 ± 0.1	18.0 ± 0.1	18.0 ± 0.1	17.9 ± 0.1	18.1 ± 0.1	17.4 ± 0.1*
Week 14	17.5 ± 0.1	17.5 ± 0.1	17.5 ± 0.1	17.4 ± 0.0	17.4 ± 0.1	16.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.3 ± 0.2	32.2 ± 0.1	31.9 ± 0.2	32.2 ± 0.3	32.2 ± 0.1	32.9 ± 0.3
Day 22	32.6 ± 0.2	32.7 ± 0.3	32.6 ± 0.1	32.4 ± 0.1	32.5 ± 0.2	32.0 ± 0.1*
Week 14	32.2 ± 0.1	32.2 ± 0.1	32.3 ± 0.1	31.9 ± 0.1	32.1 ± 0.1	31.4 ± 0.1**
Platelets (10^3/μL)						
Day 3	892 ± 23	950 ± 26	924 ± 57	834 ± 75	937 ± 22	935 ± 59
Day 22	709 ± 25	647 ± 48	766 ± 11	717 ± 22	685 ± 23	791 ± 24
Week 14	716 ± 21	665 ± 28	689 ± 24	662 ± 14	690 ± 11	743 ± 12

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Leukocytes (10^3/μL)						
Day 3	9.04 ± 0.39	9.24 ± 0.51	9.27 ± 0.37	8.54 ± 0.38	9.02 ± 0.46	8.38 ± 0.79
Day 22	8.53 ± 0.52	8.41 ± 0.36	8.26 ± 0.37	7.80 ± 0.45	8.15 ± 0.51	8.71 ± 0.48
Week 14	7.67 ± 0.62	7.40 ± 0.47	8.04 ± 0.32	7.59 ± 0.29	8.36 ± 0.46	8.52 ± 0.27
Segmented neutrophils (10^3/μL)						
Day 3	1.57 ± 0.08	1.47 ± 0.12	1.59 ± 0.15	1.36 ± 0.11	1.47 ± 0.09	1.46 ± 0.10
Day 22	1.69 ± 0.10	1.58 ± 0.13	1.59 ± 0.06	1.57 ± 0.10	1.79 ± 0.22	2.00 ± 0.17
Week 14	1.90 ± 0.18	1.60 ± 0.15	1.62 ± 0.07	1.56 ± 0.06	1.69 ± 0.13	1.86 ± 0.12
Lymphocytes (10^3/μL)						
Day 3	7.06 ± 0.38	7.31 ± 0.43	7.21 ± 0.33	6.77 ± 0.31	7.16 ± 0.38	6.53 ± 0.75
Day 22	6.45 ± 0.50	6.48 ± 0.29	6.34 ± 0.31	5.88 ± 0.41	5.98 ± 0.33	6.33 ± 0.41
Week 14	5.47 ± 0.49	5.51 ± 0.32	6.12 ± 0.28	5.74 ± 0.26	6.39 ± 0.36	6.36 ± 0.17
Monocytes (10^3/μL)						
Day 3	0.32 ± 0.02	0.33 ± 0.04	0.34 ± 0.02	0.28 ± 0.02	0.30 ± 0.03	0.29 ± 0.04
Day 22	0.25 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.20 ± 0.02	0.26 ± 0.02	0.29 ± 0.02
Week 14	0.18 ± 0.02	0.16 ± 0.02	0.16 ± 0.01	0.17 ± 0.01	0.19 ± 0.02	0.21 ± 0.01
Basophils (10^3/μL)						
Day 3	0.020 ± 0.001	0.029 ± 0.004	0.021 ± 0.002	0.023 ± 0.002	0.025 ± 0.003	0.019 ± 0.004
Day 22	0.026 ± 0.004	0.033 ± 0.004	0.024 ± 0.002	0.032 ± 0.006	0.027 ± 0.003	0.023 ± 0.004
Week 14	0.029 ± 0.005	0.027 ± 0.006	0.031 ± 0.004	0.024 ± 0.004	0.022 ± 0.004	0.017 ± 0.002
Eosinophils (10^3/μL)						
Day 3	0.07 ± 0.01	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
Day 22	0.10 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	0.10 ± 0.02	0.08 ± 0.02
Week 14	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.06 ± 0.01*	0.06 ± 0.00*
Clinical Chemistry						
n						
Day 3	10	10	10	10	10	10
Day 22	9	10	10	10	10	9
Week 14	10	10	10	10	10	7
Urea nitrogen (mg/dL)						
Day 3	13.2 ± 0.7[b]	14.0 ± 0.8	13.7 ± 0.4	14.2 ± 0.9	12.8 ± 0.6	16.6 ± 1.2[b]
Day 22	13.1 ± 0.9	12.9 ± 0.6	11.5 ± 0.3	12.6 ± 0.6	13.9 ± 0.7	13.2 ± 0.5
Week 14	13.3 ± 0.7	12.6 ± 0.4	14.7 ± 0.6	13.1 ± 0.2	13.9 ± 0.5	13.1 ± 1.1

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Creatinine (mg/dL)						
Day 3	0.61 ± 0.01	0.60 ± 0.00	0.61 ± 0.01	0.63 ± 0.02	0.60 ± 0.00	0.67 ± 0.02**
Day 22	0.61 ± 0.01	0.62 ± 0.01	0.59 ± 0.01	0.62 ± 0.01	0.61 ± 0.01	0.63 ± 0.02
Week 14	0.70 ± 0.00	0.73 ± 0.02	0.71 ± 0.01	0.71 ± 0.01	0.70 ± 0.00	0.71 ± 0.03
Glucose (mg/dL)						
Day 3	127 ± 2	128 ± 3	137 ± 6	129 ± 2	122 ± 2	128 ± 6
Day 22	143 ± 4	150 ± 4	135 ± 3	134 ± 2	138 ± 3	139 ± 3
Week 14	126 ± 3	120 ± 2	124 ± 4	128 ± 4	127 ± 4	127 ± 4
Total protein (g/dL)						
Day 3	5.7 ± 0.1[b]	5.8 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.5 ± 0.0*	5.5 ± 0.1*
Day 22	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.6 ± 0.1**
Week 14	7.0 ± 0.1	7.3 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.7 ± 0.1**	7.9 ± 0.1**
Albumin (g/dL)						
Day 3	4.1 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.0 ± 0.1	4.0 ± 0.1
Day 22	4.5 ± 0.1	4.4 ± 0.0	4.5 ± 0.1	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.1
Week 14	4.8 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	5.0 ± 0.1**	5.1 ± 0.0**
Cholesterol (mg/dL)						
Day 3	90 ± 2	90 ± 3	98 ± 2*	99 ± 2**	109 ± 3**	108 ± 4**
Day 22	88 ± 8	84 ± 4	86 ± 1	97 ± 2**	138 ± 2**	185 ± 4**
Week 14	77 ± 2	84 ± 3*	87 ± 1**	98 ± 2**	140 ± 3**	166 ± 6**
Triglycerides (mg/dL)						
Day 3	95 ± 5	85 ± 5	78 ± 3	86 ± 8	102 ± 8	209 ± 21**
Day 22	72 ± 8	67 ± 6	54 ± 5	56 ± 8	61 ± 5	94 ± 8
Week 14	50 ± 7	56 ± 4	53 ± 5	46 ± 4	67 ± 10	69 ± 5*
Alanine aminotransferase (IU/L)						
Day 3	52 ± 1	62 ± 3**	58 ± 2*	61 ± 2**	68 ± 2**	75 ± 3**
Day 22	43 ± 2	42 ± 2	41 ± 1	43 ± 1	49 ± 1**	57 ± 3**
Week 14	52 ± 2	56 ± 4	50 ± 2	47 ± 2	50 ± 2	73 ± 4*
Alkaline phosphatase (IU/L)						
Day 3	510 ± 10	555 ± 19	532 ± 13	541 ± 23	508 ± 12	488 ± 19
Day 22	374 ± 5	366 ± 6	345 ± 6**	352 ± 7*	331 ± 6**	333 ± 10**
Week 14	261 ± 6	267 ± 5	262 ± 6	223 ± 7**	193 ± 6**	246 ± 12*

