NTP

National Toxicology Program

U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology and Carcinogenesis Studies of

Methyleugenol (CAS No. 93-15-2) in F344/N Rats and B6C3F₁ Mice (Gavage Studies)

NTP TR 491

JULY 2000

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF METHYLEUGENOL

(CAS NO. 93-15-2)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

July 2000

NTP TR 491

NIH Publication No. 00-3950

National Toxicology Program

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

FOREWORD

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

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

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

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

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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CONTENTS

ABSTRACT

$$CH_2CH =\!= CH_2$$

(chemical structure of methyleugenol: benzene ring with allyl group and two OCH₃ groups)

METHYLEUGENOL

CAS No. 93-15-2

Chemical Formula: $C_{11}H_{14}O_2$ Molecular Weight: 178.2

Synonyms: 4-Allyl-1,2-dimethoxybenzene; 4-allylveratrole; 4-allyl-3,4-dimethoxy-benzene; 1,2-dimethoxy-4-allylbenzene; 3,4-dimethoxyallylbenzene ENT 21040; 1-(3,4-dimethoxyphenyl)-2-propene; eugenol methyl ether; 1,3,4-eugenol methyl ether; veratrole methyl ether

Methyleugenol is used as a flavoring agent in jellies, baked goods, nonalcoholic beverages, chewing gum, candy, pudding, relish, and ice cream. It is also used as a fragrance in perfumes, creams, lotions, detergents, and soaps. Methyleugenol has also been used as an insect attractant in eradication programs and as an anesthetic in rodents. Methyleugenol was nominated for testing because of its widespread use and because of its structural resemblance to safrole, a known carcinogen, and isosafrole and estragole. Male and female F344/N rats and B6C3F₁ mice received methyleugenol (approximately 99% pure) in 0.5% methylcellulose by gavage for 14 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and mouse peripheral blood erythrocytes.

14-WEEK STUDY IN RATS

Groups of 9 or 10 male and 10 female F344/N rats were administered 0, 10, 30, 100, 300, or 1,000 mg methyleugenol/kg body weight in 0.5% methylcellu- lose by gavage 5 days per week for 14 weeks. A water control group of 10 male and 10 female rats received deionized water by gavage. All rats survived until the end of the study. The final mean body weights of 300 and 1,000 mg/kg males and of all dosed groups of females were significantly less than those of the vehicle controls. Erythrocyte microcytosis was demonstrated by decreased mean cell volumes in 300 mg/kg males and 1,000 mg/kg males and females. There was evidence of a thrombocytosis at all time points, demonstrated by increased platelet counts in the 100 mg/kg or greater groups. The serum activities of alanine aminotransferase and sorbitol dehydrogenase were increased in the 100 mg/kg or greater rats at various time points, suggesting hepatocellular injury. Additionally, bile acid concentrations were generally increased in the 300 and 1,000 mg/kg groups at all time points, consistent with cholestasis or altered hepatic function. A hypoproteinemia and hypoalbuminemia, evidenced by decreased total protein and albumin concentrations, occurred in rats in the 300 and 1,000 mg/kg groups at all time points.

Liver weights of 100, 300, and 1,000 mg/kg males and 300 and 1,000 mg/kg females and testis weights of 1,000 mg/kg males were significantly increased. Increased incidences of liver lesions occurred in 300 and 1,000 mg/kg males and females and hepatocellular adenoma occurred in one 1,000 mg/kg male. The incidences of atrophy and chronic inflammation of the mucosa of the glandular stomach were significantly increased in rats administered 300 or 1,000 mg/kg. Increased incidences of adrenal gland cortical hypertrophy and/or cytoplasmic alteration in the submandibular gland occurred in the 100 mg/kg or greater groups.

14-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F$_1$ mice received methyleugenol in 0.5% methylcellulose by gavage at doses of 0, 10, 30, 100, 300, or 1,000 mg/kg, 5 days per week for 14 weeks. A water control group of 10 male and 10 female mice received deionized water by gavage. All but one male and all females receiving 1,000 mg/kg died before the end of the study. The mean body weight gains of mice in the 300 mg/kg groups were significantly less than those of the vehicle controls. The only clinical finding was toxicity manifested as generalized morbidity in mice administered 1,000 mg/kg. Liver weights of 30, 100, and 300 mg/kg males and of 300 mg/kg females were significantly increased. Male mice administered 10 or 30 mg/kg had significantly lower cauda epididymis, epididymis, and testis weights; males receiving 100 mg/kg had significantly lower spermatozoal concentrations. Increased incidences of liver lesions occurred in 1,000 mg/kg males and 300 and 1,000 mg/kg females. The incidences of lesions of the glandular stomach were increased in one or more groups administered 30 mg/kg or greater.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats received methyleugenol in 0.5% methylcellulose by gavage at doses of 37, 75, or 150 mg/kg, 5 days per week for 105 weeks; groups of 60 male and 60 female rats received the 0.5% methylcellulose vehicle only. Stop-exposure groups of 60 male and 60 female rats received 300 mg/kg in 0.5% methylcellulose by gavage for 52 weeks followed by just the 0.5% methylcellulose vehicle for the remaining 53 weeks of

the study. Special study groups of 10 male and 10 female rats administered 36, 75, 150, or 300 mg/kg were designated for toxicokinetic studies.

Survival and Body Weights

All 150 and 300 mg/kg males died before the end of the study, and survival of 150 mg/kg females was slightly less than that of the vehicle controls. Mean body weights of all dosed groups of rats were less than those of the vehicle controls throughout most of the 2-year study.

Pathology Findings

Chemical-related liver neoplasms occurred in all dosed groups of rats and included hepatocellular adenoma, hepatocellular carcinoma, hepatocholangioma, and hepatocholangiocarcinoma; at 2 years, there were positive trends in the incidences of hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) in core study rats and in the numbers of rats with multiple liver neoplasms. Nonneoplastic lesions included eosinophilic and mixed cell foci, hepatocellular hypertrophy, oval cell hyperplasia, cystic degeneration, and bile duct hyperplasia (females); the incidences of these lesions in dosed groups of male and female rats were increased at 6 months, 12 months, and/or 2 years.

Chemical-related neoplasms and nonneoplastic lesions of the glandular stomach included benign and malignant neuroendocrine tumors in the 150 and 300 mg/kg groups and females in the 75 mg/kg group. In all dosed groups of rats at all time points, the incidences of mucosal atrophy were significantly greater than in the vehicle controls. Neuroendocrine cell hyperplasia was observed in females at 6 months and males and females at 12 months and at 2 years. In core study female rats, there was a positive trend in the incidences of squamous cell papilloma or carcinoma (combined) of the forestomach, and the incidence in the 150 mg/kg group exceeded the historical control range.

The incidences of renal tubule proliferative lesions in male rats were suggestive of a neoplastic effect in the kidney. Therefore, additional step sections of the kidneys of male rats were prepared. The incidences of renal tubule hyperplasia and adenoma in the extended evaluation and the combined incidences of standard and step sections in the 75, 150, and 300 mg/kg

groups were greater than those in the vehicle controls. The incidences of nephropathy were increased in all dosed groups of females, and the increase was significant in the 300 mg/kg group.

In dosed groups of male rats, there was a positive trend in the incidences of malignant mesothelioma, and the incidences were significantly greater in 150 and 300 mg/kg males than in the vehicle controls. The incidences of mammary gland fibroadenoma in 75 and 150 mg/kg males were significantly increased. The incidences of fibroma of the subcutaneous tissue in 37 and 75 mg/kg males and the combined incidences of fibroma or fibrosarcoma in 37, 75, and 150 mg/kg males were significantly increased.

2-YEAR STUDY IN MICE
Groups of 50 male and 50 female mice received methyleugenol in 0.5% methylcellulose by gavage at doses of 0, 37, 75, or 150 mg/kg for 105 weeks. Special study groups of 10 male and 10 female mice administered 37, 75, or 150 mg/kg were designated for toxicokinetic studies.

Survival and Body Weights
Survival of all dosed groups of male mice was similar to that of the vehicle controls. Survival of dosed groups of females was significantly less. Mean body weights of dosed mice were generally less than those of the vehicle controls throughout the studies.

Pathology Findings
Chemical-related increases in the incidences of liver neoplasms and nonneoplastic lesions in mice included hepatocellular adenoma and carcinoma, hepatoblastoma, hepatocholangiocarcinoma, eosinophilic foci, oval cell hyperplasia, bile duct hyperplasia, hemosiderin pigmentation, chronic active inflammation, and hematopoietic cell proliferation. In all dosed groups of males and females, the incidences of hepatocellular neoplasms and the multiplicity of neoplasms were generally greater than in the vehicle controls. The incidences of hepatoblastoma were significantly increased in all dosed groups of females and slightly increased in 150 mg/kg males. Hepatocholangiocarcinoma was observed in 150 mg/kg females. The incidences of eosinophilic foci, oval cell hyperplasia, portal hypertrophy, hepatocyte necrosis, hematopoietic cell proliferation, bile duct hyperplasia, and

hemosiderin pigmentation were significantly increased in two or more dosed groups of male and/or female mice.

The incidences of glandular ectasia, mucosal atrophy, chronic active inflammation, epithelial hyperplasia, and neuroendocrine cell hyperplasia of the glandular stomach were increased in one or more dosed groups of male and female mice. In addition, malignant neuroendocrine tumors were observed in the glandular stomach of two 150 mg/kg male mice; one male in this group had a carcinoma.

TOXICOKINETIC STUDIES
Methyleugenol is rapidly absorbed following oral administration to rats and mice. The kinetic data are consistent with rapid clearance from the blood, metabolism in the liver, and excretion of the parent and various metabolites in the urine.

GENETIC TOXICOLOGY
Methyleugenol was not mutagenic in *S. typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without exogenous metabolic activation (S9). In cytogenetic tests with cultured Chinese hamster ovary cells, methyleugenol induced sister chromatid exchanges in the presence of S9, but no induction of chromosomal aberrations was noted in cultured Chinese hamster ovary cells following exposure to methyleugenol, with or without S9. *In vivo*, no increase in the frequency of micronucleated normochromatic erythrocytes was seen in male or female mice administered methyleugenol by gavage for 14 weeks.

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL
A physiologically based pharmacokinetic (PBPK) model resulting from intravenous and oral exposure was created to characterize tissue concentrations of methyleugenol in rats and mice. Data used to create the model were obtained from the literature or from current studies. The primary conclusions that can be reached from the PBPK model are: 1) absorption of oral doses of methyleugenol in rats and mice is rapid and complete, 2) distribution of methyleugenol to

tissues is not hampered by capillary permeability, and 3) metabolism of methyleugenol is saturable and must have some extrahepatic component in the mouse. Model-based plasma methyleugenol concentrations were not found to be good dosimeters for evaluating neoplasm dose-response data.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** of methyleugenol in male and female F344/N rats based on the increased incidences of liver neoplasms and neuroendocrine tumors of the glandular stomach in male and female rats and the increased incidences of kidney neoplasms, malignant mesothelioma, mammary gland fibroadenoma, and subcutaneous fibroma

and fibroma or fibrosarcoma (combined) in male rats. A marginal increase in the incidence of squamous cell neoplasms of the forestomach may have been related to methyleugenol administration in female rats. There was *clear evidence of carcinogenic activity* of methyleugenol in male and female B6C3F$_1$ mice based on the increased incidences of liver neoplasms. Neuroendocrine tumors of the glandular stomach in male mice were also considered related to methyleugenol administration.

In male and female rats and mice, methyleugenol administration caused significant increases in the incidences of nonneoplastic lesions of the liver and glandular stomach.

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*   Explanation of Levels of Evidence of Carcinogenic Activity is on page 14. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 16.

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses in methylcellulose by gavage	0, 37, 75, or 150 mg/kg or 300 mg/kg (stop-exposure)	0, 37, 75, or 150 mg/kg or 300 mg/kg (stop-exposure)	0, 37, 75, or 150 mg/kg	0, 37, 75, or 150 mg/kg
Body weights	Dosed groups less than the vehicle control group	Dosed groups less than the vehicle control group	Dosed groups less than the vehicle control group	Dosed groups less than the vehicle control group
Survival rates	20/50, 16/50, 15/50, 0/50, 0/50	22/50, 25/50, 22/50, 11/50, 16/50	38/49, 36/50, 37/50, 35/50	31/50, 18/50, 18/50, 2/50
Nonneoplastic effects	Liver: eosinophilic foci (11/50, 28/50, 43/50, 47/50, 39/50); mixed cell foci (1/50, 7/50, 14/50, 8/50, 2/50); hepatocyte hypertrophy (0/50, 13/50, 25/50, 30/50, 26/50); oval cell hyperplasia (14/50, 17/50, 24/50, 34/50, 27/50); cystic degeneration (4/50, 2/50, 25/50, 38/50, 41/50) Glandular stomach: neuroendocrine cell hyperplasia (0/50, 0/50, 1/50, 8/50, 8/50); atrophy (0/50, 14/50, 32/50, 37/50, 29/50)	Liver: eosinophilic foci (10/50, 20/50, 27/49, 31/49, 37/50); mixed cell foci (6/50, 4/50, 19/49, 9/49, 7/50); hepatocyte hypertrophy (1/50, 13/50, 16/49, 26/49, 31/50); oval cell hyperplasia (1/50, 15/50, 19/49, 35/49, 34/50); bile duct hyperplasia (11/50, 11/50, 17/49, 22/49, 30/50); cystic degeneration (0/50, 0/50, 1/49, 4/49, 29/50) Glandular stomach: neuroendocrine cell hyperplasia (0/50, 5/50, 11/50, 9/50, 3/50); atrophy (3/50, 41/50, 45/50, 39/50, 33/50)	Liver: eosinophilic foci (10/49, 20/50, 25/50, 19/50); oval cell hyperplasia (0/49, 8/50, 27/50, 46/50); hepatocyte hypertrophy (0/49, 1/50, 7/50, 46/50) Glandular stomach: atrophy (0/49, 3/48, 35/49, 45/50); hyperplasia (0/49, 1/48, 15/49, 20/50); ectasia (13/49, 25/48, 40/49, 49/50)	Liver: oval cell hyperplasia (0/50, 46/50, 36/49, 38/50); hepatocyte hypertrophy (0/50, 10/50, 7/49, 23/50); hepatocyte necrosis (5/50, 9/50, 16/49, 17/50); hematopoietic cell proliferation (4/50, 14/50, 23/49, 24/50); bile duct hyperplasia (1/50, 1/50, 11/49, 9/50); hemosiderin pigmentation (0/50, 11/50, 24/49, 19/50) Glandular stomach: atrophy (0/45, 0/49, 10/46, 10/45); ectasia (14/45, 33/49, 31/46, 38/45)

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Neoplastic effects	Liver: hepatocellular adenoma (5/50, 12/50, 23/50, 38/50, 32/50); hepatocellular carcinoma (2/50, 3/50, 14/50, 25/50, 36/50); hepatocellular adenoma or carcinoma (7/50, 14/50, 28/50, 43/50, 45/50); hepatocholangioma (0/50, 0/50, 0/50, 1/50, 6/50); hepatocholangiocarcinoma (0/50, 0/50, 1/50, 1/50, 7/50); hepatocholangioma or hepatocholangiocarcinoma (0/50, 0/50, 1/50, 2/50, 13/50)	Liver: hepatocellular adenoma (1/50, 8/50, 11/49, 33/49, 43/50); hepatocellular carcinoma (0/50, 0/50, 4/49, 8/49, 22/50); hepatocellular adenoma or carcinoma (1/50, 8/50, 14/49, 34/49, 43/50); hepatocholangioma (0/50, 0/50, 0/49, 0/49, 8/50); hepatocholangiocarcinoma (0/50, 0/50, 0/49, 3/49, 9/50); hepatocholangioma or hepatocholangiocarcinoma (0/50, 0/50, 0/49, 3/49, 17/50)	Liver: hepatocellular adenoma (26/49, 43/50, 38/50, 39/50); hepatocellular carcinoma (10/49, 20/50, 19/50, 9/50); hepatocellular adenoma or carcinoma (31/49, 47/50, 46/50, 40/50); hepatoblastoma (0/49, 0/50, 1/50, 3/50) Glandular stomach: malignant neuroendocrine tumor (0/49, 0/48, 0/49, 2/50)	Liver: hepatocellular adenoma (20/50, 48/50, 46/49, 41/50); hepatocellular carcinoma (7/50, 37/50, 47/49, 47/50); hepatocellular adenoma or carcinoma (25/50, 50/50, 49/49, 49/50); hepatoblastoma (0/50, 6/50, 11/49, 15/50); hepatocholangiocarcinoma (0/50, 0/50, 0/49, 2/50)
	Glandular stomach: benign neuroendocrine tumor (0/50, 0/50, 0/50, 3/50, 2/50); malignant neuroendocrine tumor (0/50, 0/50, 0/50, 4/50, 2/50); benign or malignant neuroendocrine tumor (0/50, 0/50, 0/50, 7/50, 4/50)	Glandular stomach: benign neuroendocrine tumor (0/50, 0/50, 13/50, 9/50, 5/50); malignant neuroendocrine tumor (0/50, 1/50, 12/50, 26/50, 36/50); benign or malignant neuroendocrine tumor (0/50, 1/50, 25/50, 34/50, 41/50)		
	Kidney: renal tubule adenoma (standard and extended evaluations combined - 4/50, 6/50, 17/50, 13/50, 20/50)			
	Malignant mesothelioma: (1/50, 3/50, 5/50, 12/50, 5/50)			
	Mammary gland: fibroadenoma (5/50, 5/50, 15/50, 13/50, 6/50)			
	Skin (subcutaneous): fibroma (1/50, 9/50, 8/50, 5/50, 4/50); fibroma or fibrosarcoma (1/50, 12/50, 8/50, 8/50, 4/50)			
Uncertain findings		Forestomach: squamous cell papilloma or carcinoma (0/50, 0/50, 1/50, 3/50, 1/50)		
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence

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

Genetic toxicology

Salmonella typhimurium gene mutations: Negative in strains TA98, TA100, TA1535, and TA1537, with and without S9

Sister chromatid exchanges
  Cultured Chinese hamster ovary cells *in vitro*:   Positive with S9, negative without S9

Chromosomal aberrations
  Cultured Chinese hamster ovary cells *in vitro*:   Negative with and without S9

Micronucleated erythrocytes
  Mouse peripheral blood *in vivo*:   Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on methyleugenol on 30 October 1998 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 30 October 1998, the draft Technical Report on the toxicology and carcinogenesis studies of methyleugenol received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of methyleugenol by discussing the uses of the chemical, describing the rationale for the study and the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in rats and mice. The proposed conclusions for the 2-year gavage studies of methyleugenol were *clear evidence of carcinogenic activity* in male and female F344/N rats and B6C3F$_1$ mice.

Dr. R.A. Herbert, NIEHS, characterized the lesions in the fundic region of the glandular stomach associated with methyleugenol administration in male and female rats and mice. These lesions included atrophy and neuroendocrine cell hyperplasia, as well as benign and malignant neuroendocrine tumors, which are rare in rats and mice as either spontaneous or chemically induced lesions. Dr. Herbert described a series of short-term (14-, 30-, and 90-day) studies providing data that supported the hypothesis that parietal cell cytotoxicity with subsequent mucosal atrophy, increased intragastric pH, and increased circulating gastrin (hypergastrinemia) is probably how methyleugenol produces neuroendocrine tumors in the glandular stomach.

Dr. M.L. Cunningham, NIEHS, presented data from work in progress that described *in vivo* and *in vitro* studies of methyleugenol metabolism in rodents and some recent results from human model systems. He began by describing the more widely studied metabolism of the close structural analogue and hepatocarcinogen, safrole, and contrasted the results with those obtained for methyleugenol. The findings to date indicate that methyleugenol can undergo a variety of Phase 1 oxidation reactions, that metabolites can be further metabolized through Phase 2 conjugations to yield reactive sulfonoxy metabolites, and that human

tissue preparations are capable of metabolizing and bioactivating the chemical. The genetic toxicity of methyleugenol is similar to safrole and for both compounds appears to be dependent on both Phase 1 and Phase 2 metabolic activation.

Dr. T.R. Devereux, NIEHS, provided information on molecular alterations in neoplasms from the NTP study, concentrating on the mouse liver and lung neoplasms for which there is a large database of genetic information. She focused on the APC/β-catenin-Wnt signaling pathways that have been implicated in various human and rodent cancers. In neoplasm cells, either a mutation in the APC gene or in β-catenin can upregulate β-catenin and the Wnt signaling pathway, leading eventually to cell proliferation. β-Catenin mutations were found in about half of the methyleugenol mouse liver neoplasms compared with mutations in only 5% of spontaneous neoplasms. Mutations were found at the same sites as those in human hepatocellular carcinomas, suggesting similar carcinogenic pathways. Genetic alterations were not found in H-*ras* or p53, suggesting that these genes are not involved in methyleugenol-induced mouse liver carcinogenesis.

Dr. G.M. Blumenthal, NIEHS, discussed the development of physiologically based pharmacokinetic models to describe and simulate the toxicokinetics of methyleugenol in rats and humans. Animal data were obtained from single-dose administration to rats at 37 mg/kg by intravenous injection and by gavage at 37, 75, and 150 mg/kg. Human data were obtained from an in-house study in which volunteers ate 12 gingersnaps; blood samples were collected prior to exposure and 15, 30, 60, and 120 minutes afterward. Data was also obtained from the NHANES database collected by the Centers for Disease Control and Prevention. The studies to date show that absorption of methyleugenol was rapid in rats and humans with a large first pass effect in rats that was also assumed in humans. Metabolism was saturated at all doses in rats, while a slower metabolism was predicted in humans. Over 90% of the doses were metabolized within 24 hours in rats, and this value was assumed for the human model. More studies are in process and should lead to an entire dose response characterization.

Dr. Hecht, a principal reviewer, agreed with the proposed conclusions. He wondered why, considering the structural similarity to safrole and human exposure to methyleugenol, the NTP had not studied this chemical earlier.

Dr. Cullen, the second principal reviewer, agreed with the proposed conclusions. He thought the study was remarkable because of the presence of two unusual neoplasms. In the liver, unusual mixed neoplasms composed of cholangiocellular and hepatocellular elements suggest a potent carcinogenic effect that affects both biliary and hepatic cell lineage or, possibly, a stem-cell population. He said that gastric neuroendocrine tumors are also rare and thought it prudent that immunohistochemical and histochemical stains were done to establish the cell type. Dr. Herbert noted that some of the liver neoplasms appeared to have a hepatocellular component and a biliary cell component; therefore, the diagnoses of hepatocholangiocellular neoplasms were most descriptive. Dr. Cullen said the dose-related increases in the incidences of oval cell hyperplasia in mice suggested further discussion of the possibility of a synergistic effect with the presence of *Helicobacter* and this lesion.

Dr. Bus, the third principal reviewer, agreed with the proposed conclusions. He complimented the NTP on the extensive toxicokinetic and disposition studies including information on how disposition may change with time and with the age of the animals. Noting that the low dose in the rodent studies, 37 mg/kg, was metabolically saturating and likely not a no-observed-effect level, Dr. Bus suggested there were lessons here for future protocol designs to provide data more valuable for future risk assessment purposes. Dr. J.R. Bucher, NIEHS, commented that methyl-

eugenol is listed as a "generally recognized as safe" substance in the United States, and although there is a 5 mg/kg limit in Europe, there are not large differences between concentrations permitted in foods and the 37 mg/kg dose used in rats and mice. Palatability may be the limiting factor.

Dr. Medinsky cautioned that bioavailability of a compound is less relevant when a metabolite is the active/toxic form. Dr. Bailer commented that he was a bit uncomfortable with the possible utility of data for risk assessment purposes when the lowest animal dose is approximately 37,000 times the human dose (the gingersnap study). Dr. G.W. Lucier, NIEHS, pointed out that the blood levels from the NHANES study were only about 1,000-fold greater than the rat blood levels.

Dr. Tim Adams, Flavor and Extract Manufacturers Association (FEMA), stated that actual exposure to methyleugenol has substantially decreased over the last 30 years, with most coming from fruits and spices. Further, FEMA estimates that exposure in the diet exceeds intentional addition by a factor of at least 100. With regard to the neuroendocrine lesions in the stomach, he noted that the agent was given by gavage in a microencapsulated form, perhaps allowing for prolonged stomach exposure. Dr. Bucher said the methyleugenol was in methylcellulose, the gavage vehicle, and not microencapsulated.

Dr. Hecht moved that the Technical Report on methyleugenol be accepted with revisions discussed and the conclusions as written for male and female rats and mice, *clear evidence of carcinogenic activity*. Dr. Cullen seconded the motion, which was accepted unanimously with six votes.

INTRODUCTION
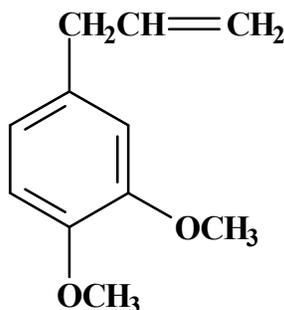
$$CH_2CH = CH_2$$

OCH$_3$

OCH$_3$

METHYLEUGENOL

CAS No. 93-15-2

Chemical Formula: $C_{11}H_{14}O_2$ Molecular Weight: 178.2

Synonyms: 4-Allyl-1,2-dimethoxybenzene; 4-allylveratrole; 4-allyl-3,4-dimethoxy-benzene; 1,2-dimethoxy-4-allylbenzene; 3,4-dimethoxyallylbenzene ENT 21040; 1-(3,4-dimethoxyphenyl)-2-propene; eugenol methyl ether; 1,3,4-eugenol methyl ether; veratrole methyl ether

CHEMICAL AND PHYSICAL PROPERTIES

Methyleugenol is a colorless to pale yellow, oily liquid with a clove-carnation odor and a bitter taste. It is soluble in ethanol, ethyl ether, chloroform, and most other organic solvents but is insoluble in water, glycol, and propylene glycol. Methyleugenol darkens and thickens slowly when exposed to air and evaporates readily at room temperature (Lide, 1998; *Sax's*, 1992). Methyleugenol has a melting point of –4° C, a boiling point of 254.7° C, a refractive index of 1.532, and a density of 1.0396 at 20° C.

PRODUCTION, USE, AND HUMAN EXPOSURE

Methyleugenol is produced by methylation of eugenol (Opdyke, 1979). The annual production in the United States is estimated at 25,000 pounds (SRI, 1990). Methyleugenol was given generally recognized as safe (GRAS) status in 1965 and is approved by the FDA for use in food (21 CFR 121.1164). Methyleugenol is a natural constituent of a large number of essential oils including rose, basil, hyacinth, pimento, citronella (FEMA, 1978), anise (400 ppm), nutmeg, mace, cinnamon leaves (*Fenaroli's*, 1975), pixuri seeds (520 ppm) (Carlini *et al.*, 1983), and laurel fruits and leaves (*Farm Chemical Handbook*, 1992). The chemical has also been identified in blackberry essence, bananas, black pepper, and bilberries (WHO, 1981). Methyleugenol was detected in the oil (240 ppm) and juice (42 ppb) of oranges treated with abscission chemicals such as cycloheximide (MacGregor *et al.*, 1974). Methyleugenol (0.02 mg/L) has been detected in wastewater effluent from a paper mill (Moshonas and Shaw, 1978).

Methyleugenol is used as a flavoring agent in jellies (52 ppm), baked goods (13 ppm), nonalcoholic beverages (10 ppm), chewing gum, candy (11 ppm), pudding, relish, and ice cream (4.8 ppm) (*Fenaroli's*, 1975). It is also used as a fragrance in perfumes (0.3% to 0.8%), creams and lotions (0.01% to 0.05%), and soaps and detergents (0.02% to 0.2%)

(Opdyke, 1979). One of the major uses for methyleugenol is as an insect attractant. In 1982, methyleugenol was used in combination with malathion to control an outbreak of oriental fruit flies in California (Hays and Laws, 1991). Methyleugenol has also been used as an anesthetic in rodents (Carlini *et al.*, 1981).

The Council of Europe (1974) has listed the acceptable daily intake of methyleugenol as 5 mg/kg. The per capita intake of methyleugenol in foods is estimated at 0.073 mg/day (WHO, 1981). More recent estimates put the daily per capita consumption of methyleugenol at 0.26 μg/kg body weight (Stofberg and Grundschober, 1987; NAS, 1989). The National Occupational Exposure Survey (1981-1983) estimated that 2,824 workers in the United States were potentially exposed to methyleugenol annually (NIOSH, 1990).

ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION

Experimental Animals
Solheim and Scheline (1976) studied the metabolism of methyleugenol (3,4-dimethoxyallylbenzene) and methylisoeugenol (3,4-dimethoxypropenylbenzene) in male albino Wistar-derived rats. The distribution of metabolites did not change significantly when these two compounds were administered neat by intraperitoneal injection or as a suspension in water by gavage. No unchanged compound was found in either urine or bile.

Humans
The major pathways of metabolism of methyleugenol include the oxidation of the allylic side chain, the formation of the hydroxy acid via epoxidation of the double bond followed by hydration, *O*-demethylation, and hydroxylation of the benzene ring.

The metabolism of allylbenzene compounds including methyleugenol to allyl epoxides has been investigated in several studies (Borchert *et al.*, 1973; Stillwell *et al.*, 1974; Solheim and Scheline, 1976; Delaforge *et al.*, 1980a,b). Epoxides, because of their high reactivity toward cell components, have been implicated as ultimate carcinogens (Sims and Grover, 1974). The epoxides of allylbenzene are relatively stable.

The conversion of safrole epoxide to catechol epoxide demonstrated that the cleavage of the methylenedioxy group may occur with epoxide function intact (Delaforge *et al.*, 1980a,b). The hydroxylation at the C-1′ position and the eventual formation of 3,4-dimethoxycinnamyl alcohol are also thought to represent a key metabolic step that accounts for 60% of the administered dose. The 1′-hydroxy metabolites of safrole and estragole are converted by hepatic sulfotransferase to sulfate conjugates that are thought to be the ultimate carcinogens (Miller *et al.*, 1983). *O*-Demethylation resulting in the formation of phenolic metabolites accounts for only 11% of the total dose of methyleugenol. These phenolic metabolites are likely to be rapidly conjugated and excreted with the allylic group intact (Solheim and Scheline, 1976).

Humans are continually exposed to low levels of allylbenzene compounds that, through their metabolism to epoxides, may be toxic. The concentration of these compounds in humans depends on the rate of synthesis and the rate of deactivation by microsomal enzymes (Delaforge *et al.*, 1980a,b). Ioannides *et al.* (1981) have reported that compounds with intact allyl and methylenedioxyphenyl groups (safrole and isosafrole) were inducers of cytochromes P_{450} and P_{448}. Compounds containing an intact allyl group only (estragole and allylbenzene) or an oxidized allyl and an intact methylenedioxyphenyl group (epoxysafrole) were inducers of P_{448} only.

Results of studies (Gardner *et al.*, 1997) with liver microsome preparations from Fischer 344 rats and humans suggest that the hydroxylation of methyleugenol to the proximate carcinogen 1′-hydroxymethyleugenol is catalyzed by P_{450} isozyme P_{450} 2E1 and by an unidentified isozyme (probably CYP2C6). These researchers also found that methyleugenol caused a dose-dependent autoinduction of this hydroxylation reaction in rats. Hydroxylation of methyleugenol activities varied considerably (37-fold) in the human liver samples tested; the highest hydroxylation activity was similar to that of the liver microsomes of control rats. These results suggest that the risk to humans ingesting methyleugenol is subject to marked inter-individual variability.

PHARMACOLOGY

Methyleugenol is a central nervous system depressant with anesthetic, hypothermic, myorelaxant, and anticonvulsant properties. Jiang *et al.* (1982) studied the pharmacologic effects of methyleugenol in various animals. As a central nervous system depressant, it acted synergistically to prolong the sedative effect of both sodium phenobarbital and sodium thiopental. Rabbits, cats, dogs, and monkeys administered a constant infusion of 50 mg methyleugenol/kg body weight lost pain sensation and the righting and hearing reflexes and had a slowing of respiration and the corneal reflex. Cats had increased salivation, and dogs exhibited vomiting and diarrhea. With slower infusion of massive doses (224 mg/kg total), respiration in the dog could be slowed or stopped altogether, but this effect could be reversed with artificial respiration. Infusion in cats caused a reversible drop in blood pressure. Infusion of 50 mg/kg for 1 minute inhibited the spontaneous electroencephalogram of the rabbit cortex and midbrain reticular activating system for 11 minutes; intraperitoneal injection lowered the body temperature of rats.

Dallmeier and Carlini (1981) found that methyleugenol injected intraperitoneally was one of the most potent of nine congeners tested for anesthetic, hypothermic, and myorelaxant effects in rats and mice. A dose-related anesthetic effect was observed with eugenol and methyleugenol at doses between 1.2 and 2.4 mmol/kg. Both of these chemicals induced hypothermia, causing a $3°$ C decrease in rectal temperature of rats administered 1.2 mmol/kg by intraperitoneal injection.

Methyleugenol administered in a rectal suppository prevented carbacol-induced spasms of the urinary bladder in humans (Deininger and Wolfe, 1977). The antispasmodic activity of methyleugenol was demonstrated on the isolated guinea pig ileum (Wagner, 1980). Methyleugenol administered by gavage at doses of 50 or 100 mg/kg increased phenobarbital- and ethanol-induced sleep time of mice (Seto and Keup, 1969).

TOXICITY

Methyleugenol is moderately toxic. The median lethal oral doses were 810 to 1,560 mg/kg for rats and 540 mg/kg for mice. The undiluted chemical (98% purity) was neither an eye irritant nor a skin irritant to rats and mice (Beroza *et al.*, 1975). No information related to the toxicity of methyleugenol in humans was found in a review of the available literature.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No information describing reproductive or developmental toxicity studies of methyleugenol was found in the literature.

CARCINOGENICITY

Experimental Animals

Miller *et al.* (1983) studied the carcinogenicity of alkylbenzenes, including methyleugenol and 1′-hydroxymethyleugenol, in male B6C3F$_1$ mice administered the chemical prior to weaning. The chemicals were dissolved in tricotanoin and injected intraperitoneally on lactation days 1, 8, 15, and 22. The total dose administered per mouse was 4.75 μmol methyleugenol and 2.85 μmol 1′-hydroxymethyleugenol. The mice were weaned at 4 weeks and maintained on a purified diet for up to 18 months. At week 13, the livers were examined by laparotomy, and mice with extensive liver tumors were sacrificed; remaining animals were sacrificed at 18 months. The results of this study, increased incidences and multiplicity of liver tumors, clearly showed that methyleugenol and the 1′-hydroxy metabolite were carcinogenic to the liver. Other alkylbenzene compounds found to be carcinogenic included safrole and estragole. Eugenol was inactive when administered in the diet for 12 months or injected intraperitoneally to nursing male mouse pups.

Estragole, safrole, isosafrole, and eugenol were investigated for carcinogenicity in long-term studies, and the results were similar to those reported by Miller *et al.* (1983). Safrole and isosafrole administered by stomach tube daily at doses of 464 mg/kg (safrole) and 215 mg/kg (isosafrole) to mice from day 7 to day 28 of age, then in the feed at concentrations of 1,112 ppm and 517 ppm, respectively, have been reported to produce liver tumors (Innes *et al.*, 1969). Osborne-Mendel rats receiving 5,000 ppm isosafrole in the diet for up to 2 years

developed adenomas and carcinomas of the liver (Hagan *et al.*, 1965). Subcutaneous administration of 0.66 to 6.6 mg safrole in tricaprylin on days 1, 7, and 21 after birth produced liver and lung tumors (IARC, 1976). Fennell *et al.* (1984) presented evidence that the sulfate conjugate of 1′-hydroxysafrole may represent the major ultimate electrophilic and carcinogenic metabolite of safrole. Estragole induced hepatomas in male and female Swiss (CD-1®) mice 11 to 14 weeks following administration by gavage twice weekly for 5 weeks (total dose 25 μmol/g) and in female Swiss (CD-1®) mice given 23% or 46% in feed for 12 months. Estragole administered intraperitoneally as a total dose of 4.75 μmol to male B6C3F$_1$ mice for 13 or 18 months and 9.45 μmol to male C3H mice for 12 months produced hepatomas (CCRIS, 1998).

Eugenol administered in feed showed equivocal evidence of carcinogenicity based on the increased incidences of liver neoplasms in 3,000 ppm male and 6,000 ppm female B6C3F$_1$ mice (NTP, 1983).

Humans
No information related to the carcinogenicity of methyleugenol in humans was found in a review of the available literature.

GENETIC TOXICITY
The published data from mutagenicity studies with methyleugenol, although limited, show no activity in most standard assays but show positive results for DNA reactivity, particularly in mammalian hepatocytes. Negative results were reported in *Salmonella typhimurium* gene mutation studies (Sekizawa and Shibamoto, 1982; Mortelmans *et al.*, 1986; Kettering and Torabinejad, 1995), and in the *E. coli* WP2 uvrA gene reversion test (Sekizawa and Shibamoto, 1982). Positive, dose-related responses were observed with methyleugenol in chromosomal recombination studies in the yeast *Saccharomyces cerevisiae* (Schiestl *et al.*, 1989; Brennan *et al.*, 1996). Methyleugenol has been shown in several studies to interact with mammalian hepatocyte DNA, inducing unscheduled DNA synthesis (UDS) in rat hepatocyte primary cultures (Howes *et al.*, 1990; Chan and Caldwell, 1992; Gardner *et al.*, 1997) or forming DNA adducts in rat and human hepatocytes *in vitro* (Gardner *et al.*, 1997) or newborn mouse (Phillips *et al.*, 1984) or rat

(Gardner *et al.*, 1997) hepatocytes *in vivo*. Metabolic studies have demonstrated the conversion of methyleugenol by cytochrome P$_{450}$ enzymes to the reactive metabolite 1′-hydroxymethyleugenol (Chan and Caldwell, 1992; Gardner *et al.*, 1997). Testing of 1′-hydroxymethyleugenol for induction of UDS in rat hepatocytes showed the metabolite to be more potent than the parent compound (Chan and Caldwell, 1992). The rate and efficiency of hydroxylation of methyleugenol were shown to be variable among human liver samples tested *in vitro*, and the most efficient conversions noted in human hepatocytes matched the rates observed in control rat hepatocytes (Gardner *et al.*, 1997).

More mutagenicity information is available for eugenol. Eugenol, like methyleugenol, was not mutagenic in *S. typhimurium*, in the presence or the absence of S9 activation (Sekizawa and Shibamoto, 1982; Haworth *et al.*, 1983), but positive, dose-related responses were observed with eugenol in chromosomal recombination studies in the yeast *S. cerevisiae* (Schiestl *et al.*, 1989). Positive results were also reported with eugenol in mammalian cell test systems. Increases in both sister chromatid exchanges and chromosomal aberrations were observed in cultured Chinese hamster ovary cells treated with eugenol (Galloway *et al.*, 1987), and increased mutation frequencies were reported in mouse lymphoma cells treated with eugenol (Myhr and Caspary, 1991; Sofuni *et al.*, 1996).

Results of *in vivo* studies with eugenol were generally negative. Eugenol did not induce sex-linked recessive mutations in germ cells of male *Drosophila melanogaster* (Foureman *et al.*, 1994), and no increase in micronucleated erythrocytes was observed in mouse bone marrow following three intraperitoneal injections of 600 mg eugenol in corn oil/kg body weight (Shelby *et al.*, 1993). Additional negative micronucleus test results were reported in mice after intraperitoneal injection of up to 800 mg eugenol/kg body weight and in female rats after oral administration of up to 1,340 mg eugenol/kg body weight (Hayashi *et al.*, 1988; Maura *et al.*, 1989). Male rats also showed no increase in micronucleated erythrocytes in bone marrow after administration of one-half of the median lethal dose of eugenol (Allavena *et al.*, 1992). In contrast to these negative micronucleus test data,

Woolverton *et al*. (1986) reported positive results in mouse bone marrow micronucleus tests with eugenol dissolved in saline and administered by either intraperitoneal injection (up to 147.9 mg/kg) or gavage (14,794 mg/kg). Data from unpublished NTP *in vivo* studies with eugenol in male B6C3F$_1$ mice showed a small increase in chromosomal aberrations in bone marrow cells that was judged to be equivocal and a positive result in a single, unconfirmed sister chromatid exchange test.

In conclusion, the mutagenicity test data for methyleugenol clearly demonstrated induction of chromosomal damage *in vitro*, but the evidence for chromosomal damage *in vivo* was weak. Results from gene mutation tests with methyleugenol in bacteria were negative.

STUDY RATIONALE

Methyleugenol was nominated by the National Cancer Institute and the FDA for toxicity and carcinogenicity testing by the NTP based on the high potential for human exposure through flavoring agents and its structural resemblance to safrole, a known carcinogen (IARC, 1976), and to eugenol. Oral administration was chosen because it is the most likely route of human exposure; gavage was chosen because of the unpalatability of methyleugenol in feed.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Methyleugenol

Methyleugenol was obtained from Elan Chemical Company (Newark, NJ) in two lots (8334801 and 9224705). Lot 8334801 was used during the 14-week studies, and lot 9224705 was used during the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) and the study laboratory (Appendix J). Reports on analyses performed in support of the methyleugenol studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a clear, colorless, or pale yellow liquid, was identified as methyleugenol by infrared, ultraviolet/visible (lot 8334801), and nuclear magnetic resonance spectroscopy and density analysis (lot 8334801). The purity of lot 8334801 was determined by elemental analyses, Karl Fisher water analysis, methoxy group determination, thin-layer chromatography (TLC), and gas chromatography. The purity of lot 9224705 was determined by high-performance liquid chromatography (HPLC). For lot 8334801, elemental analyses for carbon and hydrogen were in agreement with theoretical values for methyleugenol. Karl Fischer water analysis indicated $0.07\% \pm 0.01\%$ water. Methoxy group determination indicated a purity of $101.4\% \pm 0.3\%$. TLC indicated a major spot and a trace impurity by one solvent system and a major spot, six trace impurities, and a slight trace impurity by a second system. Gas chromatography by two systems resolved a major peak and impurities with cumulative areas of 0.38% or 0.47% relative to the major peak area. The overall purity of lot 8334801 was determined to be approximately 99%. For lot 9224705, HPLC indicated one major peak and three impurities with areas greater than 0.1% relative to the major peak area. The overall purity of lot 9224705 was determined to be approximately 99%.

Accelerated stability studies of the bulk chemical were performed by the analytical chemistry laboratory using gas chromatography. These studies indicated that methyleugenol is stable as bulk chemical for 2 weeks when stored protected from light at temperatures up to $60°$ C. To ensure stability, the bulk chemical was stored at room temperature ($22°$ C, 14-week studies; $25°$ C, 2-year studies), protected from light in amber glass bottles with Teflon®-lined caps.

Stability was monitored during the 14-week studies with gas chromatography and during the 2-year studies using HPLC. No degradation of the bulk chemical was determined.

Methylcellulose

Methylcellulose (USP/FCC grade) was obtained from Fisher Scientific Company (St. Louis, MO, and Pittsburgh, PA) in three lots. Lot 874544 was used during the 14-week studies, and lots 876672 and 946150 were used during the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and the study laboratory (Appendix J).

Lots 876672 and 946150 were identified as methylcellulose by infrared spectroscopy. Ultraviolet/visible and nuclear resonance spectroscopy were also used to confirm the identity of lot 876672. The purity of lot 876672 was determined by elemental analyses, Karl Fischer water analysis, functional group titration, HPLC, and the complete battery of United States Pharmacopeia (USP) XXI analyses. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for methylcellulose, assuming $1.8°$ of substitution and corrected for 1.94% water; elemental analyses also indicated 0.06% sodium. Karl Fischer water analysis indicated $1.94\% \pm 0.03\%$ water. Functional group titration indicated $30.62\% \pm 0.08\%$ methoxy group content; this value is consistent with the theoretical value, assuming

1.8° of substitution. HPLC indicated one major peak and no impurities with areas of 0.1% or greater relative to the major peak area. The lot met the USP specifications for methylcellulose for all analyses.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using gas chromatography. These studies indicated that methylcellulose was stable as a bulk chemical for 3 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at approximately 25° C in sealed containers protected from light. Stability of both lots was monitored during the 2-year studies by monitoring the methoxy group content. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing methyleugenol with 0.5% aqueous methylcellulose to give the required concentrations (Table J1). The dose formulations were stored at room temperature in the dark in amber glass bottles for up to 18 days (14-week studies) or 35 days (2-year studies). Homogeneity and stability studies were conducted by the study laboratory using HPLC. Homogeneity of a 200 mg/g formulation was confirmed; stability of a 1.0 mg/g formulation was confirmed for 3 weeks at room temperature and when stored protected from light. Samples stored open to air and light showed methyleugenol losses of 3%. Homogeneity of 0.8 and 60 mg/mL formulations were confirmed; stability of a 0.8 mg/mL formulation was also confirmed for 35 days when the formulation was stored in sealed containers with minimal headspace, at room temperature, protected from light.

Periodic analyses of the dose formulations of methyleugenol were conducted at the study laboratory using HPLC at the beginning, midpoint, and end of the 14-week studies (Table J2). During the 2-year studies, the dose formulations were analyzed with ultraviolet spectroscopy approximately every 8 weeks (Table J3). All dose formulations used in the 14-week and 2-year studies were within 11% of the target concentrations. Results of periodic referee analyses of the 14-week dose formulations performed by the

analytical chemistry laboratory agreed with the results obtained by the study laboratory (Table J4).

14-WEEK STUDIES

The 14-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to methyleugenol and to determine the appropriate doses to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). Upon receipt, the rats were 5 weeks old; mice were 5 (females) or 6 (males) weeks old. Animals were quarantined for 10 or 11 days and were 6 (rats and female mice) or 7 (male mice) weeks old on the first day of the studies. Before initiation of the studies, seven male and five female rats and five male and five female mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female control rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix L).

Groups of 9 or 10 male and 10 female rats and mice received methyleugenol in 0.5% methylcellulose by gavage at doses of 0, 10, 30, 100, 300 or 1,000 mg/kg, 5 days per week for 14 weeks; groups of 10 male and 10 female rats and mice received deionized water only. Groups of 10 male and 10 female special study rats received the same doses for 22 or 23 days. Feed and water were available *ad libitum*. Rats were housed five per cage; mice were housed individually. Clinical findings were recorded and the animals were weighed weekly and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Hematology and clinical chemistry analyses were performed on 10 male and 10 female special study rats per group on day 5 and at week 4 and on all core study rats at study termination. At all time points, rats were anesthetized with CO_2, and blood was collected from the retroorbital sinus. Blood samples for hematology analysis were collected in tubes containing EDTA as an anticoagulant. Erythrocyte, platelet, and leukocyte counts, hematocrit values, and hemoglobin concentrations were determined using an Ortho ELT-8 analyzer (Ortho Diagnostic Systems,

Westwood, MA). Leukocyte differentials, reticulo-cyte counts, and erythrocyte, leukocyte, and platelet morphologies were determined using light micro-scopy. Mean cell volumes, mean cell hemoglobin, and mean cell hemoglobin concentrations were calcu-lated from the analyses for hemoglobin concen-trations, hematocrit values, and erythrocyte counts. Clinical chemistry parameters were determined using the Roche Cobas Fara chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). The para-meters measured are listed in Table 1.

At the end of the 14-week studies, samples were collected for sperm motility and vaginal cytology evaluations on vehicle control, 30, 100, and 300 mg/kg rats and vehicle control, 10, 30, and 100 mg/kg mice. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP's sperm morphology and vaginal cytology eval-uations protocol (NTP, 1987). For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were iso-lated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile sper-matozoa 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 de-termined microscopically with the aid of a hema-cytometer. To quantify spermatogenesis, the testic-ular 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.

A necropsy was performed on all core study rats and mice. The heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thick-ness of 5 to 6 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on core study water control, vehicle con-trol, and 1,000 mg/kg rats and water control, vehicle control, and 300 and 1,000 mg/kg mice. Table 1 lists the tissues and organs routinely examined.

2-YEAR STUDIES
Study Design
Groups of 50 male and 50 female rats received methyleugenol in 0.5% methylcellulose by gavage at doses of 37, 75, or 150 mg/kg, 5 days per week for 105 weeks; groups of 60 male and 60 female rats received the 0.5% methylcellulose vehicle only. Stop-exposure groups of 60 male and 60 female rats received 300 mg/kg methyleugenol in 0.5% methyl-cellulose by gavage for 52 weeks followed by the 0.5% methylcellulose vehicle only for the remaining 53 weeks of the study. Five male and female vehicle control rats and five male and five female rats receiv-ing 300 mg/kg were euthanized at 6 and 12 months for histologic evaluation. Groups of 50 male and 50 female mice received methyleugenol in 0.5% methylcellulose by gavage at doses of 0, 37, 75, or 150 mg/kg for 105 weeks. Groups of 10 male and 10 female rats administered 37, 75, 150, or 300 mg/kg and 10 male and 10 female mice admin-istered 37, 75, or 150 mg/kg were designated for plasma toxicokinetic studies.

Source and Specification of Animals
Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Ser-vices (Germantown, NY) for use in the 2-year studies. Rats and mice were quarantined for 11 (males) or 12 (females) days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were 5 to 6 weeks old and mice were 6 to 7 weeks old at the

beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L).

Animal Maintenance

Rats were housed three (males) or five (females) per cage; male mice were housed individually and female mice were housed five per cage. Feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix K.

All animals were observed twice daily. Clinical findings were recorded every 4 weeks; body weights were recorded at the beginning of the studies, every 4 weeks, and at the end of the studies.

Toxicokinetics

Blood samples were collected from the retroorbital sinus of toxicokinetic study rats at 6, 12, and 18 (except 300 mg/kg) months and from toxicokinetic study mice at 12 months. Blood was collected at 7 to 10 post-dosing time points; blood was collected from two or three animals per group at each time point, and blood was collected from each animal at two time points. The time points at which blood was collected from each group are listed in Table 1. At 18 months, 12 to 15 previously undosed male and female rats and mice were given a single dose of 75 mg/kg (mice) or 150 mg/kg (rats) for toxicokinetic studies in aged animals. Blood was collected from three or four rats per group at 9 or 10 time points and from two or three mice per group at five time points. Blood was collected from each rat at two time points, and from each mouse once. The time points at which blood was collected from each group are listed in Table 1. Blood was collected via the retroorbital sinus (rats) or cardiac puncture (mice) into tubes containing EDTA as an anticoagulant. The red cell fraction was separated from the plasma by centrifugation, and the plasma was stored at -20° C until analysis for methyleugenol concentration.

Pathology

A complete necropsy and microscopic examination were performed on all core study rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Neoplasm samples were collected and frozen for oncogene analysis. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all primary lesions in potential target organs, which included the adrenal medulla, bone marrow, glandular stomach, kidney, liver, mammary gland, salivary gland, skin, and spleen of male and female rats; the epididymis and testis of male rats; the uterus of female rats; the bone marrow, glandular stomach, liver, and spleen of male and female mice; and the lung of male mice.

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

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

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Methyleugenol

14-Week Studies	2-Year Studies
Study Laboratory	
Southern Research Institute (Birmingham, AL)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species	
Rats: F344/N	Rats: F344/N
Mice: B6C3F$_1$	Mice: B6C3F$_1$
Animal Source	
Simonsen Laboratories, Inc. (Gilroy, CA)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies	
11 days (rats and female mice) or 10 days (male mice)	11 days (males) or 12 days (females)
Average Age When Studies Began	
6 weeks (rats and female mice) or 7 weeks (male mice)	Rats: 5 to 6 weeks
	Mice: 6 to 7 weeks
Date of First Dose	
Rats: 27 June 1988	Rats: 7 (males) or 8 (females) February 1994
Mice: 11 July 1988	Mice: 25 (males) or 26 (females) October 1993
Duration of Dosing	
14 weeks (5 days per week)	Rats: 105 weeks (5 days per week), except 300 mg/kg group, which received only the 0.5% methylcellulose vehicle from week 53 to study completion
	Mice: 105 weeks (5 days per week)
Date of Last Dose	
Rats: 26-29 September 1988	Stop-exposure rats: 2 (males) or 3 (females) February 1995
Mice: 10-12 October 1988	Core study animals:
	Rats: 5 (males) or 6 and 7 (females) February 1996
	Mice: 23-25 (males) or 25-26 (females) October 1995
Necropsy Dates	
Rats: 27-30 September 1988	Rats: 6 (males) or 7 and 8 (females) February 1996 (core study)
Mice: 11-13 October 1988	9 (males) or 10 (females) August 1994 (6-month interim evaluation)
	2 (males) or 3 (females) February 1995 (12-month interim evaluation)
	Mice: 24-26 (males) or 26-27 (females) October 1995
Average Age at Necropsy	
20 weeks (rats and female mice) or 21 weeks (male mice)	6-month interim evaluation: 33 weeks
	12-month interim evaluation: 58 weeks
	Terminal sacrifice:
	Rats: 111 weeks
	Mice: 110 to 112 weeks
Size of Study Groups	
Rats: 9 or 10 males and 10 females	Rats: 60 males and 60 females (vehicle control and 300 mg/kg)
Mice: 10 males and 10 females	50 males and 50 females (37, 75, and 150 mg/kg)
	Mice: 50 males and 50 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 14-week studies

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Methyleugenol

14-Week Studies	2-Year Studies
Animals per Cage	
Rats: 5	Rats: 3 (males) or 5 (females)
Mice: 1	Mice: 1 (males) or 5 (females)
Method of Animal Identification	
Rats: Tail tattoo	Tail tattoo
Mice: Toe clip	
Diet	
NIH-07 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 14-week studies
Water	
Tap water (Birmingham municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*
Cages	
Polycarbonate (Lab Products, Inc., Maywood, NJ), changed twice weekly (rats) or once weekly (mice)	Same as 14-week studies, except changed twice weekly (rats and female mice) or once weekly (male mice)
Bedding	
Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed twice weekly (rats) or once weekly (mice)	Same as 14-week studies, except changed twice weekly (rats and female mice) or once weekly (male mice)
Racks	
Stainless steel (Lab Products Inc., Maywood, NJ), changed and rotated every 2 weeks	Same as 14-week studies
Animal Room Environment	
Temperature: 72° ± 3° F	Temperature: 72° ± 3° F
Relative humidity: 50% ± 15%	Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day	Room fluorescent light: 12 hours/day
Room air changes: minimum of 10/hour	Room air changes: minimum of 10/hour
Dose Concentrations	
0, 10, 30, 100, 300, or 1,000 mg/kg body weight in 0.5% methylcellulose at a volume of 5 (rats) or 10 (mice) mL/kg body weight	Core study:
	Rats: 0, 37, 75, 150, or 300 mg/kg (Dosing volume=5 mL/kg body weight)
	Mice: 0, 37, 75, or 150 mg/kg (Dosing volume=10 mL/kg body weight)
Type and Frequency of Observation	
Observed twice daily; clinical findings were recorded and animals were weighed weekly and at the end of the studies.	Observed twice daily; animals were weighed at the beginning of the studies, every 4 weeks, and at the end of the studies. Clinical findings were recorded every 4 weeks and at the end of the studies.
Method of Sacrifice	
Carbon dioxide asphyxiation	Carbon dioxide asphyxiation

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Methyleugenol

14-Week Studies	2-Year Studies
Necropsy	
Necropsy was performed on all core study animals. Organs weighed were the heart, right kidney, liver, lung, spleen, right testis, and thymus.	Necropsy was performed on all animals.
Clinical Pathology	
Blood was collected from the retroorbital sinus of special study rats on day 5 and at week 4 and from all core study rats surviving to the end of the studies for hematology and clinical chemistry determinations. *Hematology:* hematocrit; hemoglobin concentration; erythrocyte and reticulocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count and morphology; leukocyte count and differentials *Clinical chemistry:* urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and total bile acids	None
Histopathology	
Complete histopathology was performed on all core study water control, vehicle control, and 1,000 mg/kg rats and on all water control, vehicle control, and 300 and 1,000 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (with adjacent skin), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the kidney, liver, salivary gland, and stomach of male and female rats and the adrenal gland and spleen of male rats in the 10, 30, 100, and 300 mg/kg groups, the testis and uterus of rats in the 100 and 300 mg/kg groups, the stomach of male and female mice in the 10, 30, and 100 mg/kg groups, the liver of 100 mg/kg male mice and 10, 30, and 100 mg/kg female mice, and the nose of 100 mg/kg male and female mice were examined.	Complete histopathology was performed on all 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, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (with adjacent skin) (except male mice), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology	
At the end of the studies, sperm samples were collected from vehicle control, 30, 100, and 300 mg/kg male rats and vehicle control, 10, 30, and 100 mg/kg male mice for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda epididymis, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from vehicle control, 30, 100, and 300 mg/kg female rats and vehicle control, 10, 30, and 100 mg/kg female mice for vaginal cytology evaluations. The following parameters were evaluated: the estrous cycle lengths and relative frequency of estrous stages.	None

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Methyleugenol

14-Week Studies	2-Year Studies

Toxicokinetic Studies

None

Blood was collected from the retroorbital sinus of 10 male and 10 female rats given 37, 75, 150, or 300 mg/kg and 10 male and 10 female mice given 37, 75, or 150 mg/kg for toxicokinetic studies. Blood was collected from rats at 6, 12, and 18 months and from mice at 12 months. Blood was collected at the following time points after dosing:

Rats
6 and 12 months
  37 mg/kg: 5, 15, 30, 60, 90, and/or 120 minutes
  75 mg/kg: 5, 30, 90, 120, 240, 360, and/or 450 minutes
  150 mg/kg: 5, 30, 90, 240, 360, 480, and/or 600 minutes
  300 mg/kg: 5, 60, 120, 240, 360, 540, and/or 780 minutes
18 months
  Male
    37 mg/kg: 5, 15, 30, 60, 90, 120, and 240 minutes
    75 mg/kg: 5, 15, 30, 60, 90, 120, 240, and 360 minutes
    150 mg/kg: 5, 30, 90, 240, 360, 480, and 600 minutes
  Female
    37 mg/kg: 5, 10, 15, 30, 45, 60, 90, 120, 180, and
      240 minutes
    75 mg/kg: 5, 15, 30, 60, 90, 120, 240, and 360 minutes
    150 mg/kg: 5, 30, 60, 90, 120, 240, 360, 480, and
      600 minutes

Mice
Male
  37 mg/kg: 5, 10, 20, 30, 40, 60, and 75 minutes
  75 mg/kg: 5, 15, 30, 45, 60, 90, and 150 minutes
  150 mg/kg: 5, 15, 40, 75, 120, 210, and 300 minutes
Female
  37 mg/kg: 5, 10, 20, 30, 40, 50, and 60 minutes
  75 mg/kg: 5, 15, 30, 45, 60, 90, and 150 minutes
  150 mg/kg: 5, 15, 40, 120, 180, and 240 minutes

Single-Dose Toxicokinetics in Aged Animals
Blood was collected from the retroorbital sinus of 14 male and 15 female rats and by cardiac puncture from 12 male and 14 female mice after a single gavage dose of 150 mg/kg (rats) or 75 mg/kg (mice) for determination of methyleugenol concentration in plasma, observed maximum plasma concentration (C_{max}), observed time to achieve maximum plasma concentration (T_{max}), and area under the curve plasma concentration time profile (AUC). Blood was collected at the following time points:
  Male rats: 5, 15, 30, 60, 120, 240, 360, 480, and 600 minutes
  Female rats: 5, 10, 15, 30, 45, 60, 120, 240, 360, and
    480 minutes
  Mice: 5, 20, 50, 90, and 150 minutes

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

Calculation of Incidence
The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A5, B1, B5, C1, C5, D1, and D5 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, to animals that do not reach terminal sacrifice.

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

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of $k=3$ was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F$_1$ mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of lesion incidence, and reported P values are one sided. Values of P greater than 0.5 are presented as 1-P with the letter N added to indicate a lower incidence or negative trend in neoplasm occurrence relative to the control group (e.g., $P=0.99$ is presented as $P=0.01N$). For neoplasms and nonneoplastic lesions detected at the interim evaluation, the Fisher exact test (Gart *et al.*, 1979), a procedure based on the overall proportion of affected animals, was used.

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 have approximately normal distributions, were analyzed with the

parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, plasma concentration, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses.

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of neoplasm incidence in certain instances. Consequently, neoplasm incidences from the NTP historical control database, which is updated yearly, are included in the NTP reports for neoplasms appearing to show compound-related effects.

QUALITY ASSURANCE METHODS

The 14-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of

this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of methyleugenol was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, and increases in the frequency of micronucleated erythrocytes in peripheral blood of mice. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of methyleugenol are part of a larger effort by the NTP to develop a comprehensive database that would permit a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). These short-term genetic toxicity tests were originally developed to elucidate mechanisms of chemical-induced DNA damage growing out of the earlier electrophilicity/mutagenicity relationship proposed by Miller and Miller (1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). Therefore, the information obtained from these tests applies only to mutagenic carcinogens.

For mutagenic carcinogens, there is a strong correlation between a chemical's potential for DNA reactivity, mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of DNA reactivity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in multiple species and genders of rodents and at multiple tissue sites (Ashby and Tennant, 1991). Data from NTP studies show that a positive response in *Salmonella* is the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) and that there is no complementarity among the *in vitro* genetic toxicity tests (Tennant *et al.*, 1987; Zeiger

et al., 1990). That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. Although other *in vitro* genetic toxicity tests correlate less well with rodent carcinogenicity compared with the *Salmonella* test, these other tests can provide useful information on the types of DNA and chromosomal effects induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests appears to be less than the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). Positive responses in long-term peripheral blood micronucleus tests have not been formally evaluated for their predictivity for rodent carcinogenicity. However, because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

RESULTS

RATS
14-Week Study

All rats survived until the end of the study (Table 2). Final mean body weights and body weight gains of 300 and 1,000 mg/kg males and all dosed groups of females were significantly less than those of the vehicle controls; final mean body weights of 1,000 mg/kg males and females were 30% and 16% less than the vehicle controls, respectively (Table 2). Clinical findings possibly related to chemical administration included emaciation and urine staining in 100, 300, and 1,000 mg/kg female rats.

Data for the hematology and clinical chemistry variables are listed in Table F1. At week 14, there was a minimal treatment-related decrease in hemoglobin concentrations and hematocrit values in the 300 mg/kg females and 1,000 mg/kg males and females. The hematocrit and hemoglobin decreases would be consistent with an anemia, but there was no corresponding decrease in erythrocyte counts. There was, however, an erythrocyte microcytosis demonstrated by decreased mean cell volumes in the 300 mg/kg males and 1,000 mg/kg males and females; this change also occurred on day 5 and/or week 4. Animals in the 300 and 1,000 mg/kg groups also had decreased mean cell hemoglobin values; this would be consistent with the decreases in mean cell volumes. This suggests that, while there were equal numbers of circulating erythrocytes for animals in the 300 and 1,000 mg/kg groups at week 14, the erythrocytes were smaller, resulting in lower hematocrit and hemoglobin values. There was evidence of a thrombocytosis at all time points, demonstrated by increased platelet counts in the 100 mg/kg or greater groups. The serum activities of alanine aminotransferase and sorbitol dehydrogenase were increased in the 100 mg/kg or greater male and female groups at various time points and would be consistent with hepatocellular injury or leakage. Additionally, bile acid concentrations were increased in the 300 and 1,000 mg/kg males at all time points and 300 and 1,000 mg/kg females at weeks 4 and 14 and would be consistent with cholestasis or altered hepatic function. However, serum alkaline phosphatase activity, another marker of cholestasis, was either unaffected or decreased in the same animals. A hypoproteinemia and hypoalbuminemia, evidenced by decreased total protein and albumin concentrations, occurred in rats in the 300 and 1,000 mg/kg groups at all time points. There were minimal increases of creatinine concentrations in the 300 and 1,000 mg/kg female rats at all time points. However, urea nitrogen, another marker of renal function, was either unaffected or decreased in the same animals.

TABLE 2
Survival and Body Weights of Rats in the 14-Week Gavage Study of Methyleugenol

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Vehicle Controls (%)
		Initial	Final	Change	
Male					
Water Control	10/10	114 ± 4	327 ± 6	214 ± 4	99
Vehicle Control	10/10	118 ± 4	331 ± 7	214 ± 5	
10	10/10	114 ± 4	331 ± 5	218 ± 5	100
30	10/10	117 ± 5	332 ± 8	216 ± 4	100
100	10/10	119 ± 4	322 ± 7	203 ± 4	97
300	9/9	116 ± 5	304 ± 4**	188 ± 4**	92
1,000	10/10	112 ± 4	231 ± 4**	119 ± 5**	70
Female					
Water Control	10/10	96 ± 2	192 ± 3	96 ± 3	98
Vehicle Control	10/10	97 ± 2	196 ± 4	99 ± 5	
10	10/10	94 ± 2	184 ± 1*	89 ± 2*	93
30	10/10	98 ± 3	187 ± 4*	89 ± 3*	95
100	10/10	97 ± 2	183 ± 2**	86 ± 2**	93
300	10/10	97 ± 3	180 ± 2**	83 ± 3**	92
1,000	10/10	95 ± 3	164 ± 4**	69 ± 3**	84

*   Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a]   Number of animals surviving at 14 weeks/number initially in group
[b]   Weights and weight changes are given as mean ± standard error.

Liver weights of 100, 300, and 1,000 mg/kg males and 300 and 1,000 mg/kg females and testis weights of 1,000 mg/kg males were significantly greater than those of the vehicle control groups (Table G1). Thymus weights in all dosed males and in 1,000 mg/kg females were significantly less than those of the vehicle control groups. The decreases in thymus weights and differences in other organ weights were likely secondary to body weight differences.

No significant differences in sperm motility or in vaginal cytology parameters between dosed and vehicle control groups were observed (Tables H1 and H2).

Chemical-related lesions were observed grossly in the liver of male and female rats, the testis of male rats, and the uterus of female rats administered 1,000 mg/kg. Liver foci were observed in 8 of 10 males and in 1 of 10 females. The testes were enlarged in all dosed male rats; female rats had small (3/10) or thin (3/10) uteri.

No significant differences were observed between the vehicle and water control groups. Compared to the vehicle control groups, there were significant chemical-related increases in the incidences of microscopic lesions in the liver, glandular stomach, adrenal cortex, submandibular salivary gland, testis, and uterus in dosed rats (Table 3). Incidences of hepatic lesions were significantly increased in rats administered 300 or 1,000 mg/kg, and the lesions were generally more severe in males than in females. Hepatic lesions with increased incidences included cytologic alteration, cytomegaly, Kupffer cell pigmentation, bile duct hyperplasia, and foci of cellular alteration. Cytologic alteration was the term used to describe individual hepatocytes with altered tinctorial staining qualities (eosinophilic or basophilic) and increased mitotic activity and, in general, was of minimal to moderate severity. Cytomegaly was characterized by minimal to moderate enlargement of periportal hepatocytes. Kupffer cell pigmentation was a minimal change characterized by the accumulation of yellow-gold to green pigment within the cytoplasm of periportal hepatocytes. Bile duct hyperplasia consisted of minimal to moderate proliferation of small bile ductules within portal areas. Foci of cellular alteration consisted of discrete foci of hepatocytes with altered cytoplasmic staining (eosinophilic, basophilic, or mixed) and were

morphologically similar to those that occur spontaneously or with chemical exposure. One hepatocellular adenoma was present in a male rat administered 1,000 mg/kg.

The incidences of atrophy and chronic inflammation of the mucosa of the glandular stomach were significantly increased in rats administered 300 or 1,000 mg/kg. Lesions were generally of minimal to mild severity in the 300 mg/kg groups and mild to moderate in the 1,000 mg/kg groups. Atrophy consisted of a decrease in the thickness of the gastric mucosa due to generalized loss of glandular epithelial parietal and chief cells accompanied by condensation of the lamina propria. Inflammation was of mild severity and consisted of fibrosis and a diffuse infiltration of the lamina propria by lymphocytes, neutrophils, and macrophages. In addition, there was mild glandular dilatation and increased mitotic activity in the glandular epithelial cells.

The incidences of cortical hypertrophy of the adrenal cortex were significantly increased in 100, 300, and 1,000 mg/kg males and 1,000 mg/kg females. The incidences of cytoplasmic alteration of the submandibular salivary glands were increased in rats administered 30 mg/kg or greater.

Cytoplasmic alteration of the submandibular salivary gland consisted of a loss of cytoplasmic zymogen granules with reduction in the size of serous cells and their ducts. Male rats administered 1,000 mg/kg had significantly increased incidences of moderate dilatation of the seminiferous tubules and testicular degeneration characterized by diffuse loss of spermatogenic cells within the seminiferous tubules. Spermatogonia remaining within the seminiferous and epididymal tubules were morphologically normal. The incidences of mild uterine atrophy were significantly increased in female rats administered 300 or 1,000 mg/kg.

Dose Selection Rationale: Based on lower mean body weights and increased incidences of nonneoplastic liver and glandular stomach lesions in 300 and 1,000 mg/kg rats in the 14-week study, methyleugenol doses selected for rats in the 2-year gavage study were 37, 75, and 150 mg/kg. Stop-exposure groups were given 300 mg/kg for 12 months to provide additional data on progression and regression of liver lesions.

TABLE 3
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Male and Female Rats
in the 14-Week Gavage Study of Methyleugenol

	Water Control	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	1,000 mg/kg
Male							
Liver[a]	10	10	10	10	10	9	10
Cytologic Alteration[b]	0	0	0	0	0	9** (1.2)[c]	10** (3.0)
Cytomegaly	0	0	0	0	0	9** (1.7)	10** (2.8)
Pigment, Kupffer Cell	0	0	0	0	0	0	10** (1.0)
Basophilic Focus	0	0	0	0	0	0	3 (2.3)
Mixed Cell Focus	0	0	0	0	0	2 (1.5)	9** (2.2)
Hyperplasia, Bile Duct	0	0	0	0	0	6** (1.0)	10** (2.1)
Hepatocellular Adenoma	0	0	0	0	0	0	1
Glandular Stomach	10	10	10	10	10	9	10
Atrophy	0	0	0	0	0	7** (1.7)	10** (3.0)
Inflammation, Chronic	0	0	0	0	0	9** (1.2)	10** (2.6)
Adrenal Cortex	10	10	10	10	10	9	10
Hypertrophy	0	0	0	0	4* (1.0)	9** (1.0)	10** (1.0)
Submandibular Salivary Gland	10	10	10	10	10	9	10
Cytoplasmic Alteration	0	0	0	3 (1.0)	10** (1.0)	9** (1.0)	10** (1.0)
Testis	10	10	10	10	10	9	10
Dilatation	0	0	0	0	0	0	10** (2.7)
Degeneration	0	0	0	0	0	0	10** (2.8)
Female							
Liver	10	10	10	10	10	10	10
Cytologic Alteration	0	0	0	0	0	0	10** (2.3)
Cytomegaly	0	0	0	0	0	9** (1.1)	10** (2.2)
Pigment, Kupffer Cell	0	0	0	0	0	0	9** (1.0)
Mixed Cell Focus	0	0	0	0	0	2 (1.0)	8** (1.9)
Hyperplasia, Bile Duct	0	0	0	0	0	0	9** (1.2)
Glandular Stomach	10	10	10	10	10	10	10
Atrophy	0	0	0	0	0	9** (1.0)	10** (2.9)
Inflammation, Chronic	0	0	0	0	6** (1.0)	10** (1.9)	10** (2.3)
Adrenal Cortex	10	10	10	10	10	10	10
Hypertrophy	0	2 (1.0)	0	0	0	0	9** (1.0)
Submandibular Salivary Gland	10	10	10	10	10	10	10
Cytoplasmic Alteration	0	0	0	7** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Uterus	10	10	10	10	10	10	10
Atrophy	0	0	0	0	0	4* (2.5)	10** (2.0)

* Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test
** P≤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

2-Year Study
Survival
Estimates of survival probabilities for male and female rats are shown in Table 4 and in the Kaplan-Meier survival curves (Figure 1). All 150 and 300 mg/kg males died before the end of the study, and survival of 150 mg/kg females was slightly less than that of the vehicle controls.