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Creatine kinase (IU/L)						
Day 3	422 ± 107	648 ± 122[b]	762 ± 292	682 ± 119	746 ± 171	877 ± 191
Day 22	771 ± 399	565 ± 176	314 ± 78	425 ± 116	370 ± 95	1,260 ± 406
Week 14	319 ± 64	454 ± 106	315 ± 66	373 ± 56	574 ± 131	320 ± 49
Sorbitol dehydrogenase (IU/L)						
Day 3	12 ± 1	10 ± 1	12 ± 1	12 ± 1	12 ± 1	13 ± 2
Day 22	11 ± 1	11 ± 1	11 ± 0	15 ± 1*	18 ± 0**	16 ± 1**
Week 14	13 ± 1	14 ± 1	13 ± 1	15 ± 1	16 ± 1**	29 ± 2**
Bile acids (µmol/L)						
Day 3	10.4 ± 1.5	13.2 ± 1.8	13.6 ± 1.6	12.5 ± 1.5	16.5 ± 1.7*	9.3 ± 0.9
Day 22	15.8 ± 2.4	13.4 ± 2.2	12.0 ± 1.3	9.4 ± 1.2	9.7 ± 0.5	11.0 ± 1.8
Week 14	13.5 ± 2.5	15.5 ± 2.1	14.1 ± 1.9	11.7 ± 1.5	11.2 ± 1.0	11.0 ± 0.9

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b]n = 9.
[c]n = 8.
[d]n = 7.

Table B-2. Hematology Data for Mice in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Male						
n	10	9	10	9	10	10
Hematocrit (auto) (%)	50.1 ± 0.6	50.0 ± 0.7	49.7 ± 0.5	48.2 ± 0.3	49.1 ± 0.6	48.0 ± 0.6*
Manual hematocrit (%)	50.3 ± 0.6	50.1 ± 0.7	50.1 ± 0.4	49.3 ± 0.4	50.1 ± 0.7	48.8 ± 0.5
Hemoglobin (g/dL)	16.1 ± 0.2	16.1 ± 0.2	16.1 ± 0.2	15.5 ± 0.1	16.0 ± 0.3	15.6 ± 0.2
Erythrocytes (10^6/µL)	10.72 ± 0.11	10.72 ± 0.18	10.70 ± 0.11	10.25 ± 0.06**	10.48 ± 0.14*	10.40 ± 0.15*
Reticulocytes (10^3/µL)	267 ± 6	274 ± 9	259 ± 6	261 ± 9	220 ± 8**	169 ± 21**
Mean cell volume (fL)	46.8 ± 0.2	46.6 ± 0.2	46.4 ± 0.1	47.0 ± 0.1	46.9 ± 0.1	46.2 ± 0.2
Mean cell hemoglobin (pg)	15.1 ± 0.1	15.0 ± 0.1	15.0 ± 0.0	15.1 ± 0.1	15.3 ± 0.1	15.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.2 ± 0.2	32.2 ± 0.1	32.3 ± 0.1	32.2 ± 0.1	32.6 ± 0.2	32.5 ± 0.2
Platelets (10^3/µL)	796 ± 56	775 ± 44	785 ± 26	998 ± 41**	1,076 ± 52**	1,046 ± 42**
Leukocytes (10^3/µL)	5.68 ± 0.22	4.61 ± 0.26	5.87 ± 0.84	5.61 ± 0.26	7.36 ± 0.39*	8.86 ± 0.60**
Segmented neutrophils (10^3/µL)	0.80 ± 0.04	0.74 ± 0.10	0.85 ± 0.09	0.93 ± 0.07	1.24 ± 0.14**	2.12 ± 0.30**
Lymphocytes (10^3/µL)	4.66 ± 0.19	3.68 ± 0.17	4.83 ± 0.72	4.48 ± 0.21	5.80 ± 0.29	6.36 ± 0.36*
Monocytes (10^3/µL)	0.10 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.13 ± 0.02	0.18 ± 0.04
Basophils (10^3/µL)	0.006 ± 0.002	0.002 ± 0.001	0.007 ± 0.006	0.006 ± 0.002	0.011 ± 0.003	0.012 ± 0.003
Eosinophils (10^3/µL)	0.13 ± 0.02	0.10 ± 0.02	0.09 ± 0.05	0.12 ± 0.02	0.18 ± 0.04	0.19 ± 0.03
Female						
n	10	10	10	10	10	10
Hematocrit (auto) (%)	46.9 ± 0.6	46.5 ± 0.4	46.2 ± 0.3	48.7 ± 0.5	47.0 ± 0.6	47.2 ± 0.6
Manual hematocrit (%)	48.1 ± 0.7	47.8 ± 0.5	47.6 ± 0.4	49.4 ± 0.6[b]	48.3 ± 0.5	48.1 ± 0.8
Hemoglobin (g/dL)	15.5 ± 0.2	15.4 ± 0.1	15.1 ± 0.1	16.1 ± 0.2	15.5 ± 0.2	15.4 ± 0.2
Erythrocytes (10^6/µL)	10.14 ± 0.12	9.87 ± 0.09	9.83 ± 0.08	10.35 ± 0.11	10.05 ± 0.10	10.21 ± 0.14
Reticulocytes (10^3/µL)	271 ± 9	260 ± 17	265 ± 12	244 ± 17	198 ± 6**	209 ± 12**
Mean cell volume (fL)	46.3 ± 0.2	47.1 ± 0.2*	46.9 ± 0.2	47.0 ± 0.1*	46.7 ± 0.2	46.2 ± 0.1
Mean cell hemoglobin (pg)	15.3 ± 0.1	15.6 ± 0.1**	15.4 ± 0.1	15.5 ± 0.1	15.4 ± 0.1	15.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.2	33.1 ± 0.1	32.7 ± 0.1	33.0 ± 0.1	33.0 ± 0.2	32.6 ± 0.2
Platelets (10^3/µL)	769 ± 48	730 ± 50	764 ± 34	708 ± 33	790 ± 55	852 ± 41
Leukocytes (10^3/µL)	5.16 ± 0.53	3.79 ± 0.12	4.16 ± 0.24	5.46 ± 0.27	6.12 ± 0.35	7.75 ± 0.76*
Segmented neutrophils (10^3/µL)	0.56 ± 0.08	0.52 ± 0.05	0.61 ± 0.08	0.72 ± 0.08	0.89 ± 0.08**	1.43 ± 0.23**
Lymphocytes (10^3/µL)	4.40 ± 0.47	3.19 ± 0.11	3.40 ± 0.19	4.52 ± 0.18	5.04 ± 0.32	5.97 ± 0.67
Monocytes (10^3/µL)	0.07 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.11 ± 0.03	0.09 ± 0.01	0.16 ± 0.02**
Basophils (10^3/µL)	0.005 ± 0.002	0.002 ± 0.001	0.004 ± 0.002	0.007 ± 0.003	0.004 ± 0.002	0.011 ± 0.004
Eosinophils (10^3/µL)	0.12 ± 0.02	0.03 ± 0.01*	0.08 ± 0.02	0.12 ± 0.03	0.09 ± 0.02	0.19 ± 0.03

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

Appendix C. Organ Weights and Organ-Weight-to-Body-Weight Ratios

Tables

Table C-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	331 ± 6	327 ± 5	334 ± 7	335 ± 7	317 ± 9	264 ± 6**
Heart						
Absolute	1.02 ± 0.02	1.00 ± 0.02	1.01 ± 0.01	1.01 ± 0.01	1.02 ± 0.03	0.94 ± 0.02*
Relative	3.07 ± 0.05	3.07 ± 0.06	3.03 ± 0.05	3.04 ± 0.06	3.22 ± 0.03*	3.57 ± 0.05**
R. Kidney						
Absolute	1.00 ± 0.03	0.97 ± 0.02	1.04 ± 0.03	1.09 ± 0.04*	1.15 ± 0.04**	1.09 ± 0.03**
Relative	3.00 ± 0.05	2.97 ± 0.06	3.12 ± 0.07	3.27 ± 0.09**	3.62 ± 0.05**	4.13 ± 0.07**
Liver						
Absolute	12.61 ± 0.26	12.44 ± 0.34	13.74 ± 0.35	14.42 ± 0.44**	18.97 ± 0.54**	21.71 ± 0.66**
Relative	38.07 ± 0.34	38.06 ± 0.73	41.20 ± 0.97*	43.04 ± 0.70**	59.91 ± 0.78**	82.39 ± 1.68**
Lung						
Absolute	1.99 ± 0.07	1.94 ± 0.08	2.01 ± 0.06	1.94 ± 0.09	1.80 ± 0.08	1.79 ± 0.06
Relative	6.00 ± 0.17	5.94 ± 0.27	6.02 ± 0.11	5.78 ± 0.22	5.69 ± 0.19	6.81 ± 0.24*
R. Testis						
Absolute	1.394 ± 0.022	1.268 ± 0.063	1.425 ± 0.015	1.434 ± 0.023	1.454 ± 0.036	1.374 ± 0.027
Relative	4.212 ± 0.056	3.886 ± 0.196	4.285 ± 0.111	4.295 ± 0.071	4.599 ± 0.074*	5.228 ± 0.099**
Thymus						
Absolute	0.327 ± 0.012	0.301 ± 0.013	0.327 ± 0.013	0.322 ± 0.021	0.313 ± 0.014	0.220 ± 0.006**
Relative	0.989 ± 0.035	0.922 ± 0.036	0.979 ± 0.035	0.957 ± 0.055	0.989 ± 0.042	0.837 ± 0.024*
Female						
n	10	10	10	10	10	7
Necropsy body wt	179 ± 4	182 ± 3	178 ± 4	179 ± 2	172 ± 2	172 ± 7
Heart						
Absolute	0.66 ± 0.02	0.65 ± 0.02	0.64 ± 0.01	0.64 ± 0.01	0.66 ± 0.02	0.66 ± 0.02
Relative	3.67 ± 0.08	3.57 ± 0.10	3.58 ± 0.08	3.60 ± 0.04	3.81 ± 0.07	3.84 ± 0.07
R. Kidney						
Absolute	0.64 ± 0.01	0.63 ± 0.02	0.62 ± 0.02	0.65 ± 0.01	0.66 ± 0.01	0.69 ± 0.02
Relative	3.55 ± 0.06	3.43 ± 0.04	3.51 ± 0.09	3.61 ± 0.06	3.83 ± 0.04**	4.03 ± 0.08**

	Vehicle Control	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Liver						
Absolute	6.55 ± 0.23	6.64 ± 0.18	6.81 ± 0.12	7.88 ± 0.18**	10.55 ± 0.29**	14.70 ± 0.50**
Relative	36.57 ± 0.97	36.47 ± 0.65	38.33 ± 1.04	44.12 ± 1.10**	61.34 ± 1.58**	85.76 ± 1.19**
Lung						
Absolute	1.09 ± 0.05	1.24 ± 0.06	1.16 ± 0.05	1.20 ± 0.02	1.18 ± 0.05	1.12 ± 0.07
Relative	6.09 ± 0.20	6.82 ± 0.36	6.48 ± 0.21	6.71 ± 0.17	6.86 ± 0.26	6.54 ± 0.20
Thymus						
Absolute	0.223 ± 0.005	0.221 ± 0.005	0.241 ± 0.008	0.248 ± 0.010	0.217 ± 0.008	0.225 ± 0.008
Relative	1.247 ± 0.027	1.219 ± 0.034	1.352 ± 0.041	1.390 ± 0.056*	1.257 ± 0.035	1.314 ± 0.021

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

Table C-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	38.8 ± 1.2	38.1 ± 1.1	38.3 ± 0.8	37.7 ± 0.9	34.2 ± 1.1**	29.3 ± 0.6**
Heart						
Absolute	0.18 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.16 ± 0.01**
Relative	4.72 ± 0.19	5.23 ± 0.18	5.32 ± 0.14	4.80 ± 0.30	5.31 ± 0.28	5.29 ± 0.19
R. Kidney						
Absolute	0.27 ± 0.01	0.26 ± 0.02	0.26 ± 0.00	0.26 ± 0.01	0.23 ± 0.01**	0.20 ± 0.00**
Relative	6.96 ± 0.18	6.92 ± 0.43	6.85 ± 0.13	6.80 ± 0.17	6.65 ± 0.14	6.76 ± 0.08
Liver						
Absolute	1.54 ± 0.05	1.57 ± 0.05	1.58 ± 0.04	1.73 ± 0.04**	2.32 ± 0.06**	2.69 ± 0.06**
Relative	39.67 ± 0.46	41.18 ± 0.90	41.09 ± 0.47	45.97 ± 0.54**	67.94 ± 1.05**	91.64 ± 0.79**
Lung						
Absolute	0.28 ± 0.01	0.30 ± 0.02	0.30 ± 0.01	0.25 ± 0.02[b]	0.30 ± 0.04	0.22 ± 0.01
Relative	7.30 ± 0.34	8.00 ± 0.47	7.89 ± 0.34	6.72 ± 0.47[b]	8.97 ± 1.46	7.49 ± 0.30
R. Testis						
Absolute	0.117 ± 0.001	0.131 ± 0.016	0.119 ± 0.001	0.111 ± 0.002	0.117 ± 0.002	0.111 ± 0.001
Relative	3.024 ± 0.074	3.455 ± 0.448	3.106 ± 0.062	2.940 ± 0.075	3.453 ± 0.090	3.790 ± 0.082**
Thymus						
Absolute	0.046 ± 0.003	0.046 ± 0.002	0.051 ± 0.002	0.042 ± 0.002	0.041 ± 0.002	0.036 ± 0.002**
Relative	1.208 ± 0.106	1.219 ± 0.045	1.334 ± 0.059	1.119 ± 0.036	1.212 ± 0.067	1.223 ± 0.058
Female						
Necropsy body wt	28.3 ± 0.8	31.0 ± 0.8	29.1 ± 1.0	27.2 ± 0.6	25.3 ± 0.3**	23.9 ± 0.4**
Heart						
Absolute	0.14 ± 0.01	0.15 ± 0.00	0.16 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.12 ± 0.01*
Relative	4.85 ± 0.20	5.00 ± 0.19	5.45 ± 0.20	5.40 ± 0.31	5.23 ± 0.18	4.91 ± 0.19
R. Kidney						
Absolute	0.15 ± 0.00	0.17 ± 0.00	0.16 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00
Relative	5.36 ± 0.09	5.35 ± 0.08	5.44 ± 0.18	5.68 ± 0.15	5.81 ± 0.06**	6.13 ± 0.06**
Liver						
Absolute	1.09 ± 0.02	1.29 ± 0.04**	1.21 ± 0.03**	1.27 ± 0.04**	1.76 ± 0.03**	2.49 ± 0.06**
Relative	38.77 ± 0.54	41.52 ± 0.74*	41.77 ± 0.56*	46.64 ± 0.97**	69.63 ± 1.22**	103.96 ± 1.23**

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	600 mg/kg
Lung						
Absolute	0.24 ± 0.01	0.27 ± 0.01	0.25 ± 0.01	0.28 ± 0.02	0.25 ± 0.01	0.24 ± 0.01
Relative	8.69 ± 0.63	8.92 ± 0.35	8.86 ± 0.48	10.24 ± 0.66	9.74 ± 0.48	9.85 ± 0.53
Thymus						
Absolute	0.049 ± 0.002	0.055 ± 0.002	0.053 ± 0.002	0.044 ± 0.003	0.040 ± 0.001**	0.039 ± 0.002**
Relative	1.728 ± 0.106	1.766 ± 0.049	1.833 ± 0.078	1.613 ± 0.069	1.576 ± 0.043	1.632 ± 0.069

*Significantly different ($P \leq 0.05$) from the vehicle 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.