Body Weights and Clinical Findings
Mean body weights of dosed males and females were less than the vehicle controls throughout most of the study (Figure 2 and Tables 5 and 6). There was little suggestion of recovery in mean body weights in the 300 mg/kg groups when dosing was discontinued for 12 months. There were no clinical findings that could be attributed to methyleugenol administration.

TABLE 4
Survival of Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Male					
Animals initially in study	60	50	50	50	60
6-Month interim evaluation[a]	5	0	0	0	5
12-Month interim evaluation[a]	5	0	0	0	5
Accidental deaths[a]	0	1	3	1	1
Moribund	15	18	15	20	23
Natural deaths	15	15	17	29	26
Animals surviving to study termination	20	16	15	0	0
Percent probability of survival at end of study[b]	40	33	32	0	0
Mean survival (days)[c]	678	659	646	615	538
Survival analysis[d]	P<0.001	P=0.389	P=0.294	P<0.001	P<0.001
Female					
Animals initially in study	60	50	50	50	60
6-Month interim evaluation	5	0	0	0	5
12-Month interim evaluation	5	0	0	0	5
Accidental deaths	0	0	1	1	0
Moribund	17	16	14	26	25
Natural deaths	11	9	13	12	9
Animals surviving to study termination	22	25	22	11	16
Percent probability of survival at end of study	44	50	45	23	32
Mean survival (days)	659	672	675	647	638
Survival analysis	P=0.015	P=0.640N	P=0.807N	P=0.053	P=0.343

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column [the 300 mg/kg (stop-exposure) group was excluded from the trend test], and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dose group is indicated by **N**.
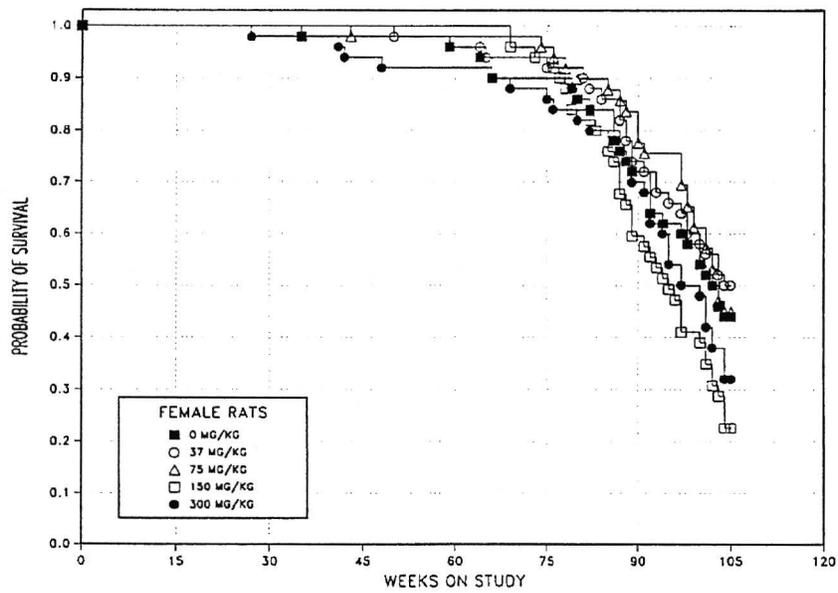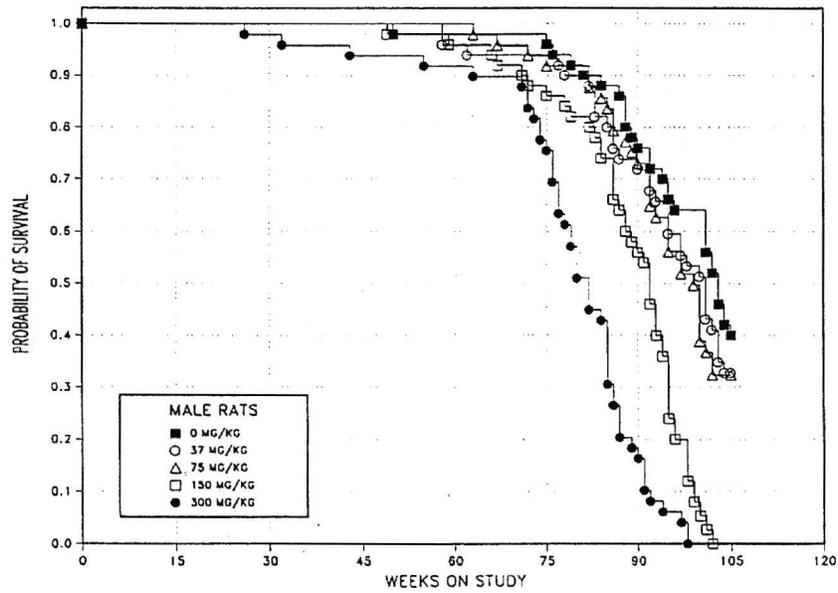
FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats
Administered Methyleugenol by Gavage for 2 Years
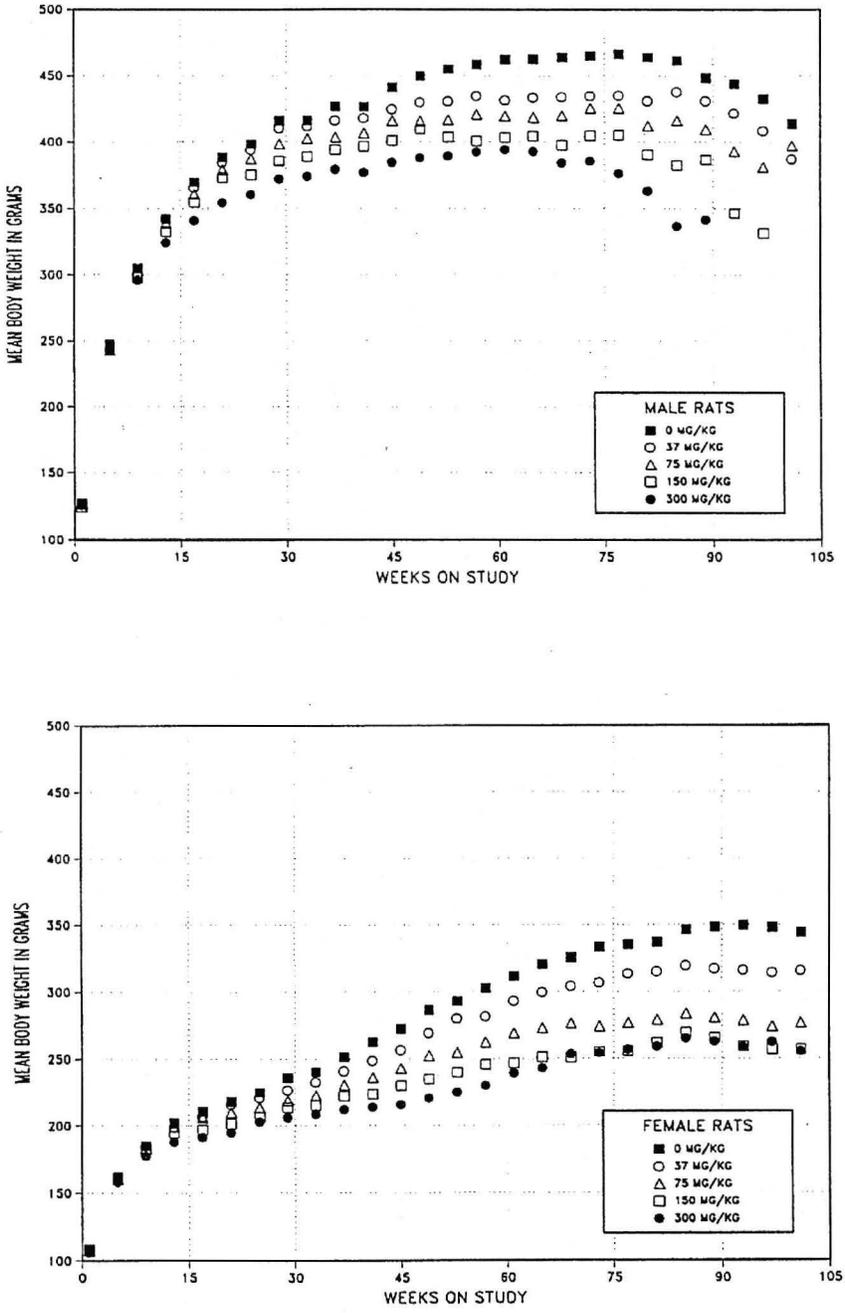
FIGURE 2
Growth Curves for Male and Female Rats
Administered Methyleugenol by Gavage for 2 Years

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of Methyleugenol

Weeks on Study	Vehicle Control		37 mg/kg			75 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	127	60	126	100	50	127	100	50
5	247	60	244	99	50	243	99	49
9	304	60	300	99	50	303	100	49
13	341	60	342	100	50	339	99	49
17	368	60	366	99	50	361	98	49
21	386	60	384	99	50	379	98	49
25	396	60	394	100	50	387	98	49
29	415	55[a]	410	99	50	398	96	49
33	414	55	412	99	50	402	97	49
37	426	55	416	98	50	403	95	49
41	427	55	418	98	50	407	95	49
45	441	55	425	96	50	416	94	49
49	449	55	430	96	50	416	93	49
53	455	49[a]	431	95	50	416	92	49
57	458	49	435	95	50	421	92	49
61	462	49	431	93	48	420	91	49
65	463	49	434	94	47	419	91	48
69	464	49	434	94	47	420	91	47
73	465	49	435	94	47	425	92	46
77	466	47	435	93	47	425	91	44
81	464	46	431	93	45	412	89	44
85	461	44	438	95	41	416	90	41
89	448	39	431	96	36	409	91	37
93	444	36	422	95	33	393	89	31
97	433	32	408	94	28	381	88	25
101	414	32	387	94	25	397	96	18
Mean for weeks								
1-13	255		253	99		253	99	
14-52	414		406	98		397	96	
53-101	454		427	94		412	91	

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of Methyleugenol

Weeks on Study	150 mg/kg				300 mg/kg (Stop-Exposure)		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors		Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	124	98	50		126	100	60
5	245	100	50		243	99	59
9	298	98	50		297	98	59
13	332	97	50		325	95	59
17	355	96	50		341	93	59
21	373	97	50		355	92	59
25	375	95	50		360	91	59
29	385	93	50		371	90	53[a]
33	389	94	50		373	90	52
37	394	92	50		379	89	52
41	397	93	50		376	88	52
45	401	91	50		384	87	51
49	409	91	49		387	86	51
53	404	89	49		389	86	46[a]
57	401	87	49		392	86	45
61	403	87	48		394	85	45
65	404	87	48		393	85	44
69	398	86	46		384	83	44
73	405	87	44		386	83	41
77	405	87	43		376	81	33
81	390	84	41		363	79	25
85	382	83	37		337	73	19
89	386	86	30		341	76	10
93	346	78	23				
97	331	77	10				
101							
Mean for weeks							
1-13	250	98			248	98	
14-52	386	93			370	89	
53-101	388	85			376	82	

[a] Interim evaluations occurred during weeks 27 and 52 for the vehicle control and 300 mg/kg (stop-exposure) groups.

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of Methyleugenol

Weeks on Study	Vehicle Control		37 mg/kg			75 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	108	60	106	98	50	107	99	50
5	162	60	161	99	50	160	99	50
9	186	60	185	100	50	183	99	50
13	203	60	199	98	50	199	98	50
17	211	60	207	98	50	206	98	50
21	219	60	216	99	50	210	96	50
25	226	60	221	98	50	214	94	50
29	236	55[a]	226	96	50	219	93	50
33	241	55	233	97	50	223	92	50
37	252	54	241	95	50	231	91	50
41	264	54	249	94	50	236	90	50
45	274	54	257	94	50	243	89	49
49	288	54	269	94	50	252	88	49
53	293	49[a]	280	96	49	255	87	49
57	303	49	282	93	49	262	87	49
61	312	48	293	94	49	269	86	49
65	321	47	300	94	48	273	85	48
69	326	45	304	93	47	276	85	48
73	334	45	307	92	47	274	82	48
77	336	45	314	93	46	277	83	46
81	337	43	315	94	46	279	83	44
85	346	42	320	92	43	284	82	44
89	348	37	317	91	39	280	81	41
93	350	32	316	91	36	279	80	37
97	348	31	314	90	32	274	79	37
101	344	27	316	92	29	277	80	30
Mean for weeks								
1-13	165		163	99		162	99	
14-52	246		235	96		226	92	
53-101	331		306	93		274	83	

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of Methyleugenol

Weeks on Study	150 mg/kg				300 mg/kg (Stop-Exposure)		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors		Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	107	99	50		107	99	60
5	161	99	50		158	97	60
9	183	98	50		178	96	60
13	195	96	50		189	93	60
17	197	93	50		192	91	60
21	201	92	50		195	89	60
25	206	91	50		203	90	60
29	213	90	50		205	87	54[a]
33	215	89	50		208	86	54
37	222	88	50		212	84	54
41	223	85	50		213	81	53
45	230	84	50		215	78	52
49	235	82	50		219	76	51
53	240	82	50		225	77	46[a]
57	246	81	50		230	76	46
61	247	79	50		239	77	46
65	251	78	50		243	76	46
69	251	77	50		254	78	44
73	255	77	48		255	76	44
77	256	76	46		257	77	42
81	262	78	42		259	77	41
85	269	78	39		265	77	40
89	266	76	32		263	76	37
93	259	74	27		259	74	31
97	257	74	23		263	76	27
101	257	75	19		256	74	24
Mean for weeks							
1-13	162	98			158	96	
14-52	216	88			207	85	
53-101	255	77			251	76	

[a] Interim evaluations occurred during weeks 27 and 52 for the vehicle control and 300 mg/kg (stop-exposure) groups.

Toxicokinetic Studies

Detailed methods and results for the single-administration intravenous and gavage studies in young rats are presented in Appendix M, and results for the core study animals that received methyleugenol by gavage 5 days per week for 6, 12, or 18 months and for the single-administration gavage studies in aged animals are presented in Appendix I. Additional chemical distribution and metabolism data are presented in an unpublished absorption, distribution, metabolism, and elimination study conducted for the NTP (Appendix N).

Absorption: Absorption from oral doses was rapid, with peak plasma levels achieved within the first 5 minutes for all doses in males and females.

Distribution: Methyleugenol and its metabolites were distributed preferentially to the liver 72 hours after gavage or intravenous administration of [^{14}C]-methyleugenol to males. Tissue:blood ratios of methyleugenol-derived radioactivity were 2 to 3 in the liver, 0.9 to 1.4 in the kidney, and significantly less than 1 in all other tissues examined after 72 hours.

Metabolism: Methyleugenol was rapidly metabolized. Approximately 85% of methyleugenol orally administered to males was eliminated in urine as metabolites by 72 hours after dosing. Bioavailability of methyleugenol was low in both males and females, with less than 6% bioavailability at 37 mg/kg. This increased to approximately 13% at 75 mg/kg and 15% to 20% at 150 mg/kg. These findings suggest a strong, but saturable, first-pass metabolic effect, leading to a nonlinear relationship between dose and parent chemical dosimetry. No parent methyleugenol was found in urine from males dosed with methyleugenol orally or by intravenous injection. Hydroxylated, sulfated, and glucuronidated metabolites constituted the majority of metabolites detected in urine.

Elimination: Approximately 85% of methyleugenol administered orally to males was eliminated in urine as parent or metabolites. Elimination of methyleugenol from the bloodstream was rapid and multiphasic, with initial half-lives on the order of 5 minutes and terminal half-lives on the order of 1 to 2 hours in males and females. No difference in the elimination of the parent compound between naive males and females was apparent with either young or aged animals. Male core study animals eliminated methyleugenol more rapidly at 6 and 12 months, with areas under the concentration versus time curve (AUCs) generally less than those for the naive animals. Females at all time points and males at 18 months had AUCs similar to those of naive animals. This suggests that metabolic induction may occur to a greater extent in males than in females. Plots of AUC versus dose were sublinear in males at 6 and 12 months, indicative of metabolic saturation at the higher doses at these time points, but approximately linear at 18 months. The increase in AUCs with age in the core study males and females is suggestive of an age-related decrease in methyleugenol metabolic capability.

Necropsy Observations

Gross lesions were observed in the liver and stomach of males and females. Focal areas of discoloration, nodules (raised lesions less than 5 mm in diameter), and masses (raised lesions greater than 5 mm in diameter) were observed in the livers of dosed rats. The incidences and multiplicity of focal areas of discoloration, nodules, and masses increased with increasing dose. Grossly, there was diffuse thickening of the entire glandular portion of the stomach with or without the presence of one or more nodules and masses.

Pathology and Statistical Analyses

This section describes the statistically significant or bi-ologically noteworthy changes in the incidences of malignant mesothelioma and neoplasms and/or non-neoplastic lesions of the liver, glandular stomach, forestomach, kidney, mammary gland, skin, bone marrow, salivary gland, adrenal medulla, and spleen. Summaries of the incidences of neoplasms and non-neoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Liver: Chemical-related lesions included hepatocel-lular adenoma; hepatocellular carcinoma; hepatochol-angioma; hepatocholangiocarcinoma; eosinophilic, basophilic, and mixed cell foci; oval cell and bile duct hyperplasia; cystic degeneration; and hepatocyte hypertrophy in core study and 300 mg/kg (stop-exposure) rats (Table 7 and Appendixes A and B).

In 300 mg/kg rats at the 12-month interim evaluation, four males had hepatocellular adenomas (including two with multiple hepatocellular adenomas), one male had a hepatocholangiocarcinoma, and one female had a hepatocellular carcinoma. At 2 years, one 150 mg/kg male had a hepatocholangioma, and one 75 mg/kg male, one 150 mg/kg male, and three 150 mg/kg females had hepatocholangiocarcinomas. The incidences of hepatocellular adenoma, hepatocel-lular carcinoma, hepatocholangioma, and hepatochol-angiocarcinoma in the 300 mg/kg male and female groups were significantly greater than those in the vehicle controls. At 2 years, there were positive trends in the incidences of hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) in core study rats, and the incidences of multiple hepato-cellular carcinomas and/or adenomas were also increased in most dosed groups of males and females. The incidences of hepatocellular adenoma or carcino-ma (combined) in all groups of males and females were equal to or exceeded the upper end of the his-torical range for corn oil vehicle controls (Tables 7, A4a, and B4a). No incidences of hepatocholangioma or hepatocholangiocarcinoma were listed in the his-torical control database. Additionally, a cholangioma was present in two males, and a cholangiocarcinoma was present in one female in the 300 mg/kg groups.

At 6 and 12 months, basophilic, eosinophilic, and mixed cell foci were observed in most 300 mg/kg rats (Tables 7, A1, and B1). At 2 years, the incidences of eosinophilic foci in all dosed groups of rats and of mixed cell foci in the 37, 75, and 150 mg/kg males and in 75 mg/kg females were significantly greater than those in the vehicle controls. All 300 mg/kg males and females had hepatocyte hypertrophy and oval cell hyperplasia at 6 and 12 months. In dosed groups at 2 years, the incidences of hepatocyte hyper-trophy and oval cell hyperplasia (except 37 mg/kg males) were significantly increased. All 300 mg/kg males had cystic degeneration at 12 months, and the incidences in 75 mg/kg or greater males and 300 mg/kg females were significantly increased at 2 years. At 2 years, the incidences of bile duct hyperplasia were significantly increased in 150 and 300 mg/kg females; however, the incidences in all dosed groups of male rats were decreased. Focal atypical bile duct hyperplasia was present in males administered 75 mg/kg or greater.

Hepatocellular adenomas were single or multiple usually discrete nodular lesions which compressed the adjacent parenchyma, and in many instances, pro-truded from the liver surface. Adenomas were characterized by altered tinctorial staining charac-teristics and loss of normal hepatic lobular archi-tecture with hepatocytes abruptly intersecting the adjacent parenchyma (Plate 1). They were composed of well-differentiated, eosinophilic, basophilic, or vacuolated hepatocytes or mixtures thereof. Minimal to mild hepatocyte pleomorphism and/or hypertrophy were evident. Many adenomas contained areas of cystic degeneration and/or vacuolation. Hepatocellu-lar carcinomas were large well- to poorly demarcated invasive masses which frequently obliterated the hepatic architecture. Carcinomas were pleomorphic, organizing in trabecular, solid or less commonly ade-noid growth patterns; multiple growth patterns occa-sionally occurred within the same neoplasm. The trabecular pattern was most common and was charac-terized by hepatic plates that were more than one cell layer thick, irregular, and composed of well- to poorly differentiated hepatocytes (Plate 2). Incidences of metastasis, most commonly to the lung, occurred with a positive trend in males (vehicle control, 0/50; 37 mg/kg, 1/50; 75 mg/kg, 2/50; 150 mg/kg, 10/50; 300 mg/kg, 22/50) and females (0/50, 0/50, 1/49, 4/49, 4/50) (Tables A1 and B1).

TABLE 7
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Male and Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Male					
6-Month Interim Evaluation					
Number Examined Microscopically	5				5
Oval Cell, Hyperplasia[a]	0				5** (1.8)[b]
Hepatocyte, Hypertrophy	0				5** (3.0)
Basophilic Focus	0				3
Eosinophilic Focus	0				3
Mixed Cell Focus	0				5**
12-Month Interim Evaluation					
Number Examined Microscopically	5				5
Oval Cell, Hyperplasia	0				5** (1.4)
Hepatocyte, Hypertrophy	0				5** (3.0)
Degeneration, Cystic, Focal	0				5** (1.0)
Basophilic Focus	1				3
Eosinophilic Focus	0				5**
Mixed Cell Focus	0				5**
Hepatocellular Adenoma, Multiple	0				2
Hepatocellular Adenoma (includes multiple)	0				4*
Hepatocholangiocarcinoma	0				1
2-Year Study					
Number Examined Microscopically	50	50	50	50	50
Bile Duct, Hyperplasia	36 (2.0)	17** (1.2)	16** (1.6)	17** (1.8)	28 (1.7)
Bile Duct, Hyperplasia, Atypical, Focal	0	0	1 (3.0)	2 (1.5)	1 (2.0)
Oval Cell, Hyperplasia	14 (2.0)	17 (1.5)	24* (1.5)	34** (1.7)	27** (1.6)
Hepatocyte, Hypertrophy	0	13** (1.7)	25** (2.7)	30** (2.8)	26** (2.5)
Degeneration, Cystic, Focal	4 (1.3)	2 (1.0)	25** (1.3)	38** (2.2)	41** (2.2)
Basophilic Focus	23	21	22	7	6
Eosinophilic Focus	11	28**	43**	47**	39**
Mixed Cell Focus	1	7**	14**	8**	2
Hepatocellular Adenoma, Multiple	0	5*	14**	24**	24**
Hepatocellular Adenoma (includes multiple)	5	12*	23**	38**	32**
Hepatocellular Carcinoma, Multiple	0	0	1	11**	23**
Hepatocellular Carcinoma (includes multiple)	2	3	14**	25**	36**
Hepatocellular Adenoma or Carcinoma[c]					
Overall rate[d]	7/50 (14%)	14/50 (28%)	28/50 (56%)	43/50 (86%)	45/50 (90%)
Adjusted rate[e]	16.6%	34.4%	64.4%	94.0%	99.4%
Terminal rate[f]	3/20 (15%)	7/16 (44%)	9/15 (60%)	0/0	0/0
First incidence (days)	529	431	502	467	437
Poly-3 test[g]	P<0.001	P=0.049	P<0.001	P<0.001	P<0.001

TABLE 7
**Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Male and Female Rats
in the 2-Year Gavage Study of Methyleugenol**

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Male (continued)					
Cholangioma	0	0	0	0	2
Hepatocholangioma, Multiple	0	0	0	0	3
Hepatocholangioma (includes multiple)	0	0	0	1	6**
Hepatocholangiocarcinoma, Multiple	0	0	0	0	1
Hepatocholangiocarcinoma (includes multiple)	0	0	1	1	7**
Hepatocholangioma or Hepatocholangiocarcinoma (includes multiple)					
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/50 (4%)	13/50 (26%)
Adjusted rate	0.0%	0.0%	2.6%	6.2%	44.4%
Terminal rate	0/20 (0%)	0/16 (0%)	0/15 (0%)	0/0	0/0
First incidence (days)	—[h]	—[i]	582	457	502
Poly-3 test	P=0.049	—[i]	P=0.482	P=0.186	P<0.001
Female					
6-Month Interim Evaluation					
Number Examined Microscopically	5				5
Oval Cell, Hyperplasia	0				5** (1.0)
Hepatocyte, Hypertrophy	0				5** (1.0)
Basophilic Focus	0				2
Mixed Cell Focus	0				3
12-Month Interim Evaluation					
Number Examined Microscopically	5				5
Oval Cell, Hyperplasia	0				5** (1.8)
Hepatocyte, Hypertrophy	0				5** (3.0)
Basophilic Focus	5				3
Eosinophilic Focus	0				3
Mixed Cell Focus	0				5**
Hepatocellular Carcinoma	0				1
2-Year Study					
Number Examined Microscopically	50	50	49	49	50
Bile Duct, Hyperplasia	11 (1.3)	11 (1.3)	17 (1.3)	22** (1.5)	30** (1.8)
Oval Cell, Hyperplasia	1 (1.0)	15** (1.3)	19** (1.5)	35** (2.2)	34** (2.1)
Hepatocyte, Hypertrophy	1 (2.0)	13** (2.1)	16** (3.1)	26** (3.2)	31** (3.3)
Degeneration, Cystic, Focal	0	0	1 (1.0)	4 (1.5)	29** (1.9)
Basophilic Focus	36	36	29	17**	10**
Eosinophilic Focus	10	20**	27**	31**	37**
Mixed Cell Focus	6	4	19**	9	7
Hepatocellular Adenoma, Multiple	0	0	5*	23**	36**
Hepatocellular Adenoma (includes multiple)	1	8*	11**	33**	43**
Hepatocellular Carcinoma, Multiple	0	0	0	2	9**
Hepatocellular Carcinoma (includes multiple)	0	0	4	8**	22**

TABLE 7
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Male and Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Female (continued)					
Hepatocellular Adenoma or Carcinoma[j]					
Overall rate	1/50 (2%)	8/50 (16%)	14/49 (29%)	34/49 (69%)	43/50 (86%)
Adjusted rate	2.5%	19.6%	33.4%	76.6%	96.5%
Terminal rate	1/22 (5%)	8/25 (32%)	7/22 (32%)	11/11 (100%)	16/16 (100%)
First incidence (days)	730 (T)	730 (T)	609	508	459
Poly-3 test	P<0.001	P=0.017	P<0.001	P<0.001	P<0.001
Cholangiocarcinoma	0	0	0	0	1
Hepatocholangioma	0	0	0	0	8**
Hepatocholangiocarcinoma, Multiple	0	0	0	0	2
Hepatocholangiocarcinoma					
(includes multiple)	0	0	0	3	9**
Hepatocholangioma or Hepatocholangiocarcinoma					
Overall rate	0/50 (0%)	0/50 (0%)	0/49 (0%)	3/49 (6%)	17/50 (34%)
Adjusted rate	0.0%	0.0%	0.0%	8.2%	43.0%
Terminal rate	0/22 (0%)	0/25 (0%)	0/22 (0%)	2/11 (18%)	6/16 (38%)
First incidence (days)	—	—	—	534	609
Poly-3 test	P=0.010	—	—	P=0.105	P<0.001

*   Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test (interim evaluations) or Poly-3 test (2-year study)
** P≤0.01
(T) Terminal sacrifice
[a]   Number of animals with lesion
[b]   Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c]   Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 16/400 (4.0% ± 3.5%); range, 0%-10%
[d]   Number of animals with neoplasm per number of animals with liver examined microscopically
[e]   Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f]   Observed incidence at terminal kill
[g]   Beneath the vehicle control incidence are the P values associated with the trend test [the 300 mg/kg (stop-exposure) group was excluded from the trend test]. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h]   Not applicable; no neoplasms in animal group
[i]   Value of statistic cannot be computed.
[j]   Historical incidence: 1/401 (0.3% ± 0.7%); range, 0%-2%

Cholangiomas and cholangiocarcinomas are neoplasms of the biliary system and are uncommon in rats as spontaneous or induced neoplasms. They have been induced in rats following exposure to hepatocarcinogens. Cholangiomas were well-circumscribed, expansive, multilocular masses that consisted of multiple empty cystic spaces of variable size (Plate 3). Cysts were separated by scant fibrous connective tissue septa and were lined by flattened or cuboidal proliferative bile duct epithelium. Cholangiocarcinomas were larger, locally invasive neoplasms composed of atypical pleomorphic proliferative biliary epithelium forming glandular or ductular structures (some of which contained mucous and cellular debris) surrounded by abundant fibrous or collagenous connective tissue stroma (Plate 4).

In addition to the hepatocellular and biliary neoplasms, neoplasms composed of both well- to poorly differentiated hepatocellular and biliary components were observed. In these cases, the diagnosis of hepatocholangioma or hepatocholangiocarcinoma was used (Plates 5 and 6). In some neoplasms, the distinction

between component cells was clear; in others, component cells occurred within less well differentiated glandular structures, and the distinction was less obvious. In the glandular structures, component cells were flattened to low cuboidal (resembling biliary epithelial cells) or were larger resembling hepatocytes.

Bile duct hyperplasia consisted of periportal proliferation of well-differentiated biliary epithelium which formed small, well- to poorly delineated ducts. Bile duct hyperplasia is a common, age-related lesion and is often seen with exposure to hepatotoxins and hepatocarcinogens. However, the lesions are thought to be reparative rather than preneoplastic, and there is no clear evidence that this change progresses to neoplasms. Atypical bile duct hyperplasia consisted of irregularly shaped, dilated ducts lined by pleomorphic atypical cuboidal to columnar cells surrounded by abundant collagenous tissue (Plate 7). In some studies, similar lesions have been diagnosed as cholangiofibrosis or cholangiofibroma. The biologic nature of these lesions is uncertain. In contrast to simple bile duct hyperplasia, they are considered by some to be preneoplastic lesions from which cholangiocellular neoplasms and even hepatocellular neoplasms may develop. In one study, after treatment was discontinued, similar lesions progressed to malignant neoplasms and continued to grow after transplantation.

Oval cell hyperplasia was characterized by the presence of small, oval, slightly basophilic cells that proliferated in rows along the sinusoids adjacent to portal tracts (Plate 8). Oval cell hyperplasia does not occur spontaneously and is often seen with chemicals that are hepatotoxic. Based on ultrastructural and antigenic studies, it has been suggested that oval cells are undifferentiated cholangiolar cells or a stem cell population for hepatocytes and biliary epithelium and that they may be the origin of chemically induced neoplasms of both cell lineages.

Cystic degeneration occurred as focal to multifocal, multilocular lesions composed of multiple irregular variably sized cystic spaces that often contained finely granular or flocculent pale eosinophilic material (Plate 9). In many of the exposed animals, cystic degeneration was extensive and often occurred within neoplasms. These lesions are thought to arise from the perisinusoidal fat-storing (Ito) cells of the liver, and large lesions are considered by some to be benign Ito cell neoplasms. Small foci of cystic degeneration may occur spontaneously in old rats. However, well-developed lesions have been observed with prolonged exposure to hepatocarcinogens and are thought to result from excess accumulation of proteoglycans and/or proteins due to overproduction or impaired degradation of these substances.

Eosinophilic foci were focal lesions up to four hepatic lobules in diameter with distinct margins and minimal to no compression of the adjacent parenchyma. Hepatocytes within the foci were larger and more eosinophilic than the adjacent hepatocytes. Hepatocyte hypertrophy involved individual mid-zonal hepatocytes and was characterized by increased cell size and cytoplasmic eosinophilia.

Glandular Stomach: Chemical-related neoplasms and nonneoplastic lesions occurred in the glandular stomach of male and female rats (Table 8 and Appendixes A and B). Lesions were confined to the fundic region and were more prevalent and severe in females than males. Lesions included benign and malignant neuroendocrine tumors, glandular epithelial atrophy, and neuroendocrine cell hyperplasia.

In all dosed groups of rats at 6 and 12 months and 2 years, the incidences of mucosal atrophy were significantly greater than in the vehicle controls. Neuroendocrine cell hyperplasia occurred in females at 6 months and in males and females at 12 months, and the incidences in 150 and 300 mg/kg males and in 37, 75, and 150 mg/kg females at 2 years were significantly increased. The incidences of benign and malignant neuroendocrine tumors were increased in 150 and 300 mg/kg males, and the incidence of malignant neuroendocrine tumors in 150 mg/kg males was significantly increased compared to the vehicle control group. There was a positive trend in the incidences of benign or malignant neuroendocrine tumors (combined) in females, and the incidences in females administered 75 mg/kg or greater were significantly greater than those in the vehicle controls (Table 8). Benign or malignant neuroendocrine tumors have not been observed in the glandular stomach of male or female historical corn oil gavage vehicle controls.

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Glandular Stomach in Male and Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Male					
6-Month Interim Evaluation					
Number Necropsied	5				5
Atrophy[a]	0				5** (2.0)[b]
12-Month Interim Evaluation					
Number Necropsied	5				5
Atrophy	0				5** (2.0)
Neuroendocrine Cell Hyperplasia	0				2 (1.0)
2-Year Study					
Number Necropsied	50	50	50	50	50
Atrophy	0	14** (1.6)	32** (1.7)	37** (2.2)	29** (2.2)
Neuroendocrine Cell, Hyperplasia	0	0	1 (1.0)	8** (1.5)	8** (1.8)
Benign Neuroendocrine Tumor	0	0	0	3	2
Malignant Neuroendocrine Tumor	0	0	0	4*	2
Benign or Malignant Neuroendocrine Tumor					
Overall rate[c]	0/50 (0%)	0/50 (0%)	0/50 (0%)	7/50 (14%)	4/50 (8%)
Adjusted rate[d]	0.0%	0.0%	0.0%	21.3%	16.5%
Terminal rate[e]	0/20	0/16 (0%)	0/15 (0%)	0/0	0/0
First incidence (days)	—[g]	—	—	642	517
Poly-3 test[f]	P<0.001	—[h]	—	P=0.002	P=0.032
Female					
6-Month Interim Evaluation					
Number Necropsied	5				5
Atrophy	0				5** (3.0)
Neuroendocrine Cell, Hyperplasia	0				1 (1.0)
12-Month Interim Evaluation					
Number Necropsied	5				5
Atrophy	0				5** (3.0)
Neuroendocrine Cell, Hyperplasia	0				1 (1.0)

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Glandular Stomach in Male and Female Rats
in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Female (continued)					
2-Year Study					
Number Necropsied	50	50	50	50	50
Atrophy	3 (1.0)	41** (1.6)	45** (2.1)	39** (2.4)	33** (2.2)
Neuroendocrine Cell, Hyperplasia	0	5* (1.0)	11** (2.0)	9** (2.2)	3 (2.0)
Benign Neuroendocrine Tumor	0	0	13**	9**	5*
Malignant Neuroendocrine Tumor	0	1	12**	26**	36**
Benign or Malignant Neuroendocrine Tumor					
Overall rate	0/50 (0%)	1/50 (2%)	25/50 (50%)	34/50 (68%)	41/50 (82%)
Adjusted rate	0.0%	2.4%	59.2%	80.3%	93.9%
Terminal rate	0/22 (0%)	0/25 (0%)	16/22 (73%)	10/11 (91%)	16/16 (100%)
First incidence (days)	—	718	676	548	477
Poly-3 test	P<0.001	P=0.508	P<0.001	P<0.001	P<0.001

* Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test (interim evaluation) or the Poly-3 test (2-year study)
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Number of animals with neoplasm per number of animals necropsied
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the vehicle control incidence are the P values associated with the trend test [the 300 mg/kg (stop-exposure) group was excluded from the trend test]. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[g] Not applicable; no neoplasms in animal group
[h] Value of statistic cannot be computed.

Mucosal atrophy was an early change observed at the 6- and 12-month evaluations and was also present at 2 years. It was confined to the epithelium of the fundic region of the glandular stomach and did not appear to involve the epithelium of the foveolar (gastric pits) region of the mucosa. Atrophy began near the limiting ridge (margo plicans) and invariably involved the entire fundic glandular mucosa. It was characterized by decreased height of the fundic mucosa due to loss of all glandular epithelial cell types, particularly parietal and chief cells (Plate 10).

Neuroendocrine proliferative lesions of the glandular stomach are extremely rare both as spontaneous and chemically induced lesions. In this study, the spectrum of proliferative neuroendocrine lesions observed

seemed to constitute a morphologic continuum that ranged from hyperplasia to benign to well- to poorly differentiated malignant neoplasms. For diagnostic purposes, specific criteria were used to separate these lesions based on morphology alone.

Neuroendocrine cell hyperplasia consisted of proliferations of cells morphologically consistent with enterochromaffin-like (ECL) cells of the glandular epithelium. These proliferations were confined to the mucosa. They tended to be focal and did not markedly expand or efface the mucosa, but did displace the adjacent fundic glands (Plate 11a). Component cells were round to polygonal, with eosinophilic to amphophilic finely granular cytoplasm and had round to oval single nuclei and usually a single

nucleolus. The cells occurred in nests, clusters, or often as infiltrations between the glandular epithelial cells extending toward the mucosal surface (Plate 11b).

Similar to neuroendocrine cell hyperplasia, benign neuroendocrine tumors were confined wholly to the mucosa and did not invade the muscularis mucosa. In contrast to hyperplasia, benign tumors were more extensive and frequently caused moderate to marked thickening of the mucosa and sometimes occurred as nodular expansions. Neoplastic cells were arranged in sheets or clusters which displaced and/or replaced the adjacent glands. The cells were mildly pleomorphic but essentially retained their ECL cell features (Plate 12). While some of these neoplasms were quite locally expansive, their biologic behavior is uncertain, and therefore, based on morphology, were considered to be benign.

Malignant neuroendocrine tumors were generally large expansive masses that morphologically exhibited malignant behavior. These neoplasms were diffuse and/or well-demarcated nodular mucosal masses that obliterated the normal mucosal architecture and frequently caused marked thickening of the mucosa (Plate 13). Invasion through the muscularis mucosa with focal nodular and/or expansive infiltrative growth within the submucosa were common. The latter characteristic was the most consistent criterium of malignancy. Most malignant tumors were solid masses consisting predominantly of ECL cells variably mixed with areas of poorly differentiated polygonal cells that had moderate to abundant amounts of eosinophilic cytoplasm. In the more malignant neoplasms, a heterogenous cell pattern was often evident and striking. These neoplasms consisted of sheets of ECL cells mixed with focal to extensive areas of atypical polygonal and sometimes spindle-shaped cells among which were occasionally interspersed variably sized nodular expansions of large polygonal cells that had brightly eosinophilic coarsely granular cytoplasm (Plate 14). A few tumors had a large component of the latter cells. Cellular anaplasia or a high mitotic rate were not characteristic of most of these neoplasms. Blood vessels distended with emboli of neoplastic cells was evidence of the potential for metastases. In female rats, metastases primarily to the liver (vehicle control, 0/50; 37 mg/kg,

0/50; 75 mg/kg,0/49; 150 mg/kg, 3/49; 300 mg/kg, 10/50) and lung (0/50, 0/50, 0/49, 0/49, 4/50) were observed (Table B1).

Immunohistochemical staining of glandular epithelial lesions with chromagranin A (CG-A) and neuron specific enolase (NSE) and the Sevier-Munger histochemical stain for argyrophylia was heterogeneous. Focal areas of neuroendocrine cell hyperplasia stained positively with CG-A and NSE, and the Sevier-Munger stain demonstrated their argyrophilic positivity. Benign neuroendocrine tumors were moderately to strongly positive with CG-A, NSE, and Sevier-Munger staining. Chromagranin-A, NSE, and Sevier-Munger staining in the more malignant tumors was variable. In general, malignant cells that retained morphologic characteristics of ECL cells often exhibited positive staining. However, staining characteristics among and within malignant tumors were heterogeneous and varied in intensity. Areas of negative staining were interspersed with areas of moderately to strongly positive staining. Areas of decreased staining intensity or negative staining were usually those composed of the more atypical cells.

Forestomach: In core study female rats, there was a positive trend in the incidences of squamous cell papilloma or carcinoma (combined) (0/50, 0/50, 1/50, 3/50; Table B3a); however, by pairwise comparisons, the incidences in the dosed groups were not significantly greater than that in the vehicle control group. The incidence in the 150 mg/kg group exceeded the historical control range for corn oil gavage vehicle controls (Table B4b).

Kidney: In the standard evaluation in core study male rats, there was a positive trend in the incidences of renal tubule adenoma (Tables 9 and A3a), and the incidence in the 300 mg/kg males was significantly greater than that in the vehicle controls. The incidences in all groups exceeded the historical control range for corn oil gavage vehicle controls (Tables 9, A3a, A3b, and A4b). The incidences of renal tubule proliferative lesions in male rats were suggestive of a neoplastic effect in the kidney. Therefore, additional step sections of the kidneys of male rats were prepared using the residual formalin-fixed wet tissue. During this evaluation, additional rats with renal tubule proliferative lesions were

T<small>ABLE</small> 9
**Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Male and Female Rats
in the 2-Year Gavage Study of Methyleugenol**

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Male					
6-Month Interim Evaluation					
Number Examined Microscopically	5				5
Nephropathy[a]	4 (1.0)[b]				5 (1.0)
12-Month Interim Evaluation					
Number Examined Microscopically	5				5
Nephropathy	5 (1.0)				5 (2.0)
2-Year Study					
Single Sections (Standard Evaluation)					
Number Examined Microscopically	50	50	50	50	50
Nephropathy	49 (2.8)	46 (2.4)	48 (2.8)	50 (3.0)	47 (3.3)*
Renal Tubule, Hyperplasia, Focal	5 (2.2)	1 (4.0)	7 (2.9)	4 (3.5)	3 (2.0)
Renal Tubule, Hyperplasia, Oncocytic	0	0	0	2 (1.5)	1 (4.0)
Renal Tubule Adenoma, Bilateral	0	0	1	0	0
Renal Tubule Adenoma (includes bilateral)[c]					
Overall rate[d]	3/50 (6%)	2/50 (4%)	6/50 (12%)	6/50 (12%)	8/50 (16%)
Adjusted rate[e]	7.2%	5.2%	15.8%	18.4%	31.2%
Terminal rate[f]	1/20 (5%)	0/16 (0%)	4/15 (27%)	0/0	0/0
First incidence (days)	712	673	596	598	528
Poly-3 test[g]	P=0.046	P=0.531N	P=0.195	P=0.135	P=0.018
Renal Tubule Carcinoma	1	0	0	0	0
Renal Tubule Adenoma or Carcinoma[h]	4	2	6	6	8*
Renal Tubule Benign Oncocytoma	0	0	1	0	1
Step Sections (Extended Evaluation)					
Number Examined Microscopically	50	50	50	50	50
Renal Tubule, Hyperplasia, Focal	10 (3.2)	13 (1.8)	20** (2.7)	20** (2.9)	21** (2.7)
Renal Tubule, Hyperplasia, Oncocytic	0	0	1	2	3
Renal Tubule Adenoma, Multiple	0	1	2	1	0
Renal Tubule Adenoma (includes multiple)					
Overall rate	2/50 (4%)	5/50 (10%)	14/50 (28%)	11/50 (22%)	13/50 (26%)
Adjusted rate	4.8%	12.9%	36.7%	31.9%	45.9%
Terminal rate	1/20 (5%)	3/16 (19%)	8/15 (53%)	0/0	0/0
First incidence (days)	712	718	673	575	537
Poly-3 test	P<0.001	P=0.186	P<0.001	P=0.002	P<0.001
Renal Tubule Benign Oncocytoma	0	0	0	1	1

TABLE 9

**Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Male and Female Rats
in the 2-Year Gavage Study of Methyleugenol**

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Male (continued)					
Single Sections and Step Sections (Combined)					
Number Examined Microscopically	50	50	50	50	50
Renal Tubule, Hyperplasia, Focal	13 (2.9)	13 (2.0)	21* (2.9)	20* (3.0)	22** (2.8)
Renal Tubule, Hyperplasia, Oncocytic	0	0	1	4	4
Renal Tubule Adenoma, Multiple	1	2	4	3	1
Renal Tubule Adenoma (includes multiple)					
Overall rate	4/50 (8%)	6/50 (12%)	17/50 (34%)	13/50 (26%)	20/50 (40%)
Adjusted rate	9.6%	15.5%	43.9%	37.3%	64.6%
Terminal rate	2/20 (10%)	3/16 (19%)	10/15 (67%)	0/0	0/0
First incidence (days)	712	673	596	575	528
Poly-3 test	P<0.001	P=0.325	P<0.001	P=0.003	P<0.001
Renal Tubule Benign Oncocytoma	0	0	1	1	2
Female					
12-Month Interim Evaluation					
Number Examined Microscopically	5				5
Nephropathy	4 (1.0)				2 (1.0)
2-Year Study					
Number Examined Microscopically	50	50	50	50	50
Nephropathy	35 (1.2)	42 (1.2)	41 (1.2)	44 (1.3)	45* (2.2)*

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test (incidences) or Mann-Whitney U test (severities)
** P≤0.01
a Number of animals with lesion
b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
c Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 3/400 (0.8% ± 1.0%); range, 0%-2%
d Number of animals with neoplasm per number of animals with kidney examined microscopically
e Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
f Observed incidence at terminal kill
g Beneath the vehicle control incidence are the P values associated with the trend test [the 300 mg/kg (stop-exposure) group was excluded from the trend test]. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposure group is indicated by **N**.
h Historical incidence: 5/400 (1.3% ± 1.5%); range, 0%-4%

identified. The incidences of renal tubule proliferative lesions identified in the step sections and the combined incidences of standard and step sections in male rats are presented in Tables 9, A3a, and A3b. The incidences of renal tubule hyperplasia and adenoma in the extended evaluation and the combined incidences of standard and step sections in the 75, 150, and 300 mg/kg groups were greater than those in the vehicle controls. Additionally, there was a slight positive trend in the incidences of renal tubule benign oncocytoma in the extended evaluation and the combined incidences of standard and step sections.

The incidences of nephropathy were increased in all dosed groups of females, and the increase was significant in the 300 mg/kg group (Tables 9 and B5). The incidences of nephropathy in dosed groups of males were similar to that in the vehicle controls (Tables 9 and A5). In dosed males, the severity of nephropathy increased with increasing dose; the severity in 300 mg/kg males was significantly greater than that in the vehicle controls.

Hyperplastic tubules varied in size from slightly greater than the diameter of normal tubules to approximately two to three times greater than a normal tubule. The hyperplastic cells usually formed solid clusters and were slightly more pleomorphic and more basophilic. Renal tubule adenomas consisted of cells that frequently were morphologically similar to those of renal tubule hyperplasia but were distinguished from hyperplasia by their larger size and more complex structure. Adenomas were discrete to well-circumscribed lesions, most of which were approximately 1 mm or greater in diameter (greater than five or more tubule diameters in size). They consisted of multiple, variably sized, tubule-like structures or solid clusters of neoplastic renal tubule epithelial cells separated by fine bands of connective tissue.

Malignant Mesothelioma: In core study males, there was a positive trend in the incidences of malignant mesothelioma (vehicle control, 1/50; 37 mg/kg, 3/50; 75 mg/kg, 5/50; 150 mg/kg, 12/50; Table A3a); the incidence was significantly greater in 150 mg/kg males than in the vehicle controls. The incidence in 300 mg/kg males (5/50; Table A3b) was also significantly greater than that in the vehicle controls (Table A3b). The incidences in the 75, 150, and 300 mg/kg groups exceeded the historical control range for corn oil gavage studies (Table A4c). Mesotheliomas were disseminated along the peritoneal surface of several organs in the abdominal cavity and/or the serosa of the testis and epididymis. The histomorphology of malignant mesotheliomas varied. Typically, most consisted of single or multiple papillary projections lined by one to several layers of mesothelial cells covering cores of fibrous stroma. Others occurred as florid papilliferous proliferations of stroma covered by several layers of proliferating cuboidal to polygonal mesothelial cells (Plate 15). The more malignant mesotheliomas consisted of pleomorphic mesothelial cells arranged in sheets or

clusters or forming tubular structures surrounded by abundant amounts of fibrous tissue stroma.

Mammary Gland: Mammary gland fibroadenoma occurred with a positive trend in male rats. The incidences of mammary gland fibroadenoma in 75 and 150 mg/kg males were significantly greater than in the vehicle controls (5/50, 5/50, 15/50, 13/50, 6/50; Tables A3a and A3b). The incidences of mammary gland fibroadenoma in all groups of males exceeded the historical control range for corn oil gavage vehicle controls [23/402 (5.7% ± 1.3%); range, 4%-8%]. Fibroadenomas were expansive nodules/masses composed of well-differentiated ducts, ductules, and/or alveoli lined by a single layer of uniformly cuboidal cells and surrounded by variable amounts of mature fibrous connective tissue. In some masses, the connective tissue was abundant and formed the predominant component. The lumens of many alveoli and ducts contained proteinaceous secretion, and alveolar epithelial cells frequently contained lipid.

Skin (subcutaneous tissue): The incidences of fibroma in 37 and 75 mg/kg males (1/50, 9/50, 8/50, 5/50, 4/50) and combined incidences of fibroma or fibrosarcoma in 37, 75, and 150 mg/kg males (1/50, 12/50, 8/50, 8/50, 4/50; Tables A3a and A3b) were significantly increased; however, the incidences did not increase with increasing dose. The incidences of these lesions in these groups exceeded the historical range for corn oil gavage vehicle controls (Table A4d). Fibromas were well-demarcated, solid, expansive masses composed of well-differentiated fibrous connective tissue. Fibrosarcomas were expansive, locally invasive masses composed of anaplastic spindle cells.

Bone Marrow: Bone marrow hyperplasia was observed at 6 months in one male and four females administered 300 mg/kg and at 12 months in one vehicle control male and five 300 mg/kg males (Tables A5 and B5). At 2 years, the incidences of bone marrow hyperplasia were increased in all groups of dosed females (4/50, 15/50, 11/49, 20/50, 25/50; Table B5), and the increases in the 37, 150, and 300 mg/kg groups were significant. Bone marrow hyperplasia consisted of a marked increase in the density of erythroid or myeloid cells or a mixture of both, frequently accompanied by proliferation of megakaryocytes. Bone marrow hyperplasia was

considered a reactive response secondary to significant hepatic and gastric pathology.

Salivary Gland: The incidences of cytoplasmic alteration of the submandibular salivary gland of all dosed male and female rats were significantly greater than those in the vehicle controls (males: vehicle control, 4/50; 37 mg/kg, 50/50; 75 mg/kg, 49/50; 150 mg/kg, 48/48; 300 mg/kg, 48/48; females: 1/50, 48/48, 49/49, 49/49, 49/50; Tables A5 and B5). This change was also present in all 300 mg/kg rats at 6 and 12 months. Cytoplasmic alteration consisted of a loss of the eosinophilic granules within the striated ducts of the submandibular salivary glands.

Adrenal Medulla: At 2 years, there was a negative trend in the incidences of benign pheochromocytoma in core study males, and the incidences were significantly decreased in the 75 and 150 mg/kg groups compared to the vehicle controls (24/50, 17/50, 11/50, 10/50, 9/50; Tables A3a and A3b). However, the incidence in the concurrent control group (48%) is high compared to the historical control rate for corn oil gavage studies (23%); the incidence in the concurrent controls is also high compared to the historical control rates for dosed feed (26%) and inhalation (32%) studies (Table A4e). The incidences of benign pheochromocytoma in the 75, 150, and 300 mg/kg groups of male rats are also within the historical control range for these routes of administration. Furthermore, there was a significant increase in the incidence of benign pheochromocytoma in 300 mg/kg females at 2 years (1/50, 1/50, 2/50, 2/49, 6/50; Tables B1 and B3b). Although 6/50 is just outside of the historical control range for female rats in corn oil gavage and dosed feed studies, as many as 6/50 have been seen in chamber control groups from inhalation studies (Table B4c). Incidences of hyperplasia did not differ between vehicle control and dosed groups of either male or female rats. The decreased incidences of benign pheochromocytoma in male rats and the increased incidence in female rats were considered variations due to chance rather than to effects associated with methyleugenol administration.

Spleen: The incidences of splenic fibrosis in 150 and 300 mg/kg females at 2 years were significantly increased (3/50, 3/50, 5/50, 12/49, 15/50; Table B5).

MICE
14-Week Study

All mice administered 1,000 mg/kg, except for one male, died before the end of the study (Table 10). Additionally, one 300 mg/kg male died during week 3 and one 10 mg/kg female died during week 12. The mean body weight gains of males and females in the 300 mg/kg groups were significantly less than those of the vehicle controls; the final mean body weights and body weight gains of other groups of mice surviving until the end of the study were similar to those of the vehicle controls (Table 10). The only clinical finding was toxicity manifested as generalized morbidity in male and female mice administered 1,000 mg/kg.

The liver weights of 30, 100, and 300 mg/kg males and of 300 mg/kg females were significantly greater than those of the vehicle controls (Table G2).

Male mice administered 10 or 30 mg/kg had significantly lower cauda epididymis, epididymis, and testis weights than did the vehicle controls (Table H3). Additionally, 100 mg/kg males had significantly decreased spermatozoal concentrations. There were no significant differences in vaginal cytology parameters between dosed and vehicle control mice (Table H4).

TABLE 10
Survival and Body Weights of Mice in the 14-Week Gavage Study of Methyleugenol

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Vehicle Controls (%)
		Initial	Final	Change	
Male					
Water Control	10/10	22.7 ± 0.5	32.1 ± 1.2	9.4 ± 1.1	96
Vehicle Control	10/10	22.3 ± 0.4	33.4 ± 0.7	11.1 ± 0.5	
10	10/10	22.9 ± 0.4	33.4 ± 0.8	10.5 ± 0.5	100
30	10/10	22.3 ± 0.3	32.6 ± 0.8	10.3 ± 0.6	98
100	10/10	23.2 ± 0.5	33.7 ± 0.9	10.5 ± 0.7	101
300	9/10[c]	22.3 ± 0.4	30.7 ± 0.6	8.4 ± 0.5**	92
1,000	1/10[d]	21.1 ± 0.7	27.3	6.2	82
Female					
Water Control	10/10	19.0 ± 0.4	30.9 ± 1.1	11.8 ± 0.9	104
Vehicle Control	10/10	19.0 ± 0.3	29.6 ± 0.6	10.7 ± 0.5	
10	9/10[e]	18.5 ± 0.3	29.2 ± 0.7	10.7 ± 0.7	99
30	10/10	19.4 ± 0.4	29.3 ± 0.7	9.9 ± 0.6	99
100	10/10	19.5 ± 0.2	29.2 ± 0.5	9.6 ± 0.5	99
300	10/10	19.4 ± 0.2	27.5 ± 0.6	8.1 ± 0.4**	93
1,000	0/10[f]	18.4 ± 0.3	—	—	—

** Significantly different (P≤0.01) from the vehicle control group by Williams' or Dunnett's test
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. No standard error was calculated for groups with high mortality. No final mean body weights or weights changes were calculated for groups with 100% mortality.
[c] Week of death: 3
[d] Weeks of death: 1, 1, 1, 1, 1, 5, 8, 9, 12
[e] Week of death: 12
[f] Weeks of death: 1, 1, 1, 1, 1, 1, 3, 4, 4, 6

Significant chemical-related gross lesions were observed in the liver of male and female mice administered 1,000 mg/kg. One male and two females had enlarged livers, one male had a liver nodule, and one female had a pale liver.

Chemical-related nonneoplastic lesions occurred in the liver, glandular stomach, and nose of male and female mice (Table 11). Hepatic changes were generally only observed in mice that survived beyond the first week of the study. Cytologic alteration, necrosis, bile duct hyperplasia, and focal subacute inflammation occurred in the liver of all 300 mg/kg females. The incidences of these lesions were also significantly increased in 1,000 mg/kg males and females (except subacute inflammation). Female mice administered 1,000 mg/kg had lower incidences of these lesions due to early mortality. Significant differences were not observed between the water and vehicle control mice. Cytologic alteration was observed primarily in periportal sites and was the term used to describe a variety of hepatocellular alterations that included mild nuclear and cytoplasmic enlargement (hypertrophy) and increased cytoplasmic eosinophilia. Necrosis of scattered individual hepatocytes occurred throughout the hepatic lobules. Bile duct hyperplasia consisted of proliferation of immature biliary cells within portal areas. Inflammation consisted of multiple small foci of primarily mononuclear inflammatory cells randomly scattered throughout the liver.

The incidences of atrophy, degeneration, necrosis, edema, mitotic alteration, and cystic glands of the fundic region of the glandular stomach were increased in one or more groups of male and female mice administered 30 mg/kg or greater compared to the vehicle controls (Table 11); these lesions were generally of minimal to mild severity. In males, the incidences of cystic glands in the 30 mg/kg group and degeneration and mitotic alteration in the 300 mg/kg group were significantly increased. In female mice administered 300 mg/kg, the incidences of atrophy, degeneration, edema, mitotic alteration, and cystic glands were significantly increased. In the 1,000 mg/kg mice, early death and subsequent autolysis significantly precluded adequate histopathologic evaluation of gastric lesions. Lesions were generally of minimal to mild severity in the 30, 100, and 300 mg/kg groups and of mild to marked severity in the 1,000 mg/kg groups. Atrophy consisted of a generalized decrease in the thickness of the mucosal epithelium due to loss of parietal and chief cells and shortening of the mucosal glands. Necrosis in the glandular epithelium was characterized by necrosis of parietal cells primarily, and to a lesser extent, chief cells. Degeneration consisted of dilated glands lined by dysplastic atypical epithelial cells and cellular detritus. Cystic glands were dilated and lined by flattened epithelium. Increased numbers of morphologically normal mitotic figures were present in regenerative areas of the glandular epithelium. Edema was of minimal to mild severity and occurred in the lamina propria.

Minimal to mild focal degeneration of the olfactory epithelium of the nose occurred in 30, 300, and 1,000 mg/kg males, 30 mg/kg or greater females, and two water control females. The incidences and severities of this lesion were not dose dependent. Degeneration consisted of unilateral or bilateral focal loss and/or disruption of the sensory olfactory epithelial cells in the dorsal meatus.

Dose Selection Rationale: Based on the high mortality rate of the 1,000 mg/kg group, the lower mean body weight gains of the 300 mg/kg groups, and the hepatotoxic effects in the 300 and 1,000 mg/kg groups, the highest dose selected in the 2-year gavage study in mice was 150 mg/kg.

TABLE 11
Incidences of Selected Nonneoplastic Lesions in Male and Female Mice
in the 14-Week Gavage Study of Methyleugenol

	Water Control		Vehicle Control		10 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		1,000 mg/kg	
Male														
Liver[a]	10		10		10		10		10		10		10	
Cytologic Alteration[b]	0		0		0		0		0		0		5*	(4.0)[c]
Necrosis	0		0		0		0		0		0		5*	(2.0)
Hyperplasia, Bile Duct	0		0		0		0		0		0		5*	(2.2)
Inflammation, Subacute	0		0		0		0		0		0		4*	(1.8)
Glandular Stomach	10		10		10		10		10		10		10	
Atrophy	0		0		0		0		0		1	(1.0)	3	(4.0)
Degeneration	0		0		0		1	(1.0)	1	(1.0)	9**	(1.0)	3	(2.0)
Necrosis	0		0		0		0		0		0		3	(3.3)
Edema	0		0		0		0		0		0		3	(1.3)
Mitotic Alteration	0		0		0		1	(1.0)	0		10**	(1.1)	0	
Cystic Glands	0		0		0		6**	(1.0)	0		2	(1.0)	1	(2.0)
Nose														
Epithelial Cell, Degeneration	0		0		0		4*	(1.5)	0		1	(1.0)	5*	(1.8)
Female														
Liver	10		10		10		10		10		10		10	
Cytologic Alteration	0		0		0		1	(1.0)	0		10**	(2.3)	4*	(3.8)
Necrosis	0		0		0		0		0		10**	(1.0)	4*	(1.8)
Hyperplasia, Bile Duct	0		0		0		0		0		10**	(2.0)	4*	(1.5)
Inflammation, Subacute	0		0		0		0		0		10**	(1.0)	1	(2.0)
Glandular Stomach	10		10		10		10		10		10		10	
Atrophy	0		0		0		0		0		10**	(1.3)	3	(4.0)
Degeneration	0		0		0		4*	(1.0)	3	(1.0)	10**	(1.7)	3	(2.0)
Necrosis	0		0		0		0		0		0		3	(4.0)
Edema	0		0		0		0		0		6**	(1.0)	3	(1.7)
Mitotic Alteration	0		0		0		4*	(1.0)	5*	(1.0)	10**	(1.2)	0	
Cystic Glands	0		0		0		4*	(1.5)	0		7**	(1.0)	0	
Nose														
Epithelial Cell, Degeneration	2	(1.0)	0		0		8**	(1.0)	4*	(1.0)	5*	(1.0)	3	(1.3)

* Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test
** P≤0.01
[a] Number of animals 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

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 12 and in the Kaplan-Meier survival curves (Figure 3). Survival of all dosed groups of male mice was similar to that of the vehicle controls. Survival rates of all dosed groups of female mice were significantly less than that of the vehicle controls. The decreased survival in female mice may have been due to the increased incidences of neoplasms and nonneoplastic lesions; these increases were greater in female mice than in males.

Body Weights and Clinical Findings

Mean body weights were generally less than those of the vehicle controls after weeks 81, 41, and 17 in males and after weeks 33, 17, and 5 in females for the 37, 75, and 150 mg/kg groups, respectively (Tables 13 and 14 and Figure 4). There were no clinical findings that could be attributed to methyleugenol administration.

TABLE 12
Survival of Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	0	1	2
Missing[a]	1	0	0	0
Moribund	5	6	5	9
Natural deaths	6	8	7	4
Animals surviving to study termination	38	36	37	35
Percent probability of survival at end of study[b]	78	72	76	73
Mean survival (days)[c]	690	705	679	686
Survival analysis[d]	P=0.815	P=0.825	P=1.000	P=0.829
Female				
Animals initially in study	50	50	50	50
Accidental deaths	0	1	1	0
Moribund	5	7	8	13
Natural deaths	14	24	23	35
Animals surviving to study termination	31	18	18[e]	2
Percent probability of survival at end of study	62	38	37	4
Mean survival (days)	696	662	664	637
Survival analysis	P<0.001	P=0.009	P=0.013	P<0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns.
[e] Includes two animals that died during the last week of the study
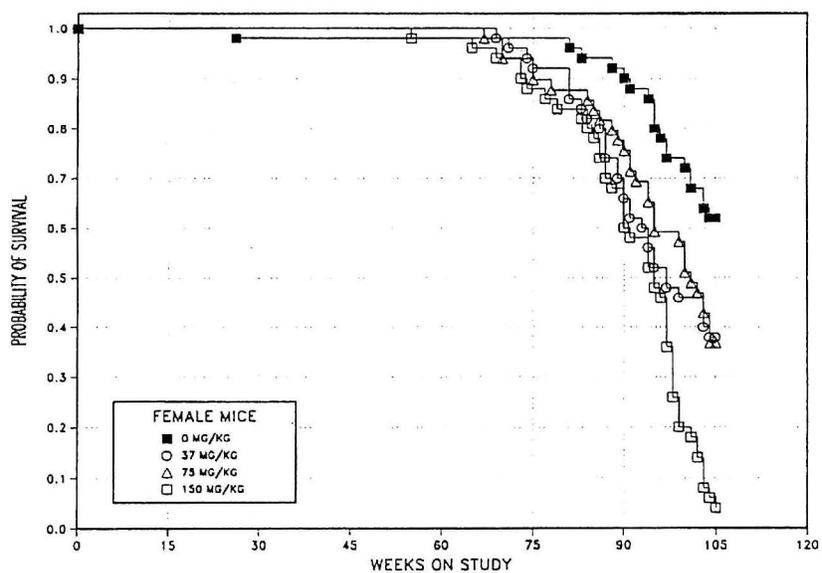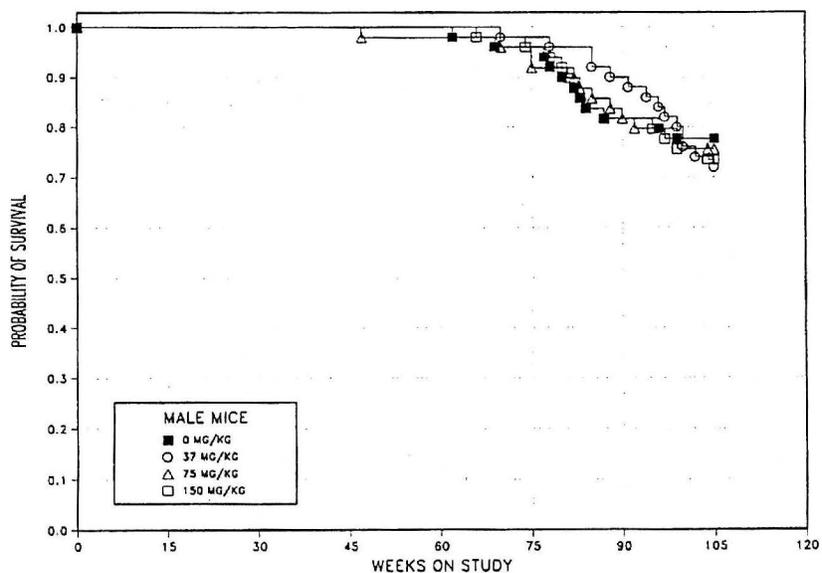
FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice
Administered Methyleugenol by Gavage for 2 Years

TABLE 13
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of Methyleugenol

Weeks on Study	Vehicle Control		37 mg/kg			75 mg/kg			150 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	24.4	50	24.2	99	50	24.2	99	50	24.2	99	50
5	28.9	50	28.9	100	50	28.4	98	50	28.5	99	50
9	33.2	50	33.6	101	50	32.9	99	49	32.7	99	50
13	37.4	50	37.6	101	50	36.8	98	49	36.0	96	50
17	41.4	50	41.8	101	50	40.4	98	49	39.4	95	50
21	45.4	50	45.0	99	50	43.4	96	49	42.1	93	50
25	47.0	50	46.5	99	50	45.0	96	49	43.6	93	50
29	48.0	50	47.8	100	50	46.1	96	49	44.8	93	50
33	49.8	50	48.8	98	50	47.5	95	49	46.8	94	50
37	50.6	50	49.8	98	50	48.1	95	49	47.3	94	50
41	50.7	50	50.2	99	50	48.7	96	49	47.8	94	50
45	51.7	50	50.3	97	50	48.8	94	49	48.7	94	50
49	52.5	50	51.0	97	50	49.3	94	48	49.3	94	50
53	52.3	50	51.5	99	50	49.7	95	48	49.4	95	49
57	52.4	50	51.1	98	50	48.9	93	48	49.4	94	49
61	52.6	50	51.7	99	50	49.4	94	48	49.1	93	49
65	52.9	49	51.8	98	50	49.6	94	48	49.9	94	49
69	52.2	48	51.5	99	50	49.1	94	48	49.7	95	48
73	51.9	48	51.0	98	49	49.4	95	47	49.2	95	48
77	51.7	47	50.5	98	49	48.9	95	45	48.5	94	47
81	52.8	44	50.4	96	48	48.9	93	45	48.6	92	44
85	53.6	41	50.5	94	48	49.6	93	42	49.9	93	41
89	53.7	40	50.3	94	45	49.3	92	41	48.8	91	40
93	53.1	40	49.1	93	44	48.3	91	39	48.2	91	40
97	52.4	39	47.8	91	41	46.1	88	39	46.9	90	39
101	52.2	38	47.1	90	38	44.0	84	38	45.4	87	37
Mean for weeks											
1-13	31.0		31.1	100		30.6	99		30.4	98	
14-52	48.6		47.9	99		46.4	96		45.5	94	
53-101	52.6		50.3	96		48.6	92		48.7	93	

TABLE 14
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of Methyleugenol

Weeks on Study	Vehicle Control		37 mg/kg			75 mg/kg			150 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.1	50	19.2	101	50	19.2	101	50	19.3	101	50
5	23.0	50	22.8	99	50	22.6	98	50	22.5	98	50
9	26.8	50	25.9	97	50	25.6	96	50	24.8	93	50
13	29.4	50	29.6	101	50	28.8	98	50	26.6	91	50
17	34.7	50	34.3	99	50	33.0	95	50	28.8	83	50
21	38.6	50	37.6	97	50	35.6	92	50	31.0	80	50
25	40.5	50	39.7	98	50	37.6	93	50	32.9	81	50
29	43.6	49	42.5	98	50	40.1	92	50	35.0	80	50
33	47.1	49	44.7	95	50	42.4	90	50	37.1	79	50
37	49.2	49	45.4	92	50	43.1	88	50	36.8	75	50
41	50.1	49	46.6	93	50	43.4	87	50	37.9	76	50
45	51.6	49	47.7	92	50	44.6	86	50	39.5	77	50
49	53.9	49	50.1	93	50	46.2	86	50	41.1	76	50
53	54.0	49	50.0	93	50	45.6	84	50	39.7	74	50
57	56.9	49	51.0	90	50	46.6	82	49	40.6	71	49
61	58.3	49	52.3	90	50	47.0	81	49	40.8	70	49
65	59.0	49	52.4	89	50	45.6	77	49	39.0	66	49
69	60.1	49	52.2	87	49	44.0	73	48	37.9	63	47
73	59.0	49	51.7	88	48	42.5	72	46	35.3	60	47
77	60.1	49	49.7	83	46	40.8	68	44	36.1	60	44
81	61.7	49	47.1	76	46	38.5	62	43	35.6	58	42
85	63.2	47	44.0	70	41	36.8	58	42	34.7	55	40
89	62.7	46	43.0	69	36	34.6	55	39	34.6	55	34
93	61.7	44	40.5	66	31	34.1	55	34	33.7	55	29
97	61.1	39	38.0	62	26	34.8	57	29	32.9	54	23
101	60.9	36	37.4	61	23	34.2	56	25	33.0	54	10
Mean for weeks											
1-13	24.6		24.4	100		24.1	98		23.3	96	
14-52	45.5		43.2	95		40.7	90		35.6	79	
53-101	59.9		46.9	79		40.4	68		36.5	61	
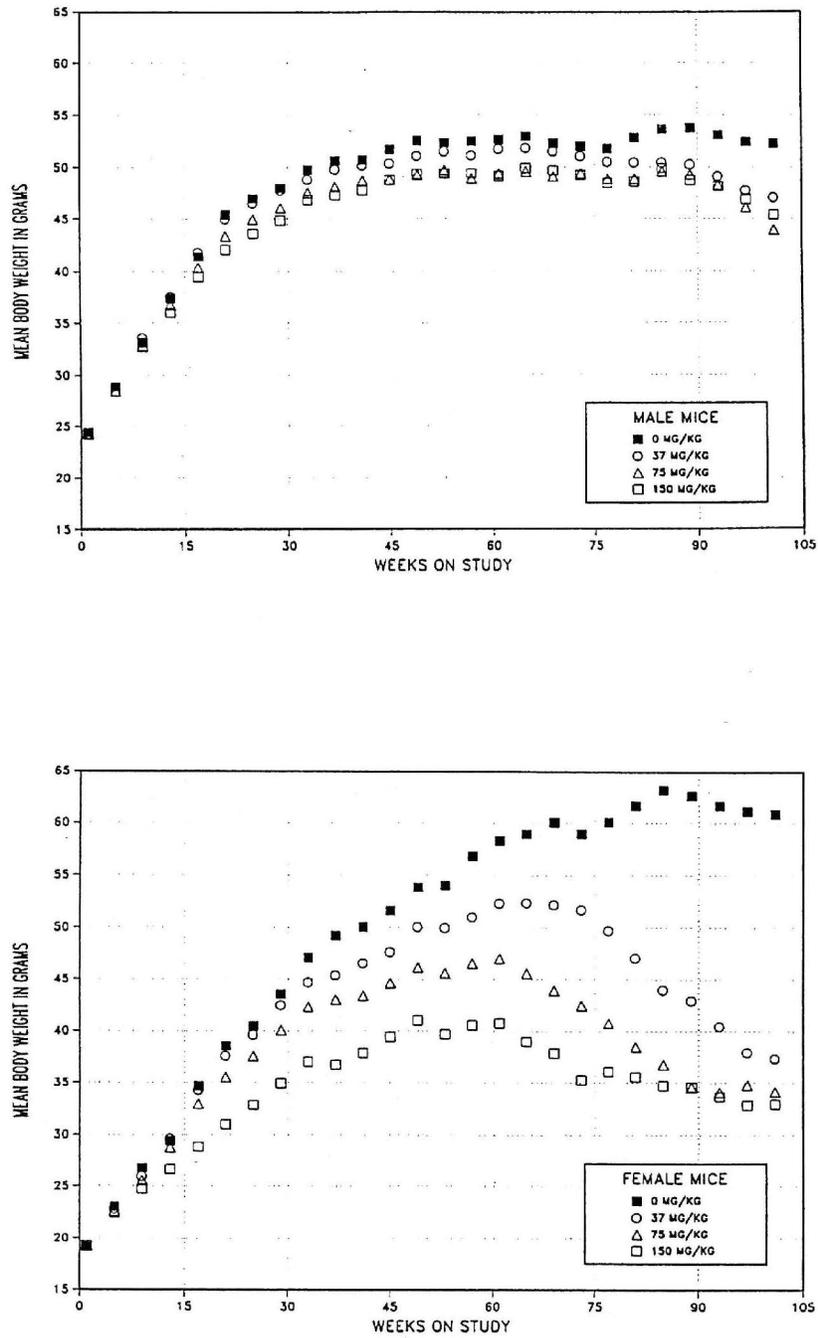
FIGURE 4
Growth Curves for Male and Female Rats
Administered Methyleugenol by Gavage for 2 Years

Toxicokinetic Studies

Detailed methods and results for the single-administration intravenous and gavage studies in young mice are presented in Appendix M, and results for the core study animals that received methyleugenol by gavage 5 days per week for 12 months and for the single-administration gavage studies in aged animals in Appendix I. Additional chemical distribution and metabolism data are presented in an unpublished absorption, distribution, metabolism, and elimination study conducted for the NTP (Appendix N).