Appendix D. Reproductive Tissue Evaluations

Tables

Table D-1. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	100 mg/kg	300 ppm	600 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	331 ± 6	335 ± 7	317 ± 9	264 ± 6**
L. Cauda epididymis	0.1365 ± 0.0033	0.1387 ± 0.0028	0.1292 ± 0.0045	0.1070 ± 0.0031**
L. Epididymis	0.4090 ± 0.0071	0.4104 ± 0.0038	0.4008 ± 0.0096	0.3444 ± 0.0080**
L. Testis	1.4464 ± 0.0217	1.4943 ± 0.0226	1.5092 ± 0.0317	1.4106 ± 0.0220
Spermatid measurements				
Spermatid heads (10^6/g testis)	163.9 ± 10.2	167.2 ± 10.4	136.6 ± 11.0	158.1 ± 19.9
Spermatid heads (10^6/testis)	236.90 ± 14.91	249.06 ± 14.58	205.87 ± 17.18	222.92 ± 28.54
Epididymal spermatozoal measurements				
Sperm motility (%)	86.0 ± 0.4	85.3 ± 0.5	85.0 ± 0.5	84.4 ± 0.5
Sperm (10^6/g cauda epididymis)	652.9 ± 32.7	643.3 ± 39.7	612.5 ± 25.3	635.8 ± 33.3
Sperm (10^6/cauda epididymis)	89.37 ± 5.49	88.63 ± 4.42	79.11 ± 4.47	67.63 ± 3.18**

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

Table D-2. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Gavage Study of Myristicin[a]

	Vehicle Control	100 mg/kg	300 mg/kg	600 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	38.8 ± 1.2	37.7 ± 0.9	34.2 ± 1.1**	29.3 ± 0.6**
L. Cauda epididymis	0.0132 ± 0.0006	0.0138 ± 0.0007	0.0138 ± 0.0007	0.0104 ± 0.0006**
L. Epididymis	0.0409 ± 0.0012	0.0414 ± 0.0007	0.0401 ± 0.0009	0.0337 ± 0.0010**
L. Testis	0.1118 ± 0.0016	0.1110 ± 0.0018	0.1147 ± 0.0014	0.1045 ± 0.0010**
Spermatid measurements				
Spermatid heads (10^6/g testis)	190.0 ± 16.2	251.4 ± 21.6	212.1 ± 10.8	177.0 ± 18.5
Spermatid heads (10^6/testis)	21.32 ± 1.93	27.93 ± 2.45	24.32 ± 1.27	18.56 ± 1.98
Epididymal spermatozoal measurements				
Sperm motility (%)	85.9 ± 0.6	86.4 ± 0.7	86.1 ± 0.5	84.8 ± 0.4
Sperm (10^6/g cauda epididymis)	1,207.5 ± 89.5	1,297.7 ± 51.8	1,298.5 ± 95.9	1,377.2 ± 57.1
Sperm (10^6/cauda epididymis)	15.89 ± 1.39	17.80 ± 0.82	17.84 ± 1.46	14.25 ± 0.85

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

Appendix E. Genetic Toxicology

Tables

Table E-1. Mutagenicity of Myristicin in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 10% rat S9	With 30% rat S9
TA100							
	0	112 ± 7	87 ± 2	118 ± 6	106 ± 9	89 ± 5	135 ± 5
	3.3	108 ± 5	91 ± 19				
	10	109 ± 1	105 ± 19	130 ± 8	120 ± 6	86 ± 3	137 ± 10
	33	111 ± 4	87 ± 13	130 ± 5	127 ± 8	92 ± 3	132 ± 5
	100	81 ± 14[b]	72 ± 3	128 ± 7	91 ± 2	97 ± 4	129 ± 16
	217	Toxic	Toxic				
	333			92 ± 8[b]	120 ± 7	54 ± 2[b]	125 ± 8
	833			87 ± 10[b]	51 ± 23[b]	Toxic	30 ± 29[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		517 ± 48	410 ± 8	251 ± 21	465 ± 4	427 ± 20	562 ± 131
TA97							
	0	153 ± 8	176 ± 10	136 ± 7	211 ± 3		
	3.3	155 ± 10	171 ± 8				
	10	150 ± 9	136 ± 8	182 ± 8	242 ± 12		
	33	154 ± 1	148 ± 11	127 ± 6	250 ± 7		
	100	88 ± 4[b]	132 ± 12	176 ± 21	202 ± 23		
	217	Toxic	Toxic				
	333			75 ± 7[b]	152 ± 8[b]		
	833			4 ± 0[b]	0 ± 0[b]		
Trial summary		Negative	Negative	Negative	Negative		
Positive control		463 ± 7	377 ± 32	455 ± 24	992 ± 28		

		With 10% rat S9	With 10% rat S9	With 30% rat S9
TA97 (continued)				
	0	130 ± 10	148 ± 8	222 ± 8
	1		143 ± 4	
	3.3		141 ± 8	
	10	200 ± 12	140 ± 6	251 ± 3
	33	199 ± 21	137 ± 10	236 ± 3
	100	183 ± 16	127 ± 10	206 ± 7
	200		81 ± 9[b]	
	333	0 ± 0[b]	0 ± 0[b]	120 ± 7[b]
	833	0 ± 0[b]		0 ± 0[b]
Trial summary		Equivocal	Negative	Negative
Positive control		1,681 ± 124	1,715 ± 61	794 ± 30

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 10% rat S9	With 30% rat S9
TA98							
	0	15 ± 2	12 ± 1	14 ± 1	20 ± 3	12 ± 1	29 ± 7
	3.3	17 ± 2	9 ± 2				
	10	14 ± 2	8 ± 1	17 ± 2	18 ± 4	15 ± 4	21 ± 3
	33	16 ± 4	13 ± 3	19 ± 4	22 ± 2	14 ± 1	22 ± 1
	100	11 ± 3[b]	10 ± 3	17 ± 3	18 ± 4	16 ± 2	17 ± 1
	217	3 ± 3[b]	0 ± 0[b]				
	333			10 ± 2[b]	17 ± 2	10 ± 4[b]	20 ± 3
	833			0 ± 0[b]	7 ± 1[b]	0 ± 0[b]	3 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		477 ± 21	71 ± 5	211 ± 16	64 ± 6	115 ± 28	226 ± 13
TA1535							
	0	14 ± 4	8 ± 1	9 ± 2	17 ± 1	12 ± 1	13 ± 2
	3.3	14 ± 2	7 ± 1				
	10	10 ± 5	9 ± 1	11 ± 1	15 ± 1	8 ± 1	18 ± 2
	33	11 ± 1	12 ± 2	11 ± 1	19 ± 3	4 ± 0	19 ± 4
	100	9 ± 1	8 ± 1	13 ± 1	14 ± 2	11 ± 3	18 ± 4
	217	Toxic	0 ± 0[b]				
	333			8 ± 2[b]	15 ± 2	7 ± 2[b]	10 ± 0
	833			1 ± 0[b]	Toxic	0 ± 0[b]	Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		336 ± 15	283 ± 12	30 ± 7	101 ± 3	156 ± 11	277 ± 17

[a]Study was performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger et al.[82] 0 µg/plate was the solvent control.
[b]Slight toxicity.
[c]The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Rats Following Administration of Myristicin by Gavage for Three Months[a]

	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)	P Value[c]
Male								
Corn oil[d]	0	5	0.44 ± 0.14		0.24 ± 0.07		1.230 ± 0.08	
Myristicin	10	5	0.38 ± 0.05	0.6132	0.18 ± 0.06	0.5967	1.209 ± 0.05	1.0000
	30	5	0.42 ± 0.08	0.7001	0.33 ± 0.04	0.4044	1.469 ± 0.06	0.0545
	100	5	0.38 ± 0.04	0.7347	0.24 ± 0.06	0.4319	1.372 ± 0.06	0.0570
	300	5	0.47 ± 0.08	0.5263	0.42 ± 0.14	0.2471	1.651 ± 0.09	0.0004
	600	5	0.75 ± 0.09	0.0109	0.26 ± 0.05	0.2528	1.789 ± 0.08	0.0001
			P = 0.001[e]		P = 0.264		P = 0.000	
Female								
Corn oil	0	5	0.24 ± 0.09		0.09 ± 0.02		1.012 ± 0.03	
Myristicin	10	5	0.24 ± 0.02	0.5014	0.07 ± 0.02	0.6367	1.053 ± 0.07	0.7446
	30	5	0.31 ± 0.02	0.3737	0.14 ± 0.02	0.3280	1.156 ± 0.08	0.2638
	100	5	0.24 ± 0.05	0.3987	0.08 ± 0.02	0.3508	1.236 ± 0.13	0.1049
	300	5	0.29 ± 0.04	0.3183	0.14 ± 0.02	0.0647	1.256 ± 0.05	0.0497
	600	5	0.41 ± 0.03	0.0139	0.23 ± 0.02	0.0000	1.599 ± 0.11	0.0002
			P = 0.006		P = 0.000		P = 0.000	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at ILS, Inc. The detailed protocol is presented by Witt et al.[83]
[b]Mean ± standard error.
[c]Pairwise comparison with the vehicle control group; dosed group values are significant when the one-sided $P \leq 0.025$ by Williams' test. P-values for %PCEs are compared to a two-sided $P < 0.05$.
[d]Vehicle control.
[e]Dose-related trend; significant when compared to one-sided $P \leq 0.025$ by linear regression.

Table E-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Myristicin by Gavage for Three Months[a]

	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)	P Value[c]
Male								
Corn oil[d]	0	5	2.67 ± 0.23		1.45 ± 0.03		1.490 ± 0.08	
Myristicin	10	5	2.29 ± 0.12	0.8147	1.47 ± 0.03	0.6573	1.439 ± 0.04	0.6890
	30	5	2.56 ± 0.11	0.8854	1.45 ± 0.03	0.7435	1.408 ± 0.04	0.6740
	100	5	2.34 ± 0.17	0.9088	1.48 ± 0.02	0.7779	1.433 ± 0.07	0.7185
	300	5	2.52 ± 0.15	0.8851	1.37 ± 0.04	0.7941	1.206 ± 0.04	0.0135
	600	5	2.49 ± 0.14	0.8944	1.39 ± 0.03	0.8073	0.736 ± 0.07	0.0000
			P = 0.471[e]		P = 0.992		P = 0.000	
Female								
Corn oil	0	5	1.87 ± 0.10		1.06 ± 0.02		1.519 ± 0.11	
Myristicin	10	5	1.82 ± 0.13	0.6273	1.07 ± 0.02	0.5436	1.583 ± 0.23	1.0000
	30	5	2.17 ± 0.04	0.7140	1.07 ± 0.02	0.6268	1.428 ± 0.18	0.7130
	100	5	1.90 ± 0.16	0.7487	1.09 ± 0.04	0.6618	1.271 ± 0.15	0.2999
	300	5	1.58 ± 0.10	0.7656	1.06 ± 0.03	0.6811	0.971 ± 0.05	0.0380
	600	5	1.53 ± 0.20	0.7785	1.00 ± 0.02	0.6939	1.149 ± 0.17	0.0389
			P = 0.997		P = 0.984		P = 0.016	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at ILS, Inc. The detailed protocol is presented by Witt et al.[83]
[b]Mean ± standard error.
[c]Pairwise comparison with the vehicle control group; dosed group values for PCEs and NCEs are significant when the one-sided $P \leq 0.025$ by Williams' test. P-values for %PCEs are compared to a two-sided $P < 0.05$.
[d]Vehicle control.
[e]Dose-related trend; significant when compared to a one-sided $P \leq 0.025$ by linear regression.

Appendix F. Chemical Characterization and Dose Formulation Studies

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F.1. Procurement and Characterization of Myristicin

Myristicin was obtained from International Specialty Chemicals, Inc. (Tarrytown, NY), in one lot (PP/MYR/03-11-01) that was used in the 3-month studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle Chemistry Support Services (Columbus, OH) for the study laboratory at Battelle Columbus Operations (Columbus, OH). Reports on the analyses performed in support of the myristicin studies are on file at the National Institute of Environmental Health Sciences.

Lot PP/MYR/03-11-01 of the chemical, a brownish orange to red, slightly sticky liquid was identified as myristicin using infrared (IR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. IR spectra were consistent with absorption assignments expected for myristicin; proton and carbon-13 nmR spectra were consistent with chemical shifts predicted for myristicin by an Advanced Chemistry Development, Inc. (ACD/Labs, Toronto, Ontario, Canada), spectral prediction program. Representative IR and proton and carbon-13 nmR spectra are presented in Figure F-1, Figure F-2, and Figure F-3, respectively.

The moisture content of lot PP/MYR/03-11-01 was determined by Galbraith Laboratories, Inc. (Knoxville, TN), using Karl Fischer titration. Elemental analyses of the bulk chemical for carbon and hydrogen were conducted by Prevalere Life Sciences, Inc. (Whitesboro, NY). The analytical chemistry laboratory determined the peroxide levels by titration and the purity of the test chemical using gas chromatography (GC) with flame ionization detection (FID), and high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. Reportable impurities were further characterized by the analytical chemistry laboratory using GC and HPLC with mass spectrometry (MS) detection. Peroxide content was determined using sodium thiosulfate titration of a solution of myristicin and potassium iodide. HPLC/UV analysis of the purity of the sample was determined using system I.

I) The system included an HPLC instrument (Agilent, Palo Alto, CA), a Luna C18, 150 mm × 4.6 mm, 5 µm particle size column (Phenomenex, Torrance, CA), mobile phases of A) 50:50:1 acetonitrile:water:acetic acid and B) 90:10:1 acetonitrile:water:acetic acid, a linear gradient of 100% A for 20 minutes to 100% B in 5 minutes, held for 20 minutes, then to 100% A in 5 minutes, held for 15 minutes, UV detection at 230 nm, and a flow rate of 0.7 mL/minute.

Karl Fischer titration indicated ≤0.16% water. Elemental analyses for carbon (68.31%) were in agreement with the theoretical values (99.4% of theoretical), but hydrogen values were slightly low (85.9% of theoretical); titration indicated the presence of no peroxide. GC/FID using system A (Table F-1) indicated one major peak with 96.5% of the total peak area and four reportable impurities with individual relative areas of 0.2%, 0.4%, 1.2%, and 1.7%. GC/MS using system B identified two of the four impurities as tributylamine and eugenol; standard addition using GC/FID by system C estimated their concentrations to be 0.31% and 1.25%, respectively. HPLC/UV using system I indicated one major peak with 94.1% of the total peak area and three reportable impurities with individual relative areas of 1.5%, 1.9%, and 2.5%. HPLC/MS conducted with a Quattro LC mass spectrometer (Waters-Micromass, Manchester, England) for detection and a system otherwise similar to system I identified two of the three impurities as 5-hydroxy-eugenol (1.9%) and eugenol (1.5%). An additional HPLC/MS analysis using this system except for reduction of the acetic acid fractions of mobile phases A and B from 1% to 0.1% and reduction of the final gradient hold to 5 minutes tentatively identified the third

impurity as an isomer of 4-allyl-2-methoxyphenyl acetate or a structurally similar compound with a molecular weight of 206. Standard addition using HPLC/UV by system I estimated the concentration of eugenol to be 1.1%. The overall purity of lot PP/MYR/03-11-01 was estimated to be greater than 94%.

Stability studies of the bulk chemical were performed using GC/FID by system C. These studies indicated that myristicin was stable as a bulk chemical for at least 14 days when stored in sealed amber glass bottles at temperatures up to 25°C. To ensure stability, the bulk chemical was stored at approximately 5°C, protected from light, in sealed glass bottles. Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 3-month studies with GC/FID by system C. No degradation of the bulk chemical was detected.

F.1.1. Corn Oil

Corn oil was obtained from Sigma-Aldrich (St. Louis, MO) in two lots (098K0008, 128K0040) and was used as the vehicle in the 3-month studies. Periodic analyses of the corn oil performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than the rejection level of 3 mEq/kg.

F.2. Preparation and Analysis of Dose Formulations

The dose formulations were prepared four times by mixing myristicin with corn oil to give the required concentrations (Table F-2). The dose formulations were stored at approximately 25°C in amber glass bottles sealed with Teflon®-lined lids for no longer than 42 days.