Absorption: Absorption from oral doses was rapid, with peak plasma levels achieved within the first 5 minutes for all doses in males and females.

Distribution: Methyleugenol and its metabolites were distributed preferentially to the ovaries, stomach, fat, spleen, and liver 72 hours after oral administration of [^{14}C]-methyleugenol to males. Tissue:blood ratios of methyleugenol-derived radioactivity were approximately 5 in the liver, 5 to 9 in the stomach, 7 in the fat and the spleen, and over 100 in the ovaries after 72 hours. Many other tissues had elevated ratios; this may represent residual binding of metabolites rather than tissue solubility.

Metabolism: Approximately 85% of methyleugenol orally administered to females was eliminated in urine as parent or metabolites by 72 hours after dosing. Bioavailability of methyleugenol was low, with 3% to 5% bioavailability at 25 mg/kg. This increased to approximately 12% at 50 mg/kg and 13% to 19% at 75 mg/kg. These findings suggest a strong, but saturable, first-pass metabolic effect, leading to a non-linear relationship between dose and parent chemical dosimetry. No unchanged methyleugenol was found in urine from females dosed with methyleugenol orally. Hydroxylated, sulfated, and glucuronidated metabolites constituted a minority of the metabolites detected in urine, with the majority unknown.

Elimination: Elimination of methyleugenol from the bloodstream was rapid and multiphasic, with terminal half-lives on the order of 15 to 30 minutes. No difference in the elimination of methyleugenol between naive males and females was apparent with either young or aged animals. Aged females exhibited a significantly higher AUC; this may be due to differences in the amount of body fat or an age-related decrease in metabolic capability. Core study animals eliminated methyleugenol with AUCs similar to those of the naive animals. Exceptions were for the low-dose females. The AUCs increased linearly with dose in females at 12 months and sublinearly in males at 12 months. The latter finding is indicative of metabolic saturation at the higher doses in males at this time point.

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

Liver: Chemical-related increases in the incidences of hepatic neoplasms and nonneoplastic lesions occurred in males and females. Lesions were frequently multifocal and coexisted within the liver. Chemical-related proliferative lesions included hepatocellular adenoma and carcinoma, hepatoblastoma, hepatocholangiocarcinoma (females), eosinophilic focus (males), oval cell hyperplasia, and bile duct hyperplasia (females). Chemical-related nonproliferative lesions included chronic active inflammation in males, and hepatocyte necrosis, hemosiderin pigmentation, and hematopoietic cell proliferation in females (Table 15, Appendixes C and D).

In all dosed groups of male and female mice, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly greater than in the vehicle controls. In 37 and 75 mg/kg male mice and in all dosed groups of females, the incidences of hepatocellular carcinoma were significantly increased. Additionally, the multiple incidences of hepatocellular adenoma in dosed males and females and of hepatocellular carcinoma in dosed females were significantly increased. The incidences of hepatocellular adenoma or carcinoma (combined) in all dosed groups of males and females exceeded the historical vehicle control ranges for corn oil gavage studies (Tables 15, C4, and D4).

The incidence of hepatoblastoma in 150 mg/kg males was slightly greater than that in the vehicle controls. In females, there was a significant, dose-related increase in the incidences of hepatoblastoma. The incidences of hepatoblastoma in 150 mg/kg male mice and in all dosed groups of female mice exceeded the historical vehicle control range for corn oil gavage

studies (Tables C4 and D4). Hepatocholangiocarcinomas were observed in two 150 mg/kg females (Table 15), and the incidence exceeded the vehicle historical control range for corn oil gavage studies (Tables 15 and D4).

The incidences of eosinophilic foci were significantly increased in dosed groups of male mice. In all dosed groups of males and females, the incidences of oval cell hyperplasia were significantly increased; in males, the incidences increased with increasing dose (Tables 15, C5, and D5). In 75 and 150 mg/kg males and in all dosed groups of females, the incidences of periportal hypertrophy were significantly greater than in the vehicle controls; in males, the incidences increased with increasing dose. The incidences of bile duct hyperplasia in 75 and 150 mg/kg female mice and of hemosiderin pigmentation in all dosed groups of female mice were significantly increased. In females, there was a dose-related increase in the incidences of hepatocyte necrosis, and the increases in the 75 and 150 mg/kg groups were significant. In females, there was also a significant, dose-related increase in the incidences of hematopoietic cell proliferation (Tables 15, C5, and D5).

Hepatocellular adenomas and carcinomas were morphologically similar to those observed in the rat study. Hepatoblastomas were generally well-demarcated masses that frequently arose within hepatocellular adenomas and/or carcinomas (Plate 16). They consisted of sheets of poorly differentiated round to spindle-shaped cells that were frequently aligned radially along numerous small blood vessels. The lung was a frequent site of metastases for hepatocellular carcinomas and hepatoblastomas. Hepatocholangiocarcinomas, bile duct hyperplasia, oval cell hyperplasia, and hepatocyte hypertrophy were histologically similar to those observed in the rat study.

Based on retrospective analyses, *Helicobacter hepaticus* was determined to have infected mice in 12 recent NTP 2-year studies (Appendix S). Of the 12 studies, mice (primarily males) from nine studies had *H. hepaticus*-associated hepatitis. Qualitatively, the hepatitis and silver-staining organisms (*H. hepaticus*) within the liver were similar among the nine studies. Using an assay based on polymerase chain

TABLE 15
**Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Male and Female Mice
in the 2-Year Gavage Study of Methyleugenol**

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Male				
Number Examined Microscopically	49	50	50	50
Oval Cell, Hyperplasia[a]	0	8** (1.1)[b]	27** (1.2)	46** (1.5)
Hepatocyte, Hypertrophy	0	1 (3.0)	7** (2.3)	46** (2.8)
Eosinophilic Focus	10	20*	25**	19*
Inflammation, Chronic Active	19 (1.3)	21 (1.3)	28* (1.3)	28* (1.3)
Hepatocellular Adenoma, Multiple	13	33**	33**	29**
Hepatocellular Adenoma (includes multiple)	26	43**	38**	39**
Hepatocellular Carcinoma, Multiple	1	3	3	2
Hepatocellular Carcinoma (includes multiple)	10	20*	19*	9
Hepatocellular Adenoma or Carcinoma[c]				
Overall rate[d]	31/49 (63%)	47/50 (94%)	46/50 (92%)	40/50 (80%)
Adjusted rate[e]	65.4%	97.4%	96.4%	85.6%
Terminal rate[f]	23/38 (61%)	36/36 (100%)	36/37 (97%)	30/35 (86%)
First incidence (days)	430	593	486	512
Poly-3 test[g]	P=0.018	P<0.001	P<0.001	P=0.016
Hepatoblastoma (includes multiple)[h]	0	0	1	3
Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	10/49 (20%)	20/50 (40%)	20/50 (40%)	11/50 (22%)
Adjusted rate	21.8%	41.8%	42.2%	24.6%
Terminal rate	5/38 (13%)	10/36 (28%)	11/37 (30%)	7/35 (20%)
First incidence (days)	477	593	486	554
Poly-3 test	P=0.490N	P=0.030	P=0.027	P=0.471
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	31/49 (63%)	47/50 (94%)	46/50 (92%)	41/50 (82%)
Adjusted rate	65.4%	97.4%	96.4%	86.7%
Terminal rate	23/38 (61%)	36/36 (100%)	36/37 (97%)	30/35 (86%)
First incidence (days)	430	593	486	512
Poly-3 test	P=0.012	P<0.001	P<0.001	P=0.011

TABLE 15
**Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Male and Female Mice
in the 2-Year Gavage Study of Methyleugenol**

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Female				
Number Examined Microscopically	50	50	49	50
Hematopoietic Cell Proliferation	4 (1.3)	14** (1.8)	23** (1.7)	24** (1.6)
Pigmentation, Hemosiderin	0	11** (1.6)	24** (1.8)	19** (1.6)
Bile Duct, Hyperplasia	1 (2.0)	1 (2.0)	11** (1.5)	9** (1.8)
Hepatocyte, Necrosis	5 (2.0)	9 (2.3)	16** (2.3)	17** (2.0)
Oval Cell, Hyperplasia	0	46** (1.9)	36** (1.9)	38** (1.9)
Hepatocyte, Hypertrophy	0	10** (2.1)	7** (2.3)	23** (3.0)
Hepatocellular Adenoma, Multiple	8	39**	38**	32**
Hepatocellular Adenoma (includes multiple)	20	48**	46**	41**
Hepatocellular Carcinoma, Multiple	0	24**	29**	38**
Hepatocellular Carcinoma (includes multiple)	7	37**	47**	47**
Hepatocellular Adenoma or Carcinoma[i]				
Overall rate	25/50 (50%)	50/50 (100%)	49/49 (100%)	49/50 (98%)
Adjusted rate	54.8%	100.0%	100.0%	99.7%
Terminal rate	18/31 (58%)	18/18 (100%)	18/18 (100%)	2/2 (100%)
First incidence (days)	665	477	465	450
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatoblastoma, Multiple	0	0	0	4
Hepatoblastoma (includes multiple)[j]	0	6**	11**	15**
Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	7/50 (14%)	38/50 (76%)	48/49 (98%)	49/50 (98%)
Adjusted rate	15.5%	81.0%	98.0%	99.7%
Terminal rate	3/31 (10%)	13/18 (72%)	17/18 (94%)	2/2 (100%)
First incidence (days)	665	493	465	450
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	25/50 (12%)	50/50 (100%)	49/49 (100%)	49/50 (98%)
Adjusted rate	54.8%	100.0%	100.0%	99.7%
Terminal rate	18/31 (58%)	18/18 (100%)	18/18 (100%)	2/2 (100%)
First incidence (days)	665	477	465	450
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocholangiocarcinoma[k]	0	0	0	2

*   Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a]   Number of animals with lesion
[b]   Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c]   Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 267/514
      (52.1% ± 14.9%); range, 25%-72%
[d]   Number of animals with neoplasm per number of animals with liver examined microscopically
[e]   Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f]   Observed incidence at terminal kill
[g]   Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values
      corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential
      mortality in animals that do not reach terminal sacrifice. A negative trend is indicated by **N**.
[h]   Historical incidence: 2/514 (0.3% ± 1.0%); range, 0%-3%
[i]   Historical incidence: 138/511 (26.8% ± 14.1%); range, 8%-58%
[j]   Historical incidence: 0/511
[k]   Historical incidence: 1/511 (0.2% ± 0.6%); range, 0%-2%

reaction-restriction fragment length polymorphism (PCR-RFLP), an organism compatible with *H. hepaticus* was identified in three other studies from which adequately preserved (frozen) liver tissue was available, including tissues from 4 of 14 mice in the methyleugenol study (Malarkey *et al.*, 1997; Fox *et al.*, 1999). However, mice from these three studies did not have *H. hepaticus*-associated hepatitis. In the methyleugenol study, the incidences of one of the lesions typically associated with *H. hepaticus* infection, oval cell hyperplasia, increased with increasing dose, which is not typical of the incidences in the other nine studies. In those studies, all groups, including control animals, were relatively equally affected. Also in the methyleugenol study, dose-related increases in the incidences of oval cell hyperplasia occurred in female mice and in the rats. F344/N rats have not been shown to be infected with *H. hepaticus*, and liver lesions in infected female B6C3F$_1$ mice from NTP studies are minimal to nonexistent; therefore, oval cell hyperplasia was attributed to administration of methyleugenol and was not considered to be related to the presence of *H. hepaticus*.

In the nine studies in which the hepatitis was present, spiral organisms consistent with *H. hepaticus* were identified within the livers of animals with liver lesions using the Warthin-Starry silver stain or Steiner's modification. However, not all animals, and especially not all females, within these nine studies had liver lesions, and organisms were not identified using these stains when hepatic lesions were not present. This was true despite positive findings with the PCR-RFLP-based assay in some of the animals. In addition, using Warthin-Starry silver stains on the livers of 12 male mice (two vehicle controls and ten 150 mg/kg males) from the methyleugenol study, no organisms consistent with *H. hepaticus* were identified. While mice from this study of methyleugenol were considered to be infected with *H. hepaticus* based on the PCR-RFLP-based assay, liver disease associated with the infection was not apparent, and the infection was not considered to have compromised the outcome of the toxicology and carcinogenesis studies of methyleugenol.

Glandular Stomach: Nonneoplastic lesions observed in male and female mice included glandular ectasia, mucosal atrophy, chronic active inflammation, epithelial hyperplasia, and neuroendocrine cell hyperplasia.

In addition, malignant neuroendocrine tumors were observed in two 150 mg/kg male mice; one male in this group had a carcinoma. Lesions coexisted and were confined to the fundic region of the glandular stomach, affecting both the more superficial foveolar epithelium of the gastric pits and the deeper epithelium of the gastric glands.

A significant, dose-related increase in the incidences of mucosal atrophy of the glandular stomach occurred in male mice; the incidences of mucosal atrophy in 75 and 150 mg/kg female mice were also significantly greater than that in the vehicle controls (Tables 16, C5, and D5). The incidences of hyperplasia in 150 mg/kg males and 75 mg/kg males and females were significantly increased. The incidences of ectasia in all dosed groups of males and females were significantly increased, and the incidences in males increased with increasing dose. There was a dose-related increase in the incidences of chronic active inflammation in male mice, and these incidences in 75 and 150 mg/kg males were significantly greater than that in the vehicle controls; the incidences of this lesion were not increased in dosed females. Four males in the 150 mg/kg group had neuroendocrine cell hyperplasia.

Neuroendocrine cell hyperplasia was similar to that observed in the glandular stomach of rats and consisted of focal proliferations of ECL cells in small nests or clusters. These proliferations were confined to the glandular mucosa infiltrating between and displacing glandular epithelial cells.

Malignant neuroendocrine tumors were locally expansive invasive masses which caused moderate to marked thickening of the mucosa (Plate 17a). The neoplastic cells formed clusters, cords, or poorly defined glandular-like structures displacing and/or replacing the normal architecture of the glandular mucosa. The cells were large, polygonal to spindle-shaped with scant to abundant finely granular amphophilic cytoplasm and large pleomorphic, mostly vesicular nuclei that had one or more prominent nucleoli (Plate 17b). Invasion into the submucosa was extensive and occurred as nests of neoplastic neuroendocrine cells surrounded by moderate amounts of fibrous tissue. Infiltrates of lymphocytes, neutrophils, and macrophages were diffusely interspersed among the tumor cells. The carcinoma was an ulcerated,

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Glandular Stomach in Male and Female Mice
in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Male				
Number Examined Microscopically	49	48	49	50
Atrophy[a]	0	3 (1.0)[b]	35** (1.5)	45** (2.6)
Ectasia	13 (1.0)	25* (1.0)	40** (1.4)	49** (1.7)
Hyperplasia	0	1 (1.0)	15** (1.7)	20** (1.8)
Inflammation, Chronic Active	10 (1.0)	11 (1.1)	25** (1.2)	33** (1.3)
Neuroendocrine Cell, Hyperplasia	0	0	0	4 (3.0)
Carcinoma	0	0	0	1
Malignant Neuroendocrine Tumor	0	0	0	2
Female				
Number Examined Microscopically	45	49	46	45
Atrophy	0	0	10** (1.6)	10** (2.0)
Ectasia	14 (1.0)	33** (1.1)	31** (1.3)	38** (1.4)
Hyperplasia	0	1 (1.0)	5* (1.6)	2 (1.5)
Inflammation, Chronic Active	17 (1.1)	21 (1.0)	12 (1.0)	14 (1.1)
Neuroendocrine Cell, Hyperplasia	0	1 (1.0)	0	0

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

locally extensive, invasive mass that obliterated the mucosal architecture extending into and replacing the submucosa and much of the muscle layers of the stomach. The neoplastic cells occurred as sheets, clusters, and poorly defined cords and were ana- plastic, polygonal to spindle-shaped cells with scant eosinophilic cytoplasm and large pleomorphic hyper- chromatic nuclei.

One lesion observed in mice but not in rats consisted of focal to multifocal hyperplasia of the columnar foveolar epithelium of the gastric pits (Plate 18). This hyperplasia often extended toward the base of the mucosa with displacement of parietal and chief cells of the mucosal glands and was often accompanied by minimal to mild chief cell hyperplasia. Ectatic muco- sal glands were sometimes present at the base of hyperplastic lesions. Ectasia was multifocal and con- sisted of variably dilated glands which were lined by

flattened, often degenerate, epithelial cells (Plate 19). Many ectatic glands contained necrotic cells and cellu- lar debris.

Atrophy of the glandular mucosa was histologically similar to that observed in the rat. It was character- ized by loss of glandular epithelial cells (chief and parietal cells) in the fundic region of the stomach with concomitant reduction in the height of the fundic mucosa. The severity of atrophy was variable. Mini- mal atrophy consisted of the focal to multifocal loss of primarily chief cells in an area equivalent to the width of three glands. Mild atrophy consisted of multifo- cally extensive to diffuse loss of chief cells, whereas moderate to marked atrophy was characterized by dif- fuse loss of parietal and chief cells accompanied by a significant reduction in the height of the fundic mucosa. Minimal to mild chronic active inflammation was a consistent change associated with the gastric lesions.

It consisted of infiltrates of mononuclear leukocytes and variable numbers of neutrophils within the deep mucosa, lamina propria, and the submucosa of the glandular stomach.

Bone Marrow: In male and female mice, the incidences of bone marrow hyperplasia were significantly greater than those in the vehicle controls (males: vehicle control, 14/49; 37 mg/kg, 26/49; 75 mg/kg, 33/50; 150 mg/kg, 35/50; females: 18/50, 47/50, 46/48, 50/50; Tables C5 and D5); the incidences in male mice increased with increasing dose. Bone marrow hyperplasia was considered to be secondary to the changes in hepatic and gastric pathology.

GENETIC TOXICOLOGY

Methyleugenol, tested up to a maximum concentration of 666 μg/plate, did not induce mutations in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without induced hamster or rat liver S9 activation enzymes (Mortelmans *et al.*, 1986; Table E1). In tests for induction of chromosomal effects in cultured Chinese hamster ovary cells, methyleugenol induced sister chromatid exchanges in each of two replicate trials conducted with induced rat liver S9; no significant increase in sister chromatid exchanges was observed without S9 (Table E2). Also in cultured Chinese hamster ovary cells, no significant induction of aberrations occurred following incubation with methyleugenol in either the presence or the absence of S9 (Table E3). The doses tested in the aberrations test were similar to those used in the sister chromatid exchange test and were limited by toxicity to 233 μg/mL. Methyleugenol, administered in doses of 10 to 1,000 mg/kg by gavage to male and female B6C3F$_1$ mice for 14 weeks, did not increase the frequency of micronucleated normochromatic erythrocytes in peripheral blood and did not alter the percentage of polychromatic erythrocytes among total erythrocytes (an indication of bone marrow toxicity) (Table E4).

In conclusion, methyleugenol was not mutagenic in *S. typhimurium* and did not induce chromosomal damage in rodent cells *in vitro* or *in vivo*. A potential for methyleugenol-induced DNA damage was indicated by the positive results seen in the *in vitro* sister chromatid exchange test with Chinese hamster ovary cells in the presence of S9 activation enzymes.

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

A physiologically based pharmacokinetic (PBPK) model was developed to represent mathematically the absorption, distribution, metabolism, and elimination of methyleugenol in rats and mice (Appendix O). Data from plasma concentration time courses following intravenous and gavage administration of methyleugenol to rats and mice were used to develop the model (Appendix M). The model contains compartments for the blood, the gastrointestinal tissue, lumen, and capillary space, as well as capillary space and tissues for liver, kidney, fat, slowly perfused tissues (skin, muscle, and bone) and rapidly perfused tissues (heart, lungs, brain, and viscera). Methyleugenol entered the blood stream either directly from intravenous injection or from absorption from the gastrointestinal lumen into the portal circulation, with equilibration into gastrointestinal tissues. Plasma concentrations were assumed to be identical with blood concentrations. The absorption of methyleugenol was modeled as occurring from a single gastrointestinal lumenal compartment into the gastrointestinal capillary space, equilibrating with gastrointestinal tissues, and then entering the liver capillary space via the portal blood flow. The distribution of methyleugenol between each tissue and its capillary bed was explicitly modeled, a departure from the more common practice of assuming instantaneous equilibration of capillary and arterial blood in PBPK models. Tissue:blood partition coefficients were estimated from the octanol-water partition coefficient of methyleugenol. Other chemical-specific parameters were estimated from the plasma concentration time course data. The metabolism of methyleugenol in rats was confined to the liver and represented as a Michaelis-Menten process. The metabolism of methyleugenol in mice was confined to the liver and kidney (as a representative tissue with significant phase 1 metabolic capacity) and was also represented as a Michaelis-Menten process, with the same K_m for both tissues. The lack of a need for extrahepatic metabolism in the rat model does not lead to the conclusion that extrahepatic metabolism of methyleugenol is not important, only that the blood flow and metabolic capacity modeled for rats was sufficient to act as a surrogate for all methyleugenol metabolism.

The rat and mouse models were used to predict methyleugenol pharmacokinetic behavior from single oral doses of 37, 75, and 150 mg/kg, doses used in the chronic study. The maximum concentration (C_{max}) and the integrated concentration of methyleugenol in the liver (AUC_{liver}) over the 24-hour period following oral dosing were simulated, as was the integrated rate of hepatic metabolism (AUC_{met}) over the same 24-hour period. In both rats and mice, over 90% of administered methyleugenol was determined to have been metabolized within 24 hours. In both rats and mice, C_{max} and AUC_{liver} increased more rapidly than administered dose, while AUC_{met} increased linearly with administered dose. These results indicate that metabolism of methyleugenol is saturating at doses used in the chronic study.
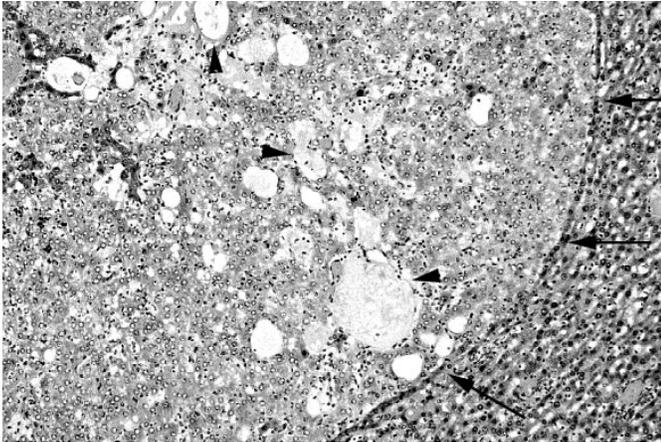
PLATE 1
Hepatocellular adenoma from a male rat exposed to 300 mg/kg methyeugenol. Note sharp demarcation from the adjacent hepatic parenchyma (arrows), loss of normal architecture and areas of cystic degeneration (arrowheads) within the adenoma. H&E; 25×.
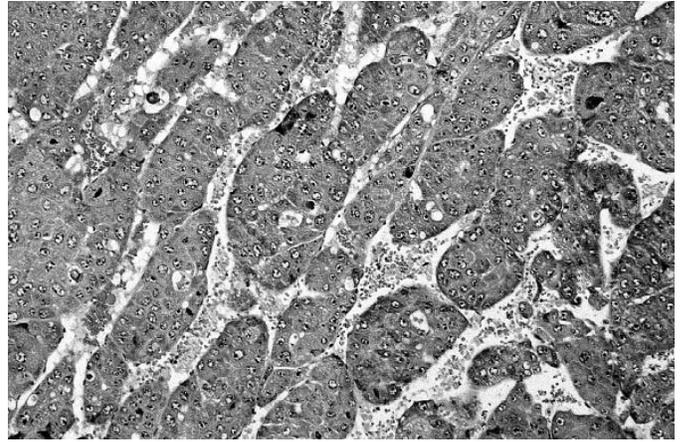
PLATE 2
Hepatocellular carcinoma from a male rat exposed to 300 mg/kg methyeugenol. Note distinct trabecular pattern characterized by thick trabeculae composed of three or more cell layers of neoplastic hepa-tocytes. H&E; 33×.
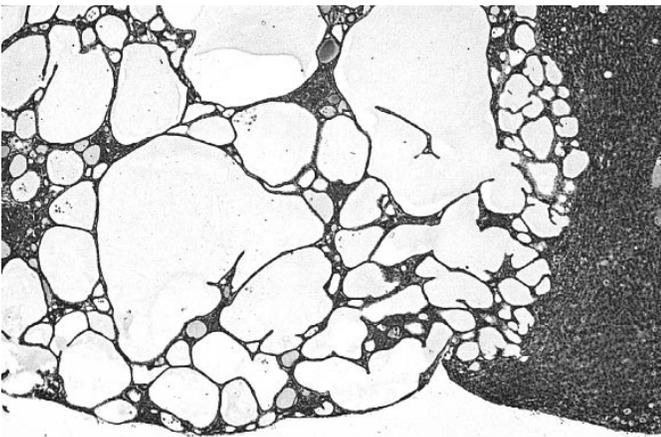
PLATE 3
Cholangioma from a male rat exposed to 300 mg/kg methyeugenol. The multilocular cholangioma is well-demarcated from the surround-ing hepatic parenchyma and is composed of multiple cystic spaces of variable sizes. H&E; 8×.
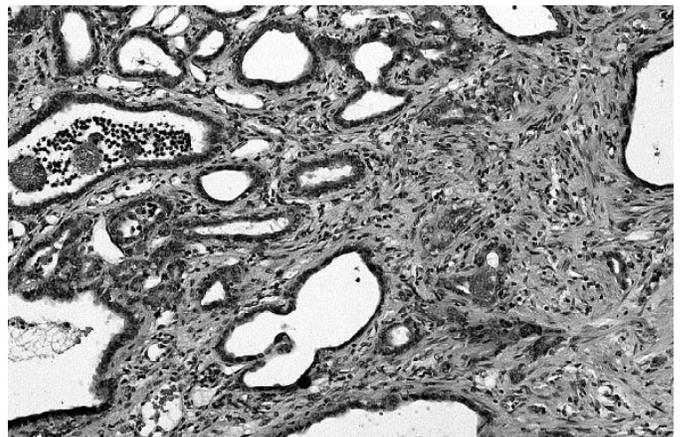
PLATE 4
Cholangiocarcinoma from a female rat exposed to 300 mg/kg methyeugenol. Note irregular ductular structures lined by cuboidal biliary-like epithelial cells and surrounded by abundant connective tissue stroma. H&E; 40×.
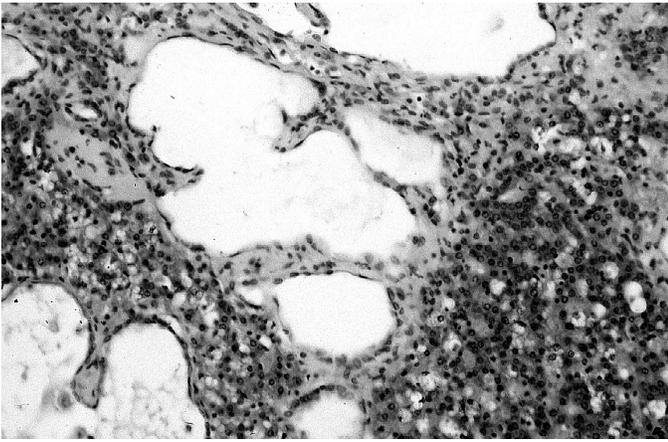
PLATE 5
Hepatocholangioma from a male rat exposed to 300 mg/kg methyeugenol. H&E; 50×.
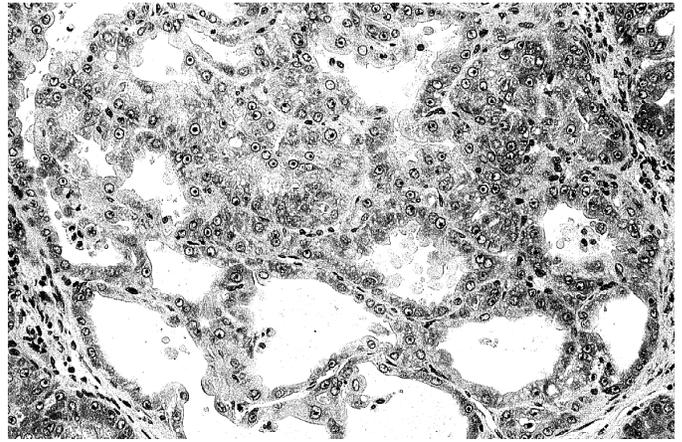
PLATE 6
Hepatocholangiocarcinoma from a male rat exposed to 300 mg/kg methyeugenol. Poorly-differentiated hepatocytes and biliary epithelium form glandular structures. H&E; 50×.
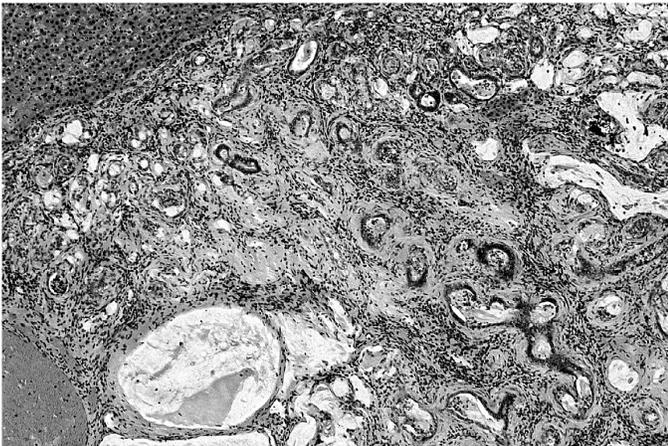
PLATE 7
Atypical bile duct hyperplasia from a male rat exposed to 3000 mg/kg. Note multiple atypical bile ducts surrounded by connective tissue stroma. H&E; 20×.
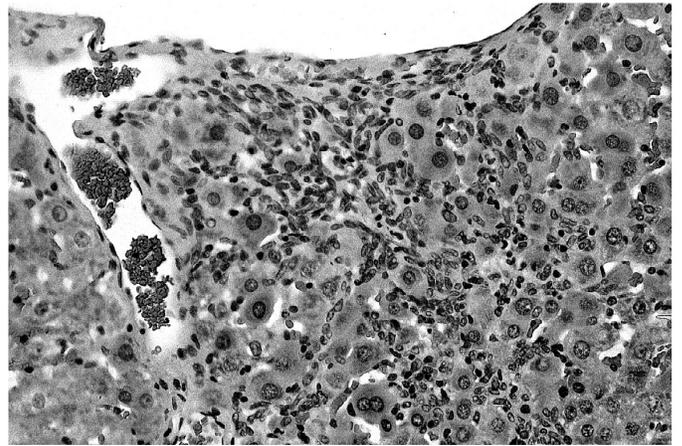
PLATE 8
Oval hyperplasia in a male rat exposed to 300 mg/kg methyleugenol. Note small ovoid cells proliferating within the hepatic sinusoids. Note also nuclei of enlarged (hypertrophic) hepatocytes. H&E; 66×.
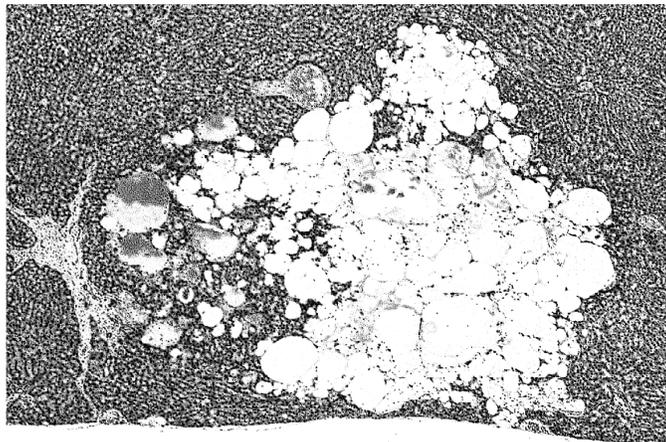
PLATE 9
Focal cystic degeneration from a male rat exposed to 300 mg/kg methyleugenol. Note characteristic multiple cyst-like spaces which contain flocculent material. H&E; 13×.
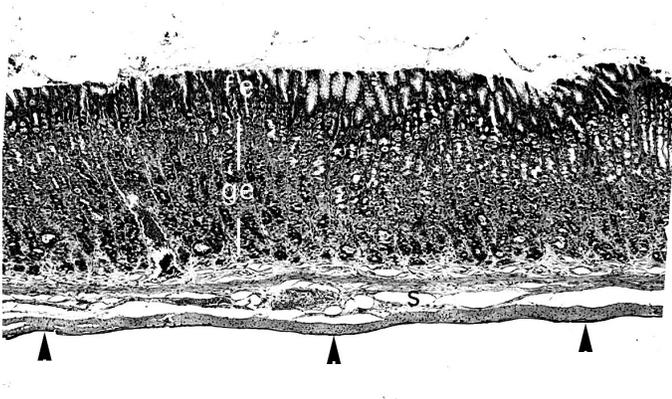
PLATE 10a
Control rat, fundic region of the glandular stomach. Foveolar epithelium of the gastric pits (fe), mucosal glandular epithelium (ge), submucosa (s), tunica muscularis (arrowheads). H&E; 16×.
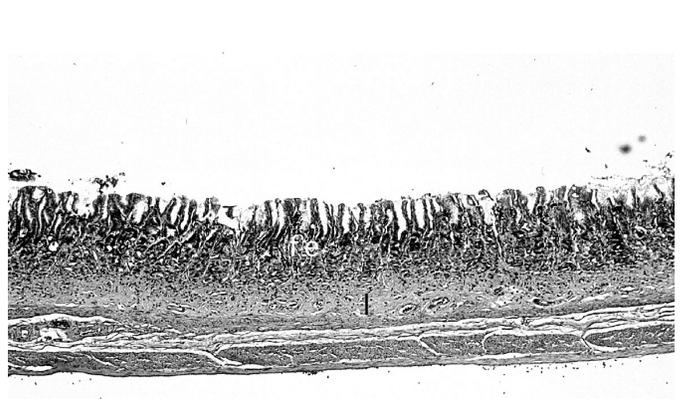
PLATE 10b
Atrophy in the glandular stomach of a female rat exposed to 300 mg/kg methyleugenol. Note decrease in the height of the mucosa due to loss of the mucosal glands with condensation and fibrosis of the lamina propria (l). H&E; 16×.
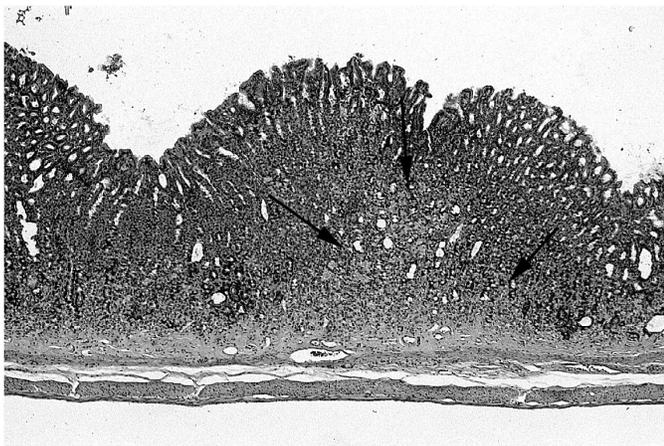
PLATE 11a
Focal neuroendocrine cell hyperplasia (arrows) in the glandular stomach of a male rat exposed to 300 mg/kg methyleugenol. H&E; 13×
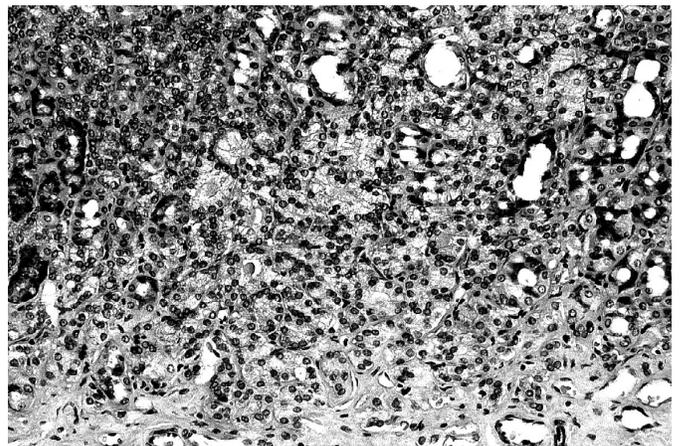
PLATE 11b
Detail of Plate 12. Hyperplastic cells have pale finely granular cytoplasm, small round nuclei and form small poorly-delineated glandular structures. H&E; 50×.
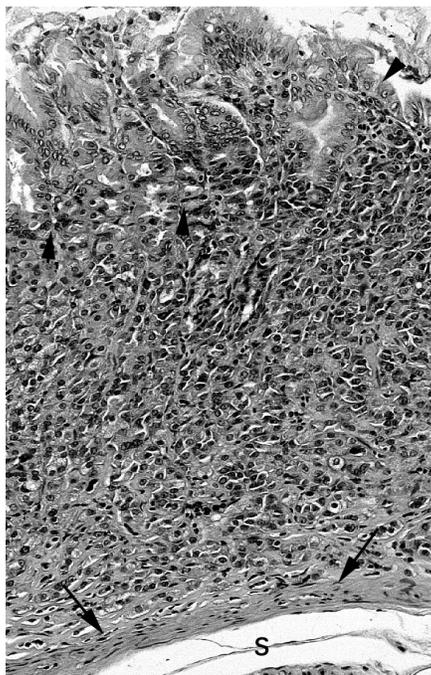
PLATE 12

Benign neuroendocrine tumor in the glandular stomach of a female rat exposed to 300 mg/kg methyleugenol. Neoplastic neuroendocrine cells have displaced and replaced glandular elements but are confined to the mucosa. Note the foveolar epithelium of the gastric pits (arrowheads) and the muscularis mucosa (arrows) separating the mucosa from the submucosa (s). H&E; 50×.
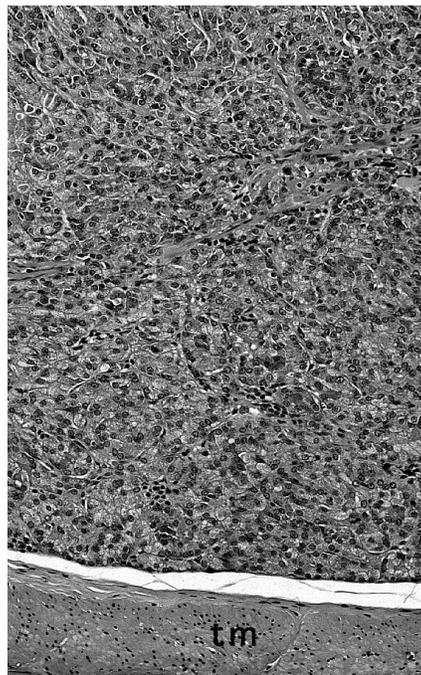
PLATE 13

Malignant neuroendocrine tumor in the glandular stomach of a female rat exposed to 150 mg/kg methyleugenol. Neoplastic neuroendocrine cells have completely replaced the gastric glands, muscularis mucosa and submucosa. Tunica muscularis (tm) H&E; 40×.
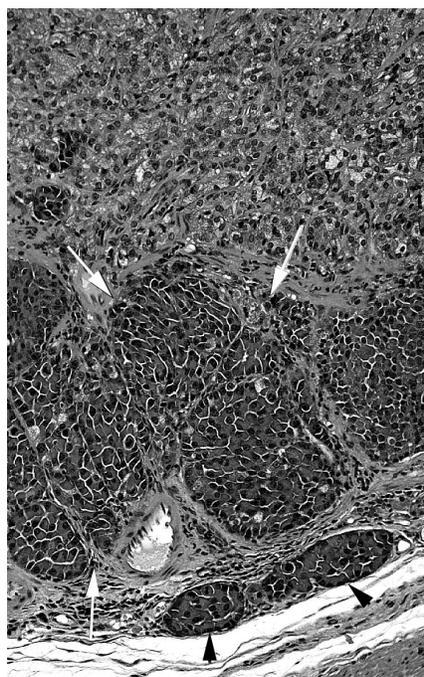
PLATE 14

Malignant neuroendocrine tumor in the glandular stomach of a female rat exposed to 300 mg/kg methyleugenol. Neoplasm is composed of a mixture of neoplastic neuroendocrine cells and nodular aggregates of atypical polygonal cells (arrows). Note neoplastic cell emboli within submucosal blood vessels (arrowheads). H&E; 40×.
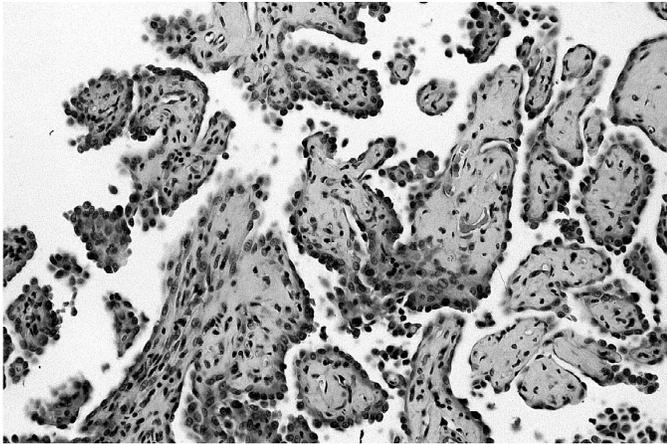
PLATE 15
Malignant mesothelioma from the mesentery of a male rat exposed to 300 mg/kg methyleugenol. Mesothelioma is composed of papilliferous proliferations of connective tissue stroma lined by cuboidal mesothelial cells. H&E; 50×.
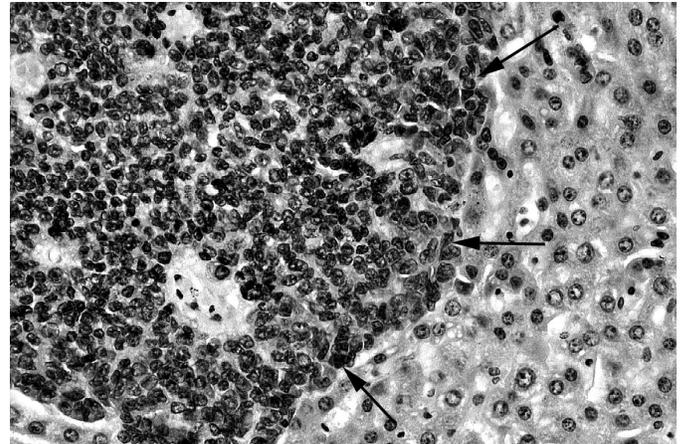
PLATE 16
Hepatoblastoma from a female mouse exposed to 150 mg/kg methyleugenol. Note sharp demarcation from the adjacent hepatic parenchyma (arrows), and characteristic cords of small round to spindyloid tumor cells with dark staining nuclei. H&E; 80×.
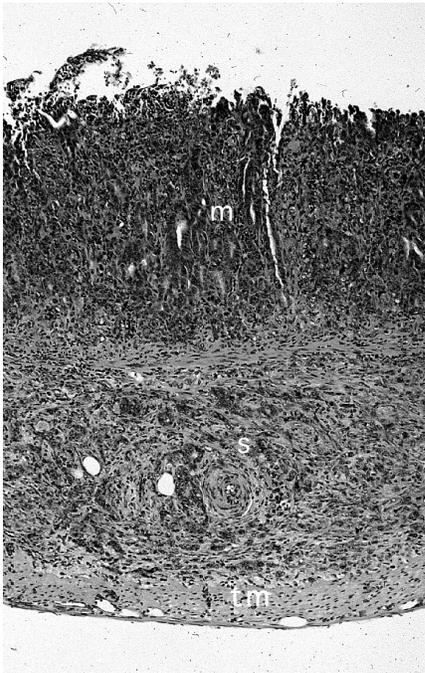
PLATE 17a
Malignant neuroendocrine tumor from the glandular stomach of a male rat exposed to 150 mg/kg methyleugenol. a) The neoplasm has effaced the mucosa (m) and has invaded and expanded the submucosa (s). Tunica muscularis (tm) H&E; 25×.
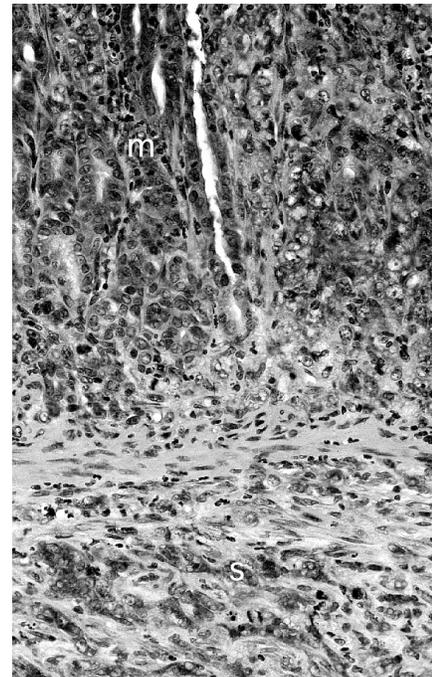
PLATE 17b
Detail of (a) showing neoplastic neuroendocrine cells within the mucosa (m) and invading submucosa (s). H&E; 66×.

PLATE 18
Focal epithelial hyperplasia in the glandular stomach of a male rat exposed to 150 mg/kg methyleugenol. The hyperplastic epithelium extends from the mucosal surface to the muscularis mucosa. Note dilated (ectatic) glands within the hyperplastic focus. H&E; 40×.
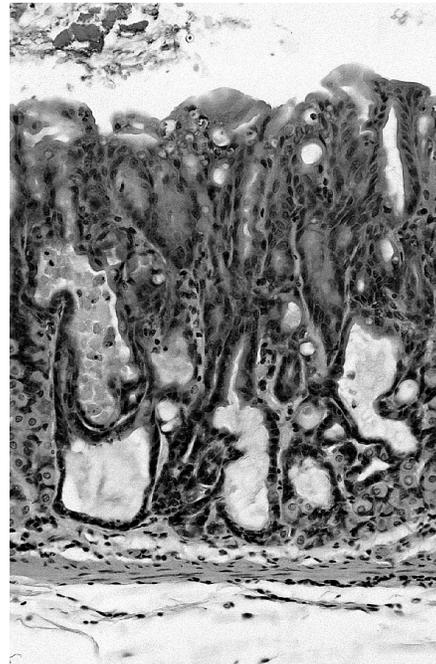
PLATE 19
Multifocal glandular ectasia in stomach of a male rat exposed to 150 mg/kg methyleugenol. The ectatic glands contain necrotic cellular debris. H&E; 50×.

DISCUSSION AND CONCLUSIONS

Toxicology and carcinogenesis studies of methyleugenol were conducted because of potential widespread human exposure and because of its structural resemblance to safrole, a known rodent carcinogen (IARC, 1976). Fourteen-week and 2-year toxicology and carcinogenicity studies were conducted by administering methyleugenol in 0.5% aqueous methylcellulose by gavage to male and female F344/N rats and B6C3F$_1$ mice. Although the principal route of human exposure is via food, the gavage route of administration was used because results of preliminary NTP studies showed methyleugenol in feed to be unpalatable to rats and mice (Battelle, 1997).

Principal findings in the 14-week and 2-year studies of methyleugenol included reduced survival rates (except in 14-week rats), depressed mean body weight gains, and marked liver and glandular stomach lesions in rats and mice at the higher doses. In the 2-year studies, the survival rates of male rats administered 150 or 300 mg/kg and of all dosed groups of female mice were reduced relative to that of the vehicle controls. However, because the majority of the deaths occurred late in the study (after week 85) and were due to liver and glandular stomach neoplasms in rats and liver neoplasms in mice, the doses used for the 2-year studies were considered adequate for evaluating the carcinogenicity of methyleugenol.

The dose-related decreases in mean body weight gain of rats and mice were likely related to the toxicity of methyleugenol to the liver and the glandular stomach. Toxic effects in the liver may cause a derangement in protein, carbohydrate, or fat metabolism with subsequent depression in body weight gain. The hypoproteinemia and hypoalbuminemia observed in dosed rats in the 14-week study suggest that methyleugenol impaired hepatic protein synthesis. Decreases in serum protein concentrations can be caused by several factors, including impaired hepatic protein synthesis (Kaneko, 1989; Nguyen, 1989). The effect of methyleugenol on body weight appears to be irreversible as indicated by the lack of recovery in rats in the stop-exposure groups when dosing was discontinued for

12 months. Methyleugenol-induced glandular stomach atrophy, characterized by loss of parietal and chief cells, may also have contributed to depression in body weight gain. Parietal cells produce gastric acid (hydrochloric acid), and G cells produce proenzymes such as pepsinogen (Guyton and Hall, 1997). The loss of parietal cells results in hypochlorhydria, increased pH, and decreased activities of acid-activated digestive enzymes such as pepsin, trypsin, and chymotrypsin, which in turn lead to inefficient utilization of food and depression in body weight. In addition, the loss of chief cells results in reduced pepsinogen (pepsin precursor) production, which will also lead to inefficient food utilization. Methyleugenol administration caused a significant increase in the stomach pH of female F344/N rats receiving 1,000 mg/kg by gavage for 30 days or 150 mg/kg or greater for 90 days (Appendix P).

In the 14-week studies, the increases in liver weights of rats and mice and the increase in testis weight of rats administered 1,000 mg/kg were the only organ weight differences considered to be related to methyleugenol administration. The increases in liver weights may have been due in part to the induction of cytochrome P$_{450}$ and P$_{448}$ enzyme systems. Allylbenzenes, including methyleugenol, are potent inducers of these enzyme systems (Ioannides *et al.*, 1981; Gardner *et al.*, 1997). The increase in liver and testis weights of dosed animals may also have been caused by toxicity related to the formation of methyleugenol adducts with proteins. A protein adduct was detected in the microsomal fraction of the liver of F344 rats administered methyleugenol intraperitoneally (Gardner *et al.*, 1996).

Hepatocellular injury caused by methyleugenol administration was evidenced by increases in serum alanine aminotransferase and sorbitol dehydrogenase activities and bile acid concentration in dosed rats in the 14-week study. Liver lesions induced by methyleugenol in rats and mice included cytologic alteration, bile duct hyperplasia, necrosis (mice), foci of hepatocellular alteration, hepatocyte hypertrophy, and oval

cell hyperplasia. The oval cell hyperplasia and hepatocellular hypertrophy observed in rats at 6 and 12 months persisted until the end of the stop-exposure study, suggesting that these lesions are irreversible.

Methyleugenol caused increased incidences of liver neoplasms in rats and mice. The increases were marked, particularly in male rats and female mice, which had high rates of metastasis of these neoplasms to the lungs. The vast majority of the neoplasms that occurred were primarily composed of neoplastic hepatocytes (hepatocellular adenomas and carcinomas). Additionally, in rats, a large group of neoplasms consisting of an admixture of what appeared to be hepatocytes and biliary epithelium were classified as hepatocholangioma or hepatocholangiocarcinoma. These are uncommon spontaneous neoplasms in F344/N rats. From a histomorphologic and biologic standpoint, classification of liver neoplasms into this particular category is not always straightforward. Some prefer to classify most neoplasms with this morphology as glandular variants of hepatocellular neoplasms. In addition, three neoplasms composed of neoplastic biliary epithelium were identified in the 300 mg/kg rats (two males with cholangioma and one female with cholangiocarcinoma). These are uncommon spontaneous neoplasms in F344/N rats.

While studied extensively, the exact histogenesis of liver neoplasms remains controversial. Some recent literature suggests that a putative stem cell is likely to play an important role in the development of some liver neoplasms (Sell and Dunsford, 1989). Oval cells are considered to represent or arise from the stem cell and are thought to have the potential to develop into hepatocytes and/or biliary epithelial cells (Factor et al., 1994). Oval cell proliferation was increased in dosed animals in the studies of methyleugenol. Some rat liver tumorigens cause an increase in the incidence of a relatively pure population of hepatocellular neoplasms, while others cause increased incidences of both hepatocellular and cholangiolar neoplasms (Maronpot et al., 1991). It is possible that the spectrum of neoplasms induced by methyleugenol represents a combination of these effects. The proliferative lesions observed in the studies are considered related to administration of methyleugenol.

As is frequently observed in many NTP studies in which robust liver neoplasm responses occur in mice, the incidences of hepatoblastoma in the liver were increased. While the increases are usually most pronounced in male mice, in the current study the increase was more pronounced in female mice. Hepatoblastomas in the mouse are uncommon neoplasms which occur spontaneously or may be chemically induced in the liver of several strains (Turusov et al., 1973; Nonoyama et al., 1988), including the B6C3F$_1$ mouse used in NTP studies. It is considered a malignant neoplasm, and in NTP studies, its metastatic potential appears similar to that of hepatocellular carcinomas. Hepatoblastomas are easily diagnosed because of their distinctive morphology on hematoxylin- and eosin-stained sections and were typical in this study.

Hepatoblastomas almost always occur within an existing proliferative lesion, most often within a hepatocellular carcinoma, and when that occurs in NTP studies, the entire proliferative lesion is diagnosed as a hepatoblastoma. The cell of origin of the hepatoblastoma has not been clearly defined in rodents or humans, but it may be a very primordial cell (Abenoza et al., 1987; van Eyken et al., 1990; Stocker, 1994). Although the histogenesis is not fully understood, the hepatoblastoma is considered to be part of the spectrum of liver neoplasms that occurs both spontaneously and as a result of chemical treatment. Whereas individual analyses are informative, the NTP considers the combinations of hepatocellular carcinoma or hepatoblastoma and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma to be the most important in evaluating the carcinogenic potential of an agent on the liver.

In the stop-exposure groups, the incidences of liver neoplasms observed after the administration of methyleugenol was discontinued were greater than those observed at 12 months or in the vehicle control groups at 2 years. Similarly, the increased incidences of oval cell hyperplasia and hepatocellular atrophy observed in rats at 6 and 12 months persisted until the end of the stop-exposure study. These results suggest that the effect of methyleugenol on the liver is irreversible and that the preneoplastic lesions produced are likely to progress and produce neoplasms in the absence of continued chemical exposure.

The finding that methyleugenol induced hepatic neoplasms is consistent with findings observed for other chemicals with structures resembling that of

methyleugenol; these chemicals include estragole, safrole, and isosafrole (IARC, 1976; Miller *et al.*, 1983; CCRIS, 1998). Methyleugenol also was found to induce hepatic neoplasms in mice (Miller *et al.*, 1983). Eugenol was not a hepatocarcinogen in the Miller *et al.* (1983) studies and gave equivocal results in the NTP (1983) studies. Miller *et al.* (1983) showed that the 1′-hydroxy metabolites of these chemicals are more potent hepatocarcinogens than the parent compounds. Studies from the same laboratory showed the importance of sulfation in the hepatotumorigenicity of the 1′-hydroxy metabolites (Boberg *et al.*, 1983). These researchers found that the tumorigenicity of 1′-hydroxysafrole can be inhibited by sulfotransferase inhibitors and can be markedly reduced in brachymorphic mice (mice with diminished sulfation capacity).

There were increased incidences of neuroendocrine tumors of the glandular stomach in dosed male and female rats in the 2-year study. Malignant neuroendocrine tumors of the glandular stomach also occurred in two male mice in the 150 mg/kg group in the 2-year study. Because these neoplasms have not been observed previously in NTP gavage studies and because the incidences of these neuroendocrine tumors occurred with a positive trend in rats and male mice and were significantly increased in dosed rats, these neoplasms in both rats and male mice were considered to be related to methyleugenol administration.

The increased incidences of neuroendocrine tumors were also generally associated with significant dose-related increases in the incidences of glandular stomach atrophy and neuroendocrine cell (enterochromaffin-like cell) hyperplasia. Gastric atrophy was observed in both the 14-week and 2-year studies. The atrophy was characterized by the thinning of the fundic mucosa due to a loss of parietal cells and/or chief cells. As mentioned previously, the loss of parietal cells results in decreased gastric acid secretion. Reduced acid production and increased pH in the stomach are known to lead to gastrin production. Increases in stomach pH and serum gastrin levels occurred in female F344/N rats receiving up to 150 mg/kg and increases in serum gastrin levels occurred in male B6C3F$_1$ mice receiving 75 mg/kg or greater (Appendix P). Many reports have shown that long-term exposure to inhibitors of gastric acid secretion leads to induction of enterochromaffin-like

cell tumors (Poynter and Selway, 1991; Johnson *et al.*, 1993; Thake *et al.*, 1995); the magnitude of the proliferative response varies with the compound. For the most part, compounds in these reports are considered nongenotoxic, and there is a direct inhibitory effect on gastric parietal cells which results in decreased hydrochloric acid production.

In a study of butachlor (Thake *et al.*, 1995), atrophy of the fundic mucosa with a loss of parietal cells occurred in rats along with hypochlorhydria and hypergastrinemia. Thake *et al.* (1995) postulated that the neuroendocrine tumors of the glandular stomach produced by butachlor were the result of long-term gastrin stimulation of the enterochromaffin-like cells. Because fundic mucosal atrophy was observed in the current studies of methyleugenol, it is probable that hypochlorhydria and hypergastrinemia played a role in the neuroendocrine cell proliferative response. In the 14-week studies of methyleugenol, there was evidence of inflammation, cellular degeneration, and necrosis in addition to gastric mucosal atrophy, suggesting that parietal cell cytotoxicity may have preceded mucosal atrophy. In additional studies of methyleugenol performed by the NTP (Appendixes P and Q), there were chemical-related increases in stomach pH, serum gastrin, and cell proliferation in the fundic glands. These changes suggest that the mechanisms for neuroendocrine tumor induction by methyleugenol and butachlor are similar. However, methyleugenol has some genotoxic activity that should also be considered when attempting to determine the pathogenesis of the neuroendocrine proliferative response.

The high lipophilicity and the extremely rapid absorption of methyleugenol may explain the toxicity of this chemical to the liver and the stomach of rats and mice. The octanol-water partition coefficient for methyleugenol was estimated to be 800, indicating that the chemical is lipophilic and can pass cell membranes easily (Battelle, 1998). Furthermore, the toxicokinetic data for rats and mice showed that the time to achieve maximum concentration in the blood was short (approximately 5 to 15 minutes). Because maximum blood concentrations were reached long before the stomach could have emptied, it is concluded that the chemical was absorbed from the stomach. This conclusion is supported by the damaging effects of

methyleugenol administration to the glandular stomach. The rapid absorption of methyleugenol suggests that the chemical was transported in a bolus dose via the portal vein to the liver, thus causing the severe hepatic effects observed in dosed rats and mice. Methyleugenol is metabolized by the cytochrome P450 system (Borchert *et al.*, 1973). According to Solheim and Scheline (1976), the metabolism involves *O*-demethylation, side-chain hydrolysis, and diol formation. Of the metabolites formed, the two reactive metabolites 1′-hydroxymethyleugenol and the epoxide diol were most likely to be responsible for the toxic effects at these two sites. Like the hepatocarcinogen safrole, methyleugenol showed DNA-binding activity in *in vitro* rat liver slices only in the presence of metabolic activation (unpublished NTP data). The adduct-forming activity of methyleugenol may have been a contributing factor to its hepatotumorigenic activity. Devereux *et al.* (1999) investigated the somatic mutations of β-catenin (a regulator of the cadherin-mediated cell-adhesion system in the Wnt signal transduction pathway) in hepatocellular adenomas and carcinomas in methyleugenol-treated and control B6C3F$_1$ mice (Appendix R). The majority of methyleugenol-induced neoplasms showed β-catenin mutations. Identical mutations have been found in human hepatocellular neoplasms (de la Coste *et al.*, 1998), suggesting similar pathways of carcinogenesis in both species. Accumulations of cytoplasmic β-catenin, which result from mutations of the β-catenin gene, are causatively associated with colon cancer (Takahashi *et al.*, 1998).

In the 2-year study, in addition to liver and glandular stomach neoplasm induction, methyleugenol caused increases in the incidences of other neoplasms in dosed rats including kidney neoplasms (males), malignant mesothelioma, mammary gland fibroadenoma, subcutaneous fibroma (males), and subcutaneous fibroma or fibrosarcoma (combined). Because the incidences of these neoplasms occurred with dose-related trends or were markedly increased in certain dosed groups at rates greater than the historical control rates for corn oil gavage studies, the increases were considered to be related to methyleugenol administration. The marked liver and stomach neoplasm responses may have limited the expression of these additional neoplasms in groups administered 75 mg/kg or greater. The possible mechanisms of tumorigenesis of methyleugenol in these organs are unknown.

The incidence of forestomach squamous cell papilloma or carcinoma (combined) in the 150 mg/kg female rats was not significantly different from that in the vehicle controls. However, because the incidence in this group exceeded the historical vehicle control range for corn oil gavage studies, the increase in the incidence of these neoplasms may have been related to methyleugenol administration.

The increased eosinophilic granularity (cytoplasmic alteration) in the submandibular salivary gland in dosed rats is considered secondary to the toxic effects of methyleugenol on the glandular stomach. Dietary factors such as protein starvation are known to cause loss of zymogen granules (McBride *et al.*, 1987). Methyleugenol administration in rats may have created such a condition due to loss of parietal and chief cells of the glandular stomach. Acid conditions and production of protein digesting enzymes are the functions of these two types of cells, respectively. Loss of these cells could inhibit proper protein utilization and essentially lead to protein deficiency.

There were increases in the incidences of bone marrow hyperplasia in dosed rats and mice and hematopoietic proliferation in the liver of mice. These increased incidences were considered to be secondary to the increased incidences of necrosis and inflammation associated with the large and multiple liver neoplasms in dosed animals.

Uterine atrophy observed in rats administered 300 or 1,000 mg/kg in the 14-week study is likely due to the depressed mean body weights of these animals. Testicular dilatation observed in all male rats in the 1,000 mg/kg group was considered to be related to chemical administration. However, the underlying mechanism of the induction of these lesions by methyleugenol is not known. None of the lesions observed in dosed rats or mice were observed in similar NTP studies in which rats and mice were given up to 12,500 ppm eugenol (a structurally related chemical) in feed (NTP, 1983). No reports indicating that the glandular stomach, adrenal gland, testis, or uterus of rats were sites of toxicity for alkenylbenzenes with structural resemblances to methyleugenol were found in the literature.

The limited genotoxicity data for methyleugenol include negative results in *Salmonella typhimurium*

gene mutation assays (Sekizawa and Shibamoto, 1982; Mortelmans *et al.*, 1986; Kettering and Torabinejad, 1995), with and without liver S9 activation enzymes, and negative results in mammalian cell chromosome damage tests *in vitro* and *in vivo*. However, there was induction of unscheduled DNA synthesis (DNA repair) in human and rodent hepatocytes exposed *in vitro* and *in vivo* (Phillips *et al.*, 1984; Howes *et al.*, 1990; Chan and Caldwell, 1992; Gardner *et al.*, 1997). This may be important in some of the neoplastic responses observed in dosed male and female rats and mice in these studies of methyleugenol. The no-observed-effect level for methyleugenol for rats and mice was not reached in either the 14-week or the 2-year studies but could be well below 37.5 mg/kg, the lowest dose used in these studies.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** of

methyleugenol in male and female F344/N rats based on the increased incidences of liver neoplasms and neuroendocrine tumors of the glandular stomach in male and female rats and the increased incidences of kidney neoplasms, malignant mesothelioma, mammary gland fibroadenoma, and subcutaneous fibroma and fibroma or fibrosarcoma (combined) in male rats. A marginal increase in the incidence of squamous cell neoplasms of the forestomach may have been related to methyleugenol administration in female rats. There was *clear evidence of carcinogenic activity* of methyleugenol in male and female B6C3F$_1$ mice based on the increased incidences of liver neoplasms. Neuroendocrine tumors of the glandular stomach in male mice were also considered related to methyleugenol administration.

In male and female rats and mice, methyleugenol administration caused significant increases in the incidences of nonneoplastic lesions of the liver and glandular stomach.