The analytical chemistry laboratory performed a gavagability study of a 200 mg/mL formulation and stability studies of the 1 mg/mL dose formulation using GC/FID by system C (Table F-1). Gavagability was confirmed for a 22 gauge or larger needle. Stability was confirmed for at least 42 days for dose formulations stored in amber glass bottles sealed with Teflon®-lined lids at ambient and refrigerated temperatures and for at least 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of myristicin were conducted by the study laboratory using GC/FID by system C. During the 3-month studies, the dose formulations were analyzed three times; all 27 dose formulations were within 10% of the target concentrations (Table F-3). Animal room samples of these dose formulations were also analyzed; 14 of 15 for rats and 14 of 15 for mice were within 10% of the target concentrations.

Table F-1. Gas Chromatography Systems Used in the Three-month Gavage Studies of Myristicin[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	RTX®-5, 30 m × 0.53 mm, 1.0 µm film (Restek, Bellefonte, PA)	Helium at 5 mL/minute	50°C for 3 minutes, then 10°C/minute to 300°C, held for 2 minutes
System B			
Mass spectrometry with electron impact ionization (50 to 500 amu)	RTX®-5, 30 m × 0.32 mm, 1.0 µm film (Restek)	Helium at 5 mL/minute	50°C for 3 minutes, then 10°C/minute to 300°C, held for 2 minutes
System C			
Flame ionization	RTX®-5, 30 m × 0.32 mm, 1.0 µm film (Restek)	Helium at 5 mL/minute	50°C for 1 minute (dose formulation analysis and stability studies) or 3 minutes (bulk chemical impurity quantification, periodic reanalysis, and stability studies), then 10°C/minute to 300°C, held for 2 minutes

[a]The gas chromatographs and mass spectrometer were manufactured by Agilent Technologies, Inc. (Santa Clara, CA).

Table F-2. Preparation and Storage of Dose Formulations in the Three-month Gavage Studies of Myristicin

Three-month Studies
Preparation
Approximately 200, 700, or 900 mL of corn oil was placed into a calibrated glass mixing container and the appropriate amount of myristicin was added (with corn oil rinsing) to achieve approximately 90% of the final volume in the mixing container for the 1, 3, 10, and 30, 2, 6, 20, and 120, and 60 mg/mL dose formulations, respectively. Each mixing container was then diluted to final volume with corn oil, and the contents were stirred using a stir bar and stir plate or an overhead stirrer for approximately 10 minutes.
Chemical Lot Number
PP/MYR/03-11-01
Maximum Storage Time
42 days
Storage Conditions
Stored in sealed glass bottles protected from light at room temperature
Study Laboratory
Battelle Columbus Operations (Columbus, OH)

Table F-3. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Three-month Gavage Studies of Myristicin

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
January 6, 2009	January 8, 2009	2	2.05	+3
		6	6.17	+3
		20	20.0	0
		60	59.6	−1
		120	119	−1
	February 24, 2009[b]	2	1.92	−4
		6	5.76	−4
		20	19.1	−4
		60	56.4	−6
		120	128	+7
February 27, 2009	March 3, 2009	2	1.94	−3
		6	5.90	−2
		20	19.9	−1
		60	59.1	−2
		120	117	−3
	April 14, 2009[b]	2	2.06	+3
		6	6.32	+5
		20	17.8	−11
		60	58.6	−2
		120	118	−2
April 1, 2009	April 6, 2009	2	1.93	−4
		6	5.85	−3
		20	19.4	−3
		60	56.6	−6
		120	118	−2
	April 24, 2009[b]	2	1.95	−3
		6	5.96	−1
		20	19.7	−2
		60	58.2	−3
		120	115	−4

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Mice				
January 6, 2009	January 8, 2009	1	0.997	0
		3	3.11	+4
		10	9.99	0
		30	30.2	+1
		60	59.6	−1
	February 24, 2009[b]	1	0.966	−3
		3	2.89	−4
		10	9.71	−3
		30	28.4	−5
		60	56.1	−7
February 27, 2009	March 3, 2009	1	0.955	−5
		3	2.84	−5
		10	9.90	−1
		30	29.8	−1
		60	59.1	−2
	April 14, 2009[b]	1	0.899	−10
		3	2.74	−9
		10	8.09	−19
		30	29.3	−2
		60	59.0	−2
April 1, 2009	April 6, 2009	1	0.916	−8
		3	2.92	−3
		10	9.53	−5
		30	28.6	−5
		60	56.6	−6
	April 24, 2009[b]	1	0.930	−7
		3	2.90	−3
		10	9.65	−4
		30	28.6	−5
		60	56.1	−7

[a]Results of duplicate analyses. For rats, dosing volume = 5 mL/kg; 2 mg/mL = 10 mg/kg, 6 mg/mL = 30 mg/kg, 20 mg/mL = 100 mg/kg, 60 mg/mL = 300 mg/kg, 120 mg/mL = 600 mg/kg. For mice, dosing volume = 10 mL/kg; 1 mg/mL = 10 mg/kg, 3 mg/mL = 30 mg/kg, 10 mg/mL = 100 mg/kg, 30 mg/mL = 300 mg/kg, 60 mg/mL = 600 mg/kg.
[b]Animal room samples.

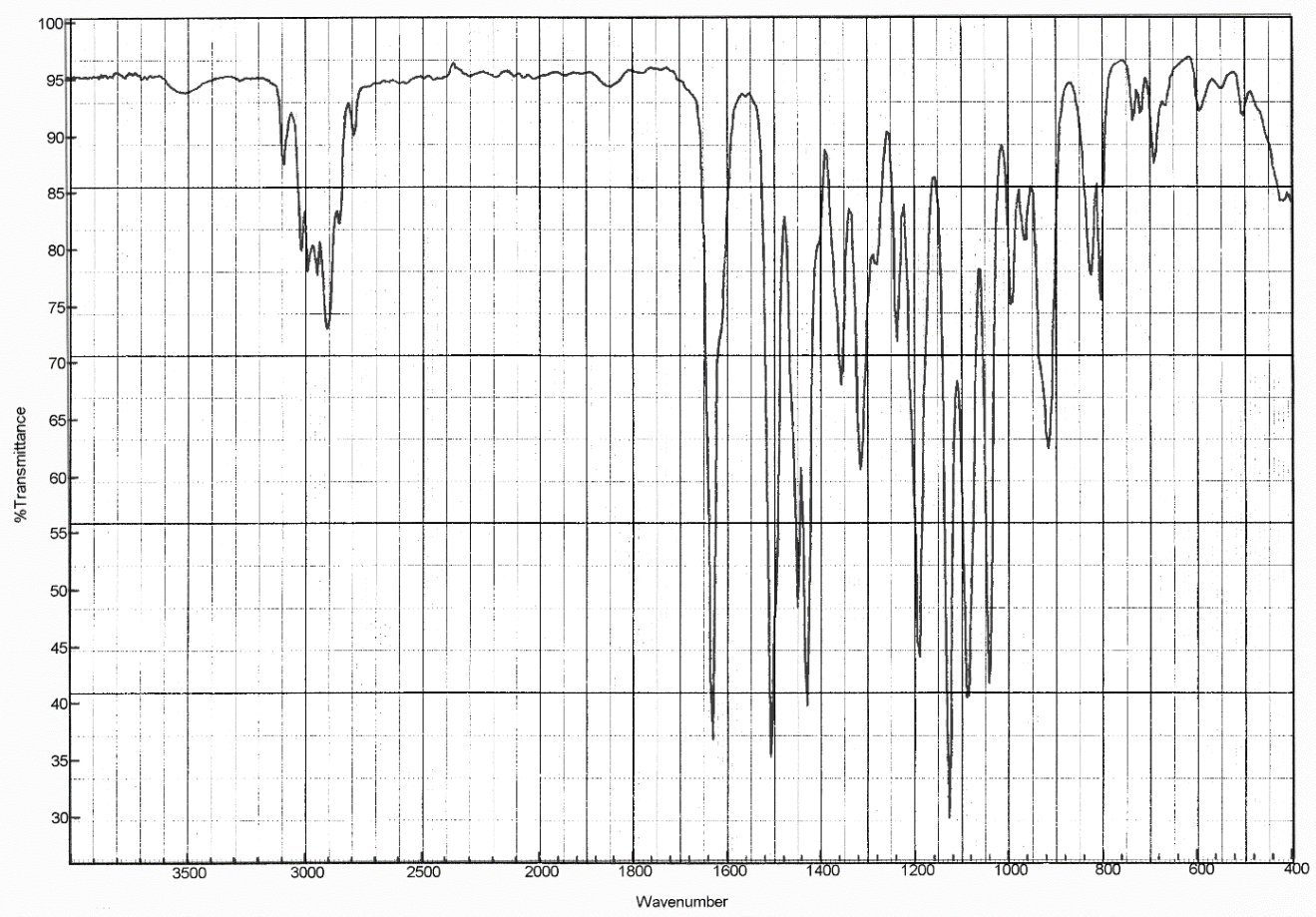

Figure F-1. Infrared Absorption Spectrum of Myristicin

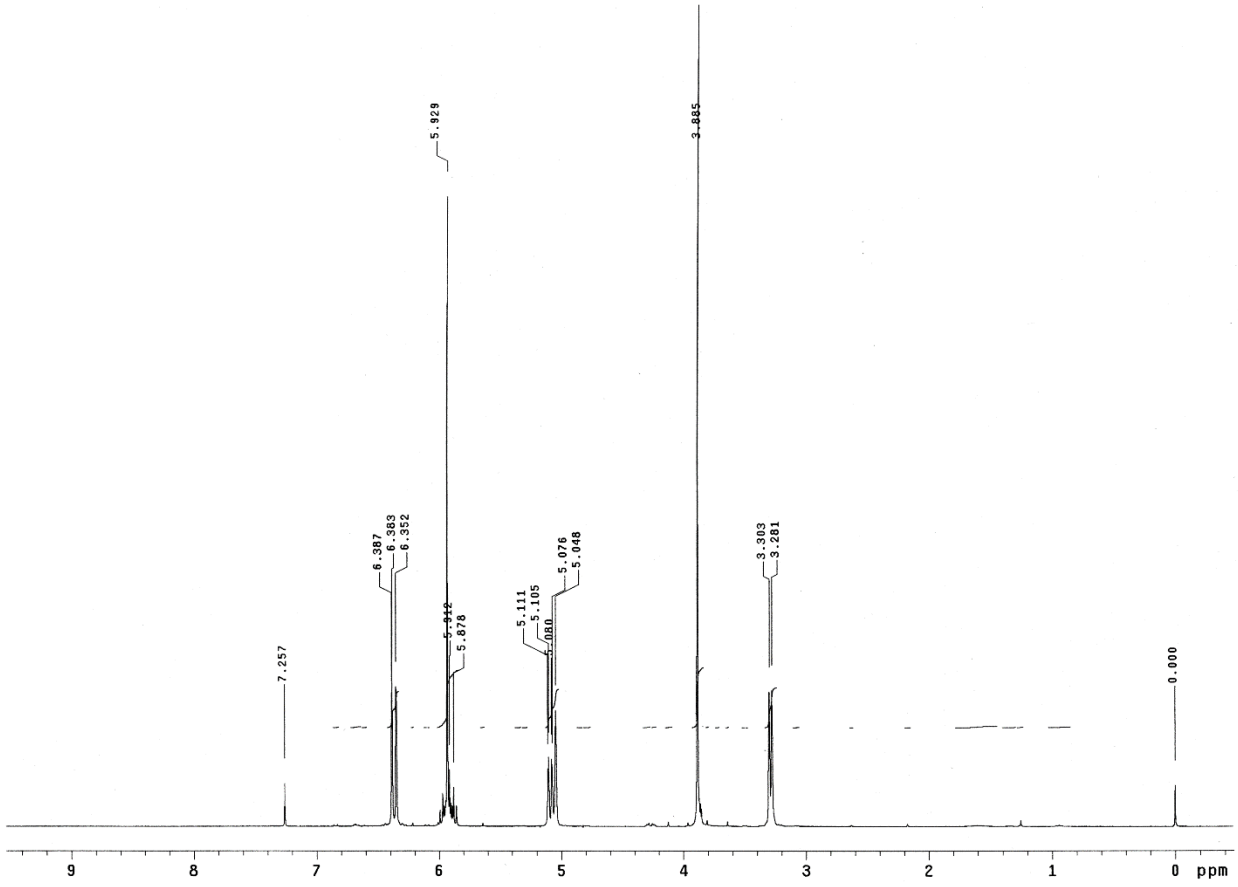

Figure F-2. Proton Nuclear Magnetic Resonance Spectrum of Myristicin

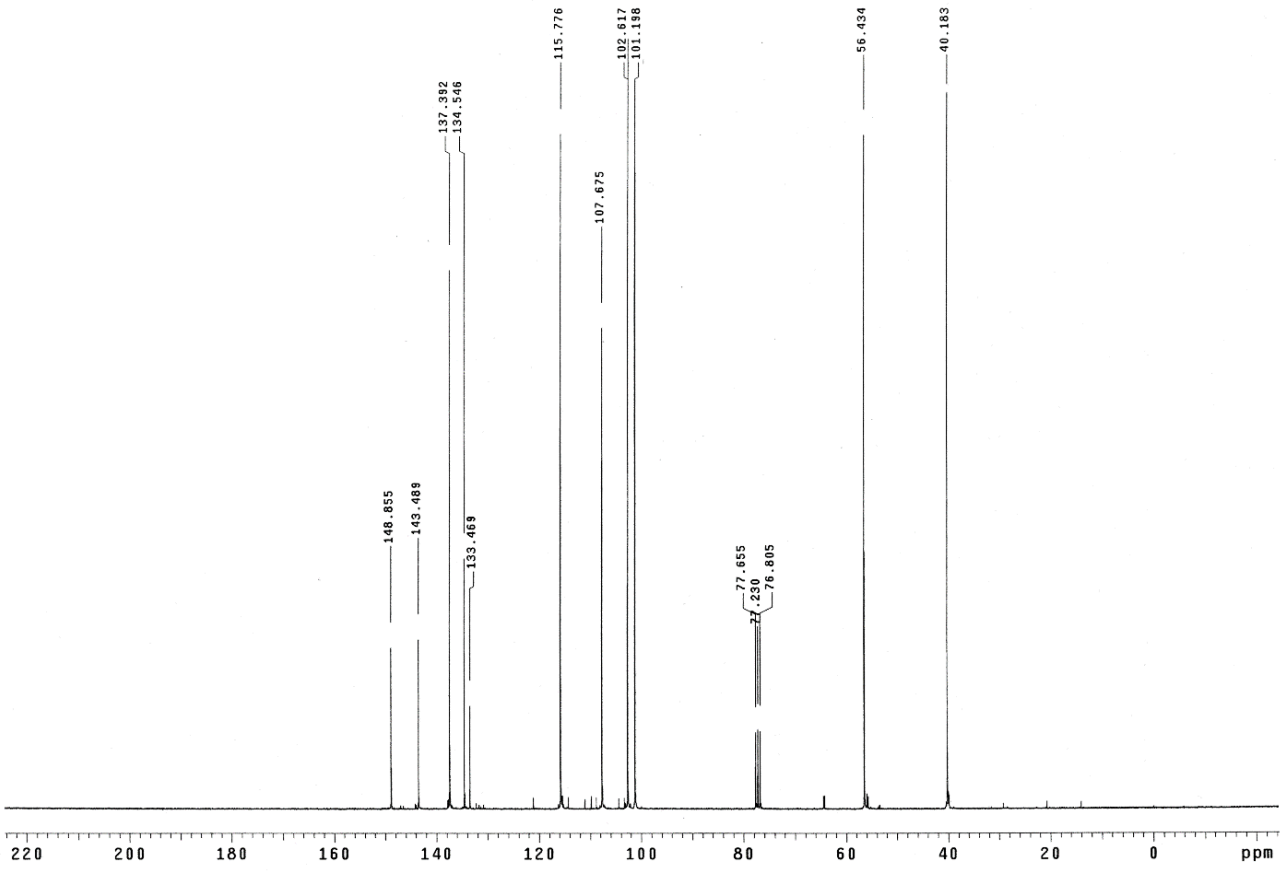

Figure F-3. Carbon-13 Nuclear Magnetic Resonance Spectrum of Myristicin

Appendix G. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

Table G-1. 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 G-2. 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 G-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.3 ± 1.07	13.7–15.9	4
Crude fat (% by weight)	8.2 ± 0.33	7.8–8.5	4
Crude fiber (% by weight)	9.1 ± 0.32	8.8–9.4	4
Ash (% by weight)	5.1 ± 0.15	4.9–5.2	4
Amino Acids (% of total diet)			
Arginine	0.783 ± 0.070	0.670–0.970	22
Cystine	0.220 ± 0.024	0.150–0.250	22
Glycine	0.701 ± 0.041	0.620–0.800	22
Histidine	0.352 ± 0.077	0.270–0.680	22
Isoleucine	0.546 ± 0.044	0.430–0.660	22
Leucine	1.095 ± 0.067	0.960–1.240	22
Lysine	0.711 ± 0.114	0.310–0.860	22
Methionine	0.409 ± 0.046	0.260–0.490	22
Phenylalanine	0.627 ± 0.040	0.540–0.720	22
Threonine	0.505 ± 0.043	0.430–0.610	22
Tryptophan	0.150 ± 0.028	0.110–0.200	22
Tyrosine	0.401 ± 0.061	0.280–0.540	22
Valine	0.665 ± 0.043	0.550–0.730	22
Essential Fatty Acids (% of total diet)			
Linoleic	3.950 ± 0.259	3.49–4.55	22
Linolenic	0.303 ± 0.032	0.21–0.35	22
Vitamins			
Vitamin A (IU/kg)	4,120 ± 152	2,770–5,720	4
Vitamin D (IU/kg)	1,000[a]	–	–
α-Tocopherol (ppm)	80.6 ± 22.03	27.0–124.0	22
Thiamine (ppm)[b]	6.7 ± 0.56	6.2–7.5	4
Riboflavin (ppm)	7.6 ± 2.89	4.20–17.50	22
Niacin (ppm)	78.9 ± 9.08	66.4–98.2	22
Pantothenic acid (ppm)	26.88 ± 12.63	17.4–81.0	22
Pyridoxine (ppm)[b]	9.54 ± 2.00	6.44–13.7	22
Folic acid (ppm)	1.62 ± 0.48	1.15–3.27	22
Biotin (ppm)	0.32 ± 0.10	0.2–0.704	22
Vitamin B_{12} (ppb)	53.6 ± 39.6	18.3–174.0	22
Choline (ppm)[b]	2,846 ± 484	1,820–3,790	22

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.91 ± 0.04	0.87–0.969	4
Phosphorus (%)	0.55 ± 0.05	0.49–0.606	4
Potassium (%)	0.666 ± 0.030	0.626–0.733	22
Chloride (%)	0.386 ± 0.039	0.300–0.474	22
Sodium (%)	0.189 ± 0.016	0.160–0.222	22
Magnesium (%)	0.216 ± 0.062	0.185–0.49	22
Sulfur (%)	0.170 ± 0.029	0.116–0.209	22
Iron (ppm)	185 ± 39.2	135–311	22
Manganese (ppm)	51.4 ± 10.28	21.0–73.1	22
Zinc (ppm)	53.4 ± 8.46	43.3–78.5	22
Copper (ppm)	7.01 ± 2.56	3.21–16.3	22
Iodine (ppm)	0.503 ± 0.206	0.158–0.972	22
Chromium (ppm)	0.694 ± 0.275	0.330–1.380	21
Cobalt (ppm)	0.26 ± 0.164	0.098–0.864	20

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

Table G-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.20 ± 0.05	0.155–0.25	4
Cadmium (ppm)	0.06 ± 0.01	0.055–0.068	4
Lead (ppm)	0.09 ± 0.01	0.081–0.097	4
Mercury (ppm)	<0.02	–	4
Selenium (ppm)	0.19 ± 0.05	0.144–0.252	4
Aflatoxins (ppb)	<5.00	–	4
Nitrate nitrogen (ppm)[c]	17.0 ± 7.84	10.0–26.5	4
Nitrite nitrogen (ppm)[c]	<0.61	–	4
BHA (ppm)[d]	<1.0	–	4
BHT (ppm)[d]	<1.0	–	4
Aerobic plate count (CFU/gm)	10 ± 0.0	10	4
Coliform (MPN/gm)	3.0 ± 0.0	3.0	4
Escherichia coli (MPN/gm)	<10	–	4
Salmonella (MPN/gm)	Negative	–	4
Total nitrosoamines (ppb)[e]	9.3 ± 2.53	5.6–11.3	4