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*   Explanation of Levels of Evidence of Carcinogenic Activity is on page 14. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 16.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS
IN THE 2-YEAR GAVAGE STUDY
OF METHYLEUGENOL

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

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Disposition Summary					
Animals initially in study	60	50	50	50	60
6-Month interim evaluation	5				5
12-Month interim evaluation	5				5
Early deaths					
Accidental deaths		1	3	1	1
Moribund	15	18	15	20	23
Natural deaths	15	15	17	29	26
Survivors					
Terminal sacrifice	20	16	15		
Animals examined microscopically	60	50	50	50	60

Systems Examined at 6 Months with No Neoplasms Observed
Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

12-Month Interim Evaluation
Alimentary System

Liver	(5)				(5)
Hepatocellular adenoma					2 (40%)
Hepatocellular adenoma, multiple					2 (40%)
Hepatocholangiocarcinoma					1 (20%)

Genital System

Testes	(5)				(5)
Bilateral, interstitial cell, adenoma	4 (80%)				5 (100%)
Interstitial cell, adenoma	1 (20%)				

Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)

12-Month Interim Evaluation (continued)
Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

2-Year Study
Alimentary System

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Intestine large, cecum	(50)	(50)	(50)	(50)	(49)
Intestine small, jejunum	(50)	(50)	(50)	(50)	(50)
Polyp adenomatous	1 (2%)				
Liver	(50)	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas					1 (2%)
Cholangioma					2 (4%)
Fibrous histiocytoma, metastatic, skin		1 (2%)			
Hepatocellular carcinoma	2 (4%)	3 (6%)	13 (26%)	14 (28%)	13 (26%)
Hepatocellular carcinoma, multiple		1 (2%)	11 (22%)	23 (46%)
Hepatocellular adenoma	5 (10%)	7 (14%)	9 (18%)	14 (28%)	8 (16%)
Hepatocellular adenoma, multiple		5 (10%)	14 (28%)	24 (48%)	24 (48%)
Hepatocholangiocarcinoma			1 (2%)	1 (2%)	6 (12%)
Hepatocholangiocarcinoma, multiple					1 (2%)
Hepatocholangioma				1 (2%)	3 (6%)
Hepatocholangioma, multiple					3 (6%)
Ito cell tumor benign					1 (2%)
Interstitial cell, carcinoma, metastatic, testes					1 (2%)
Mesentery	(5)	(6)		(1)	(1)
Hepatocellular carcinoma, metastatic, liver					1 (100%)
Leiomyosarcoma				1 (100%)	
Oral mucosa				(1)	
Pharyngeal, squamous cell papilloma				1 (100%)	
Pancreas	(50)	(50)	(50)	(50)	(49)
Adenoma			1 (2%)	1 (2%)	
Carcinoma					1 (2%)
Hepatocellular carcinoma, metastatic, liver				1 (2%)	1 (2%)
Leiomyosarcoma, metastatic, mesentery				1 (2%)	
Salivary glands	(50)	(50)	(50)	(48)	(48)
Schwannoma malignant	1 (2%)				
Stomach, forestomach	(50)	(50)	(50)	(50)	(49)
Squamous cell carcinoma					1 (2%)
Squamous cell papilloma		1 (2%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Alimentary System (continued)					
Stomach, glandular	(50)	(50)	(50)	(50)	(49)
Neuroendocrine tumor, benign				3 (6%)	2 (4%)
Neuroendocrine tumor, malignant				4 (8%)	2 (4%)
Tongue		(2)		(1)	
Squamous cell papilloma		2 (100%)		1 (100%)	
Cardiovascular System					
Heart	(50)	(50)	(50)	(50)	(49)
Endocardium, schwannoma malignant		1 (2%)			
Endocrine System					
Adrenal cortex	(50)	(50)	(50)	(50)	(50)
Adenoma		1 (2%)			
Hepatocellular carcinoma, metastatic, liver					1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)		1 (2%)	1 (2%)	
Pheochromocytoma benign	18 (36%)	12 (24%)	8 (16%)	8 (16%)	9 (18%)
Bilateral, pheochromocytoma benign	6 (12%)	5 (10%)	3 (6%)	2 (4%)	
Islets, pancreatic	(50)	(50)	(50)	(50)	(49)
Adenoma	3 (6%)	1 (2%)			
Parathyroid gland	(48)	(49)	(48)	(45)	(46)
Adenoma	1 (2%)	1 (2%)			
Pituitary gland	(50)	(50)	(50)	(50)	(50)
Oligodendroglioma malignant, metastatic, brain			1 (2%)		
Pars distalis, adenoma	7 (14%)	8 (16%)	8 (16%)	2 (4%)	3 (6%)
Thyroid gland	(50)	(50)	(50)	(48)	(48)
C-cell, adenoma	6 (12%)	4 (8%)	2 (4%)	3 (6%)	1 (2%)
Follicular cell, adenoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Follicular cell, carcinoma		1 (2%)	1 (2%)		
General Body System					
Peritoneum	(1)	(1)	(2)	(6)	(2)
Squamous cell carcinoma, metastatic, ureter					1 (50%)
Genital System					
Epididymis	(50)	(49)	(49)	(50)	(50)
Squamous cell carcinoma, metastatic, ureter					1 (2%)
Preputial gland	(50)	(50)	(49)	(50)	(50)
Adenoma	8 (16%)	12 (24%)	3 (6%)	7 (14%)	4 (8%)
Carcinoma	5 (10%)	1 (2%)	2 (4%)		
Bilateral, adenoma	1 (2%)	4 (8%)		1 (2%)	2 (4%)
Prostate	(50)	(50)	(49)	(49)	(49)
Carcinoma				1 (2%)	
Leiomyosarcoma, metastatic, mesentery				1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Genital System (continued)					
Seminal vesicle	(50)	(50)	(50)	(50)	(50)
Leiomyosarcoma, metastatic, mesentery				1 (2%)	
Squamous cell carcinoma, metastatic, ureter					1 (2%)
Testes	(50)	(49)	(49)	(50)	(50)
Squamous cell carcinoma, metastatic, ureter					1 (2%)
Bilateral, interstitial cell, adenoma	45 (90%)	46 (94%)	48 (98%)	46 (92%)	41 (82%)
Interstitial cell, adenoma	1 (2%)	2 (4%)		4 (8%)	2 (4%)
Interstitial cell, carcinoma					1 (2%)
Hematopoietic System					
Bone marrow	(50)	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)			
Lymph node	(8)	(4)	(4)	(4)	(3)
Lumbar, carcinoma, metastatic, preputial gland	1 (13%)				
Lumbar, fibrous histiocytoma, metastatic, skin		1 (25%)			
Mediastinal, carcinoma, metastatic, pancreas					1 (33%)
Mediastinal, fibrous histiocytoma, metastatic, skin		1 (25%)			
Mediastinal, hepatocellular carcinoma, metastatic, liver					1 (33%)
Renal, fibrous histiocytoma, metastatic, skin		1 (25%)			
Lymph node, mandibular	(49)	(50)	(50)	(48)	(47)
Fibrous histiocytoma, metastatic, skin		1 (2%)			
Lymph node, mesenteric	(50)	(50)	(49)	(50)	(48)
Fibrous histiocytoma, metastatic, skin		1 (2%)			
Hepatocellular carcinoma, metastatic, liver					1 (2%)
Spleen	(50)	(50)	(50)	(50)	(49)
Hemangiosarcoma					1 (2%)
Leiomyosarcoma	1 (2%)				
Thymus	(46)	(45)	(43)	(42)	(43)
Thymoma benign		1 (2%)			
Integumentary System					
Mammary gland	(50)	(49)	(49)	(45)	(46)
Adenoma, multiple			1 (2%)		
Carcinoma	1 (2%)		2 (4%)		
Fibroadenoma	5 (10%)	5 (10%)	10 (20%)	10 (22%)	6 (13%)
Fibroadenoma, multiple			5 (10%)	3 (7%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Integumentary System (continued)					
Skin	(50)	(50)	(50)	(50)	(50)
Basal cell carcinoma		1 (2%)			
Keratoacanthoma	3 (6%)	3 (6%)	4 (8%)	5 (10%)	2 (4%)
Squamous cell papilloma				1 (2%)	1 (2%)
Pinna, melanoma malignant		1 (2%)	1 (2%)		1 (2%)
Subcutaneous tissue, fibroma	1 (2%)	8 (16%)	6 (12%)	5 (10%)	4 (8%)
Subcutaneous tissue, fibroma, multiple		1 (2%)	2 (4%)		
Subcutaneous tissue, fibrosarcoma		2 (4%)		2 (4%)	
Subcutaneous tissue, fibrosarcoma, multiple		1 (2%)		1 (2%)	
Subcutaneous tissue, fibrous histiocytoma		1 (2%)			
Subcutaneous tissue, hemangioma, multiple			1 (2%)		
Subcutaneous tissue, lipoma					1 (2%)
Musculoskeletal System					
Bone	(50)	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)				
Skeletal muscle		(1)			
Sarcoma NOS		1 (100%)			
Nervous System					
Brain	(50)	(50)	(50)	(50)	(50)
Oligodendroglioma benign		1 (2%)			
Oligodendroglioma malignant	1 (2%)	1 (2%)	1 (2%)		
Spinal cord	(1)	(1)	(1)		
Respiratory System					
Lung	(50)	(50)	(50)	(50)	(49)
Alveolar/bronchiolar adenoma		1 (2%)		1 (2%)	
Chordoma, metastatic, uncertain primary site					1 (2%)
Fibrous histiocytoma, metastatic, skin		1 (2%)			
Hepatocellular carcinoma, metastatic, liver		1 (2%)	2 (4%)	10 (20%)	22 (45%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)		1 (2%)
Pheochromocytoma malignant, metastatic, adrenal medulla	1 (2%)				
Squamous cell carcinoma, metastatic, ureter					1 (2%)
Mediastinum, osteosarcoma, metastatic, bone	1 (2%)				
Special Senses System					
Zymbal's gland	(1)	(2)		(1)	(1)
Carcinoma	1 (100%)	2 (100%)		1 (100%)	1 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin		1 (2%)			
Hepatocellular carcinoma, metastatic, liver					1 (2%)
Mesenchymal tumor benign	1 (2%)				
Bilateral, renal tubule, adenoma			1 (2%)		
Renal tubule, adenoma	3 (6%)	2 (4%)	5 (10%)	6 (12%)	8 (16%)
Renal tubule, carcinoma	1 (2%)				
Renal tubule, oncocytoma benign			1 (2%)		1 (2%)
Ureter					(1)
Squamous cell carcinoma					1 (100%)
Systemic Lesions					
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)
Leukemia mononuclear	26 (52%)	27 (54%)	15 (30%)	13 (26%)	1 (2%)
Mesothelioma malignant	1 (2%)	3 (6%)	5 (10%)	12 (24%)	5 (10%)
Neoplasm Summary					
Total animals with primary neoplasms[c]					
12-Month interim evaluation	5				5
2-Year study	50	50	48	50	46
Total primary neoplasms					
12-Month interim evaluation	5				10
2-Year study	159	180	176	212	189
Total animals with benign neoplasms					
12-Month interim evaluation	5				5
2-Year study	49	50	48	50	46
Total benign neoplasms					
12-Month interim evaluation	5				9
2-Year study	117	134	133	147	129
Total animals with malignant neoplasms					
12-Month interim evaluation					1
2-Year study	33	33	34	39	43
Total malignant neoplasms					
12-Month interim evaluation					1
2-Year study	42	46	43	58	56
Total animals with metastatic neoplasms					
2-Year study	3	2	4	11	25
Total metastatic neoplasms					
2-Year study	3	10	4	14	38
Total animals with malignant neoplasms of uncertain primary site					
2-Year study					1
Total animals with uncertain neoplasms- benign or malignant					
2-Year study				7	4
Total uncertain neoplasms					
2-Year study				7	4

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	344	521	529	553	564	588	603	610	610	614	617	626	639	642	652	661	662	668	704	704	707	707	712	714	715
Carcass ID Number	025	008	055	043	031	048	040	028	039	046	034	012	019	005	047	004	011	054	021	022	018	049	060	024	003
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma			X																						
Hepatocellular adenoma																									X
Mesentery				+														+							+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																X									
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign				X	X	X					X					X	X	X			X	X		X	
Bilateral, pheochromocytoma benign																									
Islets pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X				X			
Parathyroid gland	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X													X	X		X							
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																			X						
Follicular cell, adenoma													X			X									
General Body System																									
Peritoneum						+																			

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

																										Total
Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	1	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	8	1	2	2	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Total Tissues/
	5	0	1	5	5	0	0	0	0	1	1	1	1	1	2	2	2	2	3	3	4	4	4	5	5	Tumors
	7	7	5	0	1	1	2	6	9	0	3	4	6	7	0	2	6	9	2	6	1	2	4	3	9	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous																	X									1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																							X			2
Hepatocellular adenoma		X	X		X				X																	5
Mesentery						+								+												5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant														X												1
Pheochromocytoma benign	X	X			X					X			X			X	X			X		X				18
Bilateral, pheochromocytoma benign			X	X	X				X									X			X					6
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma											X															3
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma											X															1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma																		X		X	X					7
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma												X		X		X							X	X		6
Follicular cell, adenoma																										2
General Body System																										
Peritoneum																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Number of Days on Study	344	521	529	553	564	588	603	610	610	614	617	626	639	642	652	661	662	668	704	704	707	707	712	714	715
Carcass ID Number	025	008	055	043	031	048	040	028	039	046	034	012	019	005	047	004	011	054	021	027	018	049	060	024	003
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X				X		X		X										
Carcinoma					X														X						
Bilateral, adenoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+		+	+					+						+							
Lumbar, carcinoma, metastatic, preputial gland																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma							X																		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue, fibroma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma malignant	X																								
Peripheral nerve													+												
Spinal cord													+												
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant, metastatic, adrenal medulla																									
Mediastinum, osteosarcoma, metastatic, bone																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

	718	721	722	722	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	Total
Number of Days on Study / **Carcass ID Number**	057	007	015	050	051	001	002	006	009	010	013	014	016	017	020	022	026	029	032	036	041	042	044	053	059	Tissues/Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma							X		X	X										X						8
Carcinoma					X																	X		X		5
Bilateral, adenoma		X																								1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	45
Interstitial cell, adenoma											X															1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+	+																				+			8
Lumbar, carcinoma, metastatic, preputial gland																							X			1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																							X			1
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma								X																		1
Fibroadenoma							X	X	X								X									5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma						X		X		X																3
Subcutaneous tissue, fibroma								X																		1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma		X																								1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma malignant																										1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant, metastatic, adrenal medulla										X																1
Mediastinum, osteosarcoma, metastatic, bone		X																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	3 4 4	5 2 1	5 2 9	5 5 3	5 6 4	5 8 8	6 0 3	6 1 0	6 1 0	6 1 4	6 1 7	6 2 6	6 3 9	6 4 2	6 5 2	6 6 1	6 6 2	6 6 8	7 0 4	7 0 4	7 0 7	7 0 7	7 1 2	7 1 4	7 1 5
Carcass ID Number	0 2 5	0 0 8	0 5 5	0 4 3	0 3 1	0 4 8	0 4 0	0 2 8	0 3 9	0 4 6	0 3 4	0 1 2	0 1 9	0 0 5	0 4 7	0 0 4	0 1 1	0 5 4	0 2 1	0 2 7	0 1 8	0 4 9	0 6 0	0 2 4	0 0 3

Special Senses System

Eye				+																					
Zymbal's gland																							+		
Carcinoma																							X		

Urinary System

Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenchymal tumor benign																									
Renal tubule, adenoma																						X		X	
Renal tubule, carcinoma																									
Urinary bladder	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Systemic Lesions

Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	X	X		X	X		X	X	X						X	X	X	X	X	X	X	X	
Mesothelioma malignant					X																				

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	7 1 8	7 2 1	7 2 2	7 2 2	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 5 7	0 0 7	0 1 5	0 5 0	0 5 1	0 0 1	0 0 2	0 0 6	0 0 9	0 1 0	0 1 3	0 1 4	0 1 6	0 1 7	0 2 0	0 2 2	0 2 6	0 2 9	0 3 2	0 3 6	0 4 1	0 4 2	0 4 4	0 5 3	0 5 9	Total Tissues/ Tumors

Special Senses System

Eye																										1
Zymbal's gland																										1
Carcinoma																										1

Urinary System

Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesenchymal tumor benign															X											1
Renal tubule, adenoma							X																			3
Renal tubule, carcinoma															X											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

Systemic Lesions

Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X		X					X	X			X	X	X		X					X	X			26
Mesothelioma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	4 0 3	4 0 6	4 3 1	5 3 5	5 4 2	5 7 2	5 7 5	5 7 9	5 7 9	5 8 9	5 9 1	5 9 6	6 0 2	6 0 6	6 2 5	6 3 8	6 3 9	6 5 0	6 6 0	6 6 1	6 6 4	6 7 3	6 7 4	6 8 0	6 9 5
Carcass ID Number	1 0 5	0 9 0	0 8 3	0 9 1	0 8 7	0 8 1	0 9 9	0 6 6	0 7 6	0 7 0	0 7 2	0 8 8	0 6 8	0 7 7	0 7 5	0 8 4	0 9 5	0 7 8	1 0 1	0 9 4	1 0 0	0 6 2	0 8 5	0 8 2	1 0 3

Alimentary System

Organ	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin																				X					
Hepatocellular carcinoma																									
Hepatocellular adenoma			X									X										X		X	X
Hepatocellular adenoma, multiple																	X								
Mesentery																		+							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue												+													
Squamous cell papilloma												X													

Cardiovascular System

Organ	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocardium, schwannoma malignant																									

Endocrine System

Organ	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign															X		X	X			X				
Bilateral, pheochromocytoma benign							X																	X	X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Adenoma																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X			X	X	X				X	X											
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
Follicular cell, adenoma			X																						
Follicular cell, carcinoma																									

General Body System

Peritoneum

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

	703	704	704	704	712	718	718	718	725	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	Total
Carcass ID Number	097	061	065	086	106	071	104	108	089	063	064	067	069	073	074	079	080	092	093	096	098	102	107	109	100	110	Tissues/Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																											1
Hepatocellular carcinoma						X										X							X				3
Hepatocellular adenoma													X			X											7
Hepatocellular adenoma, multiple						X					X			X											X		5
Mesentery													+	+				+	+	+							6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma															X												1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue								+																			2
Squamous cell papilloma								X																			2
Cardiovascular System																											
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocardium, schwannoma malignant															X												1
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma							X																				1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign					X	X					X				X	X		X						X	X		12
Bilateral, pheochromocytoma benign				X							X																5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																						X					1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																						X					1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X																					X					8
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma													X			X	X		X								4
Follicular cell, adenoma																											1
Follicular cell, carcinoma					X																						1
General Body System																											
Peritoneum	+																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	403	406	431	535	542	572	575	579	579	589	591	596	602	606	625	638	639	650	660	661	664	673	674	680	695
Carcass ID Number	105	090	083	091	087	081	099	066	076	070	072	088	068	077	075	084	095	078	001	194	000	162	085	082	103
Genital System																									
Epididymis	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X			X	X				X												
Carcinoma																									
Bilateral, adenoma							X																	X	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma	X		X			X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
Interstitial cell, adenoma		X																X							
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin																		X							
Lymph node																		+							
Lumbar, fibrous histiocytoma, metastatic, skin																		X							
Mediastinal, fibrous histiocytoma, metastatic, skin																		X							
Renal, fibrous histiocytoma, metastatic, skin																		X							
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin																		X							
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin																		X							
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M
Thymoma benign																		X							
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma								X												X	X				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Keratoacanthoma																									
Pinna, melanoma malignant															X										
Subcutaneous tissue, fibroma					X	X												X							
Subcutaneous tissue, fibroma, multiple																			X						
Subcutaneous tissue, fibrosarcoma																									
Subcutaneous tissue, fibrosarcoma, multiple																						X			
Subcutaneous tissue, fibrous histiocytoma																		X							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle		+																							
Sarcoma NOS		X																							

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Total Tissues/ Tumors
Number of Days on Study	703	704	704	704	712	718	718	718	725	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	097	066	065	086	100	070	104	108	089	063	064	067	069	073	074	079	080	092	093	096	098	099	102	107	109	110	
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X			X	X			X	X	X	X	X													12
Carcinoma		X																									1
Bilateral, adenoma																					X					X	4
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	46
Interstitial cell, adenoma																											2
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																											1
Lymph node		+						+																+			4
Lumbar, fibrous histiocytoma, metastatic, skin																											1
Mediastinal, fibrous histiocytoma, metastatic, skin																											1
Renal, fibrous histiocytoma, metastatic, skin																											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																											1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																											1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	45
Thymoma benign																											1
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibroadenoma																					X		X				5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma									X																		1
Keratoacanthoma			X							X												X					3
Pinna, melanoma malignant																											1
Subcutaneous tissue, fibroma						X		X								X					X	X					8
Subcutaneous tissue, fibroma, multiple																											1
Subcutaneous tissue, fibrosarcoma									X		X																2
Subcutaneous tissue, fibrosarcoma, multiple																											1
Subcutaneous tissue, fibrous histiocytoma																											1
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																											1
Sarcoma NOS																											1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	4 0 3	4 0 6	4 3 1	5 3 5	5 4 2	5 7 2	5 7 5	5 7 9	5 7 9	5 8 9	5 9 1	5 9 6	6 0 2	6 0 6	6 2 5	6 3 8	6 3 9	6 5 0	6 6 0	6 6 1	6 6 4	6 7 3	6 7 4	6 8 0	6 9 5	
Carcass ID Number	1 0 5	0 9 0	0 8 3	0 9 1	0 8 7	0 8 1	0 9 6	0 6 6	0 7 6	0 7 0	0 7 2	0 8 8	0 6 8	0 7 7	0 7 5	0 8 4	0 9 5	0 7 8	1 0 1	0 9 4	1 0 0	0 6 2	1 0 8	0 8 2	0 8 5	1 0 3

Nervous System

Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma benign																										
Oligodendroglioma malignant			X																							
Peripheral nerve																				+						
Spinal cord																				+						

Respiratory System

Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																							X			
Fibrous histiocytoma, metastatic, skin																						X				
Hepatocellular carcinoma, metastatic, liver																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Special Senses System

Eye			+																							
Zymbal's gland					+		+																			
Carcinoma					X		X																			

Urinary System

Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, skin																						X				
Renal tubule, adenoma																							X			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Systemic Lesions

Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear												X		X	X	X	X				X			X		
Mesothelioma malignant																X										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

	703	704	704	704	712	718	718	718	725	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	Total Tissues/
Carcass ID Number	097	061	065	086	106	071	104	108	089	063	064	067	069	073	074	079	080	092	093	096	098	102	107	109	110	Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma benign																							X			1
Oligodendroglioma malignant																										1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Fibrous histiocytoma, metastatic, skin																										1
Hepatocellular carcinoma, metastatic, liver														X												1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										1
Zymbal's gland																										2
Carcinoma																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, skin																										1
Renal tubule, adenoma								X																		2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X		X	X	X		X	X	X	X	X	X	X	X	X	X		X		X		X			27
Mesothelioma malignant	X																X									3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study →	015	438	467	502	521	522	568	572	582	595	596	601	610	619	630	638	639	642	642	651	658	659	661	664	673
Carcass ID Number	151	124	111	150	134	154	127	135	141	126	133	143	160	117	147	116	122	129	131	121	118	112	115	114	156
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma			X				X		X												X	X	X		X
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma					X			X										X							
Hepatocellular adenoma, multiple							X			X	X	X							X						
Hepatocholangiocarcinoma								X																	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign													X				X		X						
Bilateral, pheochromocytoma benign														X											
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma malignant, metastatic, brain		X																							
Pars distalis, adenoma	X										X	X											X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
Follicular cell, adenoma																									
Follicular cell, carcinoma																									
General Body System																									
Peritoneum									+			+													
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X						X								
Carcinoma			X												X										
Prostate	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
Number of Days on Study	674	692	695	695	695	696	696	706	709	712	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	153	157	145	152	158	125	159	155	138	140	113	119	120	123	128	130	132	136	137	139	142	144	146	148	149	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma						X			X				X	X			X			X						13
Hepatocellular carcinoma, multiple			X																							1
Hepatocellular adenoma					X	X		X					X	X						X						9
Hepatocellular adenoma, multiple	X	X		X			X			X						X	X	X		X						14
Hepatocholangiocarcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																				X						1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant									X																	1
Pheochromocytoma benign						X			X		X		X						X							8
Bilateral, pheochromocytoma benign			X															X								3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma malignant, metastatic, brain																										1
Pars distalis, adenoma									X		X												X	X		8
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																		X		X						2
Follicular cell, adenoma	X																									1
Follicular cell, carcinoma			X																							1
General Body System																										
Peritoneum																										2
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma													X													3
Carcinoma																										2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	48

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study	015	438	467	502	521	522	568	572	582	595	596	601	610	619	630	638	639	642	642	651	658	659	661	664	673
Carcass ID Number	151	124	111	150	134	154	127	135	141	126	133	143	160	117	147	116	122	129	131	121	118	112	115	114	156
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node											+				+			+	+						
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, multiple																									
Carcinoma																									
Fibroadenoma							X															X	X		
Fibroadenoma, multiple																X								X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Pinna, melanoma malignant																X									
Subcutaneous tissue, fibroma														X			X								X
Subcutaneous tissue, fibroma, multiple																						X			
Subcutaneous tissue, hemangioma, multiple		X																							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma malignant			X																						
Peripheral nerve																	+								
Spinal cord																	+								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver				X																			X		
Hepatocholangiocarcinoma, metastatic, liver									X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																	+								
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, renal tubule, adenoma																									
Renal tubule, adenoma											X														
Renal tubule, oncocytoma benign							X																		
Urinary bladder	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X			X	X			X	X		X				X	X	X				
Mesothelioma malignant								X		X			X		X					X					

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study	6 7 4	6 9 2	6 9 5	6 9 5	6 9 5	6 9 6	6 9 6	7 0 6	7 0 9	7 1 2	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 5 3	1 5 7	1 4 5	1 5 2	1 5 8	1 2 5	1 5 9	1 5 5	1 3 8	1 4 0	1 1 3	1 1 9	1 2 0	1 2 3	1 2 8	1 3 0	1 3 2	1 3 6	1 3 7	1 3 9	1 4 2	1 4 4	1 4 6	1 4 8	1 4 9	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	M	+	+	M	+	+	M	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	43
Integumentary System																										
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, multiple																X										1
Carcinoma													X											X		2
Fibroadenoma							X						X	X				X			X		X	X	X	10
Fibroadenoma, multiple										X						X				X						5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma	X						X			X														X		4
Pinna, melanoma malignant																										1
Subcutaneous tissue, fibroma		X																			X	X				6
Subcutaneous tissue, fibroma, multiple													X													2
Subcutaneous tissue, hemangioma, multiple																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma malignant																										1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic, liver																										2
Hepatocholangiocarcinoma, metastatic, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye														+												2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, renal tubule, adenoma									X																	1
Renal tubule, adenoma													X			X			X					X		5
Renal tubule, oncocytoma benign																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X									X		X		X		X				X	X			15
Mesothelioma malignant																										5

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study	337	409	457	467	495	502	523	546	547	568	575	583	584	598	598	600	602	607	610	614	621	625	633	638	642
Carcass ID Number	182	195	189	162	179	205	209	188	175	190	169	185	183	172	180	170	198	101	191	286	171	192	163	161	168
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma						X		X			X	X	X				X				X	X			
Hepatocellular carcinoma, multiple										X						X									
Hepatocellular adenoma				X			X		X	X				X	X			X							
Hepatocellular adenoma, multiple								X			X	X			X				X	X			X		X
Hepatocholangiocarcinoma		X																							
Hepatocholangioma																			X						
Mesentery																			+						
Leiomyosarcoma																			X						
Oral mucosa																			+						
Pharyngeal, squamous cell papilloma																			X						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Hepatocellular carcinoma, metastatic, liver																							X		
Leiomyosarcoma, metastatic, mesentery																			X						
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neuroendocrine tumor, benign																									
Neuroendocrine tumor, malignant																									
Tongue																									
Squamous cell papilloma																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant										X															
Pheochromocytoma benign																					X		X		
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma															X							X			
Follicular cell, adenoma								X																	
General Body System																									
Peritoneum		+	+		+														+			+			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Carcass ID Number	193	196	208	197	178	200	165	174	203	204	206	210	187	166	167	164	184	176	207	194	199	181	177	202	173	Total Tissues/Tumors
Number of Days on Study	642	642	646	648	650	654	658	659	660	660	660	660	661	669	670	680	680	683	684	688	688	699	700	704	712	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma									X			X					X	X						X	X	14
Hepatocellular carcinoma, multiple	X	X			X					X			X	X	X	X					X					11
Hepatocellular adenoma		X		X					X	X												X	X	X		14
Hepatocellular adenoma, multiple	X		X		X	X					X	X	X	X	X	X	X	X	X	X					X	24
Hepatocholangiocarcinoma																										1
Hepatocholangioma																										1
Mesentery																										1
Leiomyosarcoma																										1
Oral mucosa																										1
Pharyngeal, squamous cell papilloma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma														X												1
Hepatocellular carcinoma, metastatic, liver																										1
Leiomyosarcoma, metastatic, mesentery																										1
Salivary glands	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neuroendocrine tumor, benign		X			X				X																	3
Neuroendocrine tumor, malignant										X	X						X		X							4
Tongue																		+								1
Squamous cell papilloma																		X								1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign									X	X		X		X				X				X				8
Bilateral, pheochromocytoma benign			X									X														2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma													X			X										2
Thyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma																								X		3
Follicular cell, adenoma																										1
General Body System																										
Peritoneum							+																			6

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study	3	4	4	4	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6
	3	0	5	6	9	0	2	4	4	6	7	8	8	9	9	0	0	0	1	1	2	2	3	3	4
	7	9	7	7	5	2	3	6	7	8	5	3	4	8	8	0	2	7	0	4	1	5	3	8	2
Carcass ID Number	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1
	8	9	8	6	7	0	0	8	7	9	6	8	8	7	8	7	9	0	9	8	7	9	6	6	6
	2	5	9	2	9	5	9	8	5	0	9	5	3	2	0	0	8	1	1	6	1	2	3	1	8
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma						X					X						X	X							
Bilateral, adenoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Leiomyosarcoma, metastatic, mesentery																							X		
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma, metastatic, mesentery																							X		
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma	X		X	X	X			X	X	X	X	X	X		X	X	X	X	X	X	X	X		X	X
Interstitial cell, adenoma		X					X							X									X		
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+						+																	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	M	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	M	+	+	+	+	+
Fibroadenoma								X	X						X						X				
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma														X							X				
Squamous cell papilloma																									
Subcutaneous tissue, fibroma																		X				X			
Subcutaneous tissue, fibrosarcoma									X																
Subcutaneous tissue, fibrosarcoma, multiple																			X						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Hepatocellular carcinoma, metastatic, liver														X			X	X				X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Total
Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	
	4	4	4	4	5	5	5	5	6	6	6	6	6	6	7	8	8	8	8	8	8	9	0	0	1	
	2	2	6	8	0	4	8	9	0	0	0	0	1	9	0	0	0	3	4	8	8	9	0	4	2	
Carcass ID Number	1	1	2	1	1	2	1	1	2	2	2	2	1	1	1	1	1	1	2	1	1	1	1	2	1	Total Tissues/
	9	9	0	9	7	0	6	7	0	0	0	1	8	6	6	6	8	7	0	9	9	8	7	0	7	Tumors
	3	6	8	7	8	0	5	4	3	4	6	0	7	6	7	4	4	6	7	4	9	1	7	2	3	
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X							X	X																7
Bilateral, adenoma													X													1
Prostate	+		+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma	X																									1
Leiomyosarcoma, metastatic, mesentery																										1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma, metastatic, mesentery																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	46
Interstitial cell, adenoma																										4
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node				+				+																		4
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	M	+	M	+	+	+	M	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	45
Fibroadenoma						X	X						X		X		X	X								10
Fibroadenoma, multiple									X													X		X		3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma	X												X		X											5
Squamous cell papilloma									X																	1
Subcutaneous tissue, fibroma											X	X						X								5
Subcutaneous tissue, fibrosarcoma																				X						2
Subcutaneous tissue, fibrosarcoma, multiple																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																						X				1
Hepatocellular carcinoma, metastatic, liver	X	X							X		X		X									X				10
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study	3 3 7	4 0 9	4 5 7	4 6 7	4 9 5	5 0 2	5 2 3	5 4 6	5 4 7	5 6 8	5 7 5	5 8 3	5 8 4	5 9 8	5 9 8	6 0 0	6 0 2	6 0 7	6 1 0	6 1 4	6 2 1	6 2 5	6 3 3	6 3 8	6 4 2
Carcass ID Number	1 8 2	1 9 5	1 8 9	1 6 2	1 7 9	2 0 5	2 0 9	1 8 8	1 7 5	1 9 0	1 6 9	1 8 5	1 8 3	1 7 2	1 8 0	1 7 0	1 9 8	2 0 1	1 9 1	2 8 6	1 7 1	1 9 2	1 6 3	1 6 1	1 6 8

Special Senses System
Eye
Zymbal's gland
   Carcinoma

Urinary System

Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma														X											
Urethra																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M

Systemic Lesions

Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X			X	X								X	X	X				X			
Mesothelioma malignant		X	X		X											X	X		X		X		X		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study																										
	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	
	4	4	4	4	5	5	5	5	6	6	6	6	6	6	7	8	8	8	8	8	8	9	0	0	1	
	2	2	6	8	0	4	8	9	0	0	0	0	1	9	0	0	0	3	4	8	8	9	0	4	2	
Carcass ID Number	1	1	2	1	1	2	1	1	2	2	2	2	1	1	1	1	1	1	2	1	1	1	1	2	1	Total
	9	9	0	9	7	0	6	7	0	0	0	1	8	6	6	6	8	7	0	9	9	8	7	0	7	Tissues/
	3	6	8	7	8	0	5	4	3	4	6	0	7	6	7	4	4	6	7	4	9	1	7	2	3	Tumors
Special Senses System																										
Eye																	+									1
Zymbal's gland				+																						1
Carcinoma				X																						1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma												X	X			X	X				X					6
Urethra													+													1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X		X						X								X		X		X			13
Mesothelioma malignant							X	X			X			X												12

TABLE A2
**Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg
(Stop-Exposure)**

Number of Days on Study
0 1 2 2 3 4 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
1 7 1 9 8 3 9 9 0 0 1 1 2 2 2 3 3 3 4 4 5 5 5 5 5
8 7 9 9 0 7 5 8 2 6 3 7 1 6 8 8 3 3 7 0 8 3 6 6 7

Carcass ID Number
2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
3 2 2 1 4 4 6 2 1 6 2 2 5 4 3 3 4 6 5 1 5 3 1 3 2
0 3 2 3 2 5 9 6 4 5 1 4 6 1 8 9 4 6 8 1 0 7 6 4 5

Alimentary System

Finding	Animals
Esophagus	+ + + + + + + + + + + + + + + + M + + + + + + + +
Intestine large, colon	+ +
Intestine large, rectum	+ +
Intestine large, cecum	+ + + + + + + + + + + + + + + + M + + + + + + + +
Intestine small, duodenum	+ + + + + + + + + + + + + + + + M + + + + + + + +
Intestine small, jejunum	+ +
Intestine small, ileum	+ + + + + + + + + + + + + + + + M + + + + + + + +
Liver	+ +
Carcinoma, metastatic, pancreas	X
Cholangioma	
Hepatocellular carcinoma	X X X X X X
Hepatocellular carcinoma, multiple	X X X X X X X X X
Hepatocellular adenoma	X X X
Hepatocellular adenoma, multiple	X X X X X X X X X X
Hepatocholangiocarcinoma	X X X
Hepatocholangiocarcinoma, multiple	
Hepatocholangioma	X X
Hepatocholangioma, multiple	X
Ito cell tumor benign	X
Interstitial cell, carcinoma, metastatic, testes	X
Mesentery	+
Hepatocellular carcinoma, metastatic, liver	X
Pancreas	+ + + + + + + + + + + + + + + + M + + + + + + + +
Carcinoma	X
Hepatocellular carcinoma, metastatic, liver	X
Salivary glands	+ + + + + + + + + + + + + + + + M + + + + + + + +
Stomach, forestomach	+ + + + + + + + + + + + + + + + M + + + + + + + +
Squamous cell carcinoma	X
Stomach, glandular	+ + + + + + + + + + + + + + + + M + + + + + + + +
Neuroendocrine tumor, benign	X
Neuroendocrine tumor, malignant	X

Cardiovascular System

Finding	Animals
Blood vessel	+ +
Heart	+ + + + + + + + + + + + + + + + M + + + + + + + +

Endocrine System

Finding	Animals
Adrenal cortex	+ +
Hepatocellular carcinoma, metastatic, liver	
Adrenal medulla	+ +
Pheochromocytoma benign	X X X X
Islets, pancreatic	+ + + + + + + + + + + + + + + + M + + + + + + + +
Parathyroid gland	I + + + + + M + + + + + + + M + + + + + + + +
Pituitary gland	+ +
Pars distalis, adenoma	X X
Thyroid gland	+ + + + + + + + + + + + + + + + M + + + + + + + +
C-cell, adenoma	
Follicular cell, adenoma	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

Number of Days on Study	570	572	572	582	589	589	589	591	592	592	597	601	604	604	608	619	627	631	633	634	638	653	674	680	684	Total Tissues/ Tumors
Carcass ID Number	235	262	263	252	231	236	260	267	248	249	253	270	220	228	215	232	246	217	255	259	218	257	219	233	261	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, pancreas																										1
Cholangioma									X									X								2
Hepatocellular carcinoma							X		X		X							X	X	X			X			13
Hepatocellular carcinoma, multiple		X	X	X	X				X		X	X	X			X	X				X	X	X		X	23
Hepatocellular adenoma			X						X									X	X		X					8
Hepatocellular adenoma, multiple	X	X		X	X	X	X	X						X	X		X				X		X	X		24
Hepatocholangiocarcinoma									X								X						X			6
Hepatocholangiocarcinoma, multiple																	X									1
Hepatocholangioma							X																			3
Hepatocholangioma, multiple		X	X																							3
Ito cell tumor benign																										1
Interstitial cell, carcinoma, metastatic, testes																										1
Mesentery																										1
Hepatocellular carcinoma, metastatic, liver																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Hepatocellular carcinoma, metastatic, liver																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell carcinoma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Neuroendocrine tumor, benign																								X		2
Neuroendocrine tumor, malignant																							X			2
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic, liver																						X				1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign									X				X			X	X						X			9
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma					X																					3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	48
C-cell, adenoma																				X						1
Follicular cell, adenoma											X									X						3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

Number of Days on Study	018	177	219	299	380	437	495	498	502	506	513	517	521	526	528	538	533	533	547	540	558	553	556	556	557
Carcass ID Number	230	223	222	213	242	245	269	226	214	265	221	224	256	241	238	239	244	266	258	211	250	237	216	234	225
General Body System																									
Peritoneum				+																				+	
Squamous cell carcinoma, metastatic, ureter				X																					
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, ureter				X																					
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X																					
Bilateral, adenoma													X							X					
Prostate	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, ureter				X																					
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, ureter				X																					
Bilateral, interstitial cell, adenoma					X	X	X		X	X		X	X	X	X	X	X	X	X		X	X	X	X	
Interstitial cell, adenoma											X							X							
Interstitial cell, carcinoma																		X							
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node										+							+								
Mediastinal, carcinoma, metastatic, pancreas										X															
Mediastinal, hepatocellular carcinoma, metastatic, liver																	X								
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver																	X								
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Hemangiosarcoma											X														
Thymus	+	+	+	+	M	+	+	M	+	M	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+
Fibroadenoma				X																					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																X									
Squamous cell papilloma																									
Pinna, melanoma malignant																					X				
Subcutaneous tissue, fibroma																				X		X			
Subcutaneous tissue, lipoma																						X			
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

Number of Days on Study	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
	7	7	7	8	8	8	8	9	9	9	9	0	0	0	0	1	2	3	3	3	3	5	7	8	8	
	0	2	2	2	9	9	9	1	2	2	7	1	4	4	8	9	7	1	3	4	8	3	4	0	4	
Carcass ID Number	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	**Total**
	3	6	6	5	3	3	6	6	4	4	5	7	2	2	1	3	4	1	5	5	1	5	1	3	6	**Tissues/**
	5	2	3	2	1	6	0	7	8	9	3	0	0	8	5	2	6	7	5	9	8	7	9	3	1	**Tumors**
General Body System																										
Peritoneum																										2
Squamous cell carcinoma, metastatic, ureter																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, ureter																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X						X															X			4
Bilateral, adenoma																										2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, ureter																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, ureter																										1
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	41
Interstitial cell, adenoma																										2
Interstitial cell, carcinoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																							+			3
Mediastinal, carcinoma, metastatic, pancreas																										1
Mediastinal, hepatocellular carcinoma, metastatic, liver																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular carcinoma, metastatic, liver																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Integumentary System																										
Mammary gland	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Fibroadenoma						X	X	X												X		X				6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma		X																								2
Squamous cell papilloma					X																					1
Pinna, melanoma malignant																										1
Subcutaneous tissue, fibroma								X									X									4
Subcutaneous tissue, lipoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25
Number of Days on Study	018	177	219	299	380	437	495	498	502	506	513	517	521	526	528	538	533	533	547	540	558	553	556	556	557
Carcass ID Number	230	223	222	213	242	245	269	226	214	265	221	224	256	241	238	239	244	266	258	251	250	237	216	234	225
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Chordoma, metastatic, uncertain primary site																									
Hepatocellular carcinoma, metastatic, liver			X	X			X	X	X			X		X	X		X		X						
Hepatocholangiocarcinoma, metastatic, liver																									
Squamous cell carcinoma, metastatic, ureter				X																					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Special Senses System																									
Zymbal's gland													+												
Carcinoma													X												
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver													X												
Renal tubule, adenoma																	X								
Renal tubule, oncocytoma benign																X									
Ureter				+																					
Squamous cell carcinoma				X																					
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Mesothelioma malignant																								X	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

Number of Days on Study	5 7 0	5 7 2	5 7 2	5 8 2	5 8 9	5 8 9	5 8 9	5 9 1	5 9 2	5 9 2	5 9 7	6 0 1	6 0 4	6 0 4	6 0 8	6 1 9	6 2 7	6 3 1	6 3 3	6 3 4	6 3 8	6 5 3	6 7 4	6 8 0	6 8 4	
Carcass ID Number	2 3 5	2 6 2	2 6 3	2 5 2	2 3 1	2 3 6	2 6 0	2 6 7	2 4 8	2 4 9	2 5 3	2 7 0	2 2 0	2 2 8	2 1 5	2 3 2	2 4 6	2 1 7	2 5 5	2 5 9	2 1 8	2 5 7	2 1 9	2 3 3	2 6 1	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Chordoma, metastatic, uncertain primary site																	X									1
Hepatocellular carcinoma, metastatic, liver		X	X		X				X	X	X				X					X		X	X	X	X	22
Hepatocholangiocarcinoma, metastatic, liver																	X									1
Squamous cell carcinoma, metastatic, ureter																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Special Senses System																										
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic, liver																										1
Renal tubule, adenoma		X							X									X X		X		X X				8
Renal tubule, oncocytoma benign																										1
Ureter																										1
Squamous cell carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X																							1
Mesothelioma malignant		X				X	X																			5

TABLE A3a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	24/50 (48%)	17/50 (34%)	11/50 (22%)	10/50 (20%)
Adjusted rate[b]	54.5%	41.5%	28.2%	29.6%
Terminal rate[c]	9/20 (45%)	7/16 (44%)	4/15 (27%)	0/0
First incidence (days)	588	575	619	625
Poly-3 test[d]	P=0.007N	P=0.155N	P=0.010N	P=0.019N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	25/50 (50%)	17/50 (34%)	12/50 (24%)	11/50 (22%)
Adjusted rate	56.8%	41.5%	30.7%	32.1%
Terminal rate	10/20 (50%)	7/16 (44%)	4/15 (27%)	0/0
First incidence (days)	588	575	619	575
Poly-3 test	P=0.009N	P=0.108N	P=0.011N	P=0.019N
Kidney (Renal Tubule): Adenoma (Single Sections)				
Overall rate	3/50 (6%)	2/50 (4%)	6/50 (12%)	6/50 (12%)
Adjusted rate	7.2%	5.2%	15.8%	18.4%
Terminal rate	1/20 (5%)	0/16 (0%)	4/15 (27%)	0/0
First incidence (days)	712	673	596	598
Poly-3 test	P=0.046	P=0.531N	P=0.195	P=0.135
Kidney (Renal Tubule): Adenoma (Step Sections)				
Overall rate	2/50 (4%)	5/50 (10%)	14/50 (28%)	11/50 (22%)
Adjusted rate	4.8%	12.9%	36.7%	31.9%
Terminal rate	1/20 (5%)	3/16 (19%)	8/15 (53%)	0/0
First incidence (days)	712	718	673	575
Poly-3 test	P<0.001	P=0.186	P=0.001	P=0.002
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	4/50 (8%)	6/50 (12%)	17/50 (34%)	13/50 (26%)
Adjusted rate	9.6%	15.5%	43.9%	37.3%
Terminal rate	2/20 (10%)	3/16 (19%)	10/15 (67%)	0/0
First incidence (days)	712	673	596	575
Poly-3 test	P<0.001	P=0.325	P<0.001	P=0.003
Kidney (Renal Tubule): Adenoma or Carcinoma (Single Sections)				
Overall rate	4/50 (8%)	2/50 (4%)	6/50 (12%)	6/50 (12%)
Adjusted rate	9.6%	5.2%	15.8%	18.4%
Terminal rate	2/20 (10%)	0/16 (0%)	4/15 (27%)	0/0
First incidence (days)	712	673	596	598
Poly-3 test	P=0.090	P=0.368N	P=0.310	P=0.226
Kidney (Renal Tubule): Adenoma or Carcinoma (Single and Step Sections)				
Overall rate	5/50 (10%)	6/50 (12%)	17/50 (34%)	13/50 (26%)
Adjusted rate	12.0%	15.5%	43.9%	37.3%
Terminal rate	3/20 (15%)	3/16 (19%)	10/15 (67%)	0/0
First incidence (days)	712	673	596	575
Poly-3 test	P<0.001	P=0.452	P<0.001	P=0.008
Liver: Hepatocellular Adenoma				
Overall rate	5/50 (10%)	12/50 (24%)	23/50 (46%)	38/50 (76%)
Adjusted rate	12.0%	29.5%	55.1%	86.8%
Terminal rate	2/20 (10%)	5/16 (31%)	8/15 (53%)	0/0
First incidence (days)	715	431	522	467
Poly-3 test	P<0.001	P=0.042	P<0.001	P<0.001

TABLE A3a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Liver: Hepatocellular Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	14/50 (28%)	25/50 (50%)
Adjusted rate	4.8%	7.8%	34.7%	64.0%
Terminal rate	1/20 (5%)	2/16 (13%)	5/15 (33%)	0/0
First incidence (days)	529	718	502	523
Poly-3 test	P<0.001	P=0.461	P<0.001	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	7/50 (14%)	14/50 (28%)	28/50 (56%)	43/50 (86%)
Adjusted rate	16.6%	34.4%	64.4%	94.0%
Terminal rate	3/20 (15%)	7/16 (44%)	9/15 (60%)	0/0
First incidence (days)	529	431	502	467
Poly-3 test	P<0.001	P=0.049	P<0.001	P<0.001
Mammary Gland: Fibroadenoma				
Overall rate	5/50 (10%)	5/50 (10%)	15/50 (30%)	13/50 (26%)
Adjusted rate	12.0%	12.6%	38.3%	37.0%
Terminal rate	4/20 (20%)	2/16 (13%)	8/15 (53%)	0/0
First incidence (days)	610	579	568	546
Poly-3 test	P<0.001	P=0.596	P=0.004	P=0.008
Mammary Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.4%	0.0%	8.0%	0.0%
Terminal rate	1/20 (5%)	0/16 (0%)	3/15 (20%)	0/0
First incidence (days)	730 (T)	—[e]	730 (T)	—
Poly-3 test	P=0.611N	P=0.514N	P=0.268	P=0.555N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	5/50 (10%)	5/50 (10%)	16/50 (32%)	13/50 (26%)
Adjusted rate	12.0%	12.6%	40.8%	37.0%
Terminal rate	4/20 (20%)	2/16 (13%)	9/15 (60%)	0/0
First incidence (days)	610	579	568	546
Poly-3 test	P<0.001	P=0.596	P=0.002	P=0.008
Pancreatic Islets: Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	7.2%	2.6%	0.0%	0.0%
Terminal rate	1/20 (5%)	1/16 (6%)	0/15 (0%)	0/0
First incidence (days)	704	730 (T)	—	—
Poly-3 test	P=0.046N	P=0.332N	P=0.138N	P=0.178N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	7/50 (14%)	8/50 (16%)	8/50 (16%)	2/50 (4%)
Adjusted rate	16.3%	19.4%	20.4%	6.3%
Terminal rate	3/20 (15%)	1/16 (6%)	3/15 (20%)	0/0
First incidence (days)	521	572	438	670
Poly-3 test	P=0.185N	P=0.468	P=0.425	P=0.174N
Preputial Gland: Adenoma				
Overall rate	9/50 (18%)	16/50 (32%)	3/49 (6%)	8/50 (16%)
Adjusted rate	21.2%	38.6%	8.0%	23.3%
Terminal rate	4/20 (20%)	7/16 (44%)	1/15 (7%)	0/0
First incidence (days)	610	542	568	502
Poly-3 test	P=0.295N	P=0.061	P=0.087N	P=0.521

TABLE A3a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Preputial Gland: Carcinoma				
Overall rate	5/50 (10%)	1/50 (2%)	2/49 (4%)	0/50 (0%)
Adjusted rate	11.9%	2.6%	5.3%	0.0%
Terminal rate	2/20 (10%)	0/16 (0%)	0/15 (0%)	0/0
First incidence (days)	564	704	502	—
Poly-3 test	P=0.037N	P=0.120N	P=0.260N	P=0.064N
Preputial Gland: Adenoma or Carcinoma				
Overall rate	14/50 (28%)	17/50 (34%)	5/49 (10%)	8/50 (16%)
Adjusted rate	32.4%	40.9%	12.9%	23.3%
Terminal rate	6/20 (30%)	7/16 (44%)	1/15 (7%)	0/0
First incidence (days)	564	542	502	502
Poly-3 test	P=0.065N	P=0.276	P=0.030N	P=0.261N
Skin: Keratoacanthoma				
Overall rate	3/50 (6%)	3/50 (6%)	4/50 (8%)	5/50 (10%)
Adjusted rate	7.2%	7.8%	10.6%	15.2%
Terminal rate	3/20 (15%)	2/16 (13%)	1/15 (7%)	0/0
First incidence (days)	730 (T)	704	674	584
Poly-3 test	P=0.151	P=0.631	P=0.449	P=0.235
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Basal Cell Carcinoma				
Overall rate	3/50 (6%)	4/50 (8%)	4/50 (8%)	6/50 (12%)
Adjusted rate	7.2%	10.3%	10.6%	18.1%
Terminal rate	3/20 (15%)	2/16 (13%)	1/15 (7%)	0/0
First incidence (days)	730 (T)	704	674	584
Poly-3 test	P=0.102	P=0.462	P=0.449	P=0.141
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	1/50 (2%)	9/50 (18%)	8/50 (16%)	5/50 (10%)
Adjusted rate	2.4%	22.3%	20.6%	15.3%
Terminal rate	1/20 (5%)	3/16 (19%)	2/15 (13%)	0/0
First incidence (days)	730 (T)	535	619	607
Poly-3 test	P=0.108	P=0.006	P=0.011	P=0.055
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	7.7%	0.0%	9.3%
Terminal rate	0/20 (0%)	1/16 (6%)	0/15 (0%)	0/0
First incidence (days)	—	674	—	547
Poly-3 test	P=0.110	P=0.106	—[f]	P=0.080
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	1/50 (2%)	12/50 (24%)	8/50 (16%)	8/50 (16%)
Adjusted rate	2.4%	29.6%	20.6%	23.7%
Terminal rate	1/20 (5%)	4/16 (25%)	2/15 (13%)	0/0
First incidence (days)	730 (T)	535	619	547
Poly-3 test	P=0.037	P<0.001	P=0.011	P=0.005
Skin (Subcutaneous Tissue): Fibrosarcoma or Fibrous Histiocytoma				
Overall rate	0/50 (0%)	4/50 (8%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	10.3%	0.0%	9.3%
Terminal rate	0/20 (0%)	1/16 (6%)	0/15 (0%)	0/0
First incidence (days)	—	664	—	547
Poly-3 test	P=0.158	P=0.052	—	P=0.080

TABLE A3a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Fibrous Histiocytoma				
Overall rate	1/50 (2%)	13/50 (26%)	8/50 (16%)	8/50 (16%)
Adjusted rate	2.4%	31.9%	20.6%	23.7%
Terminal rate	1/20 (5%)	4/16 (25%)	2/15 (13%)	0/0
First incidence (days)	730 (T)	535	619	547
Poly-3 test	P=0.046	P<0.001	P=0.011	P=0.005
Stomach (Glandular): Benign Neuroendocrine Tumor				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	9.4%
Terminal rate	0/20 (0%)	0/16 (0%)	0/15 (0%)	0/0
First incidence (days)	—	—	—	642
Poly-3 test	P=0.009	—	—	P=0.079
Stomach (Glandular): Malignant Neuroendocrine Tumor				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	0.0%	12.5%
Terminal rate	0/20 (0%)	0/16 (0%)	0/15 (0%)	0/0
First incidence (days)	—	—	—	660
Poly-3 test	P=0.002	—	—	P=0.033
Stomach (Glandular): Benign or Malignant Neuroendocrine Tumor				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	7/50 (14%)
Adjusted rate	0.0%	0.0%	0.0%	21.3%
Terminal rate	0/20 (0%)	0/16 (0%)	0/15 (0%)	0/0
First incidence (days)	—	—	—	642
Poly-3 test	P<0.001	—	—	P=0.002
Testes: Adenoma				
Overall rate	46/50 (92%)	48/49 (98%)	48/49 (98%)	50/50 (100%)
Adjusted rate	96.1%	98.0%	100.0%	100.0%
Terminal rate	20/20 (100%)	15/16 (94%)	15/15 (100%)	0/0
First incidence (days)	529	403	438	337
Poly-3 test	P=0.080	P=0.529	P=0.204	P=0.194
Thyroid Gland (C-cell): Adenoma				
Overall rate	6/50 (12%)	4/50 (8%)	2/50 (4%)	3/48 (6%)
Adjusted rate	14.5%	10.4%	5.4%	9.8%
Terminal rate	5/20 (25%)	4/16 (25%)	2/15 (13%)	0/0
First incidence (days)	704	730 (T)	730 (T)	600
Poly-3 test	P=0.256N	P=0.416N	P=0.167N	P=0.409N
All Organs: Mononuclear Cell Leukemia				
Overall rate	26/50 (52%)	27/50 (54%)	15/50 (30%)	13/50 (26%)
Adjusted rate	56.0%	65.2%	36.4%	36.1%
Terminal rate	8/20 (40%)	12/16 (75%)	5/15 (33%)	0/0
First incidence (days)	521	602	521	467
Poly-3 test	P=0.008N	P=0.246	P=0.047N	P=0.051N
All Organs: Malignant Mesothelioma				
Overall rate	1/50 (2%)	3/50 (6%)	5/50 (10%)	12/50 (24%)
Adjusted rate	2.4%	7.7%	12.7%	32.9%
Terminal rate	0/20 (0%)	1/16 (6%)	0/15 (0%)	0/0
First incidence (days)	564	639	582	409
Poly-3 test	P<0.001	P=0.279	P=0.085	P<0.001

TABLE A3a
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
All Organs: Benign Neoplasms				
Overall rate	49/50 (98%)	50/50 (100%)	48/50 (96%)	50/50 (100%)
Adjusted rate	99.8%	100.0%	98.9%	100.0%
Terminal rate	20/20 (100%)	16/16 (100%)	15/15 (100%)	0/0
First incidence (days)	521	403	438	337
Poly-3 test	P=0.878	P=1.000	P=0.843N	P=1.000
All Organs: Malignant Neoplasms				
Overall rate	33/50 (66%)	32/50 (64%)	34/50 (68%)	39/50 (78%)
Adjusted rate	68.9%	73.1%	74.1%	85.7%
Terminal rate	12/20 (60%)	12/16 (75%)	10/15 (67%)	0/0
First incidence (days)	344	431	467	409
Poly-3 test	P=0.026	P=0.413	P=0.369	P=0.035
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	48/50 (96%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	98.9%	100.0%
Terminal rate	20/20 (100%)	16/16 (100%)	15/15 (100%)	0/0
First incidence (days)	344	403	438	337
Poly-3 test	P=0.861N	—	P=0.796N	—

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

TABLE A3b
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Stop-Exposure Gavage Study of Methyleugenol

	Vehicle Control	300 mg/kg
Adrenal Medulla: Benign Pheochromocytoma		
Overall rate[a]	24/50 (48%)	9/50 (18%)
Adjusted rate[b]	54.5%	33.4%
Terminal rate[c]	9/20 (45%)	0/0
First incidence (days)	588	495
Poly-3 test[d]		P=0.078N
Adrenal Medulla: Benign or Malignant Pheochromocytoma		
Overall rate	25/50 (50%)	9/50 (18%)
Adjusted rate	56.8%	33.4%
Terminal rate	10/20 (50%)	0/0
First incidence (days)	588	495
Poly-3 test		P=0.052N
Kidney (Renal Tubule): Adenoma (Single Sections)		
Overall rate	3/50 (6%)	8/50 (16%)
Adjusted rate	7.2%	31.2%
Terminal rate	1/20 (5%)	0/0
First incidence (days)	712	528
Poly-3 test		P=0.018
Kidney (Renal Tubule): Adenoma (Step Sections)		
Overall rate	2/50 (4%)	13/50 (26%)
Adjusted rate	4.8%	45.9%
Terminal rate	1/20 (5%)	0/0
First incidence (days)	712	537
Poly-3 test		P<0.001
Kidney (Renal Tubule): Adenoma (Single and Step Sections)		
Overall rate	4/50 (8%)	20/50 (40%)
Adjusted rate	9.6%	64.6%
Terminal rate	2/20 (10%)	0/0
First incidence (days)	712	528
Poly-3 test		P<0.001
Kidney (Renal Tubule): Adenoma or Carcinoma (Single Sections)		
Overall rate	4/50 (8%)	8/50 (16%)
Adjusted rate	9.6%	31.2%
Terminal rate	2/20 (10%)	0/0
First incidence (days)	712	528
Poly-3 test		P=0.039
Kidney (Renal Tubule): Adenoma or Carcinoma (Single and Step Sections)		
Overall rate	5/50 (10%)	20/50 (40%)
Adjusted rate	12.0%	64.6%
Terminal rate	3/20 (15%)	0/0
First incidence (days)	712	528
Poly-3 test		P<0.001
Liver: Hepatocellular Adenoma		
Overall rate	5/50 (10%)	32/50 (64%)
Adjusted rate	12.0%	83.0%
Terminal rate	2/20 (10%)	0/0
First incidence (days)	715	437
Poly-3 test		P<0.001

TABLE A3b
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Stop-Exposure Gavage Study of Methyleugenol

	Vehicle Control	300 mg/kg
Liver: Hepatocellular Carcinoma		
Overall rate	2/50 (4%)	36/50 (72%)
Adjusted rate	4.8%	89.1%
Terminal rate	1/20 (5%)	0/0
First incidence (days)	529	437
Poly-3 test		P<0.001
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate	7/50 (14%)	45/50 (90%)
Adjusted rate	16.6%	99.4%
Terminal rate	3/20 (15%)	0/0
First incidence (days)	529	437
Poly-3 test		P<0.001
Liver: Hepatocholangioma		
Overall rate	0/50 (0%)	6/50 (12%)
Adjusted rate	0.0%	23.1%
Terminal rate	0/20 (0%)	0/0
First incidence (days)	—e	502
Poly-3 test		P=0.004
Liver: Hepatocholangiocarcinoma		
Overall rate	0/50 (0%)	7/50 (14%)
Adjusted rate	0.0%	27.1%
Terminal rate	0/20 (0%)	0/0
First incidence (days)	—	506
Poly-3 test		P<0.001
Liver: Hepatocholangioma or Hepatocholangiocarcinoma		
Overall rate	0/50 (0%)	13/50 (26%)
Adjusted rate	0.0%	44.4%
Terminal rate	0/20 (0%)	0/0
First incidence (days)	—	502
Poly-3 test		P<0.001
Mammary Gland: Fibroadenoma		
Overall rate	5/50 (10%)	6/50 (12%)
Adjusted rate	12.0%	23.4%
Terminal rate	4/20 (20%)	0/0
First incidence (days)	610	380
Poly-3 test		P=0.231
Mammary Gland: Fibroadenoma or Carcinoma		
Overall rate	5/50 (10%)	6/50 (12%)
Adjusted rate	12.0%	23.4%
Terminal rate	4/20 (20%)	0/0
First incidence (days)	610	380
Poly-3 test		P=0.231
Pancreatic Islets: Adenoma		
Overall rate	3/50 (6%)	0/49 (0%)
Adjusted rate	7.2%	0.0%
Terminal rate	1/20 (5%)	0/0
First incidence (days)	704	—
Poly-3 test		P=0.380N

TABLE A3b
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Stop-Exposure Gavage Study of Methyleugenol

	Vehicle Control	300 mg/kg
Pituitary Gland (Pars Distalis): Adenoma		
Overall rate	7/50 (14%)	3/50 (6%)
Adjusted rate	16.3%	12.3%
Terminal rate	3/20 (15%)	0/0
First incidence (days)	521	502
Poly-3 test		P=0.530N
Preputial Gland: Adenoma		
Overall rate	9/50 (18%)	6/50 (12%)
Adjusted rate	21.2%	23.2%
Terminal rate	4/20 (20%)	0/0
First incidence (days)	610	437
Poly-3 test		P=0.586
Preputial Gland: Carcinoma		
Overall rate	5/50 (10%)	0/50 (0%)
Adjusted rate	11.9%	0.0%
Terminal rate	2/20 (10%)	0/0
First incidence (days)	564	—
Poly-3 test		P=0.190N
Preputial Gland: Adenoma or Carcinoma		
Overall rate	14/50 (28%)	6/50 (12%)
Adjusted rate	32.4%	23.2%
Terminal rate	6/20 (30%)	0/0
First incidence (days)	564	437
Poly-3 test		P=0.337N
Skin: Keratoacanthoma		
Overall rate	3/50 (6%)	2/50 (4%)
Adjusted rate	7.2%	8.4%
Terminal rate	3/20 (15%)	0/0
First incidence (days)	730 (T)	528
Poly-3 test		P=0.692
Skin: Squamous Cell Papilloma or Keratoacanthoma		
Overall rate	3/50 (6%)	3/50 (6%)
Adjusted rate	7.2%	12.4%
Terminal rate	3/20 (15%)	0/0
First incidence (days)	730 (T)	528
Poly-3 test		P=0.480
Skin (Subcutaneous Tissue): Fibroma		
Overall rate	1/50 (2%)	4/50 (8%)
Adjusted rate	2.4%	16.3%
Terminal rate	1/20 (5%)	0/0
First incidence (days)	730 (T)	548
Poly-3 test		P=0.096
Stomach (Glandular): Benign or Malignant Neuroendocrine Tumor		
Overall rate	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	16.5%
Terminal rate	0/20 (0%)	0/0
First incidence (days)	—	517
Poly-3 test		P=0.032

TABLE A3b
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Stop-Exposure Gavage Study of Methyleugenol

	Vehicle Control	300 mg/kg
Testes: Adenoma or Carcinoma		
Overall rate	46/50 (92%)	43/50 (86%)
Adjusted rate	96.1%	97.6%
Terminal rate	20/20 (100%)	0/0
First incidence (days)	529	437
Poly-3 test		P=0.615
Thyroid Gland (C-cell): Adenoma		
Overall rate	6/50 (12%)	1/48 (2%)
Adjusted rate	14.5%	4.6%
Terminal rate	5/20 (25%)	0/0
First incidence (days)	704	634
Poly-3 test		P=0.309N
Thyroid Gland (Follicular Cell): Adenoma		
Overall rate	2/50 (4%)	3/48 (6%)
Adjusted rate	4.8%	13.0%
Terminal rate	0/20 (0%)	0/0
First incidence (days)	626	513
Poly-3 test		P=0.335
All Organs: Mononuclear Cell Leukemia		
Overall rate	26/50 (52%)	1/50 (2%)
Adjusted rate	56.0%	4.3%
Terminal rate	8/20 (40%)	0/0
First incidence (days)	521	572
Poly-3 test		P<0.001N
All Organs: Malignant Mesothelioma		
Overall rate	1/50 (2%)	5/50 (10%)
Adjusted rate	2.4%	19.8%
Terminal rate	0/20 (0%)	0/0
First incidence (days)	564	556
Poly-3 test		P=0.041
All Organs: Benign Neoplasms		
Overall rate	49/50 (98%)	46/50 (92%)
Adjusted rate	99.8%	99.8%
Terminal rate	20/20 (100%)	0/0
First incidence (days)	521	380
Poly-3 test		P=1.000N
All Organs: Malignant Neoplasms		
Overall rate	33/50 (66%)	43/50 (86%)
Adjusted rate	68.9%	96.8%
Terminal rate	12/20 (60%)	0/0
First incidence (days)	344	380
Poly-3 test		P<0.001

TABLE A3b
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Stop-Exposure Gavage Study of Methyleugenol

	Vehicle Control	300 mg/kg
All Organs: Benign or Malignant Neoplasms		
Overall rate	50/50 (100%)	46/50 (92%)
Adjusted rate	100.0%	99.8%
Terminal rate	20/20 (100%)	0/0
First incidence (days)	344	380
Poly-3 test		P=1.000N

(T)Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, kidney, liver, pancreatic islets, pituitary gland, preputial gland, testis, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

TABLE A4a
Historical Incidence of Hepatocellular Neoplasms in Vehicle Control Male F344/N Rats Receiving Corn Oil by Gavage[a]

| Study | Incidence in Controls | | |
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
o-Benzyl-*p*-chlorophenol	0/50	0/50	0/50
Overall Historical Incidence			
Total (%)	12/400 (3.0%)	4/400 (1.0%)	16/400 (4.0%)
Mean ± standard deviation	3.0% ± 3.0%	1.0% ± 1.8%	4.0% ± 3.5%
Range	0%-8%	0%-4%	0%-10%

[a]   Data as of 12 November 1997

TABLE A4b
Historical Incidence of Renal Tubule Neoplasms in Vehicle Control Male F344/N Rats Receiving Corn Oil by Gavage[a]

| Study | Incidence in Controls | | |
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
o-Benzyl-*p*-chlorophenol	1/50	0/50	1/50
Overall Historical Incidence			
Total (%)	3/400 (0.8%)	2/400 (0.5%)	5/400 (1.3%)
Mean ± standard deviation	0.8% ± 1.0%	0.5% ± 1.4%	1.3% ± 1.5%
Range	0%-2%	0%-4%	0%-4%

[a]   Data as of 12 November 1997

TABLE A4c
Historical Incidence of Malignant Mesothelioma in Vehicle Control Male F344/N Rats Receiving Corn Oil by Gavage[a]

Study	Incidence in Controls
Historical Incidence at Battelle Columbus Laboratories	
o-Benzyl-*p*-chlorophenol	0/50
Overall Historical Incidence	
Total (%)	7/402 (1.7%)
Mean ± standard deviation	1.7% ± 2.2%
Range	0%-6%

[a]   Data as of 12 November 1997

TABLE A4d
Historical Incidence of Skin (Subcutaneous Tissue) Neoplasms in Vehicle Control Male F344/N Rats Receiving Corn Oil by Gavage[a]

Study	Incidence in Controls		
	Fibroma	**Fibrosarcoma**	**Fibroma or Fibrosarcoma**
Historical Incidence at Battelle Columbus Laboratories			
o-Benzyl-*p*-chlorophenol	1/50	1/50	2/50
Overall Historical Incidence			
Total (%)	17/402 (4.2%)	3/402 (0.8%)	20/402 (5.0%)
Mean ± standard deviation	4.3% ± 3.6%	0.8% ± 1.0%	5.0% ± 3.4%
Range	0%-12%	0%-2%	0%-12%

[a]   Data as of 12 November 1997

TABLE A4e
Historical Incidence of Adrenal Medulla Pheochromocytoma in Control Male F344/N Rats[a]

	Incidence in Controls		
	Benign	**Malignant**	**Benign or Malignant**[b]
Historical Incidence at Battelle Columbus Laboratories: Corn Oil Gavage Studies			
o-Benzyl-p-chlorophenol	13/50	1/50	14/50
Overall Historical Incidence: Corn Oil Gavage Studies			
Total (%)	94/401 (23.4%)	8/401 (2.0%)	101/401 (25.2%)
Mean ± standard deviation	23.5% ± 7.0%	2.0% ± 1.9%	25.2% ± 7.8%
Range	14%-34%	0%-6%	16%-36%
Overall Historical Incidence: Feed Studies			
Total (%)	228/896 (25.5%)	28/896 (3.1%)	252/896 (28.1%)
Mean ± standard deviation	25.5% ± 9.7%	3.1% ± 3.1%	28.2% ± 8.4%
Range	10%-46%	0%-12%	14%-46%
Overall Historical Incidence: Inhalation Studies			
Total (%)	285/901 (31.6%)	17/901 (1.9%)	295/901 (32.7%)
Mean ± standard deviation	31.5% ± 9.9%	1.9% ± 2.1%	32.6% ± 9.7%
Range	8%-50%	0%-6%	8%-50%

[a] Data as of 12 November 1997
[b] All overall historical incidences include benign, malignant, or complex pheochromocytoma.

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

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Disposition Summary					
Animals initially in study	60	50	50	50	60
6-Month interim evaluation	5				5
12-Month interim evaluation	5				5
Early deaths					
Accidental deaths		1	3	1	1
Moribund	15	18	15	20	23
Natural deaths	15	15	17	29	26
Survivors					
Terminal sacrifice	20	16	15		
Animals examined microscopically	60	50	50	50	60

6-Month Interim Evaluation

Alimentary System

	Vehicle Control				300 mg/kg (Stop-Exposure)
Intestine large, rectum	(5)				(5)
Parasite metazoan	1 (20%)				
Liver	(5)				(5)
Basophilic focus					3 (60%)
Eosinophilic focus					3 (60%)
Hepatodiaphragmatic nodule	1 (20%)				
Mixed cell focus					5 (100%)
Hepatocyte, hypertrophy					5 (100%)
Oval cell, hyperplasia					5 (100%)
Pancreas	(5)				(5)
Acinus, atrophy	1 (20%)				2 (40%)
Duct, hyperplasia					1 (20%)
Salivary glands	(5)				(5)
Submandibular gland, cytoplasmic alteration					5 (100%)
Stomach, glandular	(5)				(5)
Atrophy					5 (100%)

Cardiovascular System

	Vehicle Control				300 mg/kg (Stop-Exposure)
Heart	(5)				(5)
Myocardium, degeneration	3 (60%)				2 (40%)

Endocrine System

	Vehicle Control				300 mg/kg (Stop-Exposure)
Islets, pancreatic	(5)				(5)
Hyperplasia	1 (20%)				

Genital System

	Vehicle Control				300 mg/kg (Stop-Exposure)
Preputial gland	(5)				(5)
Inflammation, chronic					1 (20%)

Hematopoietic System

	Vehicle Control				300 mg/kg (Stop-Exposure)
Bone marrow	(5)				(5)
Hyperplasia					1 (20%)

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

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)

6-Month Interim Evaluation (continued)
Urinary System

Kidney	(5)				(5)
Nephropathy	4 (80%)				5 (100%)

Systems Examined with No Lesions Observed
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System

12-Month Interim Evaluation
Alimentary System

Intestine large, rectum	(5)				(5)
Parasite metazoan	1 (20%)				1 (20%)
Liver	(5)				(5)
Basophilic focus	1 (20%)				3 (60%)
Clear cell focus	1 (20%)				1 (20%)
Degeneration, cystic, focal					5 (100%)
Eosinophilic focus					5 (100%)
Hepatodiaphragmatic nodule					1 (20%)
Mixed cell focus					5 (100%)
Bile duct, hyperplasia					1 (20%)
Bile duct, hyperplasia, atypical, focal					1 (20%)
Hepatocyte, hypertrophy					5 (100%)
Oval cell, hyperplasia					5 (100%)
Salivary glands	(5)				(5)
Submandibular gland, cytoplasmic alteration					5 (100%)
Stomach, glandular	(5)				(5)
Atrophy					5 (100%)
Neuroendocrine cell, hyperplasia					2 (40%)

Cardiovascular System

Heart	(5)				(5)
Myocardium, degeneration	4 (80%)				3 (60%)

Endocrine System

Islets, pancreatic	(5)				(5)
Hyperplasia	2 (40%)				

Genital System

Testes	(5)				(5)
Interstitial cell, hyperplasia	1 (20%)				

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
12-Month Interim Evaluation (continued)					
Hematopoietic System					
Bone marrow	(5)				(5)
Hyperplasia	1 (20%)				5 (100%)
Respiratory System					
Lung	(5)				(5)
Inflammation, granulomatous					1 (20%)
Alveolar epithelium, hypertrophy, focal	1 (20%)				
Nose	(5)				(5)
Inflammation, suppurative					1 (20%)
Special Senses System					
Eye	(1)				
Cataract	1 (100%)				
Urinary System					
Kidney	(5)				(5)
Nephropathy	5 (100%)				5 (100%)
Systems Examined with No Lesions Observed					
General Body System					
Integumentary System					
Musculoskeletal System					
Nervous System					
2-Year Study					
Alimentary System					
Esophagus	(50)	(50)	(50)	(50)	(48)
Perforation			1 (2%)	1 (2%)	
Periesophageal tissue, inflammation			1 (2%)		1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)	(50)
Fibrosis					1 (2%)
Mineralization					4 (8%)
Necrosis					1 (2%)
Parasite metazoan	1 (2%)	2 (4%)	5 (10%)	1 (2%)	1 (2%)
Intestine large, rectum	(49)	(49)	(49)	(50)	(50)
Parasite metazoan	1 (2%)	3 (6%)	5 (10%)	4 (8%)	1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)	(49)
Ulcer	1 (2%)				
Intestine small, duodenum	(50)	(50)	(50)	(50)	(49)
Ulcer	3 (6%)	1 (2%)			
Peyer's patch, hyperplasia	1 (2%)				
Intestine small, jejunum	(50)	(50)	(50)	(50)	(50)
Inflammation, chronic, focal		1 (2%)		1 (2%)	
Intestine small, ileum	(50)	(49)	(50)	(50)	(49)
Mineralization				1 (2%)	
Ulcer	1 (2%)				

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Alimentary System (continued)					
Liver	(50)	(50)	(50)	(50)	(50)
Basophilic focus	23 (46%)	21 (42%)	22 (44%)	7 (14%)	6 (12%)
Clear cell focus	4 (8%)	3 (6%)	7 (14%)	3 (6%)	3 (6%)
Degeneration, cystic, focal	4 (8%)	2 (4%)	25 (50%)	38 (76%)	41 (82%)
Degeneration, fatty, focal	1 (2%)				
Eosinophilic focus	11 (22%)	28 (56%)	43 (86%)	47 (94%)	39 (78%)
Hepatodiaphragmatic nodule	4 (8%)	1 (2%)			
Inflammation, acute	1 (2%)			1 (2%)	
Mixed cell focus	1 (2%)	7 (14%)	14 (28%)	8 (16%)	2 (4%)
Necrosis, focal	2 (4%)	2 (4%)	5 (10%)	3 (6%)	2 (4%)
Bile duct, cyst					1 (2%)
Bile duct, hyperplasia	36 (72%)	17 (34%)	16 (32%)	17 (34%)	28 (56%)
Bile duct, hyperplasia, atypical, focal		1 (2%)		2 (4%)	1 (2%)
Centrilobular, degeneration	6 (12%)	1 (2%)			
Centrilobular, degeneration, fatty	2 (4%)		1 (2%)		
Centrilobular, necrosis	1 (2%)	4 (8%)		1 (2%)	1 (2%)
Hepatocyte, hypertrophy		13 (26%)	25 (50%)	30 (60%)	26 (52%)
Oval cell, hyperplasia	14 (28%)	17 (34%)	24 (48%)	34 (68%)	27 (54%)
Mesentery	(5)	(6)		(1)	(1)
Fat, necrosis	5 (100%)	6 (100%)			
Pancreas	(50)	(50)	(50)	(50)	(49)
Atrophy, acute	1 (2%)				
Hyperplasia, focal	3 (6%)	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Acinus, atrophy	20 (40%)	15 (30%)	13 (26%)	9 (18%)	10 (20%)
Acinus, hyperplasia, focal	1 (2%)	1 (2%)			
Artery, inflammation, chronic	4 (8%)				
Salivary glands	(50)	(50)	(50)	(48)	(48)
Inflammation, chronic				1 (2%)	
Parotid gland, atrophy			1 (2%)		
Submandibular gland, cytoplasmic alteration	4 (8%)	50 (100%)	49 (98%)	48 (100%)	48 (100%)
Submandibular gland, fibrosis			1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)	(49)
Hyperkeratosis			1 (2%)		
Hyperkeratosis, focal	1 (2%)				
Inflammation, chronic				1 (2%)	
Mineralization	3 (6%)		1 (2%)	6 (12%)	5 (10%)
Ulcer	7 (14%)	1 (2%)			1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)	(49)
Atrophy		14 (28%)	32 (64%)	37 (74%)	29 (59%)
Inflammation, focal, suppurative	2 (4%)	3 (6%)			1 (2%)
Mineralization	10 (20%)	4 (8%)	15 (30%)	14 (28%)	20 (41%)
Ulcer	4 (8%)	2 (4%)	3 (6%)	2 (4%)	8 (16%)
Neuroendocrine cell, hyperplasia			1 (2%)	8 (16%)	8 (16%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Cardiovascular System					
Blood vessel	(50)	(50)	(50)	(50)	(50)
Mineralization	1 (2%)	2 (4%)	3 (6%)	7 (14%)	9 (18%)
Aorta, hyperplasia					1 (2%)
Aorta, mineralization	5 (10%)		9 (18%)	6 (12%)	10 (20%)
Heart	(50)	(50)	(50)	(50)	(49)
Inflammation, focal, suppurative		1 (2%)			1 (2%)
Atrium, thrombosis	6 (12%)	5 (10%)	4 (8%)	2 (4%)	
Endocardium, fibrosis		1 (2%)			
Myocardium, degeneration	45 (90%)	41 (82%)	34 (68%)	37 (74%)	25 (51%)
Myocardium, mineralization	5 (10%)	2 (4%)	11 (22%)	9 (18%)	15 (31%)
Valve, inflammation, chronic				1 (2%)	
Valve, thrombosis		1 (2%)			
Ventricle, thrombosis				1 (2%)	
Endocrine System					
Adrenal cortex	(50)	(50)	(50)	(50)	(50)
Hyperplasia, focal	13 (26%)	12 (24%)	6 (12%)	4 (8%)	5 (10%)
Necrosis	1 (2%)	1 (2%)			
Necrosis, focal				1 (2%)	1 (2%)
Vacuolization cytoplasmic, focal					1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)	(50)
Hematocyst			1 (2%)		
Hyperplasia		1 (2%)			
Hyperplasia, focal	16 (32%)	20 (40%)	21 (42%)	21 (42%)	14 (28%)
Islets, pancreatic	(50)	(50)	(50)	(50)	(49)
Hyperplasia		4 (8%)	1 (2%)		
Parathyroid gland	(48)	(49)	(48)	(45)	(46)
Hyperplasia	7 (15%)	2 (4%)	6 (13%)	3 (7%)	7 (15%)
Hyperplasia, focal	2 (4%)				1 (2%)
Bilateral, hyperplasia	10 (21%)	6 (12%)	16 (33%)	18 (40%)	18 (39%)
Pituitary gland	(50)	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)			2 (4%)	
Cyst					1 (2%)
Hyperplasia, focal			1 (2%)		
Pars distalis, hyperplasia, focal	12 (24%)	9 (18%)	8 (16%)	2 (4%)	1 (2%)
Pars intermedia, hyperplasia, focal					1 (2%)
Pars nervosa, inflammation, focal, suppurative				1 (2%)	
Rathke's cleft, cyst			1 (2%)		
Thyroid gland	(50)	(50)	(50)	(48)	(48)
C-cell, hyperplasia	22 (44%)	25 (50%)	25 (50%)	10 (21%)	17 (35%)
Follicle, cyst	1 (2%)	1 (2%)			1 (2%)
Follicular cell, hyperplasia, focal					1 (2%)

General Body System
None

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Genital System					
Epididymis	(50)	(49)	(49)	(50)	(50)
Fibrosis			1 (2%)		
Granuloma sperm				1 (2%)	1 (2%)
Serosa, hyperplasia, mesothelium					2 (4%)
Preputial gland	(50)	(50)	(49)	(50)	(50)
Cyst	5 (10%)	2 (4%)		6 (12%)	4 (8%)
Fibrosis					1 (2%)
Hyperplasia, focal	3 (6%)	4 (8%)	6 (12%)	4 (8%)	5 (10%)
Inflammation, chronic	9 (18%)	14 (28%)	8 (16%)	12 (24%)	4 (8%)
Inflammation, chronic active		1 (2%)			
Prostate	(50)	(50)	(49)	(49)	(49)
Hyperplasia			1 (2%)	1 (2%)	
Hyperplasia, focal		1 (2%)		1 (2%)	
Inflammation, chronic	16 (32%)	18 (36%)	13 (27%)	15 (31%)	9 (18%)
Seminal vesicle	(50)	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)	2 (4%)	6 (12%)	3 (6%)
Mineralization			1 (2%)		3 (6%)
Epithelium, atrophy				1 (2%)	
Serosa, hyperplasia, mesothelium					1 (2%)
Testes	(50)	(49)	(49)	(50)	(50)
Atrophy					1 (2%)
Germinal epithelium, atrophy	3 (6%)	1 (2%)		3 (6%)	1 (2%)
Interstitial cell, hyperplasia	3 (6%)				1 (2%)
Tunic, hyperplasia, mesothelium					3 (6%)
Hematopoietic System					
Bone marrow	(50)	(50)	(50)	(50)	(50)
Fibrosis	3 (6%)		2 (4%)		
Hyperplasia	18 (36%)	14 (28%)	22 (44%)	23 (46%)	18 (36%)
Necrosis		2 (4%)	2 (4%)	1 (2%)	1 (2%)
Lymph node	(8)	(4)	(4)	(4)	(3)
Lumbar, hyperplasia, lymphoid				1 (25%)	
Mediastinal, erythrophagocytosis				1 (25%)	1 (33%)
Mediastinal, hyperplasia, lymphoid	2 (25%)				
Mediastinal, inflammation, suppurative			1 (25%)		
Lymph node, mesenteric	(50)	(50)	(49)	(50)	(48)
Ectasia	1 (2%)				
Erythrophagocytosis				1 (2%)	
Inflammation, suppurative			1 (2%)		
Spleen	(50)	(50)	(50)	(50)	(49)
Congestion		1 (2%)	1 (2%)	1 (2%)	
Fibrosis	8 (16%)	11 (22%)	12 (24%)	12 (24%)	4 (8%)
Hematopoietic cell proliferation	2 (4%)	9 (18%)	2 (4%)	13 (26%)	4 (8%)
Hemorrhage				1 (2%)	
Metaplasia, lipocyte	1 (2%)				
Lymphoid follicle, atrophy					1 (2%)
Lymphoid follicle, depletion cellular	1 (2%)				
Thymus	(46)	(45)	(43)	(42)	(43)
Cyst	1 (2%)				

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Integumentary System					
Mammary gland	(50)	(49)	(49)	(45)	(46)
Dilatation	13 (26%)	13 (27%)	4 (8%)	7 (16%)	1 (2%)
Galactocele	1 (2%)				1 (2%)
Hyperplasia, focal		1 (2%)		1 (2%)	
Duct, granuloma	1 (2%)				
Skin	(50)	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)				
Edema				1 (2%)	
Ulcer	2 (4%)				1 (2%)
Dermis, inflammation, focal, suppurative		1 (2%)			
Pinna, ulcer					1 (2%)
Subcutaneous tissue, fibrosis	1 (2%)				
Subcutaneous tissue, necrosis			1 (2%)		
Musculoskeletal System					
Bone	(50)	(50)	(50)	(50)	(50)
Fibrous osteodystrophy	8 (16%)	2 (4%)	12 (24%)	14 (28%)	21 (42%)
Osteopetrosis	1 (2%)	2 (4%)			
Cranium, hyperostosis					1 (2%)
Nervous System					
Brain	(50)	(50)	(50)	(50)	(50)
Degeneration, focal	1 (2%)				
Hydrocephalus	2 (4%)	3 (6%)	3 (6%)	1 (2%)	1 (2%)
Necrosis	1 (2%)				
Necrosis, focal		2 (4%)	1 (2%)	1 (2%)	
Hypothalamus, degeneration		1 (2%)	1 (2%)		
Spinal cord	(1)	(1)	(1)		
Axon, degeneration, focal	1 (100%)				
Respiratory System					
Lung	(50)	(50)	(50)	(50)	(49)
Congestion					1 (2%)
Erythrophagocytosis		1 (2%)			
Hemorrhage, focal			1 (2%)		
Infiltration cellular, histiocyte	1 (2%)		1 (2%)		
Inflammation, granulomatous	2 (4%)		2 (4%)	3 (6%)	2 (4%)
Inflammation, suppurative	1 (2%)	3 (6%)	2 (4%)	1 (2%)	
Alveolar epithelium, hyperplasia, focal		1 (2%)	1 (2%)	2 (4%)	1 (2%)
Alveolar epithelium, interstitium, mineralization			1 (2%)	1 (2%)	
Bronchiole, inflammation, suppurative			1 (2%)		
Bronchus, inflammation, suppurative				1 (2%)	
Interstitium, inflammation, chronic	2 (4%)	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Interstitium, mineralization	4 (8%)	1 (2%)	8 (16%)	10 (20%)	18 (37%)
Perivascular, edema		1 (2%)	1 (2%)		
Vein, thrombosis	1 (2%)				
Nose	(50)	(50)	(50)	(50)	(50)
Inflammation, suppurative	11 (22%)	14 (28%)	20 (40%)	11 (22%)	9 (18%)
Trachea	(50)	(50)	(50)	(49)	(48)
Inflammation					1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Special Senses System					
Eye	(1)	(1)	(2)	(1)	
Cataract	1 (100%)		1 (50%)	1 (100%)	
Degeneration			1 (50%)		
Cornea, inflammation, chronic		1 (100%)			
Retina, atrophy			1 (50%)		
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(50)
Hydronephrosis			2 (4%)		1 (2%)
Inflammation, focal, suppurative		1 (2%)	1 (2%)	2 (4%)	1 (2%)
Nephropathy	49 (98%)	46 (92%)	48 (96%)	50 (100%)	47 (94%)
Renal tubule, cyst		2 (4%)			2 (4%)
Renal tubule, hyperplasia, focal	5 (10%)	1 (2%)	7 (14%)	4 (8%)	3 (6%)
Renal tubule, hyperplasia, oncocytic				2 (4%)	1 (2%)
Urinary bladder	(49)	(50)	(49)	(49)	(50)
Inflammation, suppurative					1 (2%)
Ulcer	1 (2%)	1 (2%)	2 (4%)		
Transitional epithelium, hyperplasia	1 (2%)				1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS
IN THE 2-YEAR GAVAGE STUDY
OF METHYLEUGENOL

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

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Disposition Summary					
Animals initially in study	60	50	50	50	60
6-Month interim evaluation	5				5
12-Month interim evaluation	5				5
Early deaths					
Accidental deaths			1	1	
Moribund	17	16	14	26	25
Natural deaths	11	9	13	12	9
Survivors					
Terminal sacrifice	22	25	22	11	16
Animals examined microscopically	60	50	50	50	60

Systems Examined at 6 Months with No Neoplasms Observed
Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

12-Month Interim Evaluation

Alimentary System					
Liver	(5)				(5)
Hepatocellular carcinoma					1 (20%)

Endocrine System					
Pituitary gland	(5)				(5)
Pars distalis, adenoma					1 (20%)

Systems Examined with No Neoplasms Observed
Cardiovascular System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study					
Alimentary System					
Intestine large, cecum	(50)	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)				
Neuroendocrine tumor, malignant,					
metastatic, stomach, glandular				1 (2%)	
Liver	(50)	(50)	(49)	(49)	(50)
Cholangiocarcinoma					1 (2%)
Hepatocellular carcinoma			4 (8%)	6 (12%)	13 (26%)
Hepatocellular carcinoma, multiple				2 (4%)	9 (18%)
Hepatocellular adenoma	1 (2%)	8 (16%)	6 (12%)	10 (20%)	7 (14%)
Hepatocellular adenoma, multiple			5 (10%)	23 (47%)	36 (72%)
Hepatocholangiocarcinoma				3 (6%)	7 (14%)
Hepatocholangiocarcinoma, multiple					2 (4%)
Hepatocholangioma					8 (16%)
Neuroendocrine tumor, malignant,					
metastatic, stomach, glandular				3 (6%)	10 (20%)
Osteosarcoma, metastatic,					
uncertain primary site			1 (2%)		
Mesentery	(6)	(5)	(3)	(3)	(3)
Oral mucosa			(1)		(1)
Squamous cell carcinoma			1 (100%)		
Pancreas	(50)	(50)	(50)	(49)	(50)
Salivary glands	(50)	(48)	(49)	(49)	(50)
Myoepithelioma				1 (2%)	
Stomach, forestomach	(50)	(49)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)				
Squamous cell carcinoma				1 (2%)	1 (2%)
Squamous cell papilloma			1 (2%)	2 (4%)	
Stomach, glandular	(50)	(50)	(50)	(48)	(49)
Leiomyosarcoma	1 (2%)				
Neuroendocrine tumor, benign			13 (26%)	9 (19%)	5 (10%)
Neuroendocrine tumor, malignant		1 (2%)	12 (24%)	26 (54%)	36 (73%)
Cardiovascular System					
Heart	(50)	(50)	(49)	(49)	(50)
Carcinoma, metastatic, uterus		1 (2%)			
Schwannoma benign					1 (2%)
Endocrine System					
Adrenal medulla	(50)	(50)	(50)	(49)	(50)
Pheochromocytoma malignant				1 (2%)	
Pheochromocytoma benign	1 (2%)	1 (2%)	2 (4%)	2 (4%)	6 (12%)
Islets, pancreatic	(50)	(50)	(50)	(50)	(50)
Adenoma		1 (2%)			
Carcinoma	1 (2%)				
Neuroendocrine tumor, malignant,					
metastatic, stomach, glandular					1 (2%)
Pituitary gland	(50)	(50)	(50)	(50)	(49)
Pars distalis, adenoma	22 (44%)	20 (40%)	21 (42%)	22 (44%)	19 (39%)
Pars intermedia, adenoma		1 (2%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Endocrine System (continued)					
Thyroid gland	(50)	(49)	(49)	(50)	(48)
C-cell, adenoma	2 (4%)	4 (8%)	2 (4%)		2 (4%)
C-cell, carcinoma		1 (2%)	2 (4%)	2 (4%)	2 (4%)
Follicular cell, adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
General Body System					
None					
Genital System					
Clitoral gland	(48)	(46)	(49)	(46)	(48)
Adenoma	4 (8%)	6 (13%)	4 (8%)	3 (7%)	2 (4%)
Carcinoma	1 (2%)		1 (2%)		
Bilateral, adenoma	1 (2%)	2 (4%)	2 (4%)		2 (4%)
Ovary	(50)	(50)	(49)	(49)	(50)
Carcinoma, metastatic, uterus		1 (2%)			
Granulosa cell tumor malignant		2 (4%)			
Uterus	(50)	(50)	(49)	(50)	(50)
Carcinoma		1 (2%)			
Hemangioma		1 (2%)			
Hemangiosarcoma	1 (2%)				
Polyp stromal	4 (8%)	2 (4%)	3 (6%)	6 (12%)	10 (20%)
Sarcoma stromal		1 (2%)			
Vagina		(2)		(2)	
Fibrosarcoma		1 (50%)			
Leiomyosarcoma				1 (50%)	
Hematopoietic System					
Bone marrow	(50)	(50)	(49)	(50)	(50)
Lymph node	(2)	(5)	(1)	(10)	(6)
Mediastinal, carcinoma, metastatic, uterus		1 (20%)			
Mediastinal, neuroendocrine tumor, malignant, metastatic, stomach, glandular					1 (17%)
Pancreatic, neuroendocrine tumor, malignant, metastatic, stomach, glandular				1 (10%)	
Renal, neuroendocrine tumor, malignant, metastatic, stomach, glandular					1 (17%)
Lymph node, mandibular	(49)	(48)	(49)	(49)	(48)
Lymph node, mesenteric	(50)	(49)	(49)	(49)	(50)
Spleen	(50)	(50)	(50)	(49)	(50)
Leiomyosarcoma		1 (2%)		1 (2%)	
Osteosarcoma, metastatic, uncertain primary site			1 (2%)		
Thymus	(49)	(48)	(46)	(46)	(49)
Thymoma benign				1 (2%)	
Thymoma malignant		1 (2%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Integumentary System					
Mammary gland	(50)	(50)	(50)	(50)	(50)
Adenoma				1 (2%)	1 (2%)
Carcinoma	2 (4%)	3 (6%)	1 (2%)		4 (8%)
Carcinoma, multiple		1 (2%)			
Fibroadenoma	21 (42%)	23 (46%)	22 (44%)	22 (44%)	16 (32%)
Fibroadenoma, multiple	15 (30%)	13 (26%)	12 (24%)	9 (18%)	14 (28%)
Skin	(50)	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)			
Basal cell carcinoma					1 (2%)
Fibrosarcoma					1 (2%)
Trichoepithelioma		1 (2%)			
Pinna, basal cell adenoma			1 (2%)		
Pinna, melanoma malignant			1 (2%)		
Subcutaneous tissue, fibroma	1 (2%)		2 (4%)	2 (4%)	1 (2%)
Subcutaneous tissue, fibrosarcoma			2 (4%)		
Subcutaneous tissue, lipoma		1 (2%)			
Subcutaneous tissue, sarcoma NOS					1 (2%)
Subcutaneous tissue, schwannoma malignant		1 (2%)			
Musculoskeletal System					
Bone	(50)	(50)	(50)	(50)	(50)
Chordoma					1 (2%)
Skeletal muscle		(1)		(1)	(1)
Carcinoma, metastatic, uterus		1 (100%)			
Rhabdomyosarcoma				1 (100%)	
Nervous System					
Brain	(50)	(50)	(50)	(50)	(50)
Oligodendroglioma benign				1 (2%)	
Oligodendroglioma malignant	1 (2%)				
Respiratory System					
Lung	(50)	(50)	(49)	(49)	(50)
Alveolar/bronchiolar adenoma			1 (2%)		1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)				
Carcinoma, metastatic, uterus		1 (2%)			
Carcinoma, metastatic, Zymbal's gland					1 (2%)
Chordoma, metastatic, bone					1 (2%)
Hepatocellular carcinoma, metastatic, liver			1 (2%)	4 (8%)	4 (8%)
Hepatocholangiocarcinoma, metastatic, liver					1 (2%)
Neuroendocrine tumor, malignant, metastatic, stomach, glandular					4 (8%)
Squamous cell carcinoma, metastatic, oral mucosa			1 (2%)		

T<small>ABLE</small> **B1**
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Special Senses System					
Zymbal's gland				(1)	(3)
Carcinoma					3 (100%)
Urinary System					
Kidney	(50)	(50)	(50)	(49)	(50)
Transitional epithelium, carcinoma					1 (2%)
Urinary bladder	(50)	(50)	(49)	(50)	(50)
Carcinoma, metastatic, uterus		1 (2%)			
Systemic Lesions					
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)
Leukemia mononuclear	17 (34%)	20 (40%)	19 (38%)	28 (56%)	17 (34%)
Mesothelioma malignant		1 (2%)			
Neoplasm Summary					
Total animals with primary neoplasms[c]					
12-Month interim evaluation					2
2-Year study	47	49	48	47	47
Total primary neoplasms					
12-Month interim evaluation					2
2-Year study	100	121	141	187	232
Total animals with benign neoplasms					
12-Month interim evaluation					1
2-Year study	43	44	43	43	46
Total benign neoplasms					
12-Month interim evaluation					1
2-Year study	73	86	85	106	127
Total animals with malignant neoplasms					
12-Month interim evaluation					1
2-Year study	22	28	26	34	39
Total malignant neoplasms					
12-Month interim evaluation					1
2-Year study	27	34	31	46	64
Total animals with metastatic neoplasms					
2-Year study		1	3	8	16
Total metastatic neoplasms					
2-Year study		6	4	9	24
Total animals with malignant neoplasms of uncertain primary site					
2-Year study			1		
Total animals with uncertain neoplasms- benign or malignant					
2-Year study		1	25	35	41
Total uncertain neoplasms					
2-Year study		1	25	35	41

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	241	407	448	459	459	545	559	567	598	598	598	609	616	619	641	641	641	644	653	679	683	698	700	703	711
Carcass ID Number	328	322	272	308	314	271	289	319	324	275	285	290	280	315	276	281	316	279	312	327	292	309	274	307	326
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																						X			
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery								+		+	+	+													
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																						X			
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																						X			
Tongue																			+						
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																			X						
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X	X		X		X				X		X				X		X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									X
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X					
Carcinoma																									
Bilateral, adenoma																				X					
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Polyp stromal													X	X							X				

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	7 1 7	7 1 9	7 2 4	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	2 8 8	3 0 5	2 9 7	2 7 3	2 8 3	2 9 1	2 9 4	2 9 8	3 0 1	3 0 6	3 1 1	3 1 7	3 2 1	2 2 5	2 7 8	2 8 2	2 8 4	2 9 5	2 9 6	3 9 9	3 0 3	3 1 0	3 1 8	3 2 0	3 2 3	Total Tissues/ Tumor

Alimentary System

Tissue																										Total
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma					X																					1
Mesentery	+								+																	6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Tongue																										1

Cardiovascular System

Tissue																										Total
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Endocrine System

Tissue																										Total
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																				X						1
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X	X			X	X		X	X	X			X			X	X	X					X		22
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																								X		2
Follicular cell, adenoma	X																									1

General Body System
None

Genital System

Tissue																										Total
Clitoral gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma											X			X	X											4
Carcinoma												X														1
Bilateral, adenoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma					X																					1
Polyp stromal				X																						4

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	241	407	448	459	459	549	555	569	597	598	598	609	616	619	641	641	641	644	653	679	683	698	700	703	711
Carcass ID Number	328	322	272	308	314	271	289	319	324	275	285	290	280	315	276	281	316	279	312	327	292	309	274	307	326
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+																				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma		X									X	X		X		X				X		X	X	X	X
Fibroadenoma, multiple			X			X	X	X					X		X		X		X		X				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma malignant																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X	X	X	X			X		X		X		X		X			X		X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

	288	305	297	273	283	291	294	298	301	306	311	317	321	225	278	282	284	295	296	399	303	310	318	320	323	Total Tissues/ Tumor
Number of Days on Study	717	719	724	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node														+												2
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																				X				X		2
Fibroadenoma		X		X			X	X			X				X			X	X			X	X	X		21
Fibroadenoma, multiple	X		X		X				X		X		X						X							15
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma			X																							1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma malignant		X																								1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye													+													1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X		X				X									X		X				17

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

	3 4 4 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7
Number of Days on Study	4 4 5 2 6 6 8 0 0 1 1 1 2 3 4 5 6 7 8 8 9 0 1 1 2
	7 3 0 0 4 9 8 6 9 1 1 8 0 7 6 0 5 3 3 3 4 3 7 8 4

	3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
Carcass ID Number	4 6 4 7 3 4 5 5 5 4 6 7 3 7 4 4 5 7 3 6 4 3 3 3 7
	3 5 6 9 4 4 9 3 0 9 3 1 2 8 0 1 1 3 1 6 7 5 9 8 6

Alimentary System

Esophagus	+ + + + M +
Intestine large, colon	+ +
Intestine large, rectum	+ +
Intestine large, cecum	+ +
Intestine small, duodenum	+ +
Intestine small, jejunum	+ +
Intestine small, ileum	+ +
Liver	+ +
Hepatocellular adenoma	
Mesentery	+ +
Pancreas	+ +
Salivary glands	+ + + + M + + + + + + + + + + + + + M + + + + +
Stomach, forestomach	+ + + + + + + + + + + + + + + + + + + + + +
Stomach, glandular	+ +
Neuroendocrine tumor, malignant	X
Tongue	

Cardiovascular System

Blood vessel	+ +
Heart	+ +
Carcinoma, metastatic, uterus	X

Endocrine System

Adrenal cortex	+ +
Adrenal medulla	+ +
Pheochromocytoma benign	X
Islets, pancreatic	+ +
Adenoma	X
Parathyroid gland	+ + + + M + + + + + + + M M + + + + + + + M + +
Pituitary gland	+ +
Pars distalis, adenoma	X X X X X X X
Pars intermedia, adenoma	X
Thyroid gland	+ + + + M +
C-cell, adenoma	X X
C-cell, carcinoma	X
Follicular cell, adenoma	

General Body System
None

Genital System

Clitoral gland	+ M + + + +
Adenoma	X
Bilateral, adenoma	X X
Ovary	+ +
Carcinoma, metastatic, uterus	X
Granulosa cell tumor malignant	X
Uterus	+ +
Carcinoma	X
Hemangioma	
Polyp stromal	
Sarcoma stromal	
Vagina	+
Fibrosarcoma	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	Total Tissues/Tumors
Carcass ID Number	336	352	356	357	358	362	364	367	368	372	374	375	380	333	337	342	345	348	354	355	360	361	369	370	377	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma	X					X	X				X			X	X	X								X		8
Mesentery											X			X	X											5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neuroendocrine tumor, malignant																										1
Tongue														X												1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, uterus																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X				X			X					X	X	X	X	X		X	X	X	X			20
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma										X												X				4
C-cell, carcinoma																										1
Follicular cell, adenoma													X													1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	46
Adenoma	X					X			X							X		X								6
Bilateral, adenoma																										2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, uterus																										1
Granulosa cell tumor malignant														X												2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Hemangioma											X															1
Polyp stromal					X																	X				2
Sarcoma stromal					X																					1
Vagina														X												2
Fibrosarcoma														X												1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	347	443	450	520	564	569	588	606	609	611	611	618	620	637	646	650	665	673	683	683	694	703	717	718	724
Carcass ID Number	343	365	346	379	334	344	359	353	350	349	363	371	332	378	340	341	351	373	331	366	347	335	339	338	376
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+																				+		+	+
Mediastinal, carcinoma, metastatic, uterus		X																							
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma					X																				
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Thymoma malignant																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																X									
Carcinoma, multiple																									
Fibroadenoma	X		X						X	X					X	X			X	X			X	X	X
Fibroadenoma, multiple						X		X			X	X					X			X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Trichoepithelioma																									
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, schwannoma malignant																	X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle		+																							
Carcinoma, metastatic, uterus		X																							
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
None																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus		X																							
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X	X		X		X							X	X		X	X	X	X	X
Mesothelioma malignant	X																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

	Number of Days on Study																									Total Tissues/ Tumors
Carcass ID Number	336	352	356	357	358	362	364	367	368	372	374	375	380	333	337	342	345	348	354	355	360	361	369	370	377	
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																	+									5
Mediastinal, carcinoma, metastatic, uterus																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymoma malignant																	X									1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma	X																			X						3
Carcinoma, multiple							X																			1
Fibroadenoma	X	X			X		X		X		X			X		X	X	X		X			X			23
Fibroadenoma, multiple			X		X					X	X		X				X			X						13
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma														X												1
Trichoepithelioma						X																				1
Subcutaneous tissue, lipoma																					X					1
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Carcinoma, metastatic, uterus																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, uterus																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, uterus																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X					X		X		X			X							X	X	X			20
Mesothelioma malignant																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study	297	444	515	527	541	557	595	609	613	625	627	629	637	676	676	676	683	683	690	690	702	703	712	714	717
Carcass ID Number	425	418	401	430	399	415	414	382	390	395	387	397	307	496	313	417	491	306	483	310	488	400	421	427	386

Alimentary System

Esophagus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma								X										X							
Hepatocellular adenoma														X								X	X		
Hepatocellular adenoma, multiple								X								X									
Osteosarcoma, metastatic, uncertain primary site																	X								
Mesentery			+						+																
Oral mucosa													+												
Squamous cell carcinoma													X												
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neuroendocrine tumor, benign															X			X			X		X	X	
Neuroendocrine tumor, malignant																	X							X	

Cardiovascular System

Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Endocrine System

Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																					X				
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	M	M	+	+	M	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X	X			X			X		X		X	X	X			X	X			X	
Thyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma															X										
C-cell, carcinoma																									
Follicular cell, adenoma																									

General Body System
None

Genital System

Clitoral gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X										
Carcinoma																									
Bilateral, adenoma																							X		
Ovary	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal												X											X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

	405	403	426	385	394	402	409	411	416	420	422	423	424	428	429	381	384	389	392	393	398	404	408	412	419	Total Tissues/ Tumors
Number of Days on Study	717	721	724	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma			X								X															4
Hepatocellular adenoma					X			X															X			6
Hepatocellular adenoma, multiple		X					X											X								5
Osteosarcoma, metastatic, uncertain primary site																										1
Mesentery												+														3
Oral mucosa																										1
Squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma					X																					1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neuroendocrine tumor, benign		X					X			X			X	X	X								X	X		13
Neuroendocrine tumor, malignant		X		X	X	X	X		X	X		X	X						X							12
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign					X																					2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X					X	X	X		X	X					X			X		X		X	X		21
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma																							X			2
C-cell, carcinoma					X						X															2
Follicular cell, adenoma							X																			1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma		X																		X		X				4
Carcinoma	X																									1
Bilateral, adenoma																							X			2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp stromal					X																					3

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study	2 9 7	4 4 4	5 1 5	5 2 7	5 4 1	5 5 7	5 9 5	6 0 9	6 1 3	6 2 5	6 2 7	6 2 9	6 3 6	6 7 6	6 7 6	6 7 3	6 8 3	6 8 0	6 9 0	6 9 2	6 0 3	7 0 2	7 1 3	7 1 2	7 1 4	7 1 7	
Carcass ID Number	4 2 5	4 1 8	4 0 1	4 3 0	3 9 9	4 1 5	4 1 4	3 8 2	3 9 0	3 9 5	3 8 7	3 9 7	4 0 7	3 9 6	4 1 3	3 1 7	4 9 1	3 0 6	4 8 3	3 1 0	4 8 8	4 0 0	4 2 1	4 2 7	3 8 6		

Hematopoietic System

Bone marrow	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node																											
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Osteosarcoma, metastatic, uncertain primary site																	X										
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	

Integumentary System

Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Carcinoma		X																									
Fibroadenoma			X			X	X		X				X	X					X						X		
Fibroadenoma, multiple										X							X				X X X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pinna, basal cell adenoma																			X								
Pinna, melanoma malignant												X															
Subcutaneous tissue, fibroma															X												
Subcutaneous tissue, fibrosarcoma																								X			

Musculoskeletal System

Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Nervous System

Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Respiratory System

Lung	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma																	X										
Hepatocellular carcinoma, metastatic, liver																											
Squamous cell carcinoma, metastatic, oral mucosa												X															
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Special Senses System

Eye						+																					

Urinary System

Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Urinary bladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Systemic Lesions

Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Leukemia mononuclear		X	X			X	X	X						X			X	X		X	X		X				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study

	405	403	426	385	394	402	409	411	416	420	422	423	424	428	429	381	384	389	392	393	398	404	408	412	419	Total Tissues/ Tumors
Days	717	721	724	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node		+																								1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, uncertain primary site																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M		46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Fibroadenoma	X	X		X			X		X	X	X	X				X	X		X		X		X		X	22
Fibroadenoma, multiple			X			X		X					X					X				X		X		12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pinna, basal cell adenoma																										1
Pinna, melanoma malignant																										1
Subcutaneous tissue, fibroma																X										2
Subcutaneous tissue, fibrosarcoma																X										2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma																										1
Hepatocellular carcinoma, metastatic, liver										X																1
Squamous cell carcinoma, metastatic, oral mucosa																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye																							+			2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X	X	X				X			X			X				X		X						19

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study	483	483	508	527	534	541	548	553	572	576	589	590	591	600	605	609	609	611	620	620	620	632	641	647	652
Carcass ID Number	434	440	442	455	431	472	450	436	452	439	473	449	466	446	453	438	474	454	451	471	478	445	435	469	447

Alimentary System

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neuroendocrine tumor, malignant, metastatic, stomach, glandular																									
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular carcinoma, multiple												X													
Hepatocellular adenoma						X	X													X		X			
Hepatocellular adenoma, multiple		X	X		X				X	X	X			X							X			X	
Hepatocholangiocarcinoma					X																				
Neuroendocrine tumor, malignant, metastatic, stomach, glandular																									
Mesentery																				+					
Pancreas	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myoepithelioma																								X	
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Squamous cell papilloma																									
Stomach, glandular	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neuroendocrine tumor, benign								X		X							X		X		X				
Neuroendocrine tumor, malignant							X	X		X							X		X	X					

Cardiovascular System

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Endocrine System

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Adrenal cortex	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant										X															
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X	X	X					X	X			X		X	X	X						X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, carcinoma																								X	
Follicular cell, adenoma																									

General Body System
None

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study	6 6 0	6 6 7	6 7 6	6 7 9	6 7 9	6 9 4	7 0 2	7 0 3	7 1 1	7 1 1	7 1 7	7 2 3	7 2 4	7 2 4	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	4 3 7	4 5 6	4 5 8	4 4 8	4 7 9	4 4 4	4 7 5	4 6 2	4 5 7	4 6 7	4 7 6	4 6 1	4 4 3	4 6 3	4 3 2	4 4 1	4 6 0	4 6 4	4 6 5	4 7 7	4 3 3	4 5 9	4 6 8	4 7 0	4 8 0	**Total Tissues/ Tumors**

Alimentary System

Tissue / Tumor	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Total
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neuroendocrine tumor, malignant, metastatic, stomach, glandular									X																	1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma			X	X									X		X					X		X				6
Hepatocellular carcinoma, multiple																		X								2
Hepatocellular adenoma	X						X			X		X						X			X					10
Hepatocellular adenoma, multiple		X	X	X		X				X		X X X X X			X X		X					23
Hepatocholangiocarcinoma													X		X				3							
Neuroendocrine tumor, malignant, metastatic, stomach, glandular									X		X			X												3
Mesentery											+												+			3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Myoepithelioma																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																X										1
Squamous cell papilloma			X															X								2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Neuroendocrine tumor, benign	X	X	X																	X						9
Neuroendocrine tumor, malignant			X		X X X X X X X X X X X X X X X X			X		X X						26

Cardiovascular System

Tissue	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Total
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

Endocrine System

Tissue / Tumor	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Total
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant																										1
Pheochromocytoma benign									X			X														2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+				+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X			X X X		X X		X X X X				X		X					22
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, carcinoma									X																	2
Follicular cell, adenoma																X										1

General Body System
None

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study	483	483	508	527	534	541	548	553	572	576	589	590	591	600	605	609	609	611	620	620	620	632	641	647	652
Carcass ID Number	434	440	442	455	431	472	450	436	452	439	473	449	466	446	453	438	474	454	451	471	478	445	435	469	447
Genital System																									
Clitoral gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Adenoma																X		X							
Ovary	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal													X												X
Vagina				+																+					
Leiomyosarcoma				X																					
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+	+			+													+	+	
Pancreatic, neuroendocrine tumor, malignant, metastatic, stomach, glandular																									
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
Thymus	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																X									
Fibroadenoma			X					X		X		X	X			X	X	X	X			X		X	
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																		X							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Rhabdomyosarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma benign																									
Respiratory System																									
Lung	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver									X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear												+													
Lacrimal gland						+																			
Zymbal's gland																									
Urinary System																									
Kidney	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X	X		X	X		X	X	X	X			X		X	X	X			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

	660	667	676	679	679	694	702	703	711	711	717	723	724	724	730	730	730	730	730	730	731	731	731	731	731	Total Tissues/ Tumors
Carcass ID Number	437	456	458	448	479	444	475	462	457	467	476	461	443	463	432	441	460	464	465	477	433	459	468	470	480	
Genital System																										
Clitoral gland	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoma													X													3
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal									X							X					X		X			6
Vagina																										2
Leiomyosarcoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node				+		+			+		+	+														10
Pancreatic, neuroendocrine tumor, malignant, metastatic, stomach, glandular				X																						1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyosarcoma																			X							1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	46
Thymoma benign					X																					1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Fibroadenoma	X	X	X				X	X			X	X			X	X			X			X		X		22
Fibroadenoma, multiple									X	X			X	X	X		X	X			X		X			9
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma													X													2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle	+																									1
Rhabdomyosarcoma	X																									1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma benign		X																								1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma, metastatic, liver																X			X				X			4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Lacrimal gland																										1
Zymbal's gland																							+			1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X	X		X	X			X	X	X	X				X	X	X			X	X	X	28

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

	Days on Study →																								
Number of Days on Study	183	281	288	331	459	477	519	528	560	571	609	609	611	620	620	637	641	641	642	653	659	660	665	679	679
Carcass ID Number	514	490	539	487	498	492	497	532	510	482	506	536	502	495	496	491	509	520	501	521	508	585	434	503	515
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																									
Hepatocellular carcinoma									X			X	X						X			X	X		
Hepatocellular carcinoma, multiple					X						X	X													
Hepatocellular adenoma						X		X				X						X			X				
Hepatocellular adenoma, multiple					X				X		X	X		X	X	X	X	X		X	X		X		
Hepatocholangiocarcinoma																X						X		X	
Hepatocholangiocarcinoma, multiple																		X							
Hepatocholangioma									X					X		X				X			X		
Neuroendocrine tumor, malignant, metastatic, stomach, glandular											X	X		X				X							
Mesentery									+									+							
Oral mucosa																					+				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																X									
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neuroendocrine tumor, benign						X			X				X	X				X							X
Neuroendocrine tumor, malignant								X			X	X		X			X	X	X	X	X	X	X	X	
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma benign																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																	X			X					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neuroendocrine tumor, malignant, metastatic, stomach, glandular									X																
Parathyroid gland	M	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	
Pituitary gland	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma						X							X				X		X	X					
Thyroid gland	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																					X				
C-cell, carcinoma																								X	
Follicular cell, adenoma																									
General Body System																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

	486	511	516	527	519	513	518	533	540	489	493	494	499	504	522	523	530	535	488	512	517	524	525	529	531	Total Tissues/ Tumors
Number of Days on Study	694	703	703	703	709	711	724	724	724	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma																					X					1
Hepatocellular carcinoma	X	X		X			X			X	X		X													13
Hepatocellular carcinoma, multiple					X				X					X	X											9
Hepatocellular adenoma									X															X		7
Hepatocellular adenoma, multiple	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	36
Hepatocholangiocarcinoma			X										X				X	X								7
Hepatocholangiocarcinoma, multiple		X																								2
Hepatocholangioma			X						X	X						X										8
Neuroendocrine tumor, malignant, metastatic, stomach, glandular					X				X		X	X											X	X		10
Mesentery																	+									3
Oral mucosa																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Neuroendocrine tumor, benign																										5
Neuroendocrine tumor, malignant		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	36
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma benign																						X				1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign											X												X	X	X	6
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neuroendocrine tumor, malignant, metastatic, stomach, glandular																										1
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X	X		X			X	X		X	X	X			X		X		X	X				X	X	19
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma																							X			2
C-cell, carcinoma																					X					2
Follicular cell, adenoma																	X									1

General Body System
None

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

Number of Days on Study	183	281	288	331	459	477	519	528	560	571	609	609	611	620	620	637	641	641	642	653	659	660	665	679	679
Carcass ID Number	514	490	539	487	498	492	497	532	510	482	506	536	502	495	496	491	509	520	501	521	508	585	434	503	515
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Bilateral, adenoma															X										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal				X			X								X		X	X						X	
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node															+										
Mediastinal, neuroendocrine tumor, malignant, metastatic, stomach, glandular																									
Renal, neuroendocrine tumor, malignant, metastatic, stomach, glandular																									
Lymph node, mandibular	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma									X																
Fibroadenoma											X			X	X					X			X		
Fibroadenoma, multiple												X					X	X							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																				X					
Fibrosarcoma																	X								
Subcutaneous tissue, fibroma																						X			
Subcutaneous tissue, sarcoma NOS																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chordoma																				X					
Skeletal muscle																									+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

																												Total Tissues/ Tumors
Number of Days on Study	6 9 4	7 0 3	7 0 3	7 0 3	7 0 9	7 1 1	7 2 4	7 2 4	7 2 4	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	4 8 6	5 1 1	5 1 6	5 2 7	5 1 9	5 1 3	5 1 8	5 3 3	5 4 0	4 8 9	4 9 3	4 9 4	4 9 9	5 0 2	5 2 2	5 2 3	5 3 0	4 3 8	5 8 5	5 1 2	5 1 7	5 2 4	5 2 5	5 2 9	5 3 1			

Genital System

Tissue/Tumor																												Total
Clitoral gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+		48
Adenoma										X													X					2
Bilateral, adenoma																									X			2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal											X			X					X	X								10

Hematopoietic System

Tissue/Tumor																												Total
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+				+		+							+	+													6
Mediastinal, neuroendocrine tumor, malignant, metastatic, stomach, glandular					X																							1
Renal, neuroendocrine tumor, malignant, metastatic, stomach, glandular															X													1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49

Integumentary System

Tissue/Tumor																												Total
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma												X																1
Carcinoma			X	X								X																4
Fibroadenoma	X	X			X	X			X		X	X					X			X	X	X						16
Fibroadenoma, multiple			X	X	X			X			X					X	X	X		X				X	X			14
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma																												1
Fibrosarcoma																												1
Subcutaneous tissue, fibroma																												1
Subcutaneous tissue, sarcoma NOS																	X											1

Musculoskeletal System

Tissue/Tumor																												Total
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Chordoma																												1
Skeletal muscle																												1

Nervous System

Tissue/Tumor																												Total
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

Number of Days on Study	1 8 3	2 8 1	2 8 8	3 3 1	4 5 9	4 7 7	5 1 9	5 2 8	5 6 0	5 7 1	6 0 9	6 0 9	6 1 1	6 2 0	6 2 0	6 3 7	6 4 1	6 4 1	6 4 2	6 5 3	6 5 9	6 6 0	6 6 5	6 7 9	6 7 9
Carcass ID Number	5 1 4	4 9 0	5 3 9	4 8 7	4 9 8	4 9 2	4 9 7	5 3 2	5 1 0	4 8 2	5 0 6	5 3 6	5 0 2	4 9 5	4 9 6	4 9 1	5 0 9	5 2 0	5 0 1	5 2 1	5 0 8	5 8 5	4 3 4	5 0 3	5 1 5

Respiratory System

Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic, Zymbal's gland						X																			
Chordoma, metastatic, bone																						X			
Hepatocellular carcinoma, metastatic, liver											X														
Hepatocholangiocarcinoma, metastatic, liver																									
Neuroendocrine tumor, malignant, metastatic, stomach, glandular																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Special Senses System

Zymbal's gland						+	+																	+	
Carcinoma						X	X																	X	

Urinary System

Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, carcinoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Systemic Lesions

Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X				X							X	X	X			X		X			

TABLE B2

Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Methyleugenol: 300 mg/kg (Stop-Exposure)

Number of Days on Study	6 9 4	7 0 3	7 0 3	7 0 3	7 0 9	7 1 1	7 2 4	7 2 4	7 2 4	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	4 8 6	5 1 1	5 1 6	5 2 7	5 1 9	5 1 3	5 1 8	5 3 3	5 3 4	4 8 9	4 9 3	4 9 4	4 9 9	5 0 4	5 2 2	5 2 3	5 3 0	4 3 5	5 3 8	5 1 2	5 1 7	5 2 4	5 2 5	5 2 9	5 3 1		Total Tissues/ Tumors

																											Total
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	X	+	+	+	+	50
Alveolar/bronchiolar adenoma																											1
Carcinoma, metastatic, Zymbal's gland																											1
Chordoma, metastatic, bone																											1
Hepatocellular carcinoma, metastatic, liver									X		X										X						4
Hepatocholangiocarcinoma, metastatic, liver																						X					1
Neuroendocrine tumor, malignant, metastatic, stomach, glandular						X			X	X	X																4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																											
Zymbal's gland																											3
Carcinoma																											3
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, carcinoma																						X					1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X	X		X	X	X			X		X			X	X										17

TABLE B3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate[a]	1/50 (2%)	1/50 (2%)	2/50 (4%)	3/49 (6%)
Adjusted rate[b]	2.5%	2.4%	4.8%	8.2%
Terminal rate[c]	0/22 (0%)	0/25 (0%)	1/22 (5%)	0/11 (0%)
First incidence (days)	698	650	702	576
Poly-3 test[d]	P=0.141	P=0.751N	P=0.518	P=0.278
Clitoral Gland: Adenoma				
Overall rate	5/48 (10%)	8/46 (17%)	6/49 (12%)	3/46 (7%)
Adjusted rate	13.1%	21.4%	14.6%	8.8%
Terminal rate	3/21 (14%)	5/22 (23%)	3/22 (14%)	1/11 (9%)
First incidence (days)	679	620	676	609
Poly-3 test	P=0.249N	P=0.255	P=0.548	P=0.420N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	6/48 (13%)	8/46 (17%)	7/49 (14%)	3/46 (7%)
Adjusted rate	15.7%	21.4%	17.1%	8.8%
Terminal rate	4/21 (19%)	5/22 (23%)	3/22 (14%)	1/11 (9%)
First incidence (days)	679	620	676	609
Poly-3 test	P=0.195N	P=0.365	P=0.554	P=0.298N
Liver: Hepatocellular Adenoma				
Overall rate	1/50 (2%)	8/50 (16%)	11/49 (22%)	33/49 (67%)
Adjusted rate	2.5%	19.6%	26.3%	75.1%
Terminal rate	1/22 (5%)	8/25 (32%)	5/22 (23%)	11/11 (100%)
First incidence (days)	730 (T)	730 (T)	609	508
Poly-3 test	P<0.001	P=0.017	P=0.002	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	4/49 (8%)	8/49 (16%)
Adjusted rate	0.0%	0.0%	9.7%	21.8%
Terminal rate	0/22 (0%)	0/25 (0%)	2/22 (9%)	5/11 (46%)
First incidence (days)	—[e]	—	609	589
Poly-3 test	P<0.001	—[f]	P=0.066	P=0.002
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	1/50 (2%)	8/50 (16%)	14/49 (29%)	34/49 (69%)
Adjusted rate	2.5%	19.6%	33.4%	76.6%
Terminal rate	1/22 (5%)	8/25 (32%)	7/22 (32%)	11/11 (100%)
First incidence (days)	730 (T)	730 (T)	609	508
Poly-3 test	P<0.001	P=0.017	P<0.001	P<0.001
Liver: Hepatocholangiocarcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/49 (0%)	3/49 (6%)
Adjusted rate	0.0%	0.0%	0.0%	8.2%
Terminal rate	0/22 (0%)	0/25 (0%)	0/22 (0%)	2/11 (18%)
First incidence (days)	—	—	—	534
Poly-3 test	P=0.010	—	—	P=0.105
Mammary Gland: Fibroadenoma				
Overall rate	36/50 (72%)	36/50 (72%)	34/50 (68%)	31/50 (62%)
Adjusted rate	78.7%	77.9%	75.9%	73.8%
Terminal rate	15/22 (68%)	19/25 (76%)	18/22 (82%)	9/11 (82%)
First incidence (days)	407	347	527	527
Poly-3 test	P=0.306N	P=0.567N	P=0.474N	P=0.376N

TABLE B3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	36/50 (72%)	36/50 (72%)	34/50 (68%)	32/50 (64%)
Adjusted rate	78.7%	77.9%	75.9%	75.4%
Terminal rate	15/22 (68%)	19/25 (76%)	18/22 (82%)	9/11 (82%)
First incidence (days)	407	347	527	527
Poly-3 test	P=0.379N	P=0.567N	P=0.474N	P=0.451N
Mammary Gland: Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rate	5.1%	9.7%	2.4%	0.0%
Terminal rate	2/22 (9%)	3/25 (12%)	0/22 (0%)	0/11 (0%)
First incidence (days)	730 (T)	650	515	—
Poly-3 test	P=0.093N	P=0.358	P=0.476N	P=0.255N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted rate	5.1%	9.7%	2.4%	2.7%
Terminal rate	2/22 (9%)	3/25 (12%)	0/22 (0%)	0/11 (0%)
First incidence (days)	730 (T)	650	515	609
Poly-3 test	P=0.245N	P=0.358	P=0.476N	P=0.524N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	37/50 (74%)	38/50 (76%)	35/50 (70%)	32/50 (64%)
Adjusted rate	80.9%	81.7%	77.0%	75.4%
Terminal rate	16/22 (73%)	20/25 (80%)	18/22 (82%)	9/11 (82%)
First incidence (days)	407	347	515	527
Poly-3 test	P=0.243N	P=0.570	P=0.420N	P=0.344N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	22/50 (44%)	20/50 (40%)	21/50 (42%)	22/50 (44%)
Adjusted rate	51.8%	46.2%	47.6%	53.0%
Terminal rate	10/22 (46%)	13/25 (52%)	10/22 (46%)	5/11 (46%)
First incidence (days)	549	520	541	527
Poly-3 test	P=0.439	P=0.378N	P=0.429N	P=0.544
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.5%	0.0%	9.6%	5.5%
Terminal rate	0/22 (0%)	0/25 (0%)	2/22 (9%)	0/11 (0%)
First incidence (days)	724	—	676	620
Poly-3 test	P=0.195	P=0.492N	P=0.195	P=0.476
Stomach (Forestomach): Squamous Cell Papilloma or Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	2.4%	8.2%
Terminal rate	0/22 (0%)	0/25 (0%)	1/22 (5%)	2/11 (18%)
First incidence (days)	—	—	730 (T)	679
Poly-3 test	P=0.016	—	P=0.510	P=0.105
Stomach (Glandular): Benign Neuroendocrine Tumor				
Overall rate	0/50 (0%)	0/50 (0%)	13/50 (26%)	9/50 (18%)
Adjusted rate	0.0%	0.0%	31.0%	23.0%
Terminal rate	0/22 (0%)	0/25 (0%)	7/22 (32%)	1/11 (9%)
First incidence (days)	—	—	676	553
Poly-3 test	P<0.001	—	P<0.001	P<0.001

TABLE B3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Stomach (Glandular): Malignant Neuroendocrine Tumor				
Overall rate	0/50 (0%)	1/50 (2%)	12/50 (24%)	26/50 (52%)
Adjusted rate	0.0%	2.4%	28.9%	64.9%
Terminal rate	0/22 (0%)	0/25 (0%)	9/22 (41%)	9/11 (82%)
First incidence (days)	—	718	690	548
Poly-3 test	P<0.001	P=0.508	P<0.001	P<0.001
Stomach (Glandular): Benign or Malignant Neuroendocrine Tumor				
Overall rate	0/50 (0%)	1/50 (2%)	25/50 (50%)	34/50 (68%)
Adjusted rate	0.0%	2.4%	59.2%	80.3%
Terminal rate	0/22 (0%)	0/25 (0%)	16/22 (73%)	10/11 (91%)
First incidence (days)	—	718	676	548
Poly-3 test	P<0.001	P=0.508	P<0.001	P<0.001
Thyroid Gland (C-cell): Adenoma				
Overall rate	2/50 (4%)	4/49 (8%)	2/49 (4%)	0/50 (0%)
Adjusted rate	5.1%	9.8%	4.9%	0.0%
Terminal rate	1/22 (5%)	2/25 (8%)	1/22 (5%)	0/11 (0%)
First incidence (days)	711	611	683	—
Poly-3 test	P=0.128N	P=0.355	P=0.680N	P=0.255N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	5/49 (10%)	4/49 (8%)	2/50 (4%)
Adjusted rate	5.1%	12.2%	9.8%	5.5%
Terminal rate	1/22 (5%)	2/25 (8%)	3/22 (14%)	0/11 (0%)
First incidence (days)	711	611	683	647
Poly-3 test	P=0.487N	P=0.232	P=0.355	P=0.669
Uterus: Stromal Polyp				
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)	6/50 (12%)
Adjusted rate	10.0%	4.9%	7.2%	16.2%
Terminal rate	1/22 (5%)	2/25 (8%)	1/22 (5%)	3/11 (27%)
First incidence (days)	641	730 (T)	627	600
Poly-3 test	P=0.165	P=0.327N	P=0.476N	P=0.319
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	4/50 (8%)	3/50 (6%)	3/50 (6%)	6/50 (12%)
Adjusted rate	10.0%	7.4%	7.2%	16.2%
Terminal rate	1/22 (5%)	3/25 (12%)	1/22 (5%)	3/11 (27%)
First incidence (days)	641	730 (T)	627	600
Poly-3 test	P=0.207	P=0.489N	P=0.476N	P=0.319
All Organs: Mononuclear Cell Leukemia				
Overall rate	17/50 (34%)	20/50 (40%)	19/50 (38%)	28/50 (56%)
Adjusted rate	38.3%	45.3%	42.4%	64.8%
Terminal rate	5/22 (23%)	8/25 (32%)	6/22 (27%)	6/11 (55%)
First incidence (days)	448	520	515	541
Poly-3 test	P=0.007	P=0.324	P=0.426	P=0.008
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	44/50 (88%)	43/50 (86%)	43/50 (86%)
Adjusted rate	91.9%	91.2%	91.2%	92.1%
Terminal rate	20/22 (91%)	22/25 (88%)	20/22 (91%)	11/11 (100%)
First incidence (days)	407	347	527	508
Poly-3 test	P=0.553	P=0.608N	P=0.610N	P=0.661

TABLE B3a
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
All Organs: Malignant Neoplasms				
Overall rate	22/50 (44%)	28/50 (56%)	27/50 (54%)	34/50 (68%)
Adjusted rate	48.5%	60.0%	59.1%	76.9%
Terminal rate	7/22 (32%)	11/25 (44%)	10/22 (46%)	9/11 (82%)
First incidence (days)	407	443	515	534
Poly-3 test	P=0.004	P=0.178	P=0.205	P=0.003
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/50 (94%)	49/50 (98%)	48/50 (96%)	47/50 (94%)
Adjusted rate	95.8%	98.0%	99.4%	97.8%
Terminal rate	20/22 (91%)	24/25 (96%)	22/22 (100%)	11/11 (100%)
First incidence (days)	407	347	515	508
Poly-3 test	P=0.384	P=0.485	P=0.305	P=0.514

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

TABLE B3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of Methyleugenol

	Vehicle Control	300 mg/kg
Adrenal Medulla: Benign Pheochromocytoma		
Overall rate[a]	1/50 (2%)	6/50 (12%)
Adjusted rate[b]	2.5%	16.0%
Terminal rate[c]	0/22 (0%)	4/16 (25%)
First incidence (days)	698	641
Poly-3 test[d]		P=0.047
Clitoral Gland: Adenoma		
Overall rate	5/48 (10%)	4/48 (8%)
Adjusted rate	13.1%	11.3%
Terminal rate	3/21 (14%)	3/15 (20%)
First incidence (days)	679	620
Poly-3 test		P=0.556N
Clitoral Gland: Adenoma or Carcinoma		
Overall rate	6/48 (13%)	4/48 (8%)
Adjusted rate	15.7%	11.3%
Terminal rate	4/21 (19%)	3/15 (20%)
First incidence (days)	679	620
Poly-3 test		P=0.424N
Liver: Hepatocholangioma		
Overall rate	0/50 (0%)	8/50 (16%)
Adjusted rate	0.0%	20.9%
Terminal rate	0/22 (0%)	3/16 (19%)
First incidence (days)	—[e]	609
Poly-3 test		P=0.003
Liver: Hepatocholangiocarcinoma		
Overall rate	0/50 (0%)	9/50 (18%)
Adjusted rate	0.0%	23.6%
Terminal rate	0/22 (0%)	3/16 (19%)
First incidence (days)	—	641
Poly-3 test		P<0.001
Liver: Hepatocholangioma or Hepatocholangiocarcinoma		
Overall rate	0/50 (0%)	17/50 (34%)
Adjusted rate	0.0%	43.0%
Terminal rate	0/22 (0%)	6/16 (38%)
First incidence (days)	—	609
Poly-3 test		P<0.001
Liver: Hepatocellular Adenoma		
Overall rate	1/50 (2%)	43/50 (86%)
Adjusted rate	2.5%	96.5%
Terminal rate	1/22 (5%)	16/16 (100%)
First incidence (days)	730 (T)	459
Poly-3 test		P<0.001
Liver: Hepatocellular Carcinoma		
Overall rate	0/50 (0%)	22/50 (44%)
Adjusted rate	0.0%	53.8%
Terminal rate	0/22 (0%)	8/16 (50%)
First incidence (days)	—	459
Poly-3 test		P<0.001

Tᴀʙʟᴇ B3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of Methyleugenol

	Vehicle Control	300 mg/kg
Liver: Hepatocellular Adenoma or Carcinoma		
Overall rate	1/50 (2%)	43/50 (86%)
Adjusted rate	2.5%	96.5%
Terminal rate	1/22 (5%)	16/16 (100%)
First incidence (days)	730 (T)	459
Poly-3 test		P<0.001
Mammary Gland: Fibroadenoma or Adenoma		
Overall rate	36/50 (72%)	30/50 (60%)
Adjusted rate	78.7%	74.3%
Terminal rate	15/22 (68%)	14/16 (88%)
First incidence (days)	407	609
Poly-3 test		P=0.412N
Mammary Gland: Carcinoma		
Overall rate	2/50 (4%)	4/50 (8%)
Adjusted rate	5.1%	10.6%
Terminal rate	2/22 (9%)	1/16 (6%)
First incidence (days)	730 (T)	571
Poly-3 test		P=0.320
Mammary Gland: Adenoma or Carcinoma		
Overall rate	2/50 (4%)	5/50 (10%)
Adjusted rate	5.1%	13.3%
Terminal rate	2/22 (9%)	2/16 (13%)
First incidence (days)	730 (T)	571
Poly-3 test		P=0.199
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma		
Overall rate	37/50 (74%)	31/50 (62%)
Adjusted rate	80.9%	75.8%
Terminal rate	16/22 (73%)	14/16 (88%)
First incidence (days)	407	571
Poly-3 test		P=0.374N
Pituitary Gland (Pars Distalis): Adenoma		
Overall rate	22/50 (44%)	19/49 (39%)
Adjusted rate	51.8%	48.8%
Terminal rate	10/22 (46%)	9/16 (56%)
First incidence (days)	549	477
Poly-3 test		P=0.483N
Stomach (Glandular): Benign Neuroendocrine Tumor		
Overall rate	0/50 (0%)	5/50 (10%)
Adjusted rate	0.0%	12.8%
Terminal rate	0/22 (0%)	0/16 (0%)
First incidence (days)	—	477
Poly-3 test		P=0.029
Stomach (Glandular): Malignant Neuroendocrine Tumor		
Overall rate	0/50 (0%)	36/50 (72%)
Adjusted rate	0.0%	86.8%
Terminal rate	0/22 (0%)	16/16 (100%)
First incidence (days)	—	560
Poly-3 test		P<0.001

TABLE B3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of Methyleugenol

	Vehicle Control	300 mg/kg
Stomach (Glandular): Benign or Malignant Neuroendocrine Tumor		
Overall rate	0/50 (0%)	41/50 (82%)
Adjusted rate	0.0%	93.9%
Terminal rate	0/22 (0%)	16/16 (100%)
First incidence (days)	—	477
Poly-3 test		P<0.001
Thyroid Gland (C-cell): Adenoma or Carcinoma		
Overall rate	2/50 (4%)	4/48 (8%)
Adjusted rate	5.1%	10.7%
Terminal rate	1/22 (5%)	2/16 (13%)
First incidence (days)	711	660
Poly-3 test		P=0.316
Uterus: Stromal Polyp		
Overall rate	4/50 (8%)	10/50 (20%)
Adjusted rate	10.0%	25.2%
Terminal rate	1/22 (5%)	4/16 (25%)
First incidence (days)	641	331
Poly-3 test		P=0.065
Zymbal's Gland: Carcinoma		
Overall rate	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	7.8%
Terminal rate	0/22 (0%)	0/16 (0%)
First incidence (days)	—	519
Poly-3 test		P=0.115
All Organs: Mononuclear Cell Leukemia		
Overall rate	17/50 (34%)	17/50 (34%)
Adjusted rate	38.3%	42.3%
Terminal rate	5/22 (23%)	4/16 (25%)
First incidence (days)	448	459
Poly-3 test		P=0.442
All Organs: Benign Neoplasms		
Overall rate	43/50 (86%)	46/50 (92%)
Adjusted rate	91.9%	98.9%
Terminal rate	20/22 (91%)	16/16 (100%)
First incidence (days)	407	331
Poly-3 test		P=0.095
All Organs: Malignant Neoplasms		
Overall rate	22/50 (44%)	39/50 (78%)
Adjusted rate	48.5%	87.9%
Terminal rate	7/22 (32%)	13/16 (81%)
First incidence (days)	407	459
Poly-3 test		P<0.001

TABLE B3b
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Stop-Exposure Gavage Study of Methyleugenol

	Vehicle Control	300 mg/kg
All Organs: Benign or Malignant Neoplasms		
Overall rate	47/50 (94%)	47/50 (94%)
Adjusted rate	95.8%	99.7%
Terminal rate	20/22 (91%)	16/16 (100%)
First incidence (days)	407	331
Poly-3 test		P=0.275

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

TABLE B4a
Historical Incidence of Hepatocellular Neoplasms in Vehicle Control Female F344/N Rats Receiving Corn Oil by Gavage[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
o-Benzyl-p-chlorophenol	1/50	0/50	1/50
Overall Historical Incidence			
Total (%)	1/401 (0.3%)	0/401	1/401 (0.3%)
Mean ± standard deviation	0.3% ± 0.7%		0.3% ± 0.7%
Range	0%-2%		0%-2%

[a] Data as of 12 November 1997

TABLE B4b
Historical Incidence of Forestomach Neoplasms in Vehicle Control Female F344/N Rats Receiving Corn Oil by Gavage[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
o-Benzyl-p-chlorophenol	0/50	0/50	0/50
Overall Historical Incidence			
Total (%)	2/401 (0.5%)	0/401	2/401 (0.5%)
Mean ± standard deviation	0.5% ± 0.9%		0.5% ± 0.9%
Range	0%-2%		0%-2%

[a] Data as of 12 November 1997

TABLE B4c
Historical Incidence of Adrenal Medulla Pheochromocytoma in Control Female F344/N Rats[a]

	Incidence in Controls		
	Benign	**Malignant**	**Benign or Malignant[b]**
Historical Incidence at Battelle Columbus Laboratories: Corn Oil Gavage Studies			
o-Benzyl-p-chlorophenol	0/50	0/50	0/50
Overall Historical Incidence: Corn Oil Gavage Studies			
Total (%)	18/400 (4.5%)	3/400 (0.8%)	22/400 (5.5%)
Mean ± standard deviation	4.5% ± 3.4%	0.8% ± 1.5%	5.5% ± 4.3%
Range	0%-10%	0%-4%	0%-14%
Overall Historical Incidence: Feed Studies			
Total (%)	26/896 (2.9%)	4/896 (0.5%)	34/896 (3.8%)
Mean ± standard deviation	2.9% ± 1.9%	0.4% ± 0.9%	3.8% ± 1.9%
Range	0%-6%	0%-2%	0%-6%
Overall Historical Incidence: Inhalation Studies			
Total (%)	47/889 (5.3%)	5/889 (0.6%)	57/889 (6.4%)
Mean ± standard deviation	5.3% ± 3.9%	0.6% ± 1.1%	6.4% ± 3.5%
Range	0%-13%	0%-4%	2%-13%

[a] Data as of 12 November 1997
[b] All overall historical incidences include benign, malignant, NOS, or complex pheochromocytoma.