N-Ndimethylamine (ppb)[e]	1.0 ± 0.10	0.9–1.1	4
N-Npyrrolidine (ppb)[e]	8.8 ± 2.13	5.6–10.2	4
Pesticides (ppm)			
α-BHC	<0.01	–	4
β-BHC	<0.02	–	4
γ-BHC	<0.01	–	4
δ-BHC	<0.01	–	4
Heptachlor	<0.01	–	4
Aldrin	<0.01	–	4
Heptachlor epoxide	<0.01	–	4
DDE	<0.01	–	4
DDD	<0.01	–	4
DDT	<0.01	–	4
HCB	<0.01	–	4
Mirex	<0.01	–	4
Methoxychlor	<0.05	–	4
Dieldrin	<0.01	–	4
Endrin	<0.01	–	4

	Mean ± Standard Deviation[b]	Range	Number of Samples
Telodrin	<0.01	–	4
Chlordane	<0.05	–	4
Toxaphene	<0.10	–	4
Estimated PCBs	<0.20	–	4
Ronnel	<0.01	–	4
Ethion	<0.02	–	4
Trithion	<0.05	–	4
Diazinon	<0.10	–	4
Methyl chlorpyrifos	0.157 ± 0.04	0.10–0.18	4
Methyl parathion	<0.02	–	4
Ethyl parathion	<0.02	–	4
Malathion	0.058 ± 0.020	0.0324–0.079	4
Endosulfan I	<0.01	–	4
Endosulfan II	<0.01	–	4
Endosulfane sulfate	<0.03	–	4

CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride.
[a]All samples were irradiated.
[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 H. Sentinel Animal Program

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Tables

H.1. Methods

Rodents used in 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. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected and allowed to clot, and the serum was separated. All samples were processed appropriately with serology performed by the Research Animal Diagnostic Laboratory (RADIL), University of Missouri (currently IDEXX BioResearch), Columbia, MO, for determination of the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Blood was collected from five rats and five mice per sex per time point.

Table H-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program

Method and Test	Time of Collection
Rats	
Three-month Study	
Multiplex Fluorescent Immunoassay	
Kilham's rat virus (KRV)	4 weeks, Study termination
Mycoplasma pulmonis	4 weeks, Study termination
Parvo NS-1	4 weeks, Study termination
Pneumonia virus of mice (PVM)	4 weeks, Study termination
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	4 weeks, Study termination
Rat minute virus (RMV)	4 weeks, Study termination
Rat parvovirus (RPV)	4 weeks, Study termination
Rat theilovirus (RTV)	4 weeks, Study termination
Sendai	4 weeks, Study termination
Theiler's murine encephalomyelitis virus strain GDVII (TMEV GDVII)	4 weeks, Study termination
Toolan's H-1	4 weeks, Study termination
Mice	
Three-month Study	
Multiplex Fluorescent Immunoassay	
Ectromelia virus	4 weeks, Study termination
Epizootic diarrhea of infant mice (EDIM)	4 weeks, Study termination

Method and Test	Time of Collection
Lymphocytic choriomeningitis virus (LCM)	4 weeks, Study termination
Minute virus of mice (MVM)	4 weeks, Study termination
Mouse hepatitis virus (MHV)	4 weeks, Study termination
Mouse norovirus (MNV)	4 weeks, Study termination
Mouse parvovirus (MPV)	4 weeks, Study termination
M. pulmonis	4 weeks, Study termination
Parvo NS-1	4 weeks, Study termination
PVM	4 weeks, Study termination
Reovirus (REO3)	4 weeks, Study termination
Sendai	4 weeks, Study termination
TMEV GDVII	4 weeks, Study termination

H.2. Results

All test results were negative.

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