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

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
Disposition Summary					
Animals initially in study	60	50	50	50	60
6-Month interim evaluation	5				5
12-Month interim evaluation	5				5
Early deaths					
Accidental deaths			1	1	
Moribund	17	16	14	26	25
Natural deaths	11	9	13	12	9
Survivors					
Terminal sacrifice	22	25	22	11	16
Animals examined microscopically	60	50	50	50	60

6-Month Interim Evaluation
Alimentary System

	Vehicle Control				300 mg/kg (Stop-Exposure)
Liver	(5)				(5)
Basophilic focus					2 (40%)
Clear cell focus					2 (40%)
Hepatodiaphragmatic nodule	1 (20%)				2 (40%)
Mixed cell focus					3 (60%)
Hepatocyte, hypertrophy					5 (100%)
Oval cell, hyperplasia					5 (100%)
Pancreas	(5)				(5)
Acinus, atrophy	1 (20%)				1 (20%)
Salivary glands	(5)				(5)
Submandibular gland, cytoplasmic alteration					5 (100%)
Stomach, glandular	(5)				(5)
Atrophy					5 (100%)
Neuroendocrine cell, hyperplasia					1 (20%)

Endocrine System

	Vehicle Control				300 mg/kg (Stop-Exposure)
Islets, pancreatic	(5)				(5)
Hyperplasia	1 (20%)				

Genital System

	Vehicle Control				300 mg/kg (Stop-Exposure)
Ovary	(5)				(5)
Cyst	1 (20%)				
Uterus	(5)				(5)
Hyperplasia, cystic					1 (20%)

Hematopoietic System

	Vehicle Control				300 mg/kg (Stop-Exposure)
Bone marrow	(5)				(5)
Hyperplasia					4 (80%)
Lymph node	(1)				
Erythrophagocytosis	1 (100%)				

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

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
6-Month Interim Evaluation (continued)					
Respiratory System					
Lung	(5)				(5)
Inflammation, granulomatous					1 (20%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System
Urinary System

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
12-Month Interim Evaluation					
Alimentary System					
Intestine large, colon	(5)				(5)
Parasite metazoan	1 (20%)				
Liver	(5)				(5)
Basophilic focus	5 (100%)				3 (60%)
Clear cell focus	1 (20%)				2 (40%)
Eosinophilic focus					3 (60%)
Hepatodiaphragmatic nodule	2 (40%)				
Mixed cell focus					5 (100%)
Bile duct, cyst					1 (20%)
Hepatocyte, hypertrophy					5 (100%)
Oval cell, hyperplasia					5 (100%)
Salivary glands	(5)				(5)
Submandibular gland, cytoplasmic alteration					5 (100%)
Stomach, glandular	(5)				(5)
Atrophy					5 (100%)
Neuroendocrine cell, hyperplasia					1 (20%)
Cardiovascular System					
Heart	(5)				(5)
Myocardium, degeneration	2 (40%)				1 (20%)
Endocrine System					
Pituitary gland	(5)				(5)
Cyst					1 (20%)
Pars distalis, hyperplasia					1 (20%)
Genital System					
Ovary	(5)				(5)
Cyst					1 (20%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
12-Month Interim Evaluation (continued)					
Integumentary System					
Mammary gland	(5)				(5)
Dilatation	3 (60%)				
Urinary System					
Kidney	(5)				(5)
Nephropathy	4 (80%)				2 (40%)
Systems Examined with No Lesions Observed					
General Body System					
Hematopoietic System					
Musculoskeletal System					
Nervous System					
Respiratory System					
Special Senses System					
2-Year Study					
Alimentary System					
Intestine large, colon	(50)	(50)	(50)	(50)	(50)
Parasite metazoan	1 (2%)			1 (2%)	
Ulcer				1 (2%)	
Intestine large, rectum	(50)	(50)	(50)	(50)	(50)
Parasite metazoan	2 (4%)	4 (8%)	2 (4%)	2 (4%)	
Intestine large, cecum	(50)	(50)	(50)	(50)	(50)
Lymphoid tissue, hyperplasia				1 (2%)	
Intestine small, duodenum	(50)	(50)	(50)	(50)	(50)
Ulcer		1 (2%)			
Liver	(50)	(50)	(49)	(49)	(50)
Basophilic focus	36 (72%)	36 (72%)	29 (59%)	17 (35%)	10 (20%)
Clear cell focus	3 (6%)	10 (20%)	12 (24%)	4 (8%)	7 (14%)
Degeneration, cystic, focal			1 (2%)	4 (8%)	29 (58%)
Eosinophilic focus	10 (20%)	20 (40%)	27 (55%)	31 (63%)	37 (74%)
Hepatodiaphragmatic nodule	7 (14%)	2 (4%)	6 (12%)	2 (4%)	3 (6%)
Inflammation, granulomatous					1 (2%)
Mixed cell focus	6 (12%)	4 (8%)	19 (39%)	9 (18%)	7 (14%)
Necrosis, focal		1 (2%)	1 (2%)		1 (2%)
Bile duct, cyst					2 (4%)
Bile duct, hyperplasia	11 (22%)	11 (22%)	17 (35%)	22 (45%)	30 (60%)
Centrilobular, degeneration, fatty		3 (6%)			
Centrilobular, necrosis			1 (2%)	1 (2%)	
Centrilobular, necrosis, acute					1 (2%)
Hepatocyte, hypertrophy	1 (2%)	13 (26%)	16 (33%)	26 (53%)	31 (62%)
Oval cell, hyperplasia	1 (2%)	15 (30%)	19 (39%)	35 (71%)	34 (68%)
Periportal, degeneration, fatty	1 (2%)				
Mesentery	(6)	(5)	(3)	(3)	(3)
Inflammation, chronic			1 (33%)		
Artery, inflammation, chronic	1 (17%)				
Fat, fibrosis					1 (33%)
Fat, necrosis	5 (83%)	5 (100%)	2 (67%)	3 (100%)	2 (67%)
Oral mucosa			(1)		(1)
Hyperplasia, focal					1 (100%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Alimentary System (continued)					
Pancreas	(50)	(50)	(50)	(49)	(50)
Fibrosis			1 (2%)		
Hyperplasia, focal					1 (2%)
Acinus, atrophy	10 (20%)	11 (22%)	8 (16%)	6 (12%)	4 (8%)
Salivary glands	(50)	(48)	(49)	(49)	(50)
Submandibular gland, cytoplasmic alteration	1 (2%)	48 (100%)	49 (100%)	49 (100%)	49 (98%)
Stomach, forestomach	(50)	(49)	(50)	(50)	(50)
Hyperkeratosis, diffuse	1 (2%)				
Ulcer		2 (4%)			
Stomach, glandular	(50)	(50)	(50)	(48)	(49)
Atrophy	3 (6%)	41 (82%)	45 (90%)	39 (81%)	33 (67%)
Mineralization			1 (2%)		
Ulcer	3 (6%)	4 (8%)	4 (8%)		3 (6%)
Neuroendocrine cell, hyperplasia		5 (10%)	11 (22%)	9 (19%)	3 (6%)
Tongue	(1)	(1)			
Hyperkeratosis, focal	1 (100%)	1 (100%)			
Cardiovascular System					
Blood vessel	(50)	(50)	(50)	(50)	(50)
Aorta, mineralization					1 (2%)
Heart	(50)	(50)	(49)	(49)	(50)
Degeneration, mucoid	1 (2%)				
Atrium, thrombosis		2 (4%)	1 (2%)		
Myocardium, degeneration	35 (70%)	30 (60%)	26 (53%)	20 (41%)	27 (54%)
Valve, fibrosis	1 (2%)				
Endocrine System					
Adrenal cortex	(50)	(50)	(50)	(49)	(50)
Hyperplasia, focal	13 (26%)	11 (22%)	7 (14%)	9 (18%)	5 (10%)
Necrosis		2 (4%)		1 (2%)	
Bilateral, hyperplasia					1 (2%)
Adrenal medulla	(50)	(50)	(50)	(49)	(50)
Hyperplasia, focal	4 (8%)	9 (18%)	4 (8%)	3 (6%)	6 (12%)
Infarct		1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			1 (2%)	
Parathyroid gland	(44)	(45)	(41)	(46)	(45)
Hyperplasia					3 (7%)
Bilateral, hyperplasia			1 (2%)		
Pituitary gland	(50)	(50)	(50)	(50)	(49)
Angiectasis	4 (8%)	2 (4%)	2 (4%)		2 (4%)
Cyst	3 (6%)	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Pars distalis, hyperplasia, diffuse		1 (2%)			
Pars distalis, hyperplasia, focal	11 (22%)	17 (34%)	14 (28%)	15 (30%)	6 (12%)
Pars intermedia, hyperplasia, focal					1 (2%)
Rathke's cleft, cyst	1 (2%)				
Thyroid gland	(50)	(49)	(49)	(50)	(48)
Inflammation, focal, suppurative		1 (2%)			
C-cell, hyperplasia	32 (64%)	33 (67%)	30 (61%)	32 (64%)	32 (67%)
Follicle, cyst	1 (2%)				1 (2%)
Follicular cell, hyperplasia, focal		1 (2%)			

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
General Body System					
None					
Genital System					
Clitoral gland	(48)	(46)	(49)	(46)	(48)
Cyst	2 (4%)	1 (2%)	2 (4%)	1 (2%)	
Hyperplasia, focal	2 (4%)	3 (7%)	1 (2%)		1 (2%)
Inflammation, chronic		1 (2%)			
Inflammation, suppurative			1 (2%)		
Necrosis, focal	1 (2%)				
Ovary	(50)	(50)	(49)	(49)	(50)
Cyst	2 (4%)	7 (14%)	2 (4%)	2 (4%)	4 (8%)
Uterus	(50)	(50)	(49)	(50)	(50)
Hyperplasia, cystic	2 (4%)				
Cervix, hyperplasia, focal				1 (2%)	
Endometrium, degeneration			1 (2%)		
Endometrium, hyperplasia, cystic					1 (2%)
Vagina		(2)		(2)	
Infiltration cellular, polymorphonuclear		1 (50%)			
Hematopoietic System					
Bone marrow	(50)	(50)	(49)	(50)	(50)
Fibrosis	2 (4%)		2 (4%)	2 (4%)	4 (8%)
Hyperplasia	4 (8%)	15 (30%)	11 (22%)	20 (40%)	25 (50%)
Inflammation, granulomatous		2 (4%)			
Necrosis		1 (2%)		2 (4%)	
Erythroid cell, hyperplasia			1 (2%)		
Myeloid cell, hyperplasia	1 (2%)	2 (4%)			
Lymph node	(2)	(5)	(1)	(10)	(6)
Pancreatic, erythrophagocytosis	1 (50%)				
Pancreatic, hyperplasia				1 (10%)	
Pancreatic, necrosis		1 (20%)			
Renal, ectasia				1 (10%)	
Renal, inflammation, granulomatous					1 (17%)
Lymph node, mandibular	(49)	(48)	(49)	(49)	(48)
Necrosis				1 (2%)	
Lymph node, mesenteric	(50)	(49)	(49)	(49)	(50)
Erythrophagocytosis	1 (2%)				
Inflammation, granulomatous				1 (2%)	
Necrosis				1 (2%)	
Spleen	(50)	(50)	(50)	(49)	(50)
Fibrosis	3 (6%)	3 (6%)	5 (10%)	12 (24%)	15 (30%)
Hematopoietic cell proliferation		7 (14%)	6 (12%)	2 (4%)	12 (24%)
Inflammation, focal, suppurative			1 (2%)		
Inflammation, granulomatous		2 (4%)	1 (2%)	1 (2%)	
Necrosis		1 (2%)			
Lymphoid follicle, hyperplasia			1 (2%)		
Red pulp, necrosis				2 (4%)	
Thymus	(49)	(48)	(46)	(46)	(49)
Atrophy			1 (2%)		

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Integumentary System					
Mammary gland	(50)	(50)	(50)	(50)	(50)
Dilatation	27 (54%)	22 (44%)	34 (68%)	20 (40%)	10 (20%)
Galactocele	10 (20%)	10 (20%)	7 (14%)	6 (12%)	5 (10%)
Hyperplasia, cystic		2 (4%)	2 (4%)		
Hyperplasia, focal	8 (16%)	7 (14%)	9 (18%)	2 (4%)	2 (4%)
Hyperplasia, lobular		1 (2%)			
Inflammation, suppurative			1 (2%)		
Skin	(50)	(50)	(50)	(50)	(50)
Ulcer			1 (2%)		
Dermis, necrosis			1 (2%)		
Dermis, subcutaneous tissue, fibrosis	1 (2%)		1 (2%)		
Hair follicle, atrophy, focal			1 (2%)	1 (2%)	
Musculoskeletal System					
Bone	(50)	(50)	(50)	(50)	(50)
Fibrous osteodystrophy					3 (6%)
Hyperostosis	1 (2%)	1 (2%)	5 (10%)	4 (8%)	2 (4%)
Osteopetrosis		1 (2%)	2 (4%)		
Skeletal muscle		(1)		(1)	(1)
Degeneration, focal				1 (100%)	
Fibrosis					1 (100%)
Nervous System					
Brain	(50)	(50)	(50)	(50)	(50)
Congestion, focal				1 (2%)	
Hydrocephalus	4 (8%)	9 (18%)	3 (6%)	8 (16%)	9 (18%)
Cerebellum, necrosis, focal				1 (2%)	
Hypothalamus, degeneration	9 (18%)	12 (24%)	6 (12%)	7 (14%)	5 (10%)
Medulla, degeneration	1 (2%)				
Pons, degeneration				1 (2%)	
Respiratory System					
Lung	(50)	(50)	(49)	(49)	(50)
Inflammation, acute	1 (2%)		2 (4%)		
Inflammation, chronic		1 (2%)			
Inflammation, granulomatous	1 (2%)		1 (2%)		2 (4%)
Alveolar epithelium, hyperplasia, focal	2 (4%)	3 (6%)	7 (14%)	3 (6%)	5 (10%)
Interstitium, inflammation, chronic		1 (2%)			1 (2%)
Interstitium, mineralization					2 (4%)
Nose	(50)	(50)	(50)	(50)	(50)
Hyperplasia, squamous		1 (2%)			
Inflammation, suppurative	8 (16%)	10 (20%)	14 (28%)	3 (6%)	3 (6%)
Special Senses System					
Eye	(1)		(2)		
Cataract	1 (100%)		1 (50%)		
Degeneration			1 (50%)		

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg (Stop-Exposure)
2-Year Study (continued)					
Urinary System					
Kidney	(50)	(50)	(50)	(49)	(50)
Hydronephrosis		2 (4%)			
Infarct		3 (6%)	1 (2%)		
Inflammation, focal, suppurative			1 (2%)		
Necrosis	1 (2%)				
Nephropathy	35 (70%)	42 (84%)	41 (82%)	44 (90%)	45 (90%)
Renal tubule, accumulation, hyaline droplet		1 (2%)			
Renal tubule, pigmentation, lipofuscin	1 (2%)	1 (2%)	4 (8%)	3 (6%)	

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE
IN THE 2-YEAR GAVAGE STUDY
OF METHYLEUGENOL

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

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			1	2
Moribund	5	6	5	9
Natural deaths	6	8	7	4
Survivors				
Terminal sacrifice	38	36	37	35
Missing	1			
Animals examined microscopically	49	50	50	50
Alimentary System				
Intestine large, cecum	(49)	(50)	(48)	(50)
Carcinoma	1 (2%)		1 (2%)	
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Intestine small, duodenum	(47)	(47)	(48)	(50)
Polyp adenomatous		1 (2%)		
Intestine small, jejunum	(49)	(49)	(48)	(48)
Carcinoma				1 (2%)
Intestine small, ileum	(48)	(48)	(49)	(50)
Liver	(49)	(50)	(50)	(50)
Hemangiosarcoma		2 (4%)		1 (2%)
Hemangiosarcoma, multiple		1 (2%)		1 (2%)
Hemangiosarcoma, metastatic, bone marrow				1 (2%)
Hemangiosarcoma, metastatic, skeletal muscle			1 (2%)	
Hepatoblastoma			1 (2%)	2 (4%)
Hepatoblastoma, multiple				1 (2%)
Hepatocellular carcinoma	9 (18%)	17 (34%)	16 (32%)	7 (14%)
Hepatocellular carcinoma, multiple	1 (2%)	3 (6%)	3 (6%)	2 (4%)
Hepatocellular adenoma	13 (27%)	10 (20%)	5 (10%)	10 (20%)
Hepatocellular adenoma, multiple	13 (27%)	33 (66%)	33 (66%)	29 (58%)
Mast cell tumor malignant			1 (2%)	
Mesentery	(1)	(2)	(1)	(1)
Fibrous histiocytoma		1 (50%)		
Hemangiosarcoma, metastatic, lymph node, mesenteric	1 (100%)			
Oral mucosa				(1)
Pharyngeal, squamous cell carcinoma				1 (100%)
Pancreas	(49)	(49)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Fibrous histiocytoma, metastatic, lymph node			1 (2%)	
Fibrous histiocytoma, metastatic, mesentery		1 (2%)		
Mast cell tumor malignant			1 (2%)	
Salivary glands	(49)	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)		2 (4%)
Stomach, glandular	(49)	(48)	(49)	(50)
Carcinoma				1 (2%)
Neuroendocrine tumor, malignant				2 (4%)
Tooth	(23)	(13)	(4)	(5)
Odontoma	1 (4%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Cardiovascular System				
Heart	(49)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		1 (2%)	
Fibrous histiocytoma, metastatic, mesentery		1 (2%)		
Hemangiosarcoma		1 (2%)		
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Adenoma		1 (2%)		1 (2%)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Islets, pancreatic	(49)	(49)	(50)	(50)
Adenoma				1 (2%)
Thyroid gland	(49)	(50)	(50)	(50)
Follicular cell, adenoma	1 (2%)		1 (2%)	1 (2%)
Follicular cell, carcinoma	1 (2%)		1 (2%)	
General Body System				
None				
Genital System				
Epididymis	(49)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, mesentery		1 (2%)		
Preputial gland	(49)	(50)	(50)	(50)
Prostate	(49)	(50)	(50)	(50)
Carcinoma	1 (2%)			
Seminal vesicle	(49)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Fibrous histiocytoma, metastatic, mesentery		1 (2%)		
Testes	(49)	(50)	(50)	(50)
Interstitial cell, adenoma	1 (2%)		1 (2%)	
Hematopoietic System				
Bone marrow	(49)	(49)	(50)	(50)
Hemangioma			1 (2%)	
Hemangiosarcoma		1 (2%)		1 (2%)
Hemangiosarcoma, metastatic, spleen	1 (2%)	1 (2%)		
Hemangiosarcoma, metastatic, skeletal muscle	1 (2%)			
Lymph node	(3)	(4)	(5)	(3)
Lumbar, fibrous histiocytoma, metastatic, mesentery		1 (25%)		
Mediastinal, fibrous histiocytoma, metastatic, mesentery		1 (25%)		
Pancreatic, fibrous histiocytoma			1 (20%)	
Renal, fibrous histiocytoma, metastatic, mesentery		1 (25%)		
Lymph node, mandibular	(48)	(46)	(47)	(45)
Lymph node, mesenteric	(49)	(46)	(49)	(50)
Fibrous histiocytoma, metastatic, mesentery		1 (2%)		
Hemangiosarcoma	1 (2%)			
Hemangiosarcoma, metastatic, spleen	1 (2%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Hematopoietic System (continued)				
Spleen	(49)	(49)	(50)	(50)
Hemangioma			1 (2%)	
Hemangiosarcoma	2 (4%)	2 (4%)		
Hemangiosarcoma, metastatic, skeletal muscle			1 (2%)	
Mast cell tumor malignant			1 (2%)	
Thymus	(37)	(40)	(38)	(45)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (3%)			
Fibrous histiocytoma, metastatic, mesentery		1 (3%)		
Integumentary System				
Skin	(49)	(50)	(50)	(50)
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, hemangiosarcoma			1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Vertebra, osteosarcoma				1 (2%)
Skeletal muscle	(2)	(1)	(3)	(3)
Hemangiosarcoma	1 (50%)		1 (33%)	2 (67%)
Nervous System				
Brain	(49)	(50)	(50)	(50)
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	10 (20%)	9 (18%)	17 (34%)	14 (28%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Alveolar/bronchiolar carcinoma	4 (8%)	4 (8%)	5 (10%)	6 (12%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)		2 (4%)	
Fibrous histiocytoma, metastatic, mesentery		1 (2%)		
Hemangiosarcoma, metastatic, skeletal muscle			1 (2%)	
Hepatocellular carcinoma, metastatic, liver	2 (4%)	6 (12%)	7 (14%)	4 (8%)
Osteosarcoma, metastatic, uncertain primary site		1 (2%)		
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		2 (4%)	
Mediastinum, fibrous histiocytoma, metastatic, mesentery		1 (2%)		
Special Senses System				
Harderian gland	(4)	(1)	(7)	(6)
Adenoma	4 (100%)	1 (100%)	7 (100%)	6 (100%)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		1 (2%)	
Fibrous histiocytoma, metastatic, mesentery		1 (2%)		
Urinary bladder	(49)	(50)	(50)	(50)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Systemic Lesions				
Multiple organs[b]	(49)	(50)	(50)	(50)
Lymphoma malignant	3 (6%)	3 (6%)	8 (16%)	6 (12%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	41	48	49	45
Total primary neoplasms	69	92	111	102
Total animals with benign neoplasms	33	43	42	44
Total benign neoplasms	44	57	68	65
Total animals with malignant neoplasms	23	29	32	29
Total malignant neoplasms	25	35	43	35
Total animals with metastatic neoplasms	6	9	10	6
Total metastatic neoplasms	13	20	15	6
Total animals with malignant neoplasms of uncertain primary site		1		
Total animals with uncertain neoplasms- benign or malignant				2
Total uncertain neoplasms				2

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	4 3 0	4 7 7	5 3 7	5 4 0	5 5 4	5 7 0	5 8 0	5 8 3	6 0 4	6 7 0	6 9 3	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 3 1	0 4 9	0 0 5	0 2 5	0 1 0	0 4 1	0 3 3	0 1 6	0 0 1	0 4 4	0 3 4	0 0 8	0 1 2	0 1 8	0 1 9	0 2 2	0 2 3	0 2 4	0 2 6	0 2 8	0 3 5	0 3 8	0 4 2	0 4 6
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma									X															
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma		X	X				X		X	X		X												
Hepatocellular carcinoma, multiple																X								
Hepatocellular adenoma	X			X		X			X			X	X			X		X						
Hepatocellular adenoma, multiple										X						X							X	X
Mesentery																								
Hemangiosarcoma, metastatic, lymph node, mesenteric																								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung									X															
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth			+			+		+				+	+	+	+	+	+			+			+	+
Odontoma																								
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung									X															
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung									X															
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	I	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																								
Follicular cell, carcinoma																X								

General Body System
None

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 4 7	0 0 2	0 0 7	0 0 9	0 1 3	0 1 4	0 1 7	0 2 0	0 2 7	0 3 0	0 3 2	0 3 6	0 4 0	0 0 3	0 0 4	0 0 6	0 1 1	0 1 5	0 2 1	0 2 9	0 3 7	0 3 9	0 4 3	0 4 5	0 4 8	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma	X																						X		X	9
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma					X				X				X	X				X			X					13
Hepatocellular adenoma, multiple	X		X	X						X		X							X	X	X			X		13
Mesentery			+																							1
Hemangiosarcoma, metastatic, lymph node, mesenteric			X																							1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth				+			+		+				+	+			+		+	+			+			23
Odontoma									X																	1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	M	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	43
Pituitary gland	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell, adenoma														X												1
Follicular cell, carcinoma																										1

General Body System
None

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study (top to bottom, per column): 730, then 731 for columns 2–14, and 732 for columns 15–25.

Carcass ID Numbers: 047, 002, 007, 009, 013, 014, 017, 020, 027, 030, 032, 036, 040, 003, 004, 006, 011, 015, 021, 025, 037, 039, 043, 045, 048

Organ / Finding	047	002	007	009	013	014	017	020	027	030	032	036	040	003	004	006	011	015	021	025	037	039	043	045	048	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma			X																							1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Interstitial cell, adenoma	X																									1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma, metastatic, spleen																										1
Hemangiosarcoma, metastatic, skeletal muscle																										1
Lymph node																										3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma			X																							1
Hemangiosarcoma, metastatic, spleen																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma														X												2
Thymus	M	M	+	M	+	+	+	+	M	+	+	+	+	M	+	M	+	+	I	+	+	+	+	+	+	37
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skeletal muscle																							+			2
Hemangiosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spinal cord									+														+			2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma		X	X			X	X							X			X									10
Alveolar/bronchiolar adenoma, multiple																										1
Alveolar/bronchiolar carcinoma														X	X											4
Alveolar/bronchiolar carcinoma, multiple																										1
Hepatocellular carcinoma, metastatic, liver																										2
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Harderian gland														+												4
Adenoma														X												4
Zymbal's gland																					+			+		4

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	4 3 0	4 7 7	5 3 7	5 4 0	5 5 4	5 7 0	5 8 0	5 8 3	6 0 4	6 7 0	6 9 3	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 3 1	0 4 9	0 0 5	0 2 5	0 1 0	0 4 1	0 3 3	0 1 6	0 0 1	0 4 4	0 3 4	0 0 8	0 1 2	0 1 8	0 1 9	0 2 2	0 2 3	0 2 4	0 2 6	0 2 8	0 3 5	0 3 8	0 4 2	0 4 6

Urinary System

Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung								X																
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Systemic Lesions

Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant										X											X			

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 4 7	0 0 2	0 0 7	0 0 9	0 1 3	0 1 4	0 1 7	0 2 0	0 2 7	0 3 0	0 3 2	0 3 6	0 4 0	0 0 3	0 0 4	0 0 6	0 1 1	0 1 5	0 2 1	0 2 5	0 3 7	0 3 9	0 4 3	0 4 5	0 4 8	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant																							X			3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	4 8 8	5 4 6	5 9 3	5 9 3	6 1 4	6 3 6	6 5 5	6 7 0	6 7 3	6 8 7	6 9 4	6 9 4	7 1 2	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 9 8	0 9 2	0 5 2	0 8 5	0 8 9	0 6 1	0 9 3	0 5 4	0 6 7	0 8 2	0 5 7	0 7 6	0 6 9	0 7 7	0 5 5	0 5 8	0 5 9	0 6 0	0 6 3	0 6 6	0 7 1	0 7 2	0 7 3	0 7 5	0 8 0

Alimentary System

Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	A	A	M	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	A	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma				X																					
Hemangiosarcoma, multiple													X												
Hepatocellular carcinoma			X			X	X	X		X	X	X				X					X		X		X
Hepatocellular carcinoma, multiple										X				X		X									
Hepatocellular adenoma														X										X	
Hepatocellular adenoma, multiple				X		X			X	X	X		X	X	X	X	X	X	X	X		X	X	X	
Mesentery																									
Fibrous histiocytoma																									
Pancreas	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, mesentery																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth	+		+						+	+				+							+				

Cardiovascular System

Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, mesentery																									
Hemangiosarcoma													X												

Endocrine System

Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

General Body System
None

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

	081	083	084	086	100	051	053	056	064	065	070	074	079	087	088	091	094	095	096	097	062	068	078	090	099	Total Tissues/ Tumors
Number of Days on Study	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	42
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Polyp adenomatous					X																					1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																							X			2
Hemangiosarcoma, multiple																										1
Hepatocellular carcinoma		X				X	X			X			X												X	17
Hepatocellular carcinoma, multiple																										3
Hepatocellular adenoma	X				X			X			X	X				X		X			X					10
Hepatocellular adenoma, multiple		X	X	X		X	X		X	X			X	X	X	X		X		X		X	X			33
Mesentery																		+		2		+				
Fibrous histiocytoma																		X								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrous histiocytoma, metastatic, mesentery																		X								1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma					X																					1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tooth		+	+				+			+	+		+				+									13
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, mesentery																		X								1
Hemangiosarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																		X								1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	M	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

General Body System
None

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	4 8 8	5 4 6	5 9 3	5 9 3	6 1 4	6 3 6	6 5 5	6 7 0	6 7 3	6 8 7	6 9 4	6 9 4	7 1 2	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 9 8	0 9 2	0 5 2	0 8 5	0 8 9	0 6 1	0 9 3	0 5 4	0 6 7	0 8 2	0 5 7	0 7 6	0 6 9	0 7 7	0 5 5	0 5 8	0 5 9	0 6 0	0 6 3	0 6 6	0 7 1	0 7 2	0 7 3	0 7 5	0 8 0

Genital System

Organ																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, mesentery																									
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, mesentery																									
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Hematopoietic System

Organ																										
Bone marrow	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hemangiosarcoma, metastatic, spleen		X																							
Lymph node																	+			+					
Lumbar, fibrous histiocytoma, metastatic, mesentery																									
Mediastinal, fibrous histiocytoma, metastatic, mesentery																									
Renal, fibrous histiocytoma, metastatic, mesentery																									
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, mesentery																									
Spleen	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma		X																							X
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	M	M	+	+
Fibrous histiocytoma, metastatic, mesentery																									

Integumentary System

Organ																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Musculoskeletal System

Organ																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									

Nervous System

Organ																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Respiratory System

Organ																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma												X				X				X					
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma																	X								
Fibrous histiocytoma, metastatic, mesentery																									
Hepatocellular carcinoma, metastatic, liver					X		X			X	X	X					X								
Osteosarcoma, metastatic, uncertain primary site																									
Mediastinum, fibrous histiocytoma, metastatic, mesentery																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 8 1	0 8 3	0 8 4	0 8 6	1 0 0	0 5 1	0 5 3	0 5 6	0 6 4	0 6 5	0 7 0	0 7 4	0 7 9	0 8 7	0 8 8	0 9 1	0 9 4	0 9 5	0 9 6	0 9 7	0 9 2	0 6 8	0 6 8	0 7 0	0 9 9	0 9 9	**Total Tissues/ Tumors**

Genital System

Tissue / Tumor																											Total
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, mesentery																					X						1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, mesentery																					X						1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Hematopoietic System

Tissue / Tumor																											Total
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma								X																			1
Hemangiosarcoma, metastatic, spleen																											1
Lymph node																+	+										4
Lumbar, fibrous histiocytoma, metastatic, mesentery																					X						1
Mediastinal, fibrous histiocytoma, metastatic, mesentery																					X						1
Renal, fibrous histiocytoma, metastatic, mesentery																					X						1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Fibrous histiocytoma, metastatic, mesentery																					X						1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																											2
Thymus	M	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	I	+	+	+	M	+	I	+	+		40
Fibrous histiocytoma, metastatic, mesentery																					X						1

Integumentary System

Tissue / Tumor																											Total
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Musculoskeletal System

Tissue / Tumor																											Total
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																					+						1

Nervous System

Tissue / Tumor																											Total
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Respiratory System

Tissue / Tumor																											Total
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X		X			X	X				X				X				X					X		9
Alveolar/bronchiolar adenoma, multiple																								X			1
Alveolar/bronchiolar carcinoma				X						X			X														4
Fibrous histiocytoma, metastatic, mesentery																					X						1
Hepatocellular carcinoma, metastatic, liver																											6
Osteosarcoma, metastatic, uncertain primary site					X																						1
Mediastinum, fibrous histiocytoma, metastatic, mesentery																					X						1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	4 8 8	5 4 6	5 9 3	5 9 3	6 1 4	6 3 6	6 5 5	6 7 0	6 7 3	6 8 7	6 9 4	6 9 4	7 1 2	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 9 8	0 9 2	0 5 2	0 8 5	0 8 9	0 6 1	0 9 3	0 5 4	0 6 7	0 8 2	0 5 7	0 7 6	0 6 9	0 7 7	0 5 5	0 5 8	0 5 9	0 6 0	0 6 3	0 6 6	0 7 1	0 7 2	0 7 3	0 7 5	0 8 0	

Special Senses System
Harderian gland
   Adenoma

Urinary System

Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, mesentery																												
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Systemic Lesions

Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant																					X			X				

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 2	7 3 2	
Carcass ID Number	0 8 1	0 8 3	0 8 4	0 8 6	1 0 0	0 5 1	0 5 3	0 5 6	0 6 4	0 6 5	0 7 0	0 7 4	0 7 9	0 8 7	0 8 8	0 9 1	0 9 4	0 9 5	0 9 6	0 9 7	0 6 2	0 6 8	0 7 8	0 9 0	0 9 9	Total Tissues/ Tumors

Special Senses System

								+																		1
Harderian gland								+																		1
Adenoma								X																		1

Urinary System

Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, mesentery																			X							1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Systemic Lesions

Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																		X								3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

	Animal (Number of Days on Study / Carcass ID)
Number of Days on Study	037 326 486 522 522 572 575 589 614 630 639 691 722 730 730 730 730 730 730 730 730 730 730 730 730 730
Carcass ID Number	142 115 103 122 148 135 113 116 138 139 124 110 127 102 104 105 111 117 129 131 133 137 140 144 145

Alimentary System

Tissue / Lesion	Individual animal findings (left → right)
Esophagus	+ +
Gallbladder	A + + A + + + A A + + + M + + + + + + + + + + + + +
Intestine large, colon	+ + + + + A + + M + + + + + + + + + + + + + + + + +
Intestine large, rectum	+ + + + + A +
Intestine large, cecum	A + + + + + + + M + + + + + + + + + + + + + + + + +
Carcinoma	. X
Intestine small, duodenum	+ + + + + + + + + + + + + + + M M + + + + + + + + +
Intestine small, jejunum	A + + + + A +
Intestine small, ileum	A +
Liver	+ M + + +
Hemangiosarcoma, metastatic, skeletal muscle	
Hepatoblastoma	
Hepatocellular carcinoma	. X X X . X X . . X X X
Hepatocellular carcinoma, multiple	. . . . X . . X . . . . X . . . . .
Hepatocellular adenoma	 X
Hepatocellular adenoma, multiple	. . . . X X . . X X X X . . X X X X X X
Mast cell tumor malignant	
Mesentery	
Pancreas	+ +
Fibrous histiocytoma, metastatic, lymph node	 X
Mast cell tumor malignant	
Salivary glands	+ +
Stomach, forestomach	+ +
Stomach, glandular	A +
Tooth	. . . . . + ... + ... +

Cardiovascular System

Tissue / Lesion	Individual animal findings
Blood vessel	+ +
Heart	+ +
Alveolar/bronchiolar carcinoma, metastatic, lung	 X

Endocrine System

Tissue / Lesion	Individual animal findings
Adrenal cortex	+ +
Adrenal medulla	+ +
Islets, pancreatic	+ +
Parathyroid gland	+ + + + M M + + + M + + + + + + + + + + + M + + +
Pituitary gland	+ + I + I + M + + + + + + I + + + + + + + + + + +
Thyroid gland	+ +
Follicular cell, adenoma	 X
Follicular cell, carcinoma	 X

General Body System
None

Genital System

Tissue / Lesion	Individual animal findings
Coagulating gland	. . . . +
Epididymis	+ +
Preputial gland	+ +
Prostate	+ +
Seminal vesicle	+ +
Testes	+ +
Interstitial cell, adenoma	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study	730	730	731	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	Total Tissues/ Tumors
Carcass ID Number	146	149	106	107	108	112	118	121	125	126	134	136	141	143	101	109	114	119	120	123	128	130	132	147	150	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, skeletal muscle		X																								1
Hepatoblastoma																				X						1
Hepatocellular carcinoma			X	X	X			X	X		X		X							X						16
Hepatocellular carcinoma, multiple																										3
Hepatocellular adenoma						X					X						X		X							5
Hepatocellular adenoma, multiple	X	X	X	X		X	X	X	X	X	X	X		X	X	X		X		X		X	X	X	X	33
Mast cell tumor malignant																					X					1
Mesentery										+																1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, lymph node																										1
Mast cell tumor malignant																					X					1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth																					+					4
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	44
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	44
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
Follicular cell, carcinoma																										1
General Body System																										
None																										
Genital System																										
Coagulating gland																			+							2
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																					X					1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study	0 3 7	3 2 6	4 8 6	5 2 2	5 2 2	5 7 2	5 7 5	5 8 9	6 1 4	6 3 0	6 3 9	6 9 1	7 2 2	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	1 4 2	1 1 5	1 0 3	1 2 2	1 4 8	1 3 5	1 1 3	1 1 6	1 3 8	1 3 9	1 2 4	1 1 0	1 2 7	1 0 2	1 0 4	1 0 5	1 1 1	1 1 7	1 2 9	1 3 1	1 3 3	1 3 7	1 4 0	1 4 4	1 4 5	

Hematopoietic System

Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																					X					
Lymph node									+		+		+													
Pancreatic, fibrous histiocytoma											X															
Lymph node, mandibular	+	+	+	+	+	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																										
Hemangiosarcoma, metastatic, skeletal muscle																										
Mast cell tumor malignant																										
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	M	+	M	M	+	+	+	I		

Integumentary System

Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma		X																								

Musculoskeletal System

Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																										
Hemangiosarcoma																										

Nervous System

Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Respiratory System

Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X				X		X						X		X		X	X	X	X		
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma									X										X							
Alveolar/bronchiolar carcinoma, multiple							X			X																
Hemangiosarcoma, metastatic, skeletal muscle																										
Hepatocellular carcinoma, metastatic, liver							X	X			X						X									
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung							X			X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Special Senses System

Eye																										
Harderian gland																						+				
Adenoma																						X				

Urinary System

Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung						X																				
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Systemic Lesions

Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant					X				X			X								X						

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study	730	730	731	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	Total Tissues/ Tumors
Carcass ID Number	146	149	106	107	108	112	118	121	125	126	134	136	141	143	101	109	114	119	120	123	128	130	132	147	150	
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Lymph node													+								5+					
Pancreatic, fibrous histiocytoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																					X					1
Hemangiosarcoma, metastatic, skeletal muscle		X																								1
Mast cell tumor malignant																		X								1
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	M	M	+	+	+	M	+	M	M	+	+	+	38
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle		+	+								+															3
Hemangiosarcoma		X																								1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma							X		X	X	X				X					X	X			X		17
Alveolar/bronchiolar adenoma, multiple		X										X														2
Alveolar/bronchiolar carcinoma													X	X									X			5
Alveolar/bronchiolar carcinoma, multiple																										2
Hemangiosarcoma, metastatic, skeletal muscle		X																								1
Hepatocellular carcinoma, metastatic, liver				X	X				X																	7
Mediastinum, alveolar/bronchiolar carcinoma metastatic, lung																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye															+											1
Harderian gland	+						+		+	+		+											+			7
Adenoma	X						X		X	X		X											X			7
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant													X					X		X	X					8

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study	3	4	5	5	5	5	5	5	5	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7
	3	5	1	4	5	6	6	7	8	0	6	7	8	0	2	3	3	3	3	3	3	3	3	3	3
	9	9	2	3	4	1	9	9	3	4	0	3	8	5	7	0	0	0	0	0	0	0	0	0	0
Carcass ID Number	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	5	6	0	6	7	6	7	8	7	8	8	6	9	9	5	5	7	7	7	7	7	8	8	9
	5	6	1	0	3	4	9	3	0	6	5	2	2	8	4	3	9	0	2	7	8	3	6	1	7

Alimentary System

Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	+	M	A	+	+	+	+	+	A	+	+	M	+	M	M	M	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver																							X		
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Carcinoma						X																			
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																			X						
Hemangiosarcoma, multiple																									
Hemangiosarcoma, metastatic, bone marrow																									
Hepatoblastoma						X				X															
Hepatoblastoma, multiple																									
Hepatocellular carcinoma								X			X					X							X		
Hepatocellular carcinoma, multiple													X												
Hepatocellular adenoma			X			X	X	X			X		X		X	X				X					
Hepatocellular adenoma, multiple												X				X	X	X	X		X		X	X	X
Mesentery													+												
Oral mucosa														+											
Pharyngeal, squamous cell carcinoma														X											
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																				X					
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																				X					
Neuroendocrine tumor, malignant																									
Tooth							+		+											+					+

Cardiovascular System

Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Endocrine System

Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X		
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma						X																			
Parathyroid gland	+	M	+	+	+	+	+	+	M	+	+	M	M	M	+	+	+	+	+	+	M	M	+	+	M
Pituitary gland	M	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																							X		

General Body System
None

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study: 731 for all animals.

	151	152	154	157	158	164	169	179	184	188	189	192	193	195	196	199	160	165	166	168	171	175	181	187	190	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	M	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	39
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic, liver																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Hemangiosarcoma, multiple					X																					1
Hemangiosarcoma, metastatic, bone marrow														X												1
Hepatoblastoma																										2
Hepatoblastoma, multiple												X														1
Hepatocellular carcinoma								X					X						X							7
Hepatocellular carcinoma, multiple																		X								2
Hepatocellular adenoma																							X			10
Hepatocellular adenoma, multiple	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X		X	X			X	29
Mesentery																										1
Oral mucosa																										1
Pharyngeal, squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma								X																		2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Neuroendocrine tumor, malignant																							X	X		2
Tooth								+																		5
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	M	+	M	M	M	M	M	+	M	+	+	+	+	+	+	M	M		33
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	M	+	45
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1

General Body System
None

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study	339	459	512	543	554	561	569	579	583	604	660	673	688	705	727	730	730	730	730	730	730	730	730	730	730	730
Carcass ID Number	155	156	161	200	163	174	169	173	180	176	185	182	162	198	194	153	159	170	172	177	178	183	186	181	191	197
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																										
Lymph node						+						+														
Lymph node, mandibular	+	+	M	M	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M	+	+	+	+	+	+	+	+
Integumentary System																										
Mammary gland	M	M	M	M	M	M	+	M	M	M	M	+	M	M	M	M	M	M	M	+	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma									X																	
Subcutaneous tissue, hemangiosarcoma																										
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vertebra, osteosarcoma																						X				
Skeletal muscle													+								+					
Hemangiosarcoma													X													
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord		+											+													
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X		X		X		X	X					X			X						
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma								X				X			X										X	
Hepatocellular carcinoma, metastatic, liver							X							X												
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Eye											+															
Harderian gland											+															
Adenoma											X															
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant				X							X			X							X					

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

	151	152	154	157	158	164	167	179	184	188	189	192	193	195	196	199	160	165	166	168	171	175	181	187	190	Total Tissues/ Tumors
Number of Days on Study	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma												X														1
Lymph node				+																						3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	45
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, hemangiosarcoma														X												1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Vertebra, osteosarcoma																										1
Skeletal muscle		+																								3
Hemangiosarcoma		X																								2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X	X	X					X		X							X	X			14
Alveolar/bronchiolar adenoma, multiple																				X						1
Alveolar/bronchiolar carcinoma	X																								X	6
Hepatocellular carcinoma, metastatic, liver																	X		X							4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										1
Harderian gland		+		+														+			+			+		6
Adenoma		X		X														X			X			X		6
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant			X					X																		6

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	4/50 (8%)	1/50 (2%)	7/50 (14%)	6/50 (12%)
Adjusted rate[b]	9.0%	2.2%	16.2%	13.7%
Terminal rate[c]	3/38 (8%)	1/36 (3%)	7/37 (19%)	5/35 (14%)
First incidence (days)	570	730 (T)	730 (T)	569
Poly-3 test[d]	P=0.123	P=0.168N	P=0.247	P=0.362
Liver: Hemangiosarcoma				
Overall rate	0/49 (0%)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	0.0%	6.5%	0.0%	4.6%
Terminal rate	0/38 (0%)	2/36 (6%)	0/37 (0%)	2/35 (6%)
First incidence (days)	—[e]	614	—	730 (T)
Poly-3 test	P=0.351	P=0.130	—[f]	P=0.236
Liver: Hepatocellular Adenoma				
Overall rate	26/49 (53%)	43/50 (86%)	38/50 (76%)	39/50 (78%)
Adjusted rate	57.1%	91.1%	85.8%	84.0%
Terminal rate	22/38 (58%)	35/36 (97%)	35/37 (95%)	30/35 (86%)
First incidence (days)	430	614	589	512
Poly-3 test	P=0.006	P<0.001	P<0.001	P=0.003
Liver: Hepatocellular Carcinoma				
Overall rate	10/49 (20%)	20/50 (40%)	19/50 (38%)	9/50 (18%)
Adjusted rate	21.8%	41.8%	40.1%	20.4%
Terminal rate	5/38 (13%)	10/36 (28%)	10/37 (27%)	6/35 (17%)
First incidence (days)	477	593	486	579
Poly-3 test	P=0.309N	P=0.030	P=0.044	P=0.539N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	31/49 (63%)	47/50 (94%)	46/50 (92%)	40/50 (80%)
Adjusted rate	65.4%	97.4%	96.4%	85.6%
Terminal rate	23/38 (61%)	36/36 (100%)	36/37 (97%)	30/35 (86%)
First incidence (days)	430	593	486	512
Poly-3 test	P=0.018	P<0.001	P<0.001	P=0.016
Liver: Hepatoblastoma				
Overall rate	0/49 (0%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	2.3%	6.8%
Terminal rate	0/38 (0%)	0/36 (0%)	1/37 (3%)	1/35 (3%)
First incidence (days)	—	—	730 (T)	554
Poly-3 test	P=0.019	—	P=0.500	P=0.122
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	10/49 (20%)	20/50 (40%)	20/50 (40%)	11/50 (22%)
Adjusted rate	21.8%	41.8%	42.2%	24.6%
Terminal rate	5/38 (13%)	10/36 (28%)	11/37 (30%)	7/35 (20%)
First incidence (days)	477	593	486	554
Poly-3 test	P=0.490N	P=0.030	P=0.027	P=0.471
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	31/49 (63%)	47/50 (94%)	46/50 (92%)	41/50 (82%)
Adjusted rate	65.4%	97.4%	96.4%	86.7%
Terminal rate	23/38 (61%)	36/36 (100%)	36/37 (97%)	30/35 (86%)
First incidence (days)	430	593	486	512
Poly-3 test	P=0.012	P<0.001	P<0.001	P=0.011

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	11/49 (22%)	10/50 (20%)	19/50 (38%)	15/50 (30%)
Adjusted rate	24.6%	21.8%	42.5%	33.1%
Terminal rate	9/38 (24%)	9/36 (25%)	16/37 (43%)	10/35 (29%)
First incidence (days)	477	729	522	554
Poly-3 test	P=0.120	P=0.474N	P=0.056	P=0.256
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/49 (10%)	4/50 (8%)	7/50 (14%)	6/50 (12%)
Adjusted rate	11.3%	8.7%	15.8%	13.7%
Terminal rate	3/38 (8%)	4/36 (11%)	4/37 (11%)	4/35 (11%)
First incidence (days)	540	730 (T)	589	583
Poly-3 test	P=0.331	P=0.481N	P=0.377	P=0.491
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	15/49 (31%)	13/50 (26%)	25/50 (50%)	20/50 (40%)
Adjusted rate	32.8%	28.4%	54.7%	44.0%
Terminal rate	11/38 (29%)	12/36 (33%)	19/37 (51%)	14/35 (40%)
First incidence (days)	477	729	522	554
Poly-3 test	P=0.063	P=0.408N	P=0.026	P=0.187
All Organs: Hemangiosarcoma				
Overall rate	4/50 (8%)	6/50 (12%)	2/50 (4%)	6/50 (12%)
Adjusted rate	9.0%	12.8%	4.5%	13.8%
Terminal rate	3/38 (8%)	4/36 (11%)	1/37 (3%)	5/35 (14%)
First incidence (days)	570	546	326	688
Poly-3 test	P=0.383	P=0.404	P=0.337N	P=0.357
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	4/50 (8%)	6/50 (12%)	4/50 (8%)	6/50 (12%)
Adjusted rate	9.0%	12.8%	9.0%	13.8%
Terminal rate	3/38 (8%)	4/36 (11%)	3/37 (8%)	5/35 (14%)
First incidence (days)	570	546	326	688
Poly-3 test	P=0.353	P=0.404	P=0.643	P=0.357
All Organs: Malignant Lymphoma				
Overall rate	3/50 (6%)	3/50 (6%)	8/50 (16%)	6/50 (12%)
Adjusted rate	6.8%	6.6%	18.0%	13.6%
Terminal rate	2/38 (5%)	3/36 (8%)	6/37 (16%)	4/35 (11%)
First incidence (days)	693	730 (T)	522	543
Poly-3 test	P=0.124	P=0.643N	P=0.100	P=0.243
All Organs: Benign Neoplasms				
Overall rate	33/50 (66%)	43/50 (86%)	42/50 (84%)	44/50 (88%)
Adjusted rate	70.0%	91.1%	91.9%	92.1%
Terminal rate	27/38 (71%)	35/36 (97%)	36/37 (97%)	32/35 (91%)
First incidence (days)	430	614	522	512
Poly-3 test	P=0.003	P=0.005	P=0.004	P=0.004
All Organs: Malignant Neoplasms				
Overall rate	23/50 (46%)	30/50 (60%)	32/50 (64%)	29/50 (58%)
Adjusted rate	47.9%	61.4%	65.7%	61.5%
Terminal rate	14/38 (37%)	18/36 (50%)	21/37 (57%)	18/35 (51%)
First incidence (days)	477	546	326	543
Poly-3 test	P=0.138	P=0.127	P=0.057	P=0.129

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	41/50 (82%)	48/50 (96%)	49/50 (98%)	45/50 (90%)
Adjusted rate	83.1%	98.3%	100.0%	93.1%
Terminal rate	30/38 (79%)	36/36 (100%)	37/37 (100%)	32/35 (91%)
First incidence (days)	430	546	326	512
Poly-3 test	P=0.079	P=0.008	P=0.003	P=0.108

(T)Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver and lung; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE C4
Historical Incidence of Liver Neoplasms in Vehicle Control Male B6C3F$_1$ Mice Receiving Corn Oil by Gavage[a]

	Incidence in Controls		
Study	**Hepatocellular Adenoma**	**Hepatocellular Carcinoma**	**Hepatocellular Adenoma or Carcinoma**
Historical Incidence at Battelle Columbus Laboratories			
o-Benzyl-p-chlorophenol	21/50	7/50	25/50
Overall Historical Incidence			
Total (%)	201/514 (39.1%)	102/514 (19.8%)	267/514 (52.0%)
Mean ± standard deviation	39.2% ± 11.0%	20.0% ± 8.1%	52.1% ± 14.9%
Range	21%-58%	8%-38%	25%-72%
	Hepatoblastoma	**Hepatocellular Carcinoma or Hepatoblastoma**	**Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma**
Historical Incidence at Battelle Columbus Laboratories			
o-Benzyl-p-chlorophenol	0/50	7/50	25/50
Overall Historical Incidence			
Total (%)	2/514 (0.4%)	104/514 (20.2%)	267/514 (52.0%)
Mean ± standard deviation	0.3% ± 1.0%	20.3% ± 8.0%	52.1% ± 14.9%
Range	0%-3%	8%-38%	25%-72%

[a] Data as of 13 November 1997

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

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			1	2
Moribund	5	6	5	9
Natural deaths	6	8	7	4
Survivors				
Terminal sacrifice	38	36	37	35
Missing	1			
Animals examined microscopically	49	50	50	50
Alimentary System				
Intestine small, duodenum	(47)	(47)	(48)	(50)
Inflammation, chronic active		1 (2%)		
Ulcer		1 (2%)		
Intestine small, jejunum	(49)	(49)	(48)	(48)
Inflammation, chronic active	1 (2%)			
Peyer's patch, hyperplasia, lymphoid	1 (2%)	1 (2%)		
Peyer's patch, inflammation, suppurative				1 (2%)
Liver	(49)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Atrophy				1 (2%)
Basophilic focus	5 (10%)	3 (6%)	1 (2%)	3 (6%)
Clear cell focus	16 (33%)	8 (16%)	2 (4%)	6 (12%)
Eosinophilic focus	10 (20%)	20 (40%)	25 (50%)	19 (38%)
Fibrosis	1 (2%)	4 (8%)	1 (2%)	
Hematopoietic cell proliferation	3 (6%)	9 (18%)	7 (14%)	9 (18%)
Infiltration cellular, lymphocyte	6 (12%)	5 (10%)	12 (24%)	10 (20%)
Inflammation, chronic active	19 (39%)	21 (42%)	28 (56%)	28 (56%)
Mineralization		3 (6%)	1 (2%)	
Mixed cell focus	9 (18%)	13 (26%)	9 (18%)	7 (14%)
Pigmentation, hemosiderin	3 (6%)	5 (10%)	6 (12%)	5 (10%)
Tension lipidosis		1 (2%)		
Bile duct, cyst		1 (2%)	1 (2%)	
Bile duct, hyperplasia		1 (2%)		1 (2%)
Hepatocyte, hypertrophy		1 (2%)	7 (14%)	46 (92%)
Hepatocyte, necrosis	8 (16%)	14 (28%)	8 (16%)	10 (20%)
Hepatocyte, vacuolization cytoplasmic	7 (14%)	7 (14%)	2 (4%)	13 (26%)
Hepatocyte, centrilobular, atrophy			1 (2%)	
Hepatocyte, centrilobular, necrosis				2 (4%)
Oval cell, hyperplasia		8 (16%)	27 (54%)	46 (92%)
Mesentery	(1)	(2)	(1)	(1)
Thrombosis	1 (100%)			
Fat, necrosis			1 (100%)	
Pancreas	(49)	(49)	(50)	(50)
Cyst	1 (2%)		1 (2%)	
Infiltration cellular, lymphocyte	1 (2%)	4 (8%)		4 (8%)
Inflammation, chronic active	1 (2%)			
Acinus, atrophy	3 (6%)			
Artery, inflammation, chronic active				1 (2%)

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

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Alimentary System (continued)				
Salivary glands	(49)	(50)	(50)	(50)
Atrophy	1 (2%)			
Infiltration cellular, lymphocyte	31 (63%)	29 (58%)	28 (56%)	34 (68%)
Stomach, forestomach	(49)	(50)	(50)	(50)
Hyperkeratosis		1 (2%)	4 (8%)	
Hyperplasia, squamous	1 (2%)			
Inflammation, chronic active	2 (4%)		3 (6%)	
Epithelium, hyperplasia, squamous	5 (10%)	2 (4%)	3 (6%)	2 (4%)
Epithelium, ulcer			1 (2%)	
Stomach, glandular	(49)	(48)	(49)	(50)
Atrophy		3 (6%)	35 (71%)	45 (90%)
Ectasia	13 (27%)	25 (52%)	40 (82%)	49 (98%)
Hyperplasia		1 (2%)	15 (31%)	20 (40%)
Hyperplasia, atypical			1 (2%)	2 (4%)
Inflammation, chronic active	10 (20%)	11 (23%)	25 (51%)	33 (66%)
Inflammation, suppurative				1 (2%)
Mineralization		1 (2%)	1 (2%)	4 (8%)
Necrosis			1 (2%)	
Neuroendocrine cell, hyperplasia				4 (8%)
Tooth	(23)	(13)	(4)	(5)
Inflammation, chronic active	1 (4%)	1 (8%)		1 (20%)
Malformation	22 (96%)	12 (92%)	4 (100%)	4 (80%)
Cardiovascular System				
Heart	(49)	(50)	(50)	(50)
Cardiomyopathy		1 (2%)		2 (4%)
Mineralization		1 (2%)		1 (2%)
Artery, inflammation, chronic active		1 (2%)		1 (2%)
Atrium, thrombosis	1 (2%)	1 (2%)		2 (4%)
Valve, inflammation, suppurative		1 (2%)		
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Accessory adrenal cortical nodule		2 (4%)		
Angiectasis		1 (2%)	1 (2%)	1 (2%)
Degeneration, fatty	1 (2%)	1 (2%)		
Hypertrophy		5 (10%)	3 (6%)	2 (4%)
Pigmentation, hemosiderin			1 (2%)	
Subcapsular, hyperplasia	38 (78%)	42 (84%)	44 (88%)	43 (86%)
Islets, pancreatic	(49)	(49)	(50)	(50)
Hyperplasia	32 (65%)	26 (53%)	18 (36%)	17 (34%)
Pituitary gland	(45)	(48)	(44)	(45)
Pars distalis, cyst	5 (11%)	7 (15%)	3 (7%)	1 (2%)
Thyroid gland	(49)	(50)	(50)	(50)
Inflammation, chronic active		1 (2%)		3 (6%)
Follicle, cyst	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Follicular cell, hyperplasia	2 (4%)	7 (14%)	6 (12%)	5 (10%)
General Body System				
None				

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Genital System				
Epididymis	(49)	(50)	(50)	(50)
Cyst		1 (2%)		
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)		2 (4%)
Mineralization		1 (2%)		
Preputial gland	(49)	(50)	(50)	(50)
Cyst	7 (14%)	6 (12%)	10 (20%)	5 (10%)
Infiltration cellular, lymphocyte	17 (35%)	15 (30%)	14 (28%)	18 (36%)
Inflammation, chronic active	3 (6%)	4 (8%)	8 (16%)	2 (4%)
Prostate	(49)	(50)	(50)	(50)
Infiltration cellular, lymphocyte	1 (2%)			
Inflammation, chronic active	2 (4%)	2 (4%)		
Artery, inflammation, chronic active		1 (2%)		
Seminal vesicle	(49)	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)			
Testes	(49)	(50)	(50)	(50)
Germinal epithelium, atrophy		1 (2%)		1 (2%)
Hematopoietic System				
Bone marrow	(49)	(49)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia	14 (29%)	26 (53%)	33 (66%)	35 (70%)
Infiltration cellular, mast cell	1 (2%)			
Necrosis			1 (2%)	
Lymph node	(3)	(4)	(5)	(3)
Lumbar, hyperplasia, plasma cell	1 (33%)			
Mediastinal, necrosis	1 (33%)			
Renal, hyperplasia, plasma cell	1 (33%)			
Lymph node, mandibular	(48)	(46)	(47)	(45)
Hyperplasia, lymphoid			2 (4%)	
Hyperplasia, plasma cell			1 (2%)	
Necrosis		1 (2%)		
Lymph node, mesenteric	(49)	(46)	(49)	(50)
Angiectasis		1 (2%)		
Atrophy	1 (2%)			
Congestion			1 (2%)	
Ectasia		1 (2%)	1 (2%)	
Hematopoietic cell proliferation	2 (4%)		1 (2%)	
Hyperplasia			1 (2%)	
Hyperplasia, lymphoid			2 (4%)	1 (2%)
Inflammation, chronic active	1 (2%)			
Necrosis	2 (4%)	2 (4%)		1 (2%)
Spleen	(49)	(49)	(50)	(50)
Atrophy			2 (4%)	
Fibrosis		1 (2%)		
Mineralization		1 (2%)		
Necrosis		1 (2%)		
Pigmentation, hemosiderin		1 (2%)		
Lymphoid follicle, hyperplasia, lymphoid	1 (2%)		1 (2%)	3 (6%)
Lymphoid follicle, necrosis	3 (6%)	6 (12%)	1 (2%)	2 (4%)
Red pulp, erythrophagocytosis		1 (2%)		
Red pulp, hematopoietic cell proliferation	10 (20%)	20 (41%)	25 (50%)	19 (38%)
Thymus	(37)	(40)	(38)	(45)
Cyst	10 (27%)	19 (48%)	16 (42%)	12 (27%)
Ectopic parathyroid gland		1 (3%)		1 (2%)
Thymocyte, necrosis	1 (3%)	1 (3%)		1 (2%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Integumentary System				
Skin	(49)	(50)	(50)	(50)
Subcutaneous tissue, inflammation, suppurative				1 (2%)
Musculoskeletal System				
Skeletal muscle	(2)	(1)	(3)	(3)
Infiltration cellular, lymphocyte	1 (50%)		2 (67%)	1 (33%)
Nervous System				
Brain	(49)	(50)	(50)	(50)
Necrosis	1 (2%)			
Spinal cord	(2)			(2)
Hemorrhage				1 (50%)
Nerve, demyelination				1 (50%)
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Infiltration cellular, lymphocyte				1 (2%)
Inflammation, chronic active	2 (4%)	2 (4%)		
Inflammation, suppurative	1 (2%)		1 (2%)	
Thrombosis		2 (4%)		1 (2%)
Alveolar epithelium, hyperplasia	7 (14%)	6 (12%)	5 (10%)	5 (10%)
Alveolus, infiltration cellular, lymphocyte			1 (2%)	
Alveolus, infiltration cellular, histiocyte	2 (4%)	1 (2%)		2 (4%)
Bronchiole, hyperplasia	1 (2%)			
Mediastinum, inflammation, chronic active				1 (2%)
Mediastinum, necrosis				1 (2%)
Nose	(49)	(50)	(50)	(50)
Inflammation, chronic active		1 (2%)		
Inflammation, suppurative	9 (18%)	13 (26%)	11 (22%)	10 (20%)
Polyp, inflammatory	1 (2%)	1 (2%)		
Nasolacrimal duct, inflammation, suppurative	4 (8%)	2 (4%)	8 (16%)	2 (4%)
Special Senses System				
Eye			(1)	(1)
Cornea, inflammation, chronic active			1 (100%)	1 (100%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Infiltration cellular, lymphocyte	29 (59%)	32 (64%)	27 (54%)	40 (80%)
Inflammation, chronic active				1 (2%)
Inflammation, suppurative	1 (2%)	1 (2%)		1 (2%)
Metaplasia, osseous	1 (2%)	3 (6%)		1 (2%)
Mineralization		1 (2%)	3 (6%)	4 (8%)
Necrosis				1 (2%)
Nephropathy	43 (88%)	45 (90%)	45 (90%)	48 (96%)
Artery, inflammation, chronic active		1 (2%)		
Cortex, cyst	10 (20%)	27 (54%)	20 (40%)	14 (28%)
Papilla, mineralization		1 (2%)		
Papilla, necrosis		1 (2%)		
Pelvis, transitional epithelium, hyperplasia		1 (2%)		
Renal tubule, pigmentation, hemosiderin				1 (2%)
Urinary bladder	(49)	(50)	(50)	(50)
Calculus gross observation		1 (2%)		
Infiltration cellular, lymphocyte	6 (12%)	5 (10%)	9 (18%)	14 (28%)
Inflammation, chronic active	1 (2%)			

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE
IN THE 2-YEAR GAVAGE STUDY
OF METHYLEUGENOL

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

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1	1	
Moribund	5	7	8	13
Natural deaths	14	24	23	35
Survivors				
Died last week of study			2	
Terminal sacrifice	31	18	16	2
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(38)	(28)	(30)	(24)
Intestine large, colon	(50)	(50)	(48)	(47)
Intestine large, rectum	(50)	(50)	(49)	(48)
Leiomyosarcoma				1 (2%)
Intestine small, duodenum	(46)	(49)	(46)	(43)
Intestine small, jejunum	(46)	(46)	(40)	(40)
Carcinoma			1 (3%)	
Liver	(50)	(50)	(49)	(50)
Carcinoma, metastatic, ureter				1 (2%)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Fibrosarcoma, metastatic, skin		1 (2%)	1 (2%)	
Hemangioma		1 (2%)		
Hemangiosarcoma, metastatic, spleen		1 (2%)		
Hepatoblastoma		6 (12%)	11 (22%)	11 (22%)
Hepatoblastoma, multiple				4 (8%)
Hepatocellular carcinoma	7 (14%)	13 (26%)	18 (37%)	9 (18%)
Hepatocellular carcinoma, multiple		24 (48%)	29 (59%)	38 (76%)
Hepatocellular adenoma	12 (24%)	9 (18%)	8 (16%)	9 (18%)
Hepatocellular adenoma, multiple	8 (16%)	39 (78%)	38 (78%)	32 (64%)
Hepatocholangiocarcinoma				2 (4%)
Histiocytic sarcoma	1 (2%)	1 (2%)		1 (2%)
Mast cell tumor malignant	1 (2%)			
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Plasma cell tumor malignant	1 (2%)			
Schwannoma malignant, metastatic, mesentery		1 (2%)		
Mesentery	(13)	(7)	(3)	(3)
Fibrosarcoma	1 (8%)			
Fibrosarcoma, metastatic, skin		1 (14%)	1 (33%)	
Schwannoma malignant		1 (14%)	1 (33%)	
Pancreas	(49)	(50)	(46)	(46)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Fibrosarcoma, metastatic, skin		1 (2%)	1 (2%)	
Schwannoma malignant, metastatic, mesentery		1 (2%)	1 (2%)	
Salivary glands	(50)	(45)	(44)	(42)
Stomach, forestomach	(50)	(50)	(48)	(46)
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma		1 (2%)	1 (2%)	
Stomach, glandular	(45)	(49)	(46)	(45)
Tooth	(2)	(1)		
Odontoma	1 (50%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Cardiovascular System				
Heart	(50)	(50)	(48)	(44)
Histiocytic sarcoma	1 (2%)			1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Adenoma				1 (2%)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Fibrosarcoma, metastatic, skin		1 (2%)		
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Histiocytic sarcoma				1 (2%)
Plasma cell tumor malignant	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(49)
Pheochromocytoma benign			2 (4%)	1 (2%)
Bilateral, pheochromocytoma benign			1 (2%)	
Islets, pancreatic	(49)	(50)	(46)	(47)
Adenoma				1 (2%)
Pituitary gland	(48)	(44)	(44)	(40)
Pars distalis, adenoma	5 (10%)	1 (2%)		1 (3%)
Thyroid gland	(50)	(46)	(46)	(42)
Follicular cell, adenoma	2 (4%)		1 (2%)	1 (2%)
General Body System				
Peritoneum		(1)		
Schwannoma malignant, metastatic, mesentery		1 (100%)		
Genital System				
Ovary	(50)	(49)	(48)	(41)
Cystadenoma	3 (6%)			
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Fibrosarcoma, metastatic, skin		1 (2%)		
Histiocytic sarcoma	1 (2%)			1 (2%)
Luteoma	1 (2%)		1 (2%)	
Schwannoma malignant, metastatic, mesentery		1 (2%)		
Uterus	(50)	(50)	(49)	(47)
Histiocytic sarcoma	1 (2%)			1 (2%)
Polyp stromal			1 (2%)	
Vagina				(1)
Leiomyosarcoma, metastatic, intestine large, rectum				1 (100%)
Hematopoietic System				
Bone marrow	(50)	(50)	(48)	(50)
Hemangiosarcoma			1 (2%)	
Mast cell tumor malignant	1 (2%)			
Plasma cell tumor malignant	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Hematopoietic System (continued)				
Lymph node	(13)	(7)	(8)	(4)
Mediastinal, fibrosarcoma, metastatic, skin			1 (13%)	
Mediastinal, hepatocellular carcinoma,				
metastatic, liver				1 (25%)
Mediastinal, histiocytic sarcoma				1 (25%)
Lymph node, mandibular	(49)	(43)	(41)	(32)
Histiocytic sarcoma				1 (3%)
Lymph node, mesenteric	(47)	(48)	(43)	(36)
Fibrosarcoma, metastatic, mesentery	1 (2%)			
Fibrosarcoma, metastatic, skin			1 (2%)	
Schwannoma malignant, metastatic, mesentery		1 (2%)		
Spleen	(50)	(50)	(46)	(46)
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma		1 (2%)		
Mast cell tumor malignant	1 (2%)			
Plasma cell tumor malignant	1 (2%)			
Thymus	(45)	(43)	(40)	(33)
Integumentary System				
Mammary gland	(50)	(48)	(50)	(37)
Adenoma	1 (2%)			
Carcinoma	1 (2%)			
Skin	(50)	(50)	(50)	(49)
Subcutaneous tissue, fibrosarcoma		4 (8%)	1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)			
Subcutaneous tissue, pinna, fibrosarcoma		1 (2%)		
Musculoskeletal System				
Skeletal muscle	(2)	(8)	(3)	(3)
Fibrosarcoma, metastatic, skin		1 (13%)		
Hemangiosarcoma			1 (33%)	
Schwannoma malignant, metastatic, skin	1 (50%)			
Nervous System				
Brain	(50)	(47)	(46)	(41)
Respiratory System				
Lung	(50)	(50)	(48)	(45)
Alveolar/bronchiolar adenoma	5 (10%)	4 (8%)	1 (2%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Alveolar/bronchiolar carcinoma	1 (2%)			1 (2%)
Carcinoma, metastatic, harderian gland	1 (2%)			
Carcinoma, metastatic, ureter				1 (2%)
Hepatoblastoma, metastatic, liver			3 (6%)	4 (9%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	7 (14%)	23 (48%)	26 (58%)
Histiocytic sarcoma	1 (2%)	1 (2%)		1 (2%)
Osteosarcoma, metastatic, uncertain primary				
site			1 (2%)	
Mediastinum, fibrosarcoma, metastatic,				
mesentery	1 (2%)			
Nose	(50)	(50)	(49)	(47)
Carcinoma, metastatic, harderian gland	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Special Senses System				
Eye	(2)		(1)	
Carcinoma, metastatic, harderian gland	1 (50%)			
Harderian gland	(5)	(2)	(2)	
Adenoma		2 (100%)	2 (100%)	
Carcinoma	5 (100%)			
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Hemangioma			1 (2%)	
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Histiocytic sarcoma	1 (2%)			
Ureter				(1)
Transitional epithelium, carcinoma				1 (100%)
Urinary bladder	(50)	(49)	(47)	(46)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)		1 (2%)
Lymphoma malignant	15 (30%)	8 (16%)	8 (16%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	41	50	49	49
Total primary neoplasms	79	116	128	116
Total animals with benign neoplasms	29	49	46	42
Total benign neoplasms	39	57	57	47
Total animals with malignant neoplasms	27	42	48	49
Total malignant neoplasms	40	59	71	69
Total animals with metastatic neoplasms	5	10	26	30
Total metastatic neoplasms	11	19	36	34
Total animals with malignant neoplasms				
of uncertain primary site			1	

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

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Number of Days on Study	1	5	5	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7
	7	6	7	1	3	3	5	6	6	6	7	7	7	9	0	0	1	1	2	3	3	3	3	3	3
	8	4	9	0	0	2	2	3	5	5	0	3	3	5	2	6	7	8	8	1	1	1	1	1	1
Carcass ID Number	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	3	3	0	3	4	2	3	1	0	0	2	0	4	0	1	0	1	3	2	0	1	1	2	3	4
	0	6	8	1	4	9	4	0	1	5	8	7	6	9	9	2	7	5	7	3	2	3	2	2	7
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	M	+	A	+	+	A	+	+	+	+	+	+	A	A	A	+	A	M	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	M	+	A	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	A	+	A	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery																				X					
Hepatocellular carcinoma								X		X			X	X						X		X			
Hepatocellular adenoma												X			X		X	X							
Hepatocellular adenoma, multiple									X							X									
Histiocytic sarcoma													X												
Mast cell tumor malignant																									
Plasma cell tumor malignant											X														
Mesentery						+			+		+		+		+			+		+		+	+	+	
Fibrosarcoma																				X					
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery																				X					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Stomach, glandular	+	A	A	+	+	+	+	+	+	+	+	+	+	+	A	+	A	A	+	+	+	+	+	+	+
Tooth													+	+											
Odontoma													X												
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery																				X					
Plasma cell tumor malignant											X														
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	M	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X															X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma					X																				
General Body System																									
None																									

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

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

	248	204	206	211	214	215	216	218	220	221	223	224	225	226	233	237	238	239	240	241	242	243	245	249	250	Total Tissues/ Tumors
Number of Days on Study	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M	+	+	+	+	+	+	+	38
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Hepatocellular carcinoma			X																							7
Hepatocellular adenoma		X		X		X							X		X			X		X			X			12
Hepatocellular adenoma, multiple			X		X			X		X	X						X									8
Histiocytic sarcoma																										1
Mast cell tumor malignant													X													1
Plasma cell tumor malignant																										1
Mesentery	+								+																+	13
Fibrosarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrosarcoma, metastatic, mesentery																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma				X																						1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Tooth																										2
Odontoma																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Plasma cell tumor malignant																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	M	+	M	M	M	M	+	+	+	+	M	+	+	M	M	M	+	+	+	+	+	+	+	36
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma						X								X								X				5
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma								X																		2

General Body System
None

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	178	564	579	610	630	632	652	663	665	665	670	673	673	695	702	706	717	718	728	731	731	731	731	731	731
Carcass ID Number	230	236	208	231	244	229	234	210	201	205	228	207	246	209	219	202	217	235	227	203	212	213	222	232	247
Genital System																									
Clitoral gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									
Fibrosarcoma, metastatic, mesentery																							X		
Histiocytic sarcoma													X												
Luteoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant																									
Plasma cell tumor malignant													X												
Lymph node			+			+		+			+	+	+		+	+				+					
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+
Fibrosarcoma, metastatic, mesentery																							X		
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant																									
Plasma cell tumor malignant													X												
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X																	
Carcinoma									X																
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, schwannoma malignant											X														
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle											+	+													
Schwannoma malignant, metastatic, skin											X														
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X		X							X										
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma																								X	
Carcinoma, metastatic, harderian gland																									
Hepatocellular carcinoma, metastatic, liver																								X	
Histiocytic sarcoma													X												
Mediastinum, fibrosarcoma, metastatic, mesentery																							X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, harderian gland																		X							
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

| | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | |
| Number of Days on Study | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | |
	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Total
Carcass ID Number	4	0	0	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	4	4	4	4	4	4	5	Tissues/
	8	4	6	1	4	5	6	8	0	1	3	4	5	6	3	7	8	9	0	1	2	3	5	9	0	Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	47
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma									X												X			X		3
Fibrosarcoma, metastatic, mesentery																										1
Histiocytic sarcoma																										1
Luteoma												X														1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mast cell tumor malignant														X												1
Plasma cell tumor malignant																										1
Lymph node			+				+			+								+								13
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Fibrosarcoma, metastatic, mesentery																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mast cell tumor malignant														X												1
Plasma cell tumor malignant																										1
Thymus	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Schwannoma malignant, metastatic, skin																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X		X																					5
Alveolar/bronchiolar adenoma, multiple																					X					1
Alveolar/bronchiolar carcinoma																										1
Carcinoma, metastatic, harderian gland												X														1
Hepatocellular carcinoma, metastatic, liver																										1
Histiocytic sarcoma																										1
Mediastinum, fibrosarcoma, metastatic, mesentery																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	X	+	+	50
Carcinoma, metastatic, harderian gland																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	1 7 8	5 6 4	5 7 9	6 1 0	6 3 0	6 3 2	6 5 2	6 6 3	6 6 5	6 6 5	6 7 0	6 7 3	6 7 3	6 9 5	7 0 2	7 0 6	7 1 7	7 1 8	7 2 8	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	2 3 0	2 3 6	2 0 8	2 3 1	2 4 4	2 2 9	2 3 4	2 1 0	2 0 1	2 0 5	2 2 8	2 0 7	2 4 6	2 0 9	2 1 9	2 0 2	2 1 7	2 3 5	2 2 7	2 0 3	2 1 2	2 1 3	2 2 2	2 3 2	2 4 7

Special Senses System

	230	236	208	231	244	229	234	210	201	205	228	207	246	209	219	202	217	235	227	203	212	213	222	232	247
Eye									+															+	
Carcinoma, metastatic, harderian gland																								X	
Harderian gland							+		+										+					+	
Carcinoma							X		X										X					X	

Urinary System

	230	236	208	231	244	229	234	210	201	205	228	207	246	209	219	202	217	235	227	203	212	213	222	232	247
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma														X											
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Systemic Lesions

	230	236	208	231	244	229	234	210	201	205	228	207	246	209	219	202	217	235	227	203	212	213	222	232	247
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma														X											
Lymphoma malignant		X	X			X		X			X		X		X	X	X					X		X	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: Vehicle Control

Number of Days on Study	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	2 4 8	2 0 4	2 0 6	2 1 1	2 1 4	2 1 5	2 1 6	2 1 8	2 2 0	2 2 1	2 2 3	2 2 4	2 2 5	2 2 6	2 3 3	2 3 7	2 3 8	2 3 9	2 4 0	2 4 1	2 4 2	2 4 3	2 4 5	2 4 9	2 5 0	Total Tissues/ Tumors

Special Senses System

Eye — Total: 2
  Carcinoma, metastatic, harderian gland — Total: 1

Harderian gland

													+													5

  Carcinoma

													X													5

Urinary System

Kidney

+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

  Histiocytic sarcoma — Total: 1

Urinary bladder

+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

Systemic Lesions

Multiple organs

+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

  Histiocytic sarcoma — Total: 1

  Lymphoma malignant

		X	X					X									X								15

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

Number of Days on Study	477	493	516	519	562	562	567	578	582	596	604	605	605	617	620	627	629	632	633	649	652	658	661	664	675
Carcass ID Number	251	263	268	279	267	289	280	275	277	296	276	253	284	272	298	261	285	278	291	290	264	281	292	295	270
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Gallbladder	A	+	M	+	+	A	M	+	M	+	M	+	+	A	A	A	M	M	A	A	M	A	A	A	A
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	+	+	A	+	+
Intestine small, ileum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	M	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin								X																	
Hemangioma																				X					
Hemangiosarcoma, metastatic, spleen																									
Hepatoblastoma																X		X							
Hepatocellular carcinoma		X									X	X		X	X	X					X				
Hepatocellular carcinoma, multiple			X		X			X	X	X			X									X	X	X	X
Hepatocellular adenoma			X							X	X	X				X			X						
Hepatocellular adenoma, multiple	X	X		X	X	X	X	X	X				X	X	X		X	X			X	X	X		X
Histiocytic sarcoma																									
Schwannoma malignant, metastatic, mesentery											X														
Mesentery								+			+			+		+		+							
Fibrosarcoma, metastatic, skin								X																	
Schwannoma malignant											X														
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin								X																	
Schwannoma malignant, metastatic, mesentery											X														
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	M	+	+	+	M	+	M
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma								X																	
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin								X																	
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	+	+	M	+	+	+	+	+	M	+	+	M	M	+	M	+	+	M	M	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	I	+	+	I	I	+	+	M
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	M	+	M
General Body System																									
Peritoneum											+														
Schwannoma malignant, metastatic, mesentery											X														

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

	297	256	255	265	299	252	283	254	266	274	257	258	259	260	262	269	271	273	282	286	287	288	293	294	300	Total Tissues/ Tumors
Number of Days on Study	675	687	719	720	720	721	726	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	A	+	A	A	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	28
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Hemangioma																										1
Hemangiosarcoma, metastatic, spleen														X												1
Hepatoblastoma				X	X					X								X								6
Hepatocellular carcinoma		X		X	X			X							X			X			X			X		13
Hepatocellular carcinoma, multiple	X			X		X			X		X			X	X	X	X	X		X	X			X	X	24
Hepatocellular adenoma																		X			X	X				9
Hepatocellular adenoma, multiple	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	X	39
Histiocytic sarcoma		X																								1
Schwannoma malignant, metastatic, mesentery																										1
Mesentery							+					+														7
Fibrosarcoma, metastatic, skin																										1
Schwannoma malignant																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Schwannoma malignant, metastatic, mesentery																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth							+																			1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Pars distalis, adenoma														X												1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
General Body System																										
Peritoneum																										1
Schwannoma malignant, metastatic, mesentery																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

	477	493	516	519	562	562	567	578	582	596	604	605	605	617	620	627	629	632	633	649	652	658	661	664	675
Carcass ID Number	251	263	268	279	267	289	280	275	277	296	276	253	284	272	298	261	285	278	291	290	264	281	292	295	270
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin								X																	
Schwannoma malignant, metastatic, mesentery													X												
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+									+												
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	M	+	M	+	+	M	M	+	M	+
Lymph node, mesenteric	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Schwannoma malignant, metastatic, mesentery													X												
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Histiocytic sarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	M	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma						X		X																	
Subcutaneous tissue, pinna, fibrosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																			+						
Fibrosarcoma, metastatic, skin																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	M	+	+
Peripheral nerve																			+						
Spinal cord																			+						
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X									X				X						
Hepatocellular carcinoma, metastatic, liver													X				X								X
Histiocytic sarcoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M
Special Senses System																									
Harderian gland																									
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymphoma malignant						X					X														

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 37 mg/kg

	297	256	255	265	299	252	283	254	266	274	257	258	259	260	262	269	271	273	282	286	287	288	293	294	300	Total Tissues/ Tumors
Number of Days on Study	675	687	719	720	720	721	726	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Genital System																										
Clitoral gland	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	49
Fibrosarcoma, metastatic, skin																										1
Schwannoma malignant, metastatic, mesentery																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+			+		+			+	+															7
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Schwannoma malignant, metastatic, mesentery																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma													X													1
Histiocytic sarcoma			X																							1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	M	43
Integumentary System																										
Mammary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma		X																				X				4
Subcutaneous tissue, pinna, fibrosarcoma																		X								1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle		+		+			+			+		+					+			+						8
Fibrosarcoma, metastatic, skin		X																								1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma													X													4
Hepatocellular carcinoma, metastatic, liver				X	X	X																X				7
Histiocytic sarcoma			X																							1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Special Senses System																										
Harderian gland				+				+																		2
Adenoma				X				X																		2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma			X																							1
Lymphoma malignant		X			X				X					X	X								X			8

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

| Number of Days on Study | 3 7 1 | 4 6 5 | 4 8 4 | 4 8 9 | 5 2 0 | 5 2 1 | 5 4 5 | 5 8 2 | 5 9 0 | 5 9 7 | 6 1 1 | 6 1 9 | 6 2 8 | 6 3 3 | 6 3 7 | 6 4 1 | 6 5 2 | 6 5 8 | 6 5 9 | 6 5 9 | 6 6 1 | 6 9 0 | 6 9 4 | 6 9 8 |

| Carcass ID Number | 3 4 4 | 3 3 5 | 3 4 2 | 3 1 4 | 3 1 2 | 3 0 3 | 3 0 8 | 3 1 5 | 3 1 8 | 3 2 0 | 3 1 3 | 3 3 4 | 3 3 9 | 3 0 2 | 3 0 7 | 3 2 3 | 3 1 0 | 3 0 5 | 3 0 6 | 3 3 8 | 3 3 1 | 3 0 9 | 3 1 9 | 3 2 1 | 3 3 7 |

Alimentary System
```
Esophagus                          + + + + + + + + + + + + + M + + + + + + + + + +
Gallbladder                        A + + M A + A A + M A + A + A + + + + A + A A +
Intestine large, colon             + + + A + + + + + + + + + + + + + + + + + + + +
Intestine large, rectum            + + + A + + + + + + + + + + + + + + + + + + + +
Intestine large, cecum             A + + A + + + + + + + + + A + + A + + + + + + +
Intestine small, duodenum          A + + M + + + + + + + + + + M + + + + + + + + +
Intestine small, jejunum           A + + A + + A + A + + + + A M + + + + + + + + A
   Carcinoma
Intestine small, ileum             A + + A + + + + + + A + + A A + + A + + + + + +
Liver                              A + + + + + + + + + + + + + + + + + + + + + + +
   Fibrosarcoma, metastatic, skin       X
   Hepatoblastoma                                                    X       X   X                   X
   Hepatocellular carcinoma              X X X X X X X       X       X       X       X           X       X
   Hepatocellular carcinoma, multiple  X                         X       X       X       X       X       X   X X X
   Hepatocellular adenoma                                          X               X       X               X X X
   Hepatocellular adenoma, multiple      X X X X X X       X X X X X X X       X X       X                       X
   Osteosarcoma, metastatic, uncertain primary site                            X
Mesentery                                 +                                       +           +
   Fibrosarcoma, metastatic, skin        X
   Schwannoma malignant                                                               X
Pancreas                           A + + A + + + + + + + A + + + + + + + + + + + +
   Fibrosarcoma, metastatic, skin        X
   Schwannoma malignant, metastatic, mesentery                                   X
Salivary glands                    + + + + + + + + + + + + M + M + + M + + + + + M M
Stomach, forestomach               A + + A + + + + + + + + + + + + + + + + + + + +
   Squamous cell papilloma
Stomach, glandular                 A + + A + + + + + + + + A + + + + + + + + + + +
```

Cardiovascular System
```
Blood vessel                       + + + + + + + + + + + + + + + + + + + + + + + +
Heart                              + + + + + + + + + + + + + M + + + + + + + + + +
```

Endocrine System
```
Adrenal cortex                     + + + + + + + + + + + + + + + + + + + + + + + +
   Hepatocellular carcinoma, metastatic, liver                               X
Adrenal medulla                    + + + + + + + + + + + + + + + + + + + + + + + +
   Pheochromocytoma benign                                                      X
   Bilateral, pheochromocytoma benign
Islets, pancreatic                 A + + A + + + + + + + A + + + + + + + + + + + +
Parathyroid gland                  M + + + + + + M + + + + + M M + M + M M + + + M
Pituitary gland                    + + + M + + + + + + + + + M + + + + + + + + M M
Thyroid gland                      + + + + + + + + + + + + + M + + M + + + + + + M
   Follicular cell, adenoma
```

General Body System
None

Genital System
```
Clitoral gland                     + + M + + + + + + + + + + + + + + + + + + + + M
Ovary                              + + + + + + + + + + + + + + + + + + + + + + + M
   Luteoma
Uterus                             + + + + + + + + + + + + + + + + + + + + + + + +
   Polyp stromal                                                    X
```

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study	7 0 4	7 1 4	7 1 7	7 2 0	7 2 2	7 2 7	7 2 8	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	3 2 6	3 4 8	3 2 2	3 3 0	3 4 6	3 3 2	3 0 4	3 0 1	3 1 1	3 4 7	3 4 9	3 1 6	3 1 7	3 2 4	3 2 5	3 2 7	3 2 8	3 2 9	3 3 3	3 3 6	3 4 0	3 4 1	3 4 3	3 4 5	3 5 0	Total Tissues/ Tumors

Alimentary System

Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	A	A	M	A	M	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	30
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, duodenum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Carcinoma													X													1
Intestine small, ileum	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrosarcoma, metastatic, skin																										1
Hepatoblastoma				X					X		X			X	X				X				X			11
Hepatocellular carcinoma	X			X									X				X		X	X						18
Hepatocellular carcinoma, multiple		X	X		X	X	X	X	X	X	X	X				X	X				X	X	X	X	X	29
Hepatocellular adenoma						X		X																		8
Hepatocellular adenoma, multiple	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X		X	X	X		38
Osteosarcoma, metastatic, uncertain primary site																										1
Mesentery																										3
Fibrosarcoma, metastatic, skin																										1
Schwannoma malignant																										1
Pancreas	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Fibrosarcoma, metastatic, skin																										1
Schwannoma malignant, metastatic, mesentery																										1
Salivary glands	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Squamous cell papilloma						X																				1
Stomach, glandular	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46

Cardiovascular System

Blood vessel	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Heart	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

Endocrine System

Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic, liver																										
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign							X																			2
Bilateral, pheochromocytoma benign										X																1
Islets, pancreatic	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Parathyroid gland	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+			38
Pituitary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+		44
Thyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Follicular cell, adenoma																			X							1

General Body System

None

Genital System

Clitoral gland	+	+	A	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	46
Ovary	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Luteoma																				X						1
Uterus	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp stromal																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

	Number of Days on Study →																								
Days on Study	371	465	484	489	520	521	545	582	590	597	611	619	628	633	637	641	652	658	659	659	661	690	694	694	698
Carcass ID Number	344	335	342	314	312	303	308	315	318	320	313	334	339	302	307	323	310	305	306	338	331	309	319	321	337

Hematopoietic System

Lesion																									
Bone marrow	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Lymph node		+									+											+		+	
Mediastinal, fibrosarcoma, metastatic, skin		X																							
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	M	+	M	+	+	M	+	M	+	+	+	M	M
Lymph node, mesenteric	A	+	+	A	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	M	+	+	+	+	+
Fibrosarcoma, metastatic, skin		X																							
Spleen	A	+	+	A	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	+	M	+	+	M	+	+	M	M	M	+	+	+	+	+	+

Integumentary System

Lesion																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma		X																							

Musculoskeletal System

Lesion																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Hemangiosarcoma																									

Nervous System

Lesion																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	M

Respiratory System

Lesion																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma														X											
Hepatoblastoma, metastatic, liver																	X								
Hepatocellular carcinoma, metastatic, liver			X					X		X		X	X		X	X		X	X	X		X		X	
Osteosarcoma, metastatic, uncertain primary site														X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	M

Special Senses System

Lesion																									
Eye																									
Harderian gland																	+								
Adenoma																	X								

Urinary System

Lesion																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Hepatocellular carcinoma, metastatic, liver																									
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+

Systemic Lesions

Lesion																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant														X			X		X		X				

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 75 mg/kg

Number of Days on Study	704	714	717	720	722	727	728	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	Total Tissues/Tumors
Carcass ID Number	326	348	322	330	346	332	304	301	311	347	349	316	317	324	325	327	328	329	333	336	340	341	343	345	350	
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma													X													1
Lymph node	+			+	+							+														8
Mediastinal, fibrosarcoma, metastatic, skin																										1
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Lymph node, mesenteric	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Fibrosarcoma, metastatic, skin																										1
Spleen	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	M	+	+	40
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle			+		+																			+		3
Hemangiosarcoma					X																					1
Nervous System																										
Brain	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Respiratory System																										
Lung	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma																										1
Hepatoblastoma, metastatic, liver														X		X										3
Hepatocellular carcinoma, metastatic, liver									X	X		X	X		X				X	X		X		X	X	23
Osteosarcoma, metastatic, uncertain primary site																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Special Senses System																										
Eye																					+					1
Harderian gland						+																				2
Adenoma						X																				2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																							X			1
Hepatocellular carcinoma, metastatic, liver																						X				1
Urinary bladder	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant	X			X	X						X															8

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

	Animals (Days on Study / Carcass ID)
Number of Days on Study	379 450 477 505 505 518 537 548 577 588 592 596 601 603 607 615 624 626 626 627 634 652 652 654 659
Carcass ID Number	396 397 386 354 385 352 356 384 369 361 381 351 390 377 376 367 353 363 370 394 366 362 383 365 364

Alimentary System

Lesion	396	397	386	354	385	352	356	384	369	361	381	351	390	377	376	367	353	363	370	394	366	362	383	365	364
Esophagus	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	A	+	+	+	+	A	+	A	A	A	M	M	A	A	A	A	+	+	+	+	+	M	+	A
Intestine large, colon	+	+	A	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma			X																						
Intestine large, cecum	+	+	+	+	+	+	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Intestine small, jejunum	+	A	A	+	+	+	+	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	A	+	+	+	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, ureter													X												
Hepatoblastoma			X	X						X	X	X													
Hepatoblastoma, multiple																									
Hepatocellular carcinoma				X	X	X							X											X	X
Hepatocellular carcinoma, multiple		X	X				X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X		
Hepatocellular adenoma					X			X	X								X							X	
Hepatocellular adenoma, multiple		X		X		X	X			X	X	X	X	X	X			X	X	X	X			X	
Hepatocholangiocarcinoma																									
Histiocytic sarcoma			X																						
Mesentery							+			+											+				
Pancreas	+	+	A	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	M	+	+	M	+	M	M	+	+	+	M	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	A	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Cardiovascular System

Lesion	396	397	386	354	385	352	356	384	369	361	381	351	390	377	376	367	353	363	370	394	366	362	383	365	364
Blood vessel	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						

Endocrine System

Lesion	396	397	386	354	385	352	356	384	369	361	381	351	390	377	376	367	353	363	370	394	366	362	383	365	364
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Histiocytic sarcoma			X																						
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	M	M	+	+	+	M	+	+	M	+	M	M	+	M	M	M	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	M	+	+	M	+	M	M	+	M	+	M	+	+	+	+	+	+	+	+
Pars distalis, adenoma																		X							
Thyroid gland	+	+	+	+	+	+	M	+	+	M	+	M	M	+	+	+	M	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									

General Body System
None

Genital System

Lesion	396	397	386	354	385	352	356	384	369	361	381	351	390	377	376	367	353	363	370	394	366	362	383	365	364
Clitoral gland	M	+	+	+	+	+	+	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	A	+	+	M	+	+	M	M	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study (read vertically, three digits per column):

```
6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7
6 6 7 7 7 7 7 8 8 8 8 8 8 9 0 1 1 1 1 1 2 3 3 3
2 6 6 6 6 8 9 0 1 6 6 6 7 9 2 5 1 2 7 7 8 3 0 1 1
```

Carcass ID Number (read vertically, three digits per column):

```
3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 4 3 3 3 3
5 9 8 8 8 9 7 5 9 5 7 7 7 9 8 9 9 7 6 6 0 5 7 7 8
7 2 2 8 9 3 1 8 8 5 4 9 3 9 0 1 5 8 0 8 0 9 5 2 7
```

Tissue / Tumor	357	392	382	388	389	393	371	358	398	355	374	379	373	399	380	391	395	378	360	368	400	359	375	372	387	Total Tissues/Tumors
Alimentary System																										
Esophagus	M	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Gallbladder	A	+	+	+	M	+	A	+	M	+	A	A	+	A	+	M	M	A	+	+	+	A	M	+	+	24
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leiomyosarcoma																										1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	45
Intestine small, duodenum	+	+	+	+	+	+	M	+	+	+	M	+	+	A	+	+	+	+	+	+	+	+	+	+	+	43
intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	A	+	+	+	A	A	+	+	+	40
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	43
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, ureter																										1
Hepatoblastoma	X		X													X		X	X	X						11
Hepatoblastoma, multiple									X			X					X							X		4
Hepatocellular carcinoma						X										X						X				9
Hepatocellular carcinoma, multiple	X	X	X	X	X		X		X	X	X		X	X	X	X	X		X	X	X	X		X		38
Hepatocellular adenoma													X		X		X			X						9
Hepatocellular adenoma, multiple	X		X			X	X	X	X	X	X	X	X	X		X						X	X	X		32
Hepatocholangiocarcinoma		X							X																	2
Histiocytic sarcoma																										1
Mesentery																										3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Salivary glands	M	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Stomach, forestomach	+	+	+	+	+	+	M	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Stomach, glandular	+	+	+	+	+	+	M	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Heart	M	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Histiocytic sarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma		X																								1
Histiocytic sarcoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																							X			1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma																		X								1
Parathyroid gland	M	+	+	M	+	+	+	M	+	+	+	M	+	+	M	+	+	+	+	+	+	+	M	+	+	36
Pituitary gland	M	+	+	+	+	+	+	M	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Pars distalis, adenoma																										1
Thyroid gland	M	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Follicular cell, adenoma														X												1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	44
Ovary	+	+	+	+	+	+	M	+	+	+	M	A	+	+	+	+	+	M	M	+	+	+	+	+	+	41
Histiocytic sarcoma																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

Number of Days on Study	379	450	477	505	505	518	537	548	577	588	592	596	601	603	607	615	624	626	626	627	634	652	652	654	659
Carcass ID Number	396	397	386	354	385	352	356	384	369	361	381	351	390	377	376	367	353	363	370	394	366	362	363	385	364
Genital System (continued)																									
Uterus	+	+	+	+	+	+	+	+	+	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Vagina				+																					
Leiomyosarcoma, metastatic, intestine large, rectum				X																					
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+																		+					
Mediastinal, hepatocellular carcinoma, metastatic, liver																									
Mediastinal, histiocytic sarcoma		X																							
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	M	+	M	M	M	M	M	M	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Lymph node, mesenteric	M	+	M	+	+	+	M	+	+	A	+	+	M	+	M	+	+	+	+	+	+	+	+	+	M
Spleen	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	+	+	+	+	M	M	+	+	A	M	+	M	+	+	M	M	M	+	+	+	+	+	+	M
Integumentary System																									
Mammary gland	+	+	M	+	+	+	+	+	+	A	M	+	+	+	M	M	+	+	+	+	+	+	+	M	M
Skin	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle													+							+					
Nervous System																									
Brain	+	+	+	+	+	+	M	+	+	M	+	M	M	+	M	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, ureter													X												
Hepatoblastoma, metastatic, liver														X											
Hepatocellular carcinoma, metastatic, liver		X			X			X	X						X	X	X	X	X			X	X		
Histiocytic sarcoma		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	M	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
None																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ureter													+												
Transitional epithelium, carcinoma													X												
Urinary bladder	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Lymphoma malignant																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of Methyleugenol: 150 mg/kg

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Total
Number of Days on Study	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	
	6	6	7	7	7	7	7	8	8	8	8	8	8	9	0	1	1	1	1	1	2	3	3	3	3	
	2	6	6	6	6	8	9	0	1	6	6	6	7	9	2	5	1	2	7	7	8	3	0	1	1	
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	3	3	3	Total
	5	9	8	8	8	9	7	5	9	5	7	7	7	9	8	9	9	7	6	6	0	5	7	7	8	Tissues/
	7	2	2	8	9	3	1	8	8	5	4	9	3	9	0	1	5	8	0	8	0	9	5	2	7	Tumors
Genital System (continued)																										
Uterus	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																										1
Vagina																										1
Leiomyosarcoma, metastatic, intestine large, rectum																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																		+				+				4
Mediastinal, hepatocellular carcinoma, metastatic, liver																						X				1
Mediastinal, histiocytic sarcoma																										1
Lymph node, mandibular	M	+	M	+	M	+	+	M	+	+	M	M	+	M	+	+	M	+	+	+	M	M	+	+	+	32
Histiocytic sarcoma																										1
Lymph node, mesenteric	+	M	+	M	+	M	M	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	M	+	+	36
Spleen	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	46
Thymus	M	+	M	+	+	+	+	M	+	M	M	M	+	+	+	+	+	+	+	+	+	M	+	+	+	33
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	M	+	+	A	+	+	+	M	+	+	+	+	+	M	M	M	37
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																		+								3
Nervous System																										
Brain	M	+	+	+	+	+	+	M	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Respiratory System																										
Lung	M	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Alveolar/bronchiolar adenoma								X																		1
Alveolar/bronchiolar carcinoma						X																				1
Carcinoma, metastatic, ureter																										1
Hepatoblastoma, metastatic, liver																		X				X		X		4
Hepatocellular carcinoma, metastatic, liver			X	X	X	X				X	X	X		X		X	X	X	X	X	X			X		26
Histiocytic sarcoma																										1
Nose	I	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Trachea	M	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Ureter																										1
Transitional epithelium, carcinoma																										1
Urinary bladder	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant																	X									1

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	0/50 (0%)	0/50 (0%)	3/50 (6%)	1/49 (2%)
Adjusted rate[b]	0.0%	0.0%	7.6%	3.0%
Terminal rate[c]	0/31 (0%)	0/18 (0%)	2/18 (11%)	1/2 (50%)
First incidence (days)	—[e]	—	659	731 (T)
Poly-3 test[d]	P=0.149	—[f]	P=0.098	P=0.445
Harderian Gland: Carcinoma				
Overall rate	5/50 (10%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	11.1%	0.0%	0.0%	0.0%
Terminal rate	2/31 (7%)	0/18 (0%)	0/18 (0%)	0/2 (0%)
First incidence (days)	652	—	—	—
Poly-3 test	P=0.006N	P=0.046N	P=0.044N	P=0.061N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	2/50 (4%)	2/50 (4%)	0/50 (0%)
Adjusted rate	11.1%	5.2%	5.1%	0.0%
Terminal rate	2/31 (7%)	1/18 (6%)	0/18 (0%)	0/2 (0%)
First incidence (days)	652	720	659	—
Poly-3 test	P=0.033N	P=0.285N	P=0.274N	P=0.061N
Liver: Hepatocellular Adenoma				
Overall rate	20/50 (40%)	48/50 (96%)	46/49 (94%)	41/50 (82%)
Adjusted rate	44.4%	96.6%	94.4%	86.5%
Terminal rate	16/31 (52%)	18/18 (100%)	16/18 (89%)	1/2 (50%)
First incidence (days)	665	477	465	450
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rate	7/50 (14%)	37/50 (74%)	47/49 (96%)	47/50 (94%)
Adjusted rate	15.5%	79.5%	96.5%	96.4%
Terminal rate	3/31 (10%)	13/18 (72%)	17/18 (94%)	2/2 (100%)
First incidence (days)	665	493	465	450
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	25/50 (50%)	50/50 (100%)	49/49 (100%)	49/50 (98%)
Adjusted rate	54.8%	100.0%	100.0%	99.7%
Terminal rate	18/31 (58%)	18/18 (100%)	18/18 (100%)	2/2 (100%)
First incidence (days)	665	477	465	450
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Hepatoblastoma				
Overall rate	0/50 (0%)	6/50 (12%)	11/49 (22%)	15/50 (30%)
Adjusted rate	0.0%	15.3%	27.4%	39.3%
Terminal rate	0/31 (0%)	2/18 (11%)	6/18 (33%)	2/2 (100%)
First incidence (days)	—	627	628	505
Poly-3 test	P<0.001	P=0.009	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	7/50 (14%)	38/50 (76%)	48/49 (98%)	49/50 (98%)
Adjusted rate	15.5%	81.0%	98.0%	99.7%
Terminal rate	3/31 (10%)	13/18 (72%)	17/18 (94%)	2/2 (100%)
First incidence (days)	665	493	465	450
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	25/50 (50%)	50/50 (100%)	49/49 (100%)	49/50 (98%)
Adjusted rate	54.8%	100.0%	100.0%	99.7%
Terminal rate	18/31 (58%)	18/18 (100%)	18/18 (100%)	2/2 (100%)
First incidence (days)	665	477	465	450
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/50 (12%)	4/50 (8%)	1/48 (2%)	1/45 (2%)
Adjusted rate	13.2%	10.1%	2.6%	3.2%
Terminal rate	3/31 (10%)	1/18 (6%)	0/18 (0%)	0/2 (0%)
First incidence (days)	632	562	628	681
Poly-3 test	P=0.045N	P=0.455N	P=0.089N	P=0.140N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	7/50 (14%)	4/50 (8%)	1/48 (2%)	2/45 (4%)
Adjusted rate	15.4%	10.1%	2.6%	6.3%
Terminal rate	4/31 (13%)	1/18 (6%)	0/18 (0%)	0/2 (0%)
First incidence (days)	632	562	628	676
Poly-3 test	P=0.071N	P=0.341N	P=0.053N	P=0.199N
Ovary: Cystadenoma				
Overall rate	3/50 (6%)	0/49 (0%)	0/48 (0%)	0/41 (0%)
Adjusted rate	6.7%	0.0%	0.0%	0.0%
Terminal rate	3/31 (10%)	0/17 (0%)	0/18 (0%)	0/2 (0%)
First incidence (days)	731 (T)	—	—	—
Poly-3 test	P=0.047N	P=0.153N	P=0.153N	P=0.221N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	5/48 (10%)	1/44 (2%)	0/44 (0%)	1/40 (3%)
Adjusted rate	11.6%	2.9%	0.0%	3.5%
Terminal rate	4/30 (13%)	1/18 (6%)	0/17 (0%)	0/2 (0%)
First incidence (days)	665	731 (T)	—	626
Poly-3 test	P=0.071N	P=0.161N	P=0.053N	P=0.225N
Skin (Subcutaneous Skin): Fibrosarcoma				
Overall rate	0/50 (0%)	5/50 (10%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	12.6%	2.5%	0.0%
Terminal rate	0/31 (0%)	2/18 (11%)	0/18 (0%)	0/2 (0%)
First incidence (days)	—	578	484	—
Poly-3 test	P=0.359N	P=0.021	P=0.478	—
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	5.2%	7.6%	0.0%
Terminal rate	0/31 (0%)	1/18 (6%)	2/18 (11%)	0/2 (0%)
First incidence (days)	—	652	727	—
Poly-3 test	P=0.544	P=0.207	P=0.097	—
All Organs: Malignant Lymphoma				
Overall rate	15/50 (30%)	8/50 (16%)	8/50 (16%)	1/50 (2%)
Adjusted rate	31.9%	20.1%	19.8%	2.9%
Terminal rate	6/31 (19%)	4/18 (22%)	1/18 (6%)	0/2 (0%)
First incidence (days)	564	562	628	705
Poly-3 test	P<0.001N	P=0.159N	P=0.150N	P<0.001N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
All Organs: Benign Neoplasms				
Overall rate	29/50 (58%)	49/50 (98%)	46/50 (92%)	42/50 (84%)
Adjusted rate	62.3%	98.5%	94.1%	88.5%
Terminal rate	19/31 (61%)	18/18 (100%)	16/18 (89%)	1/2 (50%)
First incidence (days)	630	477	465	450
Poly-3 test	P=0.004	P<0.001	P<0.001	P=0.002
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	42/50 (84%)	48/50 (96%)	49/50 (98%)
Adjusted rate	56.0%	88.6%	97.7%	99.7%
Terminal rate	11/31 (36%)	15/18 (83%)	17/18 (94%)	2/2 (100%)
First incidence (days)	564	493	465	450
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	41/50 (82%)	50/50 (100%)	49/50 (98%)	49/50 (98%)
Adjusted rate	84.4%	100.0%	99.7%	99.7%
Terminal rate	24/31 (77%)	18/18 (100%)	18/18 (100%)	2/2 (100%)
First incidence (days)	564	477	465	450
Poly-3 test	P<0.001	P=0.004	P=0.005	P=0.005

(T)Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE D4
Historical Incidence of Liver Neoplasms in Vehicle Control Female B6C3F$_1$ Mice Receiving Corn Oil by Gavage[a]

Study	Incidence in Controls			
	Hepatocellular Adenoma	**Hepatocellular Carcinoma**	**Hepatocellular Adenoma or Carcinoma**	**Hepatoblastoma**
Historical Incidence at Battelle Columbus Laboratories				
o-Benzyl-p-chlorophenol	11/50	2/50	13/50	0/50
Overall Historical Incidence				
Total (%)	108/511 (21.1%)	37/511 (7.2%)	138/511 (27.0%)	0/511
Mean ± standard deviation	21.0% ± 9.5%	7.2% ± 6.7%	26.8% ± 14.1%	
Range	6%-40%	0%-22%	8%-58%	

		Hepatocellular Carcinoma or Hepatoblastoma	**Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma**	**Hepatocholangiocarcinoma**
Historical Incidence at Battelle Columbus Laboratories				
o-Benzyl-p-chlorophenol		2/50	13/50	0/50
Overall Historical Incidence				
Total (%)		37/511 (7.2%)	138/511 (27.0%)	1/511 (0.2%)
Mean ± standard deviation		7.2% ± 6.7%	26.8% ± 14.1%	0.2% ± 0.6%
Range		0%-22%	8%-58%	0%-2%

[a] Data as of 13 November 1997

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

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1	1	
Moribund	5	7	8	13
Natural deaths	14	24	23	35
Survivors				
Died last week of study			2	
Terminal sacrifice	31	18	16	2
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(38)	(28)	(30)	(24)
Infiltration cellular, lymphocyte		1 (4%)		
Intestine small, duodenum	(46)	(49)	(46)	(43)
Inflammation, chronic active		1 (2%)		
Parasite metazoan		1 (2%)		
Ulcer			1 (2%)	
Epithelium, necrosis		1 (2%)		
Intestine small, jejunum	(46)	(46)	(40)	(40)
Inflammation, suppurative		1 (2%)		
Ulcer		1 (2%)		
Liver	(50)	(50)	(49)	(50)
Atrophy			1 (2%)	
Basophilic focus	5 (10%)	2 (4%)		3 (6%)
Clear cell focus	1 (2%)	2 (4%)		1 (2%)
Cyst	1 (2%)			
Eosinophilic focus	5 (10%)	9 (18%)	4 (8%)	9 (18%)
Fibrosis	1 (2%)	2 (4%)	3 (6%)	
Hematopoietic cell proliferation	4 (8%)	14 (28%)	23 (47%)	24 (48%)
Infiltration cellular, lymphocyte	23 (46%)	18 (36%)	21 (43%)	19 (38%)
Inflammation, chronic active	24 (48%)	25 (50%)	16 (33%)	10 (20%)
Inflammation, suppurative				1 (2%)
Mineralization	1 (2%)	2 (4%)		
Mixed cell focus	7 (14%)	3 (6%)		
Pigmentation, hemosiderin		11 (22%)	24 (49%)	19 (38%)
Tension lipidosis	2 (4%)			
Bile duct, cyst		1 (2%)	6 (12%)	3 (6%)
Bile duct, cyst, multiple	1 (2%)			
Bile duct, hyperplasia	1 (2%)	1 (2%)	11 (22%)	9 (18%)
Hepatocyte, atypia cellular		1 (2%)		
Hepatocyte, hyperplasia, adenomatous				1 (2%)
Hepatocyte, hypertrophy		10 (20%)	7 (14%)	23 (46%)
Hepatocyte, necrosis	5 (10%)	9 (18%)	16 (33%)	17 (34%)
Hepatocyte, vacuolization cytoplasmic	20 (40%)	14 (28%)	11 (22%)	12 (24%)
Hepatocyte, centrilobular, atrophy				1 (2%)
Oval cell, hyperplasia		46 (92%)	36 (73%)	38 (76%)
Mesentery	(13)	(7)	(3)	(3)
Inflammation, chronic active	1 (8%)			
Inflammation, suppurative				1 (33%)
Artery, inflammation, chronic active		1 (14%)		
Artery, necrosis, fibrinoid		1 (14%)		
Fat, necrosis	6 (46%)	3 (43%)	1 (33%)	1 (33%)

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

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Alimentary System (continued)				
Pancreas	(49)	(50)	(46)	(46)
Infiltration cellular, lymphocyte	10 (20%)	15 (30%)	13 (28%)	2 (4%)
Acinus, atrophy		1 (2%)		
Artery, inflammation, chronic active		1 (2%)		
Artery, necrosis, fibrinoid		1 (2%)		
Salivary glands	(50)	(45)	(44)	(42)
Infiltration cellular, lymphocyte	33 (66%)	29 (64%)	27 (61%)	18 (43%)
Stomach, forestomach	(50)	(50)	(48)	(46)
Diverticulum			1 (2%)	
Hyperkeratosis	1 (2%)	3 (6%)		3 (7%)
Inflammation, chronic active		2 (4%)	1 (2%)	
Epithelium, hyperplasia, squamous	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Epithelium, ulcer		2 (4%)		
Stomach, glandular	(45)	(49)	(46)	(45)
Atrophy			10 (22%)	10 (22%)
Ectasia	14 (31%)	33 (67%)	31 (67%)	38 (84%)
Hyperplasia		1 (2%)	5 (11%)	2 (4%)
Hyperplasia, atypical				1 (2%)
Inflammation, chronic active	17 (38%)	21 (43%)	12 (26%)	14 (31%)
Mineralization	2 (4%)	1 (2%)	2 (4%)	3 (7%)
Necrosis		1 (2%)	3 (7%)	2 (4%)
Neuroendocrine cell, hyperplasia		1 (2%)		
Tooth	(2)	(1)		
Inflammation, chronic active	1 (50%)	1 (100%)		
Cardiovascular System				
Blood vessel	(50)	(50)	(49)	(47)
Inflammation, chronic active		1 (2%)		
Mineralization	1 (2%)			
Heart	(50)	(50)	(48)	(44)
Inflammation, chronic active	1 (2%)			
Mineralization	2 (4%)	1 (2%)	1 (2%)	
Artery, inflammation, chronic active		1 (2%)		
Atrium, thrombosis	1 (2%)	3 (6%)	6 (13%)	9 (20%)
Valve, inflammation, suppurative				1 (2%)
Valve, thrombosis				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Degeneration, fatty	1 (2%)			1 (2%)
Hematopoietic cell proliferation	2 (4%)	8 (16%)	17 (34%)	14 (29%)
Pigmentation, hemosiderin				1 (2%)
Subcapsular, hyperplasia	47 (94%)	48 (96%)	49 (98%)	48 (98%)
Adrenal medulla	(50)	(50)	(50)	(49)
Hyperplasia		1 (2%)	7 (14%)	5 (10%)
Islets, pancreatic	(49)	(50)	(46)	(47)
Hyperplasia	8 (16%)	3 (6%)	1 (2%)	2 (4%)
Parathyroid gland	(36)	(40)	(38)	(36)
Cyst	1 (3%)	1 (3%)		
Pituitary gland	(48)	(44)	(44)	(40)
Pars distalis, angiectasis	7 (15%)			
Pars distalis, cyst	1 (2%)		1 (2%)	1 (3%)
Pars distalis, hyperplasia	6 (13%)	8 (18%)	3 (7%)	3 (8%)

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Endocrine System (continued)				
Thyroid gland	(50)	(46)	(46)	(42)
Infiltration cellular, lymphocyte		1 (2%)		
Inflammation, chronic active		1 (2%)		
Follicle, cyst		1 (2%)	1 (2%)	
Follicular cell, hyperplasia	8 (16%)	10 (22%)	7 (15%)	5 (12%)
General Body System				
None				
Genital System				
Clitoral gland	(47)	(47)	(46)	(44)
Cyst		2 (4%)		
Infiltration cellular, lymphocyte		1 (2%)		
Ovary	(50)	(49)	(48)	(41)
Angiectasis	1 (2%)	1 (2%)		
Cyst	12 (24%)	7 (14%)	7 (15%)	4 (10%)
Infiltration cellular, lymphocyte		1 (2%)	1 (2%)	
Mineralization	1 (2%)			
Pigmentation, hemosiderin	1 (2%)			
Thrombosis				2 (5%)
Uterus	(50)	(50)	(49)	(47)
Hemorrhage				1 (2%)
Hydrometra	25 (50%)	24 (48%)	15 (31%)	5 (11%)
Hyperplasia, cystic	19 (38%)	10 (20%)		2 (4%)
Infiltration cellular, lymphocyte	1 (2%)		1 (2%)	
Inflammation, chronic active		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(48)	(50)
Hyperplasia	18 (36%)	47 (94%)	46 (96%)	50 (100%)
Myelofibrosis	10 (20%)	7 (14%)	3 (6%)	3 (6%)
Lymph node	(13)	(7)	(8)	(4)
Inguinal, infiltration cellular, mast cell				1 (25%)
Lumbar, ectasia		1 (14%)		
Mediastinal, hyperplasia, lymphoid	2 (15%)			
Mediastinal, necrosis		1 (14%)		
Pancreatic, hyperplasia		1 (14%)		
Renal, ectasia	1 (8%)			
Lymph node, mandibular	(49)	(43)	(41)	(32)
Hyperplasia, lymphoid	1 (2%)			
Hyperplasia, plasma cell	1 (2%)			
Lymph node, mesenteric	(47)	(48)	(43)	(36)
Angiectasis		1 (2%)	1 (2%)	
Ectasia	1 (2%)	2 (4%)	1 (2%)	
Hematopoietic cell proliferation		1 (2%)	1 (2%)	
Infiltration cellular, lymphocyte	1 (2%)			
Necrosis	1 (2%)	1 (2%)		

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Hematopoietic System (continued)				
Spleen	(50)	(50)	(46)	(46)
Atrophy				1 (2%)
Fibrosis			1 (2%)	
Necrosis	1 (2%)			
Pigmentation, hemosiderin	1 (2%)			
Lymphoid follicle, depletion cellular		1 (2%)		2 (4%)
Lymphoid follicle, hyperplasia, lymphoid	8 (16%)	3 (6%)	2 (4%)	
Lymphoid follicle, necrosis		2 (4%)	4 (9%)	1 (2%)
Red pulp, hematopoietic cell proliferation	29 (58%)	40 (80%)	45 (98%)	44 (96%)
Thymus	(45)	(43)	(40)	(33)
Cyst	14 (31%)	9 (21%)	5 (13%)	7 (21%)
Ectopic parathyroid gland				1 (3%)
Thymocyte, necrosis		2 (5%)	3 (8%)	
Integumentary System				
Mammary gland	(50)	(48)	(50)	(37)
Hyperplasia, cystic	1 (2%)	1 (2%)		
Skin	(50)	(50)	(50)	(49)
Subcutaneous tissue, infiltration cellular, lymphocyte	1 (2%)		2 (4%)	2 (4%)
Subcutaneous tissue, inflammation, chronic active	1 (2%)			
Musculoskeletal System				
Skeletal muscle	(2)	(8)	(3)	(3)
Infiltration cellular, lymphocyte		5 (63%)	1 (33%)	2 (67%)
Artery, inflammation, chronic active		1 (13%)		
Nervous System				
Peripheral nerve		(1)		
Demyelination		1 (100%)		
Spinal cord		(1)		
Demyelination		1 (100%)		
Artery, inflammation, chronic active		1 (100%)		
Artery, necrosis, fibrinoid		1 (100%)		
Respiratory System				
Lung	(50)	(50)	(48)	(45)
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic active		1 (2%)		2 (4%)
Inflammation, suppurative				1 (2%)
Metaplasia, osseous				1 (2%)
Alveolar epithelium, hyperplasia	2 (4%)	1 (2%)		
Alveolus, infiltration cellular, histiocyte	1 (2%)	1 (2%)	2 (4%)	4 (9%)
Bronchiole, exudate, organized			1 (2%)	
Mediastinum, inflammation, chronic active			1 (2%)	
Mediastinum, necrosis			1 (2%)	
Nose	(50)	(50)	(49)	(47)
Inflammation, suppurative	8 (16%)	11 (22%)	13 (27%)	11 (23%)
Nasolacrimal duct, inflammation, suppurative	4 (8%)	15 (30%)	11 (22%)	12 (26%)

Table D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Methyleugenol

	Vehicle Control	37 mg/kg	75 mg/kg	150 mg/kg
Special Senses System				
Eye	(2)		(1)	
Atrophy			1 (100%)	
Hemorrhage	1 (50%)			
Inflammation, suppurative	1 (50%)			
Cornea, inflammation, chronic active	1 (50%)			
Harderian gland	(5)	(2)	(2)	
Inflammation, suppurative			1 (50%)	
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Accumulation, hyaline droplet	1 (2%)	3 (6%)		
Hydronephrosis				2 (4%)
Infiltration cellular, lymphocyte	28 (56%)	29 (58%)	29 (58%)	23 (47%)
Metaplasia, osseous	2 (4%)	1 (2%)		1 (2%)
Mineralization	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Necrosis			1 (2%)	
Nephropathy	10 (20%)	13 (26%)	9 (18%)	10 (20%)
Artery, inflammation, chronic active		2 (4%)		
Cortex, cyst		1 (2%)		2 (4%)
Glomerulus, amyloid deposition				1 (2%)
Renal tubule, hyperplasia			1 (2%)	
Renal tubule, pigmentation, hemosiderin			2 (4%)	2 (4%)
Urinary bladder	(50)	(49)	(47)	(46)
Infiltration cellular, lymphocyte	20 (40%)	29 (59%)	27 (57%)	25 (54%)
Inflammation, chronic active	2 (4%)			

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Mortelmans *et al*. (1986). Methyleugenol was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of methyleugenol. 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.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al*. (1987). Methyleugenol was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of methyleugenol; the high dose was limited by toxicity. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with methyleugenol in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing methyleugenol was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with methyleugenol, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no methyleugenol. Incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity;

increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P < 0.005$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with methyleugenol for 14.7 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with methyleugenol and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10.5 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. Two hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 14-week toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with a chromatin-specific fluorescent dye mixture of Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983) and coded. Slides were scanned to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes (NCEs) in each of ten animals per dose group.

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

EVALUATION PROTOCOL

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

RESULTS

Methyleugenol, tested up to a maximum concentration of 666 μg/plate, did not induce mutations in *S. typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without induced hamster or rat liver S9 activation enzymes (Table E1). In tests for induction of chromosomal effects in cultured CHO cells, methyleugenol induced SCEs in each of two replicate trials conducted with induced rat liver S9; no significant increase in SCEs was observed without S9 (Table E2). Also in cultured CHO cells, no significant induction of Abs occurred following incubation with methyleugenol in either the presence or the absence of S9 (Table E3). The doses tested in the Abs test were similar to those used in the SCE test and were limited by toxicity to 233 μg/mL. Methyleugenol, administered in doses of 10 to 1,000 mg/kg by gavage to male and female B6C3F$_1$ mice for 14 weeks, did not increase the frequency of micronucleated NCEs in peripheral blood and did not alter the percentage of PCEs among total erythrocytes (an indication of bone marrow toxicity) (Table E4).

In conclusion, methyleugenol was not mutagenic in *S. typhimurium* and did not induce chromosomal damage in rodent cells *in vitro* or *in vivo*. A potential for methyleugenol-induced DNA damage was indicated by the positive results seen in the *in vitro* SCE test with CHO cells in the presence of S9 activation enzymes.

TABLE E1
Mutagenicity of Methyleugenol in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	-S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
				Revertants/plate[b]			
TA100	0	120 ± 3.2	90 ± 6.4	106 ± 4.4	103 ± 8.7	111 ± 7.8	98 ± 8.1
	3	101 ± 0.0	86 ± 3.5		90 ± 8.0		95 ± 5.3
	10	100 ± 6.4	93 ± 4.0	106 ± 4.6	89 ± 6.1	93 ± 3.3	94 ± 2.7
	33	114 ± 8.3	93 ± 10.7	109 ± 4.6	90 ± 6.8	109 ± 4.4	92 ± 4.3
	100	105 ± 11.6	96 ± 2.7	116 ± 7.0	80 ± 14.4	110 ± 0.7	91 ± 7.6
	333	29 ± 8.2[c]	16 ± 13.1[c]	99 ± 4.2	78 ± 1.0	95 ± 9.4	97 ± 2.6
	666			38 ± 38.0[c]		0 ± 0.0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		358 ± 7.0	388 ± 4.3	1,469 ± 61.2	1,111 ± 49.2	534 ± 46.0	351 ± 22.9
TA1535	0	24 ± 2.6	20 ± 3.5	10 ± 2.4	12 ± 2.1	10 ± 2.2	9 ± 0.6
	3	37 ± 0.6	20 ± 2.3		8 ± 0.9		6 ± 0.0
	10	33 ± 2.0	21 ± 2.3	13 ± 3.5	8 ± 2.3	8 ± 0.9	7 ± 0.3
	33	37 ± 7.5	22 ± 3.3	14 ± 1.5	9 ± 2.8	9 ± 0.0	9 ± 2.6
	100	32 ± 3.5	26 ± 2.7	11 ± 2.2	10 ± 3.7	6 ± 1.5	7 ± 1.0
	333	5 ± 5.0[c]	2 ± 0.7[c]	10 ± 2.1	9 ± 2.3	4 ± 0.3	8 ± 0.9
	666			4 ± 2.6[c]		0 ± 0.0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		375 ± 15.5	410 ± 15.2	381 ± 7.9	369 ± 20.8	146 ± 2.8	168 ± 21.2
TA1537	0	5 ± 0.6	5 ± 0.3	6 ± 1.0	5 ± 0.3	7 ± 0.7	6 ± 0.9
	3	6 ± 1.5	3 ± 0.9		9 ± 1.5		8 ± 2.1
	10	4 ± 1.2	3 ± 0.9	5 ± 1.2	6 ± 0.9	5 ± 0.9	4 ± 1.0
	33	5 ± 0.3	4 ± 1.2	5 ± 1.5	5 ± 1.2	4 ± 0.3	9 ± 1.5
	100	4 ± 1.5	4 ± 0.6	6 ± 1.5	5 ± 1.0	7 ± 0.0	7 ± 1.2
	333	0 ± 0.0[c]	3 ± 0.03[c]	3 ± 1.2	4 ± 1.3	6 ± 0.3	5 ± 2.2
	666			Toxic		0 ± 0.0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		184 ± 4.7	521 ± 48.1	424 ± 75.9	426 ± 15.5	157 ± 8.7	124 ± 11.9
TA98	0	15 ± 0.6	16 ± 1.7	25 ± 2.2	31 ± 3.7	17 ± 3.6	20 ± 4.1
	3	15 ± 0.7	13 ± 2.2		31 ± 4.0		27 ± 0.9
	10	15 ± 1.0	14 ± 0.9	27 ± 3.5	28 ± 2.3	24 ± 2.7	23 ± 2.6
	33	15 ± 0.9	13 ± 1.8	31 ± 5.8	26 ± 1.2	29 ± 2.0	20 ± 2.3
	100	14 ± 3.5	13 ± 0.9	28 ± 0.7	29 ± 6.0	20 ± 3.5	29 ± 5.5
	333	0 ± 0.0[c]	3 ± 3.0[c]	15 ± 7.7[c]	21 ± 2.7	29 ± 5.0	19 ± 0.3
	666			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		626 ± 20.6	412 ± 8.2	1,274 ± 85.7	1,362 ± 55.5	473 ± 34.3	444 ± 76.4

[a] Study performed at SRI International. The detailed protocol is presented by Mortelmans *et al*. (1986). 0 µg/plate dose was the solvent control.

[b] Revertants are presented as mean ± the standard error from three plates.

[c] Slight toxicity

[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 4-nitro-o-phenylenediamine (TA98), and 9-aminoacridine (TA1537). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Methyleugenol[a]

Compound	Concen-tration (μg/mL)	Total Cells Scored	Total Chromo-somes	Total SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Summary: Negative								
Dimethylsulfoxide[c]		50	1,044	392	0.37	7.8	26.0	
Mitomycin-C[d]	0.001	50	1,046	549	0.52	11.0	26.0	39.78
	0.004	10	210	182	0.86	18.2	26.0	130.82
Methyleugenol								
	5	50	1,046	373	0.35	7.5	26.0	–5.03
	17	50	1,039	405	0.38	8.1	26.0	3.81
	50	50	1,044	448	0.42	9.0	26.0	14.29
	167	0						
				$P=0.013$[e]				
+S9								
Trial 1								
Summary: Positive								
Dimethylsulfoxide		50	1,047	359	0.34	7.2	26.0	
Cyclophosphamide[d]	0.125	50	1,048	675	0.64	13.5	26.0	87.84
	0.500	10	208	204	0.98	20.4	26.0	186.04
Methyleugenol								
	17	50	1,048	469	0.44	9.4	26.0	30.52*
	50	50	1,045	421	0.40	8.4	26.0	17.50*
	167	50	1,044	607	0.58	12.1	26.0	69.57*
	500	0						
				$P\leq0.000$				
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,050	398	0.37	8.0	26.0	
Cyclophosphamide	0.125	50	1,046	588	0.56	11.8	26.0	48.31
	0.500	10	208	207	0.99	20.7	26.0	162.56
Methyleugenol								
	50	50	1,047	430	0.41	8.6	26.0	8.35
	167	50	1,047	477	0.45	9.5	26.0	20.19*
	250	50	1,048	548	0.52	11.0	26.0	37.95*
				$P\leq0.000$				

* Positive response ($P\geq20\%$ increase over solvent control)
[a] Study was performed at Sitek Research Laboratories. The detailed protocol is presented by Galloway *et al*. (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Solvent control
[d] Positive control
[e] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Methyleugenol[a]

Compound	Concentration (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/Cell	Cells with Aberrations (%)
-S9					
Harvest time: 16.7 hours					
Summary: Negative					
Dimethylsulfoxide[b]		200	1	0.01	0.5
Mitomycin-C[c]	0.4	25	30	1.20	56.0
Methyleugenol					
	50	200	2	0.01	1.0
	108	200	2	0.01	1.0
	233	200	0	0.00	0.0
	500	0			
					P=0.726[d]
+S9					
Harvest time: 12.5 hours					
Summary: Negative					
Dimethylsulfoxide		200	3	0.02	1.5
Cyclophosphamide	20	25	15	0.60	40.0
Methyleugenol					
	50	200	3	0.02	1.5
	108	200	8	0.04	4.0
	233	200	10	0.05	4.5
	500	0			
					P=0.015

[a] Study was performed at Sitek Research Laboratories. The detailed protocol is presented by Galloway *et al*. (1987).
[b] Solvent control
[c] Positive control
[d] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose

TABLE E4
**Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Gavage Administration
of Methyleugenol for 14 Weeks[a]**

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	PCEs (%)
Male				
Methyleugenol				
	0	10	0.81 ± 0.10	1.40 ± 0.05
	10	10	0.70 ± 0.10	1.02 ± 0.06
	30	10	0.78 ± 0.10	1.51 ± 0.08
	100	10	0.84 ± 0.05	1.61 ± 0.11
	300	10	0.63 ± 0.06	1.18 ± 0.12
	1,000	10	0.65 ± 0.02	1.16 ± 0.11
			P=0.915[c]	
Female				
Methyleugenol				
	0	10	0.46 ± 0.09	1.27 ± 0.15
	10	10	0.45 ± 0.06	1.15 ± 0.13
	30	10	0.43 ± 0.06	1.38 ± 0.13
	100	10	0.54 ± 0.08	1.41 ± 0.09
	300	10	0.45 ± 0.08	1.37 ± 0.10
	1,000	10	0.63 ± 0.08	1.21 ± 0.13
			P=0.027	

[a]   Study was performed at SRI International. The detailed protocol is presented by MacGregor *et al*. (1990). NCE=normochromatic
      erythrocyte; PCE=polychromatic erythrocyte
[b]   Mean ± standard error
[c]   Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025 (ILS, 1990)

APPENDIX F
HEMATOLOGY AND CLINICAL CHEMISTRY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Methyleugenol[a]

	Water Control	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	1,000 mg/kg
Male							
Hematology							
n							
Day 5	10	10	10	10	10	10	10
Week 4	10	9	9	10	10	10	10
Week 14	10	10	10	10	10	9	10
Hematocrit (%)							
Day 5	41.0 ± 0.4	40.5 ± 0.4	39.5 ± 0.5	40.5 ± 0.6	39.8 ± 0.5	40.7 ± 0.6	40.7 ± 0.5
Week 4	43.7 ± 0.5	43.7 ± 0.5	43.8 ± 0.6	46.2 ± 0.4*	44.9 ± 0.4	45.6 ± 0.6	41.2 ± 1.1
Week 14	45.6 ± 0.5	45.8 ± 0.7	45.5 ± 0.3	45.4 ± 0.3	47.0 ± 0.3	46.5 ± 0.4	40.0 ± 1.4**
Hemoglobin (g/dL)							
Day 5	13.5 ± 0.1	13.5 ± 0.2	13.1 ± 0.2	13.4 ± 0.2	13.3 ± 0.1	13.4 ± 0.2	13.5 ± 0.2
Week 4	15.3 ± 0.2	15.2 ± 0.2	15.2 ± 0.2	16.0 ± 0.2*	15.5 ± 0.1	15.7 ± 0.2	14.4 ± 0.4
Week 14	14.7 ± 0.1	14.9 ± 0.2	14.8 ± 0.1	14.7 ± 0.1	15.3 ± 0.1	15.3 ± 0.1	13.3 ± 0.4**
Erythrocytes ($10^6/\mu L$)							
Day 5	6.99 ± 0.13	6.94 ± 0.08	6.73 ± 0.11	6.90 ± 0.13	6.82 ± 0.10	7.01 ± 0.11	7.09 ± 0.12
Week 4	7.73 ± 0.14	7.75 ± 0.10	7.74 ± 0.14	8.26 ± 0.08**	8.01 ± 0.09*	8.24 ± 0.12**	8.68 ± 0.07**
Week 14	9.14 ± 0.09	9.08 ± 0.13	9.03 ± 0.07	8.96 ± 0.06	9.32 ± 0.05	9.32 ± 0.08	9.22 ± 0.10
Reticulocytes ($10^6/\mu L$)							
Day 5	0.34 ± 0.02	0.34 ± 0.02	0.44 ± 0.04	0.32 ± 0.03	0.33 ± 0.04	0.40 ± 0.05	0.25 ± 0.04
Week 4	0.33 ± 0.06	0.24 ± 0.03	0.31 ± 0.05	0.27 ± 0.04	0.25 ± 0.03	0.28 ± 0.04	0.31 ± 0.03
Week 14	0.15 ± 0.02	0.12 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.15 ± 0.01	0.15 ± 0.01	0.11 ± 0.02
Mean cell volume (fL)							
Day 5	58.9 ± 0.5	58.3 ± 0.4	58.9 ± 0.4	58.8 ± 0.4	58.2 ± 0.3	58.0 ± 0.3	57.4 ± 0.3
Week 4	56.4 ± 0.5	56.3 ± 0.2	56.7 ± 0.3	55.9 ± 0.3	56.1 ± 0.2	55.3 ± 0.2**	47.5 ± 1.2**
Week 14	49.9 ± 0.1	50.6 ± 0.2▲▲	50.6 ± 0.2	50.7 ± 0.2	50.3 ± 0.2	49.9 ± 0.1**	43.3 ± 1.6**
Mean cell hemoglobin (pg)							
Day 5	19.4 ± 0.2	19.5 ± 0.1	19.5 ± 0.2	19.5 ± 0.1	19.5 ± 0.2	19.1 ± 0.1	19.1 ± 0.1*
Week 4	19.8 ± 0.2	19.5 ± 0.2	19.7 ± 0.2	19.3 ± 0.2	19.3 ± 0.1	19.0 ± 0.1*	16.6 ± 0.4**
Week 14	16.1 ± 0.1	16.4 ± 0.1▲	16.4 ± 0.1	16.4 ± 0.1	16.4 ± 0.0	16.4 ± 0.1	14.4 ± 0.5**
Mean cell hemoglobin concentration (g/dL)							
Day 5	32.9 ± 0.2	33.4 ± 0.2	33.2 ± 0.1	33.1 ± 0.1	33.4 ± 0.2	33.0 ± 0.1	33.2 ± 0.2
Week 4	35.0 ± 0.2	34.7 ± 0.4	34.8 ± 0.2	34.5 ± 0.3	34.5 ± 0.2	34.4 ± 0.2	35.0 ± 0.2
Week 14	32.4 ± 0.1	32.6 ± 0.1	32.4 ± 0.1	32.4 ± 0.2	32.5 ± 0.1	32.8 ± 0.1	33.2 ± 0.2
Platelets ($10^3/\mu L$)							
Day 5	885.6 ± 11.7	903.8 ± 20.8	893.2 ± 13.9	897.2 ± 26.4	889.0 ± 16.9	953.8 ± 14.7	1,030.5 ± 39.2
Week 4	783.6 ± 29.3	767.9 ± 12.7	778.6 ± 18.2	817.7 ± 16.3*	870.2 ± 14.0**	983.3 ± 7.2**[b]	1,045.6 ± 56.9**
Week 14	635.4 ± 10.2	681.9 ± 27.8	650.4 ± 8.4	663.1 ± 12.7	716.8 ± 7.8**	781.9 ± 10.9**	937.9 ± 78.4**
Leukocytes ($10^3/\mu L$)							
Day 5	5.78 ± 0.67	6.91 ± 0.58	5.13 ± 0.78	5.94 ± 0.52	5.13 ± 0.50	6.67 ± 0.61	5.91 ± 0.70
Week 4	8.40 ± 0.45	6.13 ± 0.49▲	8.10 ± 0.64	8.12 ± 0.48	7.53 ± 0.56	7.59 ± 0.72	7.77 ± 0.79
Week 14	8.81 ± 0.58	9.14 ± 0.58	10.26 ± 0.50	9.44 ± 0.63	11.99 ± 0.43*	8.98 ± 1.10	10.13 ± 1.01
Segmented neutrophils ($10^3/\mu L$)							
Day 5	0.88 ± 0.11	1.18 ± 0.11▲	0.91 ± 0.21	0.90 ± 0.13	0.83 ± 0.11	0.98 ± 0.14	0.91 ± 0.13
Week 4	0.84 ± 0.12	0.78 ± 0.11	1.07 ± 0.14	0.75 ± 0.06	0.94 ± 0.15	1.01 ± 0.11	1.32 ± 0.20
Week 14	1.35 ± 0.13	1.50 ± 0.22	1.51 ± 0.09	1.52 ± 0.25	2.43 ± 0.32	1.87 ± 0.33	1.80 ± 0.23
Lymphocytes ($10^3/\mu L$)							
Day 5	4.46 ± 0.52	5.30 ± 0.47	3.85 ± 0.52	4.66 ± 0.43	4.01 ± 0.41	5.14 ± 0.48	4.61 ± 0.56
Week 4	7.37 ± 0.43	5.18 ± 0.44▲▲	6.78 ± 0.50	7.15 ± 0.47*	6.31 ± 0.47	6.43 ± 0.63	6.20 ± 0.62
Week 14	7.04 ± 0.55	7.40 ± 0.43	8.27 ± 0.50	7.49 ± 0.48	9.01 ± 0.41	6.78 ± 0.77	8.14 ± 0.84

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Methyleugenol

	Water Control	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	1,000 mg/kg
Male (continued)							
Hematology (continued)							
n							
Day 5	10	10	10	10	10	10	10
Week 4	10	9	9	10	10	10	10
Week 14	10	10	10	10	10	9	10
Monocytes ($10^3/\mu$L)							
Day 5	0.42 ± 0.08	0.36 ± 0.08	0.32 ± 0.09	0.35 ± 0.07	0.26 ± 0.05	0.46 ± 0.11	0.41 ± 0.10
Week 4	0.17 ± 0.05	0.17 ± 0.04	0.17 ± 0.08	0.19 ± 0.04	0.21 ± 0.05	0.13 ± 0.04	0.21 ± 0.04
Week 14	0.31 ± 0.04	0.15 ± 0.04▲	0.36 ± 0.07	0.30 ± 0.07	0.42 ± 0.12	0.29 ± 0.10	0.14 ± 0.03
Eosinophils ($10^3/\mu$L)							
Day 5	0.02 ± 0.01	0.06 ± 0.03	0.03 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.01 ± 0.01
Week 4	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.03	0.02 ± 0.01	0.05 ± 0.03
Week 14	0.11 ± 0.03	0.09 ± 0.04	0.12 ± 0.04	0.14 ± 0.06	0.09 ± 0.03	0.06 ± 0.03	0.03 ± 0.02
Clinical Chemistry							
n							
Day 5	10	10	10	10	10	10	10
Week 4	10	10	10	10	10	10	10
Week 14	10	10	10	10	10	9	10
Urea nitrogen (mg/dL)							
Day 5	19.3 ± 0.6	19.7 ± 0.7	20.0 ± 0.7	20.6 ± 0.8	20.5 ± 0.6	21.5 ± 0.8	19.4 ± 0.8
Week 4	20.2 ± 0.3	22.3 ± 0.5▲▲	22.4 ± 0.3	22.7 ± 0.9	19.3 ± 0.3**	21.6 ± 0.6*	19.6 ± 0.7**
Week 14	21.1 ± 0.8	21.1 ± 0.5	20.4 ± 0.4	20.2 ± 0.6	21.5 ± 0.5	19.5 ± 0.7	19.3 ± 1.0
Creatinine (mg/dL)							
Day 5	0.50 ± 0.02	0.45 ± 0.60[b]	0.54 ± 0.02	0.49 ± 0.02[b]	0.53 ± 0.02	0.57 ± 0.03[b]	0.61 ± 0.03**[b]
Week 4	0.68 ± 0.02	0.68 ± 0.02	0.66 ± 0.03	0.66 ± 0.02	0.69 ± 0.01	0.68 ± 0.01	0.71 ± 0.01
Week 14	0.64 ± 0.04	0.66 ± 0.02	0.64 ± 0.01	0.62 ± 0.03	0.66 ± 0.02	0.71 ± 0.03	0.71 ± 0.02
Total protein (g/dL)							
Day 5	5.71 ± 0.06	5.67 ± 0.06	5.58 ± 0.08	5.33 ± 0.35	5.65 ± 0.06	5.44 ± 0.06*	5.00 ± 0.07**
Week 4	5.99 ± 0.19	6.30 ± 0.05	6.21 ± 0.07	6.36 ± 0.07	6.03 ± 0.14	6.22 ± 0.12	6.09 ± 0.12
Week 14	6.89 ± 0.10	7.18 ± 0.06▲	7.01 ± 0.08	6.96 ± 0.04*	7.01 ± 0.08	6.81 ± 0.11**	6.36 ± 0.10**
Albumin (g/dL)							
Day 5	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.0	3.3 ± 0.2	3.4 ± 0.0	3.1 ± 0.2*	3.1 ± 0.1**
Week 4	3.7 ± 0.0	3.7 ± 0.1	3.6 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	3.7 ± 0.1	3.6 ± 0.1
Week 14	3.9 ± 0.1	4.0 ± 0.0	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	3.6 ± 0.1**
Alanine aminotransferase (IU/L)							
Day 5	48 ± 2	50 ± 2	57 ± 3	50 ± 2	54 ± 2	70 ± 4**	96 ± 4**
Week 4	42 ± 2	45 ± 1	47 ± 2	50 ± 4	47 ± 2	48 ± 2	55 ± 4*
Week 14	50 ± 3	47 ± 1	45 ± 2	49 ± 2	60 ± 2**	69 ± 4**	82 ± 5**
Alkaline phosphatase (IU/L)							
Day 5	753 ± 17	761 ± 21	765 ± 18	749 ± 27	757 ± 19	759 ± 22	723 ± 28
Week 4	573 ± 14	577 ± 20	572 ± 12	554 ± 21	557 ± 18	545 ± 18	509 ± 13**
Week 14	270 ± 6	264 ± 6	264 ± 7	258 ± 7	238 ± 7*	207 ± 6**	263 ± 13
Creatine kinase (IU/L)							
Day 5	492 ± 74	498 ± 52[b]	540 ± 71	462 ± 57	570 ± 105	643 ± 68	611 ± 62
Week 4	432 ± 56	414 ± 34	415 ± 38[b]	522 ± 80	375 ± 20	481 ± 61	673 ± 94
Week 14	599 ± 130[b]	634 ± 155[b]	482 ± 105	529 ± 79	489 ± 77	593 ± 85	531 ± 68

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Methyleugenol

	Water Control	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	1,000 mg/kg
Male (continued)							
Clinical Chemistry (continued)							
n							
Day 5	10	10	10	10	10	10	10
Week 4	10	10	10	10	10	10	10
Week 14	10	10	10	10	10	9	10
Sorbitol dehydrogenase (IU/L)							
Day 5	5 ± 1^c	5 ± 1^c	5 ± 1	5 ± 1^b	5 ± 1^d	6 ± 1^c	6 ± 1^b
Week 4	8 ± 1	7 ± 1	7 ± 1	8 ± 1	7 ± 0	7 ± 0	11 ± 1
Week 14	10 ± 1	10 ± 1	9 ± 0	10 ± 0	11 ± 1	$15 \pm 1^{**}$	$15 \pm 1^{**}$
Bile acids (μmol/L)							
Day 5	24.9 ± 2.6	21.9 ± 2.9^c	24.9 ± 4.6	26.6 ± 2.9^b	21.5 ± 3.6^c	$39.4 \pm 7.8^{*b}$	$48.0 \pm 8.0^{**c}$
Week 4	13.0 ± 3.0	11.2 ± 1.2	11.5 ± 0.9	12.1 ± 1.9	10.4 ± 0.6	$15.6 \pm 1.9^*$	$46.2 \pm 12.9^{**}$
Week 14	12.5 ± 2.6	9.5 ± 1.2	10.4 ± 1.5	10.2 ± 1.7	8.5 ± 0.7	$19.7 \pm 3.3^{**}$	$89.4 \pm 26.6^{**}$
Female							
Hematology							
n							
Day 5	10	10	10	10	10	10	10
Week 4	10	10	10	10	10	9	10
Week 14	10	10	10	10	10	10	10
Hematocrit (%)							
Day 5	41.7 ± 0.6	41.2 ± 0.5	40.3 ± 0.8	41.7 ± 0.6	41.6 ± 0.6	40.2 ± 0.7	39.9 ± 0.4
Week 4	44.9 ± 0.4	44.7 ± 0.4	44.7 ± 0.6	45.3 ± 0.5	45.4 ± 0.6	45.4 ± 0.4	44.2 ± 0.9
Week 14	45.8 ± 0.5	45.7 ± 0.6	45.2 ± 0.6	45.3 ± 0.3	45.0 ± 0.6	44.4 ± 0.3	$42.9 \pm 0.6^{**}$
Hemoglobin (g/dL)							
Day 5	13.9 ± 0.2	13.7 ± 0.1	13.4 ± 0.3	14.0 ± 0.2	13.8 ± 0.2	13.3 ± 0.3	13.3 ± 0.2
Week 4	14.9 ± 0.2	14.9 ± 0.1	15.0 ± 0.2	15.1 ± 0.2	15.1 ± 0.2	15.1 ± 0.2	14.7 ± 0.3
Week 14	15.1 ± 0.1	15.1 ± 0.1	14.9 ± 0.1	14.9 ± 0.1	14.8 ± 0.1	$14.5 \pm 0.1^{**}$	$14.1 \pm 0.2^{**}$
Erythrocytes ($10^6/\mu$L)							
Day 5	7.38 ± 0.12	7.27 ± 0.11	7.01 ± 0.15	7.36 ± 0.15	7.32 ± 0.13	7.17 ± 0.15	7.17 ± 0.08
Week 4	7.87 ± 0.10	7.81 ± 0.09	7.81 ± 0.10	7.89 ± 0.08	7.85 ± 0.10	8.06 ± 0.09	$8.73 \pm 0.10^{**}$
Week 14	8.60 ± 0.10	8.51 ± 0.11	8.48 ± 0.10	8.45 ± 0.10	8.37 ± 0.10	8.35 ± 0.08	8.45 ± 0.11
Reticulocytes ($10^6/\mu$L)							
Day 5	0.23 ± 0.03	0.24 ± 0.03	0.16 ± 0.03	0.21 ± 0.02	0.20 ± 0.02	0.17 ± 0.02	$0.13 \pm 0.01^*$
Week 4	0.09 ± 0.04	$0.04 \pm 0.01^{\blacktriangle}$	0.07 ± 0.01	0.06 ± 0.02	$0.08 \pm 0.02^*$	$0.10 \pm 0.01^{**}$	$0.10 \pm 0.01^{**}$
Week 14	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.16 ± 0.03	0.15 ± 0.02	0.17 ± 0.03	0.17 ± 0.02
Mean cell volume (fL)							
Day 5	56.5 ± 0.3	56.9 ± 0.3	57.6 ± 0.3	56.7 ± 0.3	57.0 ± 0.3	56.3 ± 0.3	$55.4 \pm 0.2^{**}$
Week 4	57.0 ± 0.3	57.1 ± 0.2	57.1 ± 0.3	57.5 ± 0.3	57.8 ± 0.4	56.4 ± 0.2	$50.6 \pm 0.9^{**}$
Week 14	53.4 ± 0.2	53.7 ± 0.2	53.4 ± 0.2	53.5 ± 0.2	53.7 ± 0.2	53.3 ± 0.2	$50.7 \pm 0.2^{**}$
Mean cell hemoglobin (pg)							
Day 5	18.8 ± 0.1	18.9 ± 0.2	19.2 ± 0.1	19.0 ± 0.1	18.8 ± 0.1	18.6 ± 0.1	18.6 ± 0.1
Week 4	19.0 ± 0.1	19.1 ± 0.2	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	18.8 ± 0.2	$16.8 \pm 0.2^{**}$
Week 14	17.6 ± 0.1	17.7 ± 0.1	17.6 ± 0.1	17.7 ± 0.1	17.7 ± 0.1	$17.3 \pm 0.1^{**}$	$16.8 \pm 0.1^{**}$

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Methyleugenol

	Water Control	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	1,000 mg/kg
Female (continued)							
Hematology (continued)							
n							
Day 5	10	10	10	10	10	10	10
Week 4	10	10	10	10	10	9	10
Week 14	10	10	10	10	10	10	10
Mean cell hemoglobin concentration (g/dL)							
Day 5	33.2 ± 0.1	33.3 ± 0.2	33.3 ± 0.2	33.5 ± 0.1	33.1 ± 0.2	33.1 ± 0.1	33.5 ± 0.2
Week 4	33.3 ± 0.1	33.3 ± 0.2	33.5 ± 0.1	33.4 ± 0.2	33.2 ± 0.1	33.3 ± 0.3	33.3 ± 0.3
Week 14	33.0 ± 0.1	33.1 ± 0.1	33.0 ± 0.2	32.9 ± 0.1	32.9 ± 0.2	32.6 ± 0.1	33.0 ± 0.2
Platelets ($10^3/\mu L$)							
Day 5	897.2 ± 36.0	837.9 ± 23.1	917.1 ± 24.7*	865.3 ± 29.0	913.8 ± 15.7*	1,003.3 ± 32.0**	1,057.4 ± 30.8**
Week 4	795.3 ± 24.7	764.3 ± 22.6	760.5 ± 34.6	816.1 ± 37.8	852.4 ± 38.8	846.3 ± 51.8[c]	977.9 ± 42.9**
Week 14	616.2 ± 8.8	664.6 ± 27.4▲	631.8 ± 14.1	662.2 ± 13.7	718.2 ± 28.5*	738.5 ± 19.0**	842.6 ± 37.6**
Leukocytes ($10^3/\mu L$)							
Day 5	4.58 ± 0.65	5.31 ± 0.65	4.52 ± 0.66	5.92 ± 0.78	4.97 ± 0.52	5.19 ± 0.92	7.03 ± 0.85
Week 4	6.26 ± 0.94	5.53 ± 0.74	6.06 ± 0.78	5.15 ± 0.72	6.05 ± 0.92	7.66 ± 0.60	7.19 ± 1.13
Week 14	6.43 ± 0.42	5.50 ± 0.58	5.62 ± 0.65	6.33 ± 0.52	6.23 ± 0.62	7.74 ± 0.72*	8.78 ± 0.47**
Segmented neutrophils ($10^3/\mu L$)							
Day 5	0.58 ± 0.08	0.77 ± 0.10	0.44 ± 0.07	0.68 ± 0.11	0.72 ± 0.11	0.65 ± 0.11	1.10 ± 0.16
Week 4	0.76 ± 0.11	0.61 ± 0.07	0.78 ± 0.08	0.60 ± 0.09	0.89 ± 0.16	0.92 ± 0.10	0.84 ± 0.12
Week 14	0.87 ± 0.08	0.88 ± 0.15	1.13 ± 0.23	0.96 ± 0.11	1.17 ± 0.18	1.35 ± 0.34	0.91 ± 0.07
Lymphocytes ($10^3/\mu L$)							
Day 5	3.86 ± 0.56	4.38 ± 0.56	3.99 ± 0.60	5.08 ± 0.69	4.10 ± 0.45	4.35 ± 0.79	5.73 ± 0.69
Week 4	5.19 ± 0.77	4.64 ± 0.63	5.06 ± 0.69	4.32 ± 0.58	4.94 ± 0.75	6.48 ± 0.56	6.10 ± 0.95
Week 14	5.33 ± 0.37	4.44 ± 0.45	4.30 ± 0.47	5.12 ± 0.43	4.86 ± 0.50	6.12 ± 0.39*	7.64 ± 0.43**
Monocytes ($10^3/\mu L$)							
Day 5	0.10 ± 0.03	0.13 ± 0.04	0.05 ± 0.03	0.14 ± 0.04	0.11 ± 0.03	0.13 ± 0.08	0.17 ± 0.06
Week 4	0.22 ± 0.07	0.24 ± 0.07	0.14 ± 0.04	0.15 ± 0.06	0.12 ± 0.04	0.19 ± 0.08	0.17 ± 0.06
Week 14	0.17 ± 0.05	0.14 ± 0.03	0.09 ± 0.02	0.17 ± 0.06	0.16 ± 0.02	0.19 ± 0.07	0.17 ± 0.04
Eosinophils ($10^3/\mu L$)							
Day 5	0.04 ± 0.02	0.03 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
Week 4	0.07 ± 0.03	0.05 ± 0.02	0.05 ± 0.03	0.06 ± 0.03	0.05 ± 0.02	0.03 ± 0.02	0.05 ± 0.03
Week 14	0.07 ± 0.02	0.07 ± 0.03	0.08 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	0.07 ± 0.03	0.04 ± 0.02
Clinical Chemistry							
n	10	10	10	10	10	10	10
Urea nitrogen (mg/dL)							
Day 5	19.3 ± 0.9	19.5 ± 0.5	20.5 ± 0.5	19.5 ± 0.4	20.0 ± 0.5	19.6 ± 0.6	19.9 ± 0.6
Week 4	22.5 ± 0.8	20.6 ± 0.4▲	22.6 ± 0.7	21.8 ± 0.7	20.8 ± 0.5	19.2 ± 0.5	19.3 ± 0.9
Week 14	23.5 ± 0.5	24.6 ± 0.5	23.5 ± 0.9	21.1 ± 0.6**	21.9 ± 0.6**	23.5 ± 0.5*	20.5 ± 0.8**
Creatinine (mg/dL)							
Day 5	0.51 ± 0.04	0.49 ± 0.02[b]	0.48 ± 0.02[b]	0.47 ± 0.03	0.55 ± 0.01*	0.57 ± 0.02**	0.61 ± 0.01**
Week 4	0.59 ± 0.01	0.59 ± 0.01	0.58 ± 0.02	0.62 ± 0.01	0.64 ± 0.01**	0.67 ± 0.01**	0.71 ± 0.01**
Week 14	0.59 ± 0.04	0.61 ± 0.03	0.62 ± 0.02	0.67 ± 0.02	0.64 ± 0.01	0.72 ± 0.03**	0.75 ± 0.03**
Total protein (g/dL)							
Day 5	5.8 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	4.9 ± 0.1**
Week 4	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	5.8 ± 0.2	5.7 ± 0.1**	5.7 ± 0.1**
Week 14	7.4 ± 0.1	7.1 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	6.7 ± 0.1*	6.4 ± 0.1**	6.2 ± 0.1**

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Gavage Study of Methyleugenol

	Water Control	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	1,000 mg/kg
Female (continued)							
Clinical Chemistry (continued)							
n	10	10	10	10	10	10	10
Albumin (g/dL)							
Day 5	4.1 ± 0.1	4.0 ± 0.1[b]	4.0 ± 0.1[b]	3.9 ± 0.1	4.0 ± 0.1	3.8 ± 0.1	3.3 ± 0.1**
Week 4	3.8 ± 0.1	3.8 ± 0.1	3.6 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	3.5 ± 0.1*
Week 14	4.5 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	3.9 ± 0.1**	3.7 ± 0.1**
Alanine aminotransferase (IU/L)							
Day 5	44 ± 1	43 ± 1	44 ± 2	44 ± 1	49 ± 2**	61 ± 3**	68 ± 7**
Week 4	34 ± 1	34 ± 1	35 ± 1	36 ± 1	37 ± 1*	45 ± 1**	62 ± 3**
Week 14	45 ± 1	49 ± 3	44 ± 2	45 ± 2	46 ± 2	54 ± 2	77 ± 4**
Alkaline phosphatase (IU/L)							
Day 5	619 ± 21	616 ± 13	627 ± 22	644 ± 25	612 ± 24	621 ± 17	489 ± 20**
Week 4	458 ± 16	407 ± 11▲	438 ± 11	424 ± 11	402 ± 11	357 ± 12*	387 ± 12
Week 14	222 ± 10	245 ± 10	229 ± 6	228 ± 6	211 ± 7	194 ± 10**	277 ± 17
Creatine kinase (IU/L)							
Day 5	643 ± 111	580 ± 62	600 ± 100	488 ± 73	666 ± 111	524 ± 54[b]	614 ± 74
Week 4	359 ± 35	308 ± 40	405 ± 39	318 ± 38	363 ± 49	507 ± 65*	481 ± 76
Week 14	365 ± 50	423 ± 65	305 ± 31	403 ± 58	415 ± 64	486 ± 83	513 ± 67
Sorbitol dehydrogenase (IU/L)							
Day 5	4 ± 0	4 ± 0	4 ± 0	4 ± 0	4 ± 0	6 ± 0*	5 ± 0*
Week 4	6 ± 0	5 ± 0▲	5 ± 0	5 ± 0	6 ± 0**	6 ± 0**	8 ± 0**
Week 14	7 ± 0	8 ± 0▲	7 ± 0	7 ± 0	7 ± 0	9 ± 1	11 ± 1
Bile acids (μmol/L)							
Day 5	24.4 ± 3.7	27.1 ± 4.4[b]	18.4 ± 1.8[b]	23.3 ± 2.4	25.0 ± 2.5	34.3 ± 3.3[b]	44.1 ± 7.3
Week 4	16.6 ± 4.6	14.2 ± 2.0	15.5 ± 2.6	14.3 ± 2.3	18.7 ± 3.2	33.4 ± 5.1**	66.7 ± 6.5**
Week 14	15.6 ± 3.1	15.0 ± 2.5	14.3 ± 2.2[b]	17.0 ± 2.5	24.4 ± 5.8	33.3 ± 7.1*	180.7 ± 39.4**

*   Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** (P≤0.01)
▲ Vehicle controls significantly different (P≤0.05) from the water control group by Dunn's or Shirley's test
▲▲ P≤0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=8
[d] n=6

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

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Gavage Study of Methyleugenol[a]

	Water Control	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	1,000 mg/kg
Male							
n	10	10	10	10	10	9	10
Necropsy body wt	340 ± 6	343 ± 8	343 ± 5	345 ± 8	336 ± 7	314 ± 4**	236 ± 5**
Heart							
Absolute	0.971 ± 0.020	0.981 ± 0.025	0.935 ± 0.020	1.011 ± 0.017	0.944 ± 0.023	0.929 ± 0.015	0.778 ± 0.021**
Relative	2.86 ± 0.05	2.86 ± 0.04	2.73 ± 0.05	2.94 ± 0.04	2.81 ± 0.05	2.96 ± 0.04	3.30 ± 0.06**
R. Kidney							
Absolute	1.215 ± 0.029	1.150 ± 0.030	1.186 ± 0.019	1.236 ± 0.030	1.219 ± 0.026	1.258 ± 0.037	1.112 ± 0.034
Relative	3.58 ± 0.06	3.35 ± 0.03▲▲	3.46 ± 0.05	3.59 ± 0.05*	3.63 ± 0.05**	4.00 ± 0.09**	4.71 ± 0.12**
Liver							
Absolute	13.555 ± 0.471	12.872 ± 0.428	13.462 ± 0.224	14.082 ± 0.430	14.548 ± 0.453*[b]	15.106 ± 0.495**	13.885 ± 0.448
Relative	39.86 ± 0.92	37.42 ± 0.42▲	39.27 ± 0.32	40.82 ± 0.47*	43.65 ± 0.85**[b]	47.99 ± 1.23**	58.83 ± 1.51**
Lung							
Absolute	1.315 ± 0.031	1.336 ± 0.029	1.308 ± 0.046	1.401 ± 0.031	1.377 ± 0.041	1.343 ± 0.029	1.122 ± 0.036**
Relative	3.88 ± 0.09	3.90 ± 0.07	3.82 ± 0.12	4.08 ± 0.08	4.10 ± 0.09	4.28 ± 0.11*	4.76 ± 0.14**
Spleen							
Absolute	0.739 ± 0.009	0.780 ± 0.025	0.758 ± 0.011	0.803 ± 0.027	0.820 ± 0.016	0.780 ± 0.013	0.563 ± 0.013**
Relative	2.18 ± 0.03	2.27 ± 0.03▲	2.22 ± 0.04	2.33 ± 0.06	2.44 ± 0.03**	2.48 ± 0.05**	2.39 ± 0.04**
R. Testis							
Absolute	1.412 ± 0.032	1.437 ± 0.031	1.411 ± 0.019	1.440 ± 0.031	1.466 ± 0.028	1.419 ± 0.031	2.357 ± 0.195**
Relative	4.16 ± 0.08	4.19 ± 0.04	4.12 ± 0.03	4.18 ± 0.05	4.37 ± 0.05	4.51 ± 0.09	9.94 ± 0.74**
Thymus							
Absolute	0.312 ± 0.016	0.355 ± 0.016	0.297 ± 0.007**	0.314 ± 0.012**	0.296 ± 0.014**	0.264 ± 0.018**	0.176 ± 0.007**
Relative	0.92 ± 0.05	1.04 ± 0.04	0.87 ± 0.02*	0.91 ± 0.03*	0.88 ± 0.04**	0.84 ± 0.06**	0.75 ± 0.03**

Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Gavage Study of Methyleugenol

	Water Control	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	1,000 mg/kg
Female							
n	10	10	10	10	10	10	10
Necropsy							
body wt	195 ± 3	200 ± 4	185 ± 1**	188 ± 3**	182 ± 3**	185 ± 3**	164 ± 4**
Heart							
Absolute	0.638 ± 0.013	0.634 ± 0.018[b]	0.627 ± 0.017	0.607 ± 0.012	0.584 ± 0.012	0.602 ± 0.012	0.565 ± 0.018**
Relative	3.28 ± 0.06	3.15 ± 0.06[b]	3.38 ± 0.09	3.23 ± 0.05	3.20 ± 0.06	3.26 ± 0.08	3.44 ± 0.07*
R. Kidney							
Absolute	0.690 ± 0.015	0.683 ± 0.015	0.677 ± 0.025	0.699 ± 0.013	0.702 ± 0.012	0.743 ± 0.013	0.705 ± 0.022
Relative	3.55 ± 0.06	3.41 ± 0.05	3.65 ± 0.12*	3.72 ± 0.05**	3.85 ± 0.05**	4.03 ± 0.09**	4.30 ± 0.10**
Liver							
Absolute	6.679 ± 0.117	6.585 ± 0.168	6.318 ± 0.158	6.366 ± 0.163	6.701 ± 0.115	8.369 ± 0.212**	9.517 ± 0.267**
Relative	34.35 ± 0.45	32.85 ± 0.43▲	34.09 ± 0.70	33.89 ± 0.70	36.73 ± 0.34**	45.30 ± 1.03**	58.05 ± 1.06**
Lung							
Absolute	0.896 ± 0.028	0.939 ± 0.029	0.923 ± 0.028	0.934 ± 0.022	0.894 ± 0.013	0.947 ± 0.032	0.850 ± 0.025
Relative	4.60 ± 0.10	4.68 ± 0.11	4.98 ± 0.15	4.98 ± 0.13	4.91 ± 0.09	5.14 ± 0.20*	5.18 ± 0.07*
Spleen							
Absolute	0.571 ± 0.013	0.449 ± 0.017▲▲	0.574 ± 0.014**	0.499 ± 0.015	0.506 ± 0.014	0.517 ± 0.019*	0.494 ± 0.019
Relative	2.95 ± 0.09	2.24 ± 0.08▲▲	3.10 ± 0.07**	2.66 ± 0.06**	2.78 ± 0.07**	2.80 ± 0.11**	3.01 ± 0.07**
Thymus							
Absolute	0.248 ± 0.014	0.268 ± 0.015	0.238 ± 0.007	0.238 ± 0.012	0.244 ± 0.015	0.248 ± 0.009	0.165 ± 0.009**
Relative	1.27 ± 0.05	1.33 ± 0.06	1.29 ± 0.04	1.26 ± 0.06	1.33 ± 0.07	1.34 ± 0.04	1.01 ± 0.06**

* Significantly different (P≤0.05) from the vehicle control group by Williams' or Dunnett's test
** P≤0.01
▲ Vehicle controls significantly different (P≤0.05) from the water control group by a *t*-test
▲▲ P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study
of Methyleugenol[a]

	Water Control	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Male						
n	10	10	10	10	10	9
Necropsy						
body wt	33.9 ± 1.2	34.0 ± 0.7	34.1 ± 0.8	33.7 ± 0.7	34.2 ± 0.9	32.0 ± 0.6
Heart						
Absolute	0.154 ± 0.004	0.160 ± 0.006	0.158 ± 0.007	0.148 ± 0.004	0.150 ± 0.007	0.146 ± 0.009
Relative	4.57 ± 0.14	4.72 ± 0.20	4.63 ± 0.17	4.39 ± 0.11	4.38 ± 0.15	4.56 ± 0.31
R. Kidney						
Absolute	0.316 ± 0.10	0.317 ± 0.007	0.312 ± 0.010	0.320 ± 0.008	0.305 ± 0.012	0.288 ± 0.010
Relative	9.36 ± 0.27	9.33 ± 0.20	9.14 ± 0.18	9.50 ± 0.13	8.91 ± 0.22	8.99 ± 0.28
Liver						
Absolute	1.602 ± 0.051	1.514 ± 0.057	1.651 ± 0.055	1.722 ± 0.038*	1.687 ± 0.069*	1.818 ± 0.058**
Relative	47.41 ± 1.17	44.51 ± 1.47	48.36 ± 1.04*	51.15 ± 0.87**	49.29 ± 1.54**	56.81 ± 1.75**
Lung						
Absolute	0.195 ± 0.015	0.191 ± 0.007	0.176 ± 0.007	0.180 ± 0.006	0.182 ± 0.010	0.177 ± 0.011
Relative	5.73 ± 0.36	5.62 ± 0.18	5.16 ± 0.15	5.37 ± 0.23	5.32 ± 0.25	5.53 ± 0.36
Spleen						
Absolute	0.079 ± 0.004	0.075 ± 0.003	0.079 ± 0.004	0.076 ± 0.003	0.077 ± 0.005	0.073 ± 0.002
Relative	2.36 ± 0.14	2.21 ± 0.11	2.31 ± 0.10	2.26 ± 0.09	2.24 ± 0.11	2.29 ± 0.07
R. Testis						
Absolute	0.118 ± 0.002	0.120 ± 0.003	0.117 ± 0.002	0.118 ± 0.004	0.117 ± 0.004	0.117 ± 0.001
Relative	3.51 ± 0.09	3.54 ± 0.08	3.46 ± 0.09	3.49 ± 0.10	3.41 ± 0.10	3.67 ± 0.07
Thymus						
Absolute	0.041 ± 0.004	0.044 ± 0.002	0.042 ± 0.003	0.039 ± 0.002	0.039 ± 0.004	0.036 ± 0.002*
Relative	1.20 ± 0.11	1.30 ± 0.05	1.22 ± 0.07	1.17 ± 0.08	1.13 ± 0.09	1.12 ± 0.08

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Gavage Study of Methyleugenol

	Water Control	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Female						
n	10	10	9	10	10	10
Necropsy						
body wt	31.4 ± 1.1	30.0 ± 0.7	30.7 ± 0.8	30.7 ± 0.6	29.6 ± 0.6	27.6 ± 0.4**
Heart						
Absolute	0.131 ± 0.005	0.136 ± 0.007	0.130 ± 0.003	0.136 ± 0.007	0.123 ± 0.005	0.115 ± 0.005*
Relative	4.20 ± 0.15	4.55 ± 0.23	4.27 ± 0.19	4.43 ± 0.17	4.19 ± 0.24	4.17 ± 0.21
R. Kidney						
Absolute	0.232 ± 0.009	0.223 ± 0.009	0.221 ± 0.007	0.226 ± 0.007	0.222 ± 0.013	0.209 ± 0.005
Relative	7.41 ± 0.17	7.45 ± 0.26	7.22 ± 0.18	7.37 ± 0.16	7.56 ± 0.52	7.58 ± 0.16
Liver						
Absolute	1.425 ± 0.047	1.345 ± 0.041	1.457 ± 0.062	1.514 ± 0.047	1.395 ± 0.041	1.530 ± 0.058*
Relative	45.56 ± 1.04	44.90 ± 1.10	47.59 ± 1.92	49.44 ± 1.32	47.27 ± 1.42	55.35 ± 1.50**
Lung						
Absolute	0.200 ± 0.023	0.192 ± 0.008	0.180 ± 0.007	0.180 ± 0.005	0.173 ± 0.010	0.171 ± 0.007
Relative	6.33 ± 0.56	6.43 ± 0.30	5.86 ± 0.15	5.88 ± 0.16	5.87 ± 0.37	6.20 ± 0.24
Spleen						
Absolute	0.094 ± 0.003	0.095 ± 0.007	0.090 ± 0.003	0.088 ± 0.005	0.081 ± 0.002	0.082 ± 0.002
Relative	3.02 ± 0.12	3.17 ± 0.21	2.96 ± 0.17	2.87 ± 0.16	2.75 ± 0.09	2.97 ± 0.08
Thymus						
Absolute	0.054 ± 0.003	0.060 ± 0.004	0.056 ± 0.004	0.055 ± 0.003	0.049 ± 0.003*	0.044 ± 0.002**
Relative	1.73 ± 0.09	2.00 ± 0.14	1.81 ± 0.11	1.77 ± 0.10	1.66 ± 0.08*	1.58 ± 0.06**

* Significantly different (P≤0.05) from the vehicle control group by Williams' test
** P≤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). Vehicle control values were not significantly different from water control group by a *t*-test. All 1,000 mg/kg mice died before the end of the study.

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS
AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Gavage Study of Methyleugenol[a]

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
n	10	10	10	9
Weights (g)				
Necropsy body wt	343 ± 8	345 ± 8	336 ± 7	314 ± 4**
L. cauda epididymis	0.1573 ± 0.0050	0.1433 ± 0.0093	0.1564 ± 0.0027	0.1532 ± 0.0027
L. epididymis	0.4347 ± 0.0084	0.4243 ± 0.0115	0.4332 ± 0.0054	0.4214 ± 0.0113
L. testis	1.5113 ± 0.0279	1.5297 ± 0.0366	1.5095 ± 0.0348	1.4970 ± 0.0338
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.35 ± 0.28	9.86 ± 0.35	9.98 ± 0.38	10.10 ± 0.18
Spermatid heads (10^7/testis)	14.10 ± 0.37	15.06 ± 0.59	14.98 ± 0.40	15.12 ± 0.46
Spermatid count				
(mean/10^{-4} mL suspension)	70.48 ± 1.86	75.30 ± 2.97	74.90 ± 1.99	75.61 ± 2.31
Epididymal spermatozoal measurements				
Motility (%)	93.39 ± 0.89	93.69 ± 0.36	94.32 ± 0.56	93.22 ± 1.43
Concentration				
(10^6/g cauda epididymal tissue)	538 ± 30	638 ± 40	513 ± 19	580 ± 34

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

TABLE H2
Summary of Estrous Cycle Characterization for Female Rats in the 14-Week Gavage Study of Methyleugenol[a]

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
n	10	10	10	10
Necropsy body wt (g)	200 ± 4	188 ± 3**	182 ± 3**	185 ± 3**
Estrous cycle length (days)	5.06 ± 0.11[b]	4.95 ± 0.12	5.30 ± 0.31	5.55 ± 0.30
Estrous stages (% of cycle)				
Diestrus	38.3	40.8	37.5	43.3
Proestrus	15.0	15.0	15.0	16.7
Estrus	32.5	30.0	31.7	30.8
Metestrus	10.0	14.2	15.8	9.2
Uncertain diagnosis	4.2	0.0	0.0	0.0

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Gavage Study of Methyleugenol[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	34.0 ± 0.7	34.1 ± 0.8	33.7 ± 0.7	34.2 ± 0.9
L. cauda epididymis	0.0159 ± 0.0006	0.0111 ± 0.0009**	0.0099 ± 0.0012**	0.0159 ± 0.0006
L. epididymis	0.0470 ± 0.0012	0.0347 ± 0.0028**	0.0307 ± 0.0032**	0.0452 ± 0.0012
L. testis	0.1163 ± 0.0018	0.1028 ± 0.0022**	0.1011 ± 0.0025**	0.1161 ± 0.0030
Spermatid measurements				
Spermatid heads (10^7/g testis)	20.00 ± 0.97	22.99 ± 1.05	22.00 ± 0.94	19.27 ± 0.67
Spermatid heads (10^7/testis)	2.33 ± 0.12	2.36 ± 0.12	2.22 ± 0.09	2.24 ± 0.12
Spermatid count (mean/10^{-4} mL suspension)	72.75 ± 3.76	73.80 ± 3.60	69.28 ± 2.77	70.15 ± 3.74
Epididymal spermatozoal parameters				
Motility (%)	90.40 ± 0.86	88.91 ± 0.73	90.78 ± 1.10	90.10 ± 0.73
Concentration (10^6/g cauda epididymal tissue)	1,466 ± 90	2,153 ± 268	2,129 ± 309	967 ± 50*

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

TABLE H4
Summary of Estrous Cycle Characterization for Female Mice in the 14-Week Gavage Study of Methyleugenol[a]

	Vehicle Control	10 mg/kg	30 mg/kg	100 mg/kg
n	10	9	10	10
Necropsy body wt (g)	30.0 ± 0.7	30.7 ± 0.8	30.7 ± 0.6	29.6 ± 0.6
Estrous cycle length (days)	4.10 ± 0.10	4.19 ± 0.13[b]	4.40 ± 0.15	4.40 ± 0.15
Estrous stages (% of cycle)				
Diestrus	30.8	30.6	36.7	28.3
Proestrus	15.0	13.9	15.8	18.3
Estrus	35.0	38.0	37.5	37.5
Metestrus	19.2	16.7	10.0	15.0
Uncertain diagnosis	0.0	0.9	0.0	0.8

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

APPENDIX I
TOXICOKINETIC RESULTS

RATS

Samples of blood for toxicokinetics were collected at 6, 12, and 18 months from the toxicokinetic study group animals and at 18 months from the sentinel animals (for the aged-animal, single-administration study). Average plasma concentration values by time point are presented in Table I1. Methyleugenol toxicokinetic parameters for rats administered single daily doses of methyleugenol for 6, 12, or 18 months are summarized in Table I2. Plots of log concentration versus time are included in Figures I1 (6 months), I2 (12 months), and I3 (18 months). Area under the plasma concentration-time curve (AUC) versus dose is illustrated in Figure I4.

The toxicokinetic parameters were limited to observed results only. Interpretation of these results was based only on the observed peak plasma concentration (C_{max}), observed time to peak concentration (T_{max}), shape of the plasma concentration-time profile, and area under the curve (AUC). In addition, calculation of a half-life ($t_{1/2}$) was attempted, but regression of the terminal linear portion of the curves resulted in very poor R^2 values, i.e., less than 0.90, and some of the curves did not have plasma concentrations above the limit of detection beyond 4 hours after dosing (approximately four half-lives). Thus, modeling of the profiles will be necessary to obtain reliable $t_{1/2}$ information.

6-Month Toxicokinetic Results

There was no absorption phase that could be characterized by the plasma concentration-time profiles, even though the first time point was 5 minutes after administration. Absorption was extremely rapid. The T_{max} values were observed at 5 minutes after dosing for all dose groups of males and females (Table I2). In general, the C_{max} values increased with increasing dose for most groups. The male 75 mg/kg per day group and female 150 mg/kg per day group had C_{max} values that were lower than expected. Female C_{max} values were higher than male values for the 37 and 75 mg/kg per day groups, but male values were higher for the 150 and 300 mg/kg per day groups.

Values for AUC increased with increasing dose for all groups except the female 150 mg/kg per day group, which had a lower AUC value than expected (possibly due to the short terminal linear phase). The increases in AUC values were proportional with dose except for the male 150 and 300 mg/kg per day groups. For the males, a four- or eightfold increase in dose, i.e., from 37 to 150 mg/kg per day or from 37 to 300 mg/kg per day, increased the AUC values 8- or 19-fold, respectively. In contrast, the AUC value for the female 300 mg/kg per day group only increased sixfold when compared to the AUC value for the 37 mg/kg per day group (an eightfold lower dose).

The multiple exposures to methyleugenol for 6 months generally decreased the C_{max} and AUC values when compared to the single-administration toxicokinetic study results. For example, the C_{max} values for the single administration of 37, 75, or 150 mg/kg per day to males were 0.66, 1.52, and 3.84 μg/mL, respectively (Table M5), whereas after 6 months of dosing, the C_{max} values for the same dose groups were 0.51, 0.43, and 1.34 μg/mL, respectively (Table I2). For the females, single-administration toxicokinetic C_{max} values for the 37, 75, and 150 mg/kg per day groups were 1.14, 3.22, and 8.25 μg/mL, respectively (Table M5), and were 1.41, 2.46, and 0.84 μg/mL, respectively, after 6 months of dosing (Table I2). AUC values from repeated-exposure animals generally exhibited a similar decrease when compared to their respective counterparts from the single-administration toxicokinetic study.

12-Month Toxicokinetic Results

There was no absorption phase that could be characterized by the plasma concentration-time profiles. The T_{max} values were 5 minutes after dosing for all groups except the male 37 mg/kg per day group, which had a T_{max} value of 15 minutes (Table I2). The C_{max} values were highly variable and difficult to correlate with dose. For example, the female 37 and 150 mg/kg per day groups had C_{max} values of 0.89 and 0.79 μg/mL, respectively, whereas the 75 and 300 mg/kg per day groups had C_{max} values of 2.05 and 1.78 μg/mL,

respectively. Although somewhat similar results were observed for the male groups, attention to the C_{max} value for the 300 mg/kg per day group is noteworthy because the mean value was 22.5 μg/mL (range, 9.4 to 43.3 μg/mL). Female C_{max} values were higher than male values for the 37 and 75 mg/kg per day groups but not for the higher dose groups. The male 150 and 300 mg/kg per day groups had higher C_{max} values than did the females at 12 months, as was also observed at 6 months.

Values for AUC increased with increasing dose for both male and female rats. For the males (37 and 75 mg/kg per day groups) and females (all dose groups), the increase was proportional with dose. This was not so for the male 150 and 300 mg/kg per day groups. A twofold increase in dose, i.e., from 75 to 150 mg/kg per day, increased the AUC value fivefold, and a fourfold increase in dose, i.e., from 75 to 300 mg/kg per day, increased the AUC value 14-fold.

6- and 12-month C_{max} and AUC values were similar for the male 37 and 75 mg/kg per day and female dose groups, but a shift from the earlier results occurred for the male 150 and 300 mg/kg per day groups. For example, AUC values at 6 months were 3.11 μg/mL●hr (150 mg/kg per day group) and 7.57 μg/mL●hr (300 mg/kg per day group) but increased at 12 months to 4.96 μg/mL●hr (150 mg/kg per day group) and 14.3 μg/mL●hr (300 mg/kg per day group). The increases in C_{max} and AUC values for the 300 mg/kg per day group from months 6 to 12 was substantial.

18-Month Toxicokinetic Results

There was no absorption phase that could be characterized by the plasma concentration-time profiles. The T_{max} values were 5 minutes for all groups except the male 37 mg/kg per day group, which had a T_{max} value of 30 minutes (Table I2). The C_{max} values did not correlate well with dose for any of the dose groups (37, 75, or 150 mg/kg per day) of males and females. The male 75 mg/kg per day group had a disparate value of 14.6 μg/mL, which may have skewed the C_{max} value. There were no results for the 300 mg/kg per day group because the toxicokinetic-study animals for this group were terminated after 12 months, and core-study animals were administered vehicle in order to evaluate whether any recovery would occur.

Values for AUC increased with increasing dose. For males, the increase was proportional with dose. For females, AUC values were similar for the 37 and 75 mg/kg per day groups but increased proportionally when the dose was increased from 75 to 150 mg/kg per day.

Comparison of 12- and 18-month data indicated that C_{max} values remained relatively unchanged for the male 37 mg/kg per day and female dose groups. The C_{max} values for the male 75 and 150 mg/kg per day groups increased approximately threefold or more. Values for AUC remained relatively unchanged during this time period.

Aged-Animal Toxicokinetic Results

Average plasma concentrations by time point are presented in Table I3. Methyleugenol toxicokinetic parameters for aged rats administered a single methyleugenol dose of 150 mg/kg are summarized in Table I4. Plots of log plasma concentration versus time are included in Figure I5.

For aged animals, C_{max} values were 7.4 μg/mL for males and 13.0 μg/mL for females after a single oral gavage administration of 150 mg/kg (Table I4). The T_{max} value was 15 minutes for males and 5 minutes for females. Values for AUC were similar for males and females.

Comparison of the aged-animal results (Table I4) with the results for the 13-week-old animals used in the single-administration toxicokinetic study (Table M5) reveals similar T_{max} values. The C_{max} and AUC values were higher for the aged animals than for the 13-week-old animals. The C_{max} values were 7.44 μg/mL (males) and 13.0 μg/mL (females) for the aged animals, whereas 13-week-old animals had C_{max} values of

3.84 μg/mL (males) and 8.25 μg/mL (females). Furthermore, the AUC values of the aged rats were 11.1 μg/mL•hr (males) and 12.5 μg/mL•hr (females), while the 13-week-old animals had AUC values of 7.6 μg/mL•hr (males) and 5.1 μg/mL•hr (females).

MICE
Specimens of blood for toxicokinetics were collected at 12 months from the toxicokinetic study group animals and at 18 months from the sentinel animals (aged-animal, single-administration study).

12-Month Toxicokinetic Results
Average plasma concentrations by time point are presented in Table I5. Methyleugenol toxicokinetic parameters for mice administered single daily doses of methyleugenol for 12 consecutive months are summarized in Table I6. Plots of log plasma concentration versus time are included in Figures I6 to I8. Area under the plasma concentration-time curve (AUC) versus dose is illustrated in Figure I9.

Peak plasma concentrations (C_{max}) were observed at 5 minutes (T_{max}) after dosing for all dose levels of males and females (Table I6). There was no absorption phase that could be characterized by the plasma concentration-time profiles. The peak plasma concentrations increased with increasing dose for males and females. Female peak plasma concentrations were slightly higher than those for males at any given dose.

The $t_{1/2}$ values increased with increasing dose for dosed groups of males and females. These results suggest that elimination was saturated. Values for AUC increased with increasing dose for male and female mice. For females, the increase was proportional with dose. This was not so for males. Twofold increases in dose, i.e., from 37 to 75 mg/kg per day or from 75 to 150 mg/kg per day, increased AUC values threefold or more. Furthermore, a fourfold increase in dose, i.e., from 37 to 150 mg/kg per day, increased AUC values 11-fold.

The 75 mg/kg per day dose was also administered to animals in the single-administration toxicokinetic study, thereby making it useful for comparing the toxicokinetics of methyleugenol following a single administration (Table M6) to that following multiple administrations (Table I6). The observed C_{max} values following a single administration were higher for males (3.10 μg/mL) and females (4.39 μg/mL) than following multiple administration (2.53 μg/mL for males; 3.03 μg/mL for females). The observed T_{max} and AUC values were similar following both a single administration and multiple dosing (12 months).

Aged-Animal Toxicokinetic Results
Average plasma concentration values by time point for 18-month-old (aged) mice are presented in Table I7. Methyleugenol toxicokinetic parameters for aged mice administered a single dose of methyleugenol are summarized in Table I8. Plots of log concentration versus time are included in Figure I10.

For aged animals, peak plasma concentrations were observed 5 minutes after dosing in both sexes. The peak plasma concentrations and AUC values were approximately twofold higher for females than for males. Values for $t_{1/2}$ were similar for both sexes.

Comparison of the aged-animal results (Table I8) with the results for the approximately 13-week-old animals used in the single-administration toxicokinetic study (Table M6) revealed similar T_{max} values. The C_{max} values were 1.54 μg/mL (males) and 3.30 μg/mL (females) for the aged animals, whereas 13-week-old animals had C_{max} values of 3.10 μg/mL (males) and 4.39 μg/mL (females). In addition, the AUC value for the older female mice (119.4 μg/mL•min) increased twofold relative to the 13-week-old mice (60.5 μg/mL•min). The AUC values for the different male groups were similar. In addition, the $t_{1/2}$ values for the older mice increased approximately twofold.

TABLE I1
Plasma Concentrations of Methyleugenol in Rats at the 6-, 12-, and 18-Month Interim Evaluations
in the 2-Year Gavage Study of Methyleugenol[a]

	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Male				
n	3	3	3	3
Month 6				
Time after Dosing (minutes)				
5	0.505 ± 0.073	0.431 ± 0.187	1.346 ± 0.529	4.025 ± 2.836
15	0.453 ± 0.182	—[b]	—	—
30	0.218 ± 0.048^c	0.278 ± 0.034^c	0.941 ± 0.264	—
60	0.128 ± 0.033	—	—	1.156 ± 0.321
90	0.075 ± 0.021	0.166 ± 0.036	0.583 ± 0.216	—
120	0.036 ± 0.004	0.094 ± 0.042	—	1.118 ± 0.246
240	—	0.064 ± 0.012^c	0.273 ± 0.123	0.572 ± 0.264
360	—	0.024^d	0.122 ± 0.049^c	0.131 ± 0.033^c
450	—	0.045 ± 0.021^c	—	—
480	—	—	0.100 ± 0.034	—
540	—	—	—	0.349 ± 0.094
600	—	—	0.032 ± 0.011^c	—
780	—	—	—	0.061^d
Month 12				
Time after Dosing (minutes)				
5	0.504 ± 0.232	0.508 ± 0.046	0.967 ± 0.155	22.480 ± 10.520
15	0.574 ± 0.229	—	—	—
30	0.206 ± 0.012^c	0.349 ± 0.107^c	0.726 ± 0.195	—
60	0.088 ± 0.010	—	—	1.180 ± 0.453
90	0.046 ± 0.002^c	0.247 ± 0.088	0.600 ± 0.021	—
120	—	0.159 ± 0.125^c	—	0.762 ± 0.283
240	—	0.031 ± 0.001^c	0.603 ± 0.301	0.140 ± 0.044
360	—	—	0.614 ± 0.063^c	0.171 ± 0.039^c
450	—	0.057 ± 0.024^c	—	—
480	—	—	0.179 ± 0.090	—
540	—	—	—	0.241 ± 0.100
600	—	—	0.093 ± 0.046	—
780	—	—	—	0.027^d
Month 18				
Time after Dosing (minutes)				
5	0.602 ± 0.244	8.323 ± 3.409	2.697 ± 1.372	—
15	0.661 ± 0.143	1.310 ± 0.255	—	—
30	0.711 ± 0.391	0.529 ± 0.063	1.414 ± 0.502	—
60	0.077 ± 0.006	0.254 ± 0.069	—	—
90	0.062 ± 0.005	0.289 ± 0.188	0.491 ± 0.214	—
120	0.166 ± 0.122	0.090 ± 0.018	—	—
240	0.024 ± 0.002^c	0.089 ± 0.017	0.197 ± 0.005	—
360	—	0.288 ± 0.263^c	0.259 ± 0.120	—
480	—	—	0.167 ± 0.014	—
600	—	—	0.072 ± 0.008	—

TABLE I1
Plasma Concentrations of Methyleugenol in Rats at the 6-, 12-, and 18-Month Interim Evaluations in the 2-Year Gavage Study of Methyleugenol

	37 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Female				
n	3	3	3	3
Month 6				
Time after Dosing (minutes)				
5	1.410 ± 0.858	2.461 ± 1.197	0.843 ± 0.379	3.110 ± 1.616
15	0.642 ± 0.064	—	—	—
30	0.243 ± 0.062[c]	0.582 ± 0.458[c]	0.623 ± 0.034	—
60	0.171 ± 0.071	—	—	0.639 ± 0.266
90	0.118 ± 0.071[c]	0.114 ± 0.032	0.238 ± 0.063	—
120	0.033 ± 0.006	0.151 ± 0.028	—	0.139 ± 0.057
240	—	0.026 ± 0.008[c]	0.026 ± 0.006[c]	0.193 ± 0.048
360	—	0.034[d]	0.084[d]	0.048 ± 0.002[c]
480	—	—	0.033 ± 0.008	—
540	—	—	—	0.070 ± 0.023
780	—	—	—	0.062 ± 0.021
Month 12				
Time after Dosing (minutes)				
5	0.891 ± 0.111	2.055 ± 0.952	0.789 ± 0.267	1.780 ± 0.261
15	0.651 ± 0.186[c]	—	—	—
30	0.825 ± 0.556[c]	0.853 ± 0.238[c]	0.590 ± 0.116	—
60	0.094 ± 0.017	—	—	0.436 ± 0.107
90	0.032 ± 0.005	0.137 ± 0.064	0.221 ± 0.072	—
120	0.058 ± 0.017	0.198 ± 0.093	—	0.767 ± 0.409
240	—	0.038[d]	0.095 ± 0.040[c]	0.137 ± 0.051
360	—	0.055[d]	0.109 ± 0.066[c]	0.070[d]
450	—	0.072[d]	—	—
480	—	—	0.245 ± 0.077[c]	—
540	—	—	—	0.373 ± 0.120
600	—	—	0.078 ± 0.019[c]	—
780	—	—	—	0.034[d]
Month 18				
Time after Dosing (minutes)				
5	1.152 ± 0.483	1.289 ± 0.345	0.750 ± 0.190	—
10	1.10 ± 0.23	—	—	—
15	0.918 ± 0.066	0.837 ± 0.020	—	—
30	0.178 ± 0.034	0.355 ± 0.063	0.509 ± 0.260	—
45	0.18 ± 0.01	—	—	—
60	0.115 ± 0.001	0.110 ± 0.021	0.456 ± 0.038	—
90	0.212 ± 0.084	0.130 ± 0.029	0.134 ± 0.002	—
120	0.078 ± 0.011	0.096 ± 0.058[c]	0.152 ± 0.055	—
180	0.07 ± 0.03	—	—	—
240	0.051[d]	0.039 ± 0.011[c]	0.177 ± 0.026	—
360	—	0.025[d]	0.060 ± 0.035	—
480	—	—	0.044 ± 0.014	—
600	—	—	0.167 ± 0.134[c]	—

[a] Data are given in μg/mL as mean ± standard error.
[b] Plasma concentrations were not determined at this time point for this dose group.
[c] n=2
[d] n=1; no standard errors calculated

TABLE I2
**Toxicokinetic Parameters in Rats at the 6-, 12-, and 18-Month Interim Evaluations
in the 2-Year Gavage Study of Methyleugenol**[a]

	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (minutes)	AUC (μg/mL•hour)
Male				
Month 6				
	37	0.51	5	0.40
	75	0.43	5	0.82
	150	1.34	5	3.11
	300	4.03	5	7.57
Month 12				
	37	0.57	15	0.39
	75	0.51	5	1.03
	150	0.97	5	4.96
	300	22.5	5	14.3
Month 18				
	37	0.71	30	0.80
	75	8.31	5	2.55
	150	2.70	5	3.92
Female				
Month 6				
	37	1.41	5	0.55
	75	2.46	5	1.34
	150	0.84	5	1.35
	300	3.11	5	3.24
Month 12				
	37	0.89	5	0.71
	75	2.05	5	1.74
	150	0.79	5	2.11
	300	1.78	5	4.26
Month 18				
	37	1.15	5	0.77
	75	1.29	5	0.81
	150	0.75	5	1.92

[a] C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration; $t_{1/2}$=elimination half-life; AUC=area under the curve
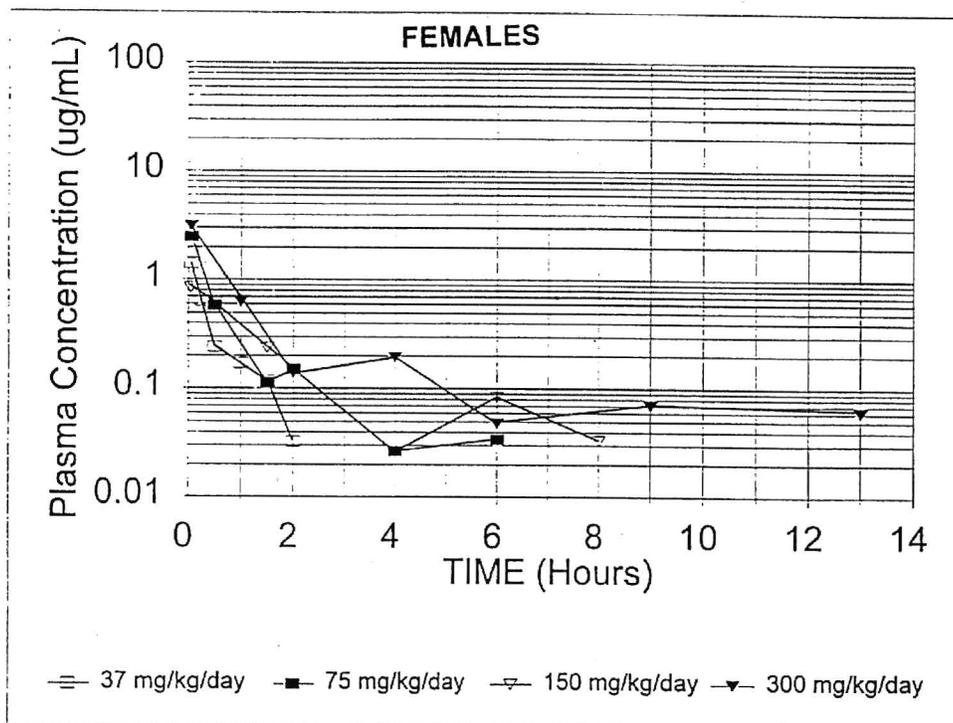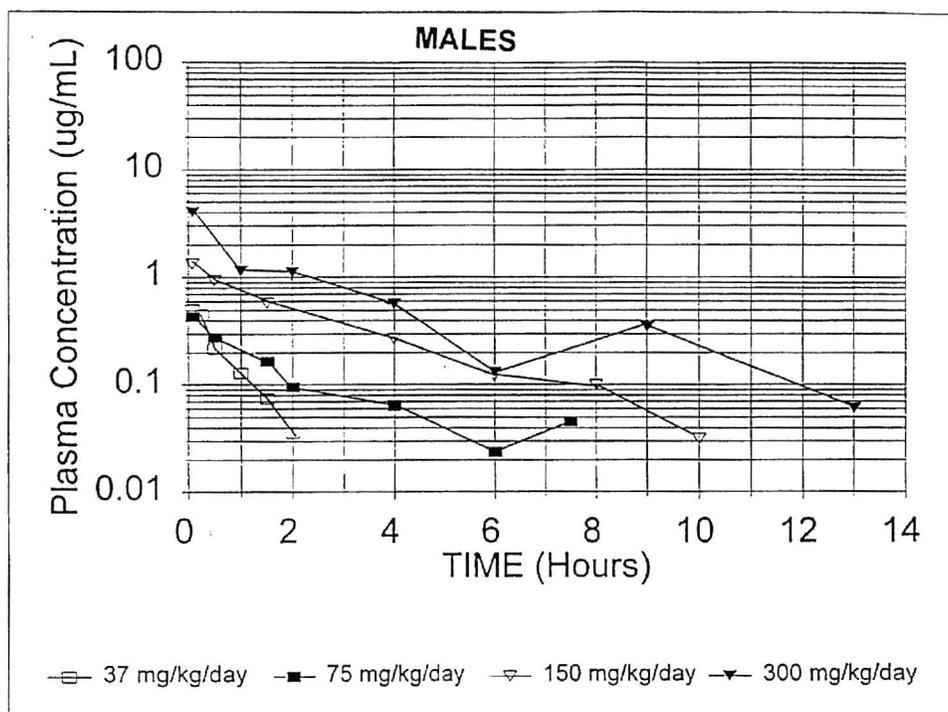
FIGURE I1
Plasma Concentrations of Methyleugenol in Rats
at the 6-Month Interim Evaluation in the 2-Year Gavage Study of Methyleugenol
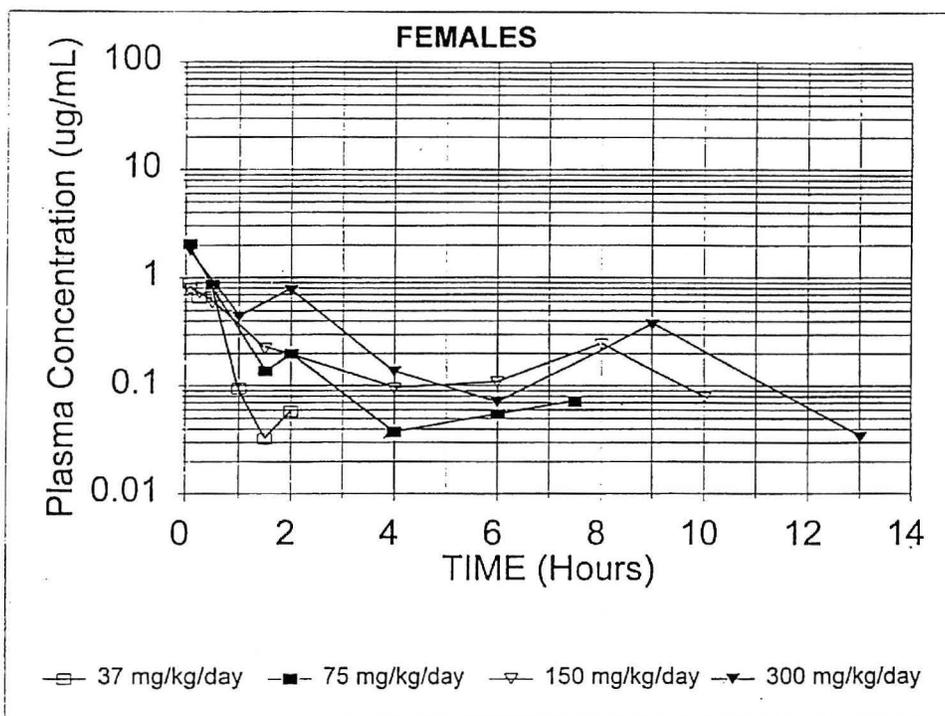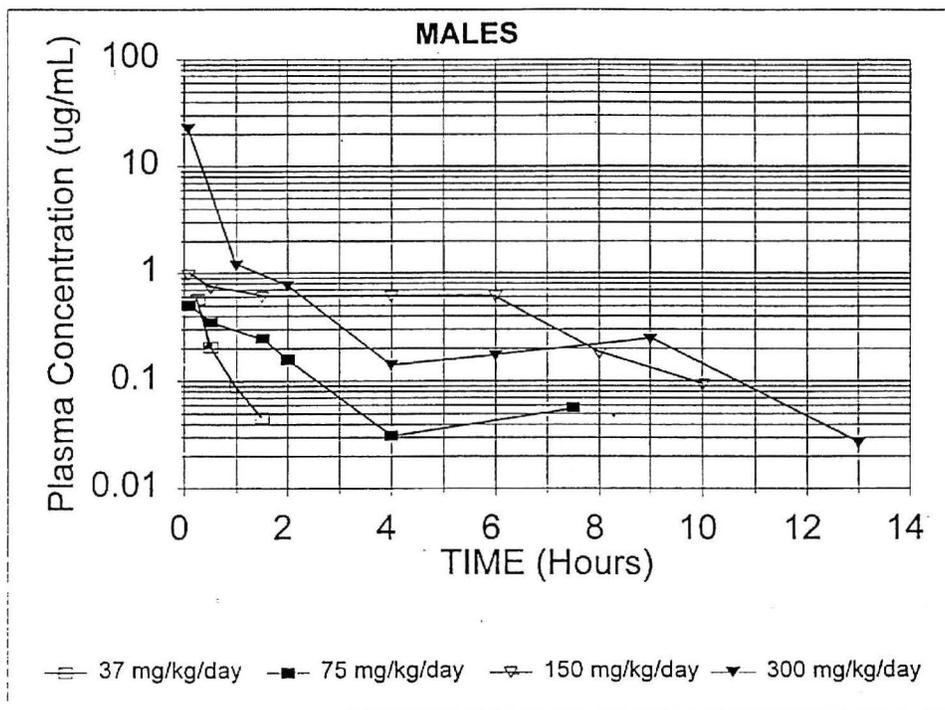
FIGURE I2
Plasma Concentrations of Methyleugenol in Rats
at the 12-Month Interim Evaluation in the 2-Year Gavage Study of Methyleugenol
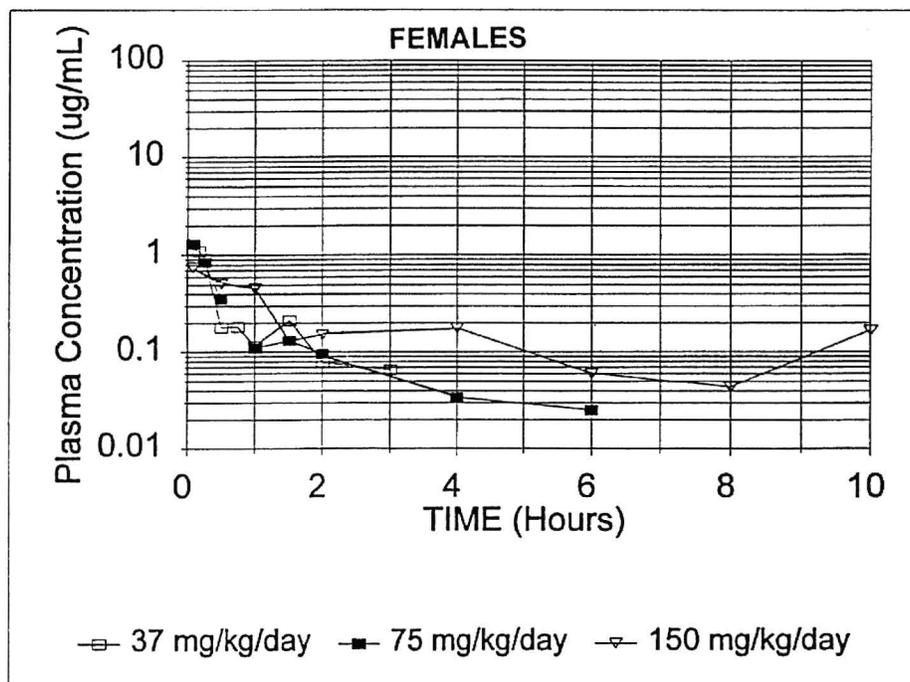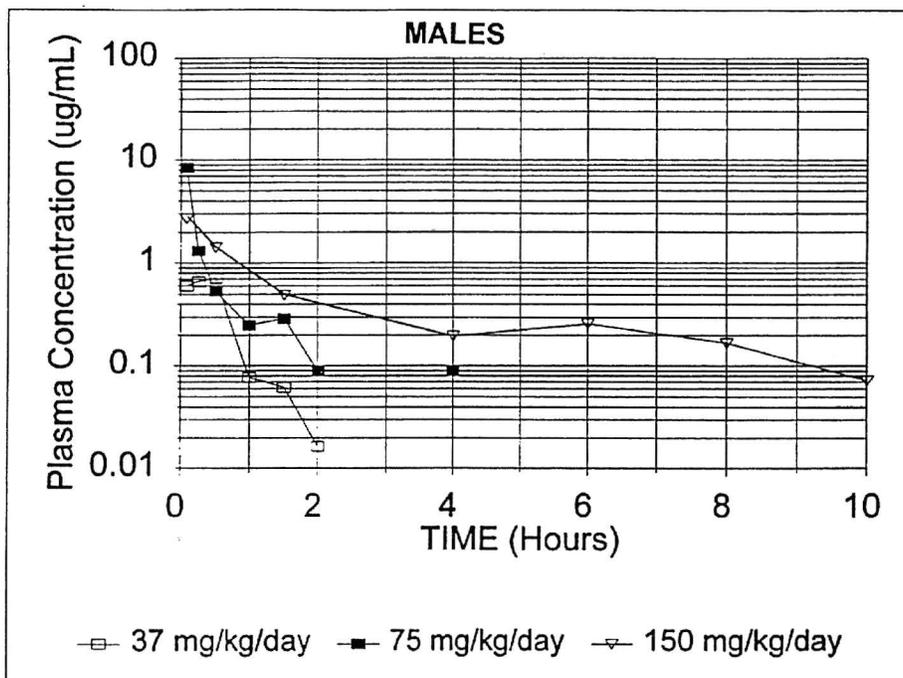
FIGURE I3
Plasma Concentrations of Methyleugenol in Rats
at the 18-Month Interim Evaluation in the 2-Year Gavage Study of Methyleugenol
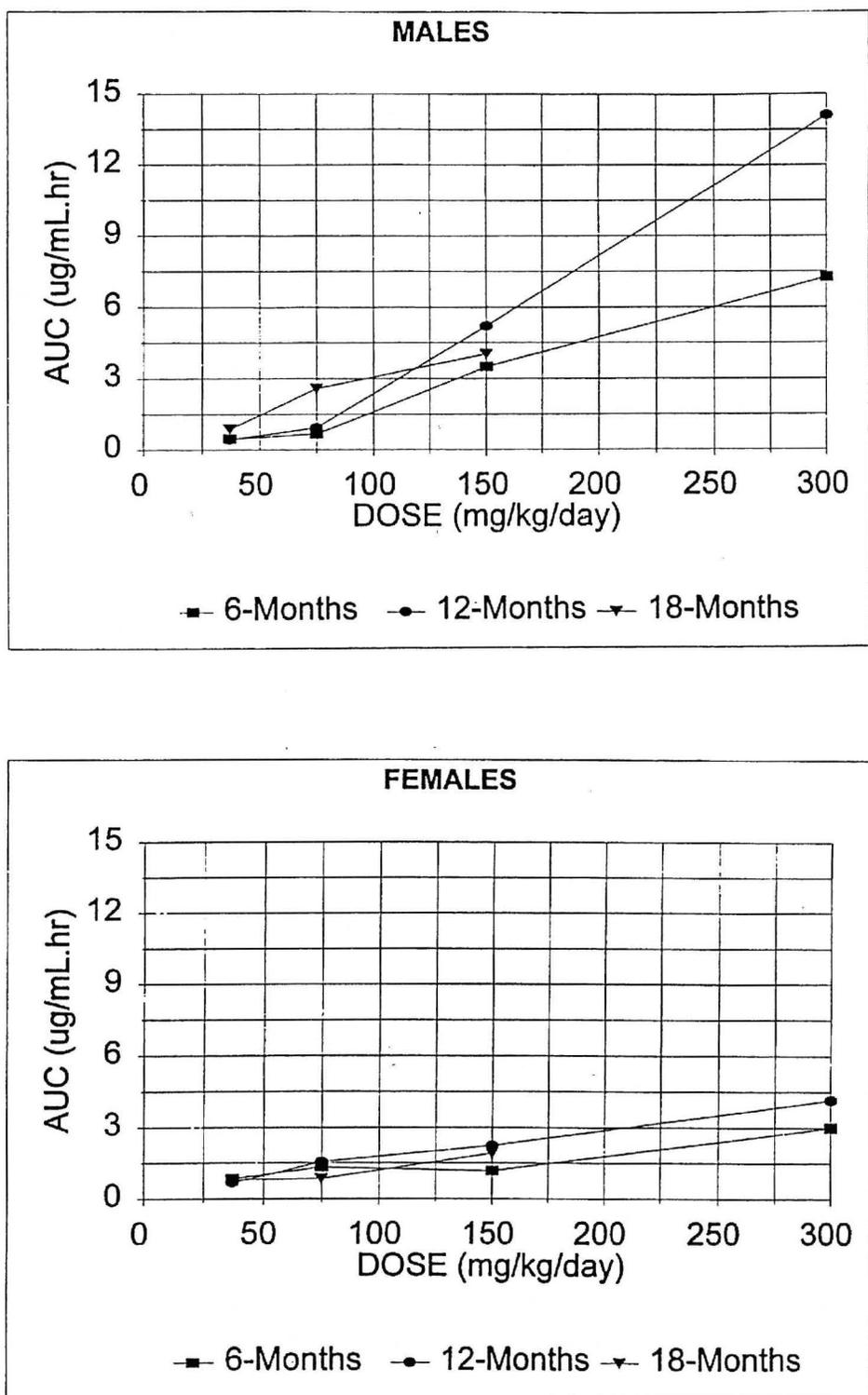
FIGURE I4
Plots of AUC Versus Dose for Methyleugenol in Rats
at the 6-, 12-, and 18-Month Interim Evaluations in the 2-Year Gavage Study of Methyleugenol

TABLE I3
Plasma Concentrations of Methyleugenol in Aged Rats after a Single Gavage Dose
of 150 mg/kg Methyleugenol[a]

Time after Dosing (minutes)	Concentration[b] (μg/mL)
Male	
5	6.58 ± 3.57
15	7.44 ± 3.79
30	2.00 ± 0.17
60	1.11 ± 0.17
120	1.41 ± 0.27
240	0.888 ± 0.104
360	1.007 ± 0.248
480	0.373 ± 0.114
600	0.252 ± 0.040[c]
Female	
5	13.00 ± 1.10
10	8.12 ± 0.65
15	6.47 ± 1.86
30	2.14 ± 0.31
45	3.25 ± 0.53
60	2.69 ± 0.48
120	1.83 ± 0.30
240	0.849 ± 0.215
360	0.507 ± 0.137
480	0.605 ± 0.182

[a]   Three animals were bled at each time point.
[b]   Data are given in μg/mL as the mean ± standard error.
[c]   Four animals bled

TABLE I4
Toxicokinetic Parameters in Aged Rats after a Single Gavage Dose of 150 mg/kg Methyleugenol[a]

	C_{max} (μg/mL)	T_{max} (minutes)	AUC (μg/mL•hour)
Male			
	7.44	15	11.1
Female			
	13.0	5	12.5

[a] C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration; $t_{1/2}$=elimination half-life; AUC=area under the curve
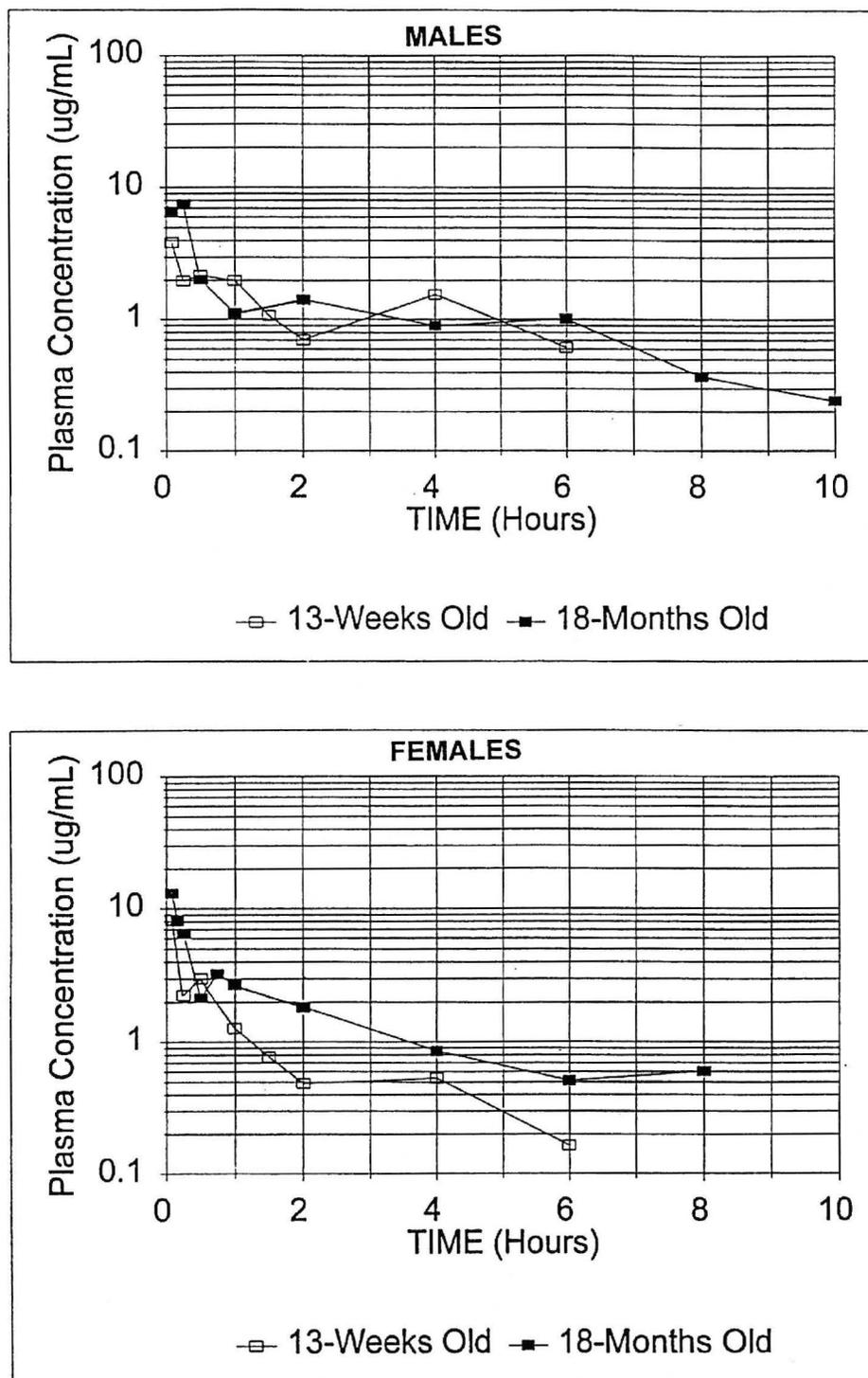
FIGURE I5
Plasma Concentrations of Methyleugenol after a Single Gavage Dose
of 150 mg/kg Methyleugenol in Aged and 13-Week-Old Rats

TABLE I5
Plasma Concentrations of Methyleugenol in Mice at the 12-Month Interim Evaluation
in the 2-Year Gavage Study of Methyleugenol[a]

	37 mg/kg	75 mg/kg	150 mg/kg
n	3	3	3
Male			
Time after Dosing (minutes)			
5	0.789 ± 0.304	2.530 ± 0.195	4.030 ± 1.783
10	0.417 ± 0.128	—[b]	—
15	—	0.528 ± 0.052	1.907 ± 0.641
20	0.099 ± 0.010	—	—
30	0.309 ± 0.284[c]	0.195 ± 0.065[c]	—
40	0.208 ± 0.084	—	0.905 ± 0.318
45	—	0.244 ± 0.076	—
60	0.062 ± 0.021	0.292 ± 0.080	—
75	0.061 ± 0.007	—	0.368 ± 0.034
90	—	0.164 ± 0.019	—
120	—	—	0.648 ± 0.054[c]
150	—	0.102 ± 0.019	—
210	—	—	0.186 ± 0.012
300	—	—	0.181 ± 0.021
Female			
Time after Dosing (minutes)			
5	2.030 ± 0.511	3.030 ± 0.360[c]	4.693 ± 1.252
10	0.681 ± 0.050	—	—
15	—	0.837 ± 0.159	2.747 ± 1.934[c]
20	0.231 ± 0.022	—	—
30	0.631 ± 0.259	0.311 ± 0.017	—
40	0.306 ± 0.031	—	0.283 ± 0.035
45	—	0.335 ± 0.133[c]	—
50	0.19 ± 0.05	—	—
60	0.167 ± 0.064[c]	0.330 ± 0.032	—
90	—	0.184 ± 0.021	—
120	—	—	0.280 ± 0.052
150	—	0.110 ± 0.001[c]	—
180	—	—	0.122 ± 0.020
240	—	—	0.141 ± 0.049

[a] Data are given in μg/mL as mean $\pm$ standard error.
[b] Plasma concentrations were not determined at this time point for this dose group.
[c] n=2

TABLE I6
**Toxicokinetic Parameters in Mice at the 12-Month Interim Evaluation
in the 2-Year Gavage Study of Methyleugenol[a]**

	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (minutes)	$t_{1/2}$ (minutes)	AUC (μg/mL•minute)
Male					
	37	0.79	5	49.4	15.1
	75	2.53	5	99.3	45.0
	150	4.03	5	174.1	167.9
Female					
	37	2.03	5	45.3	28.3
	75	3.03	5	87.8	56.6
	150	4.69	5	120.7	123.2

[a] C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration; $t_{1/2}$=elimination half-life; AUC=area under the curve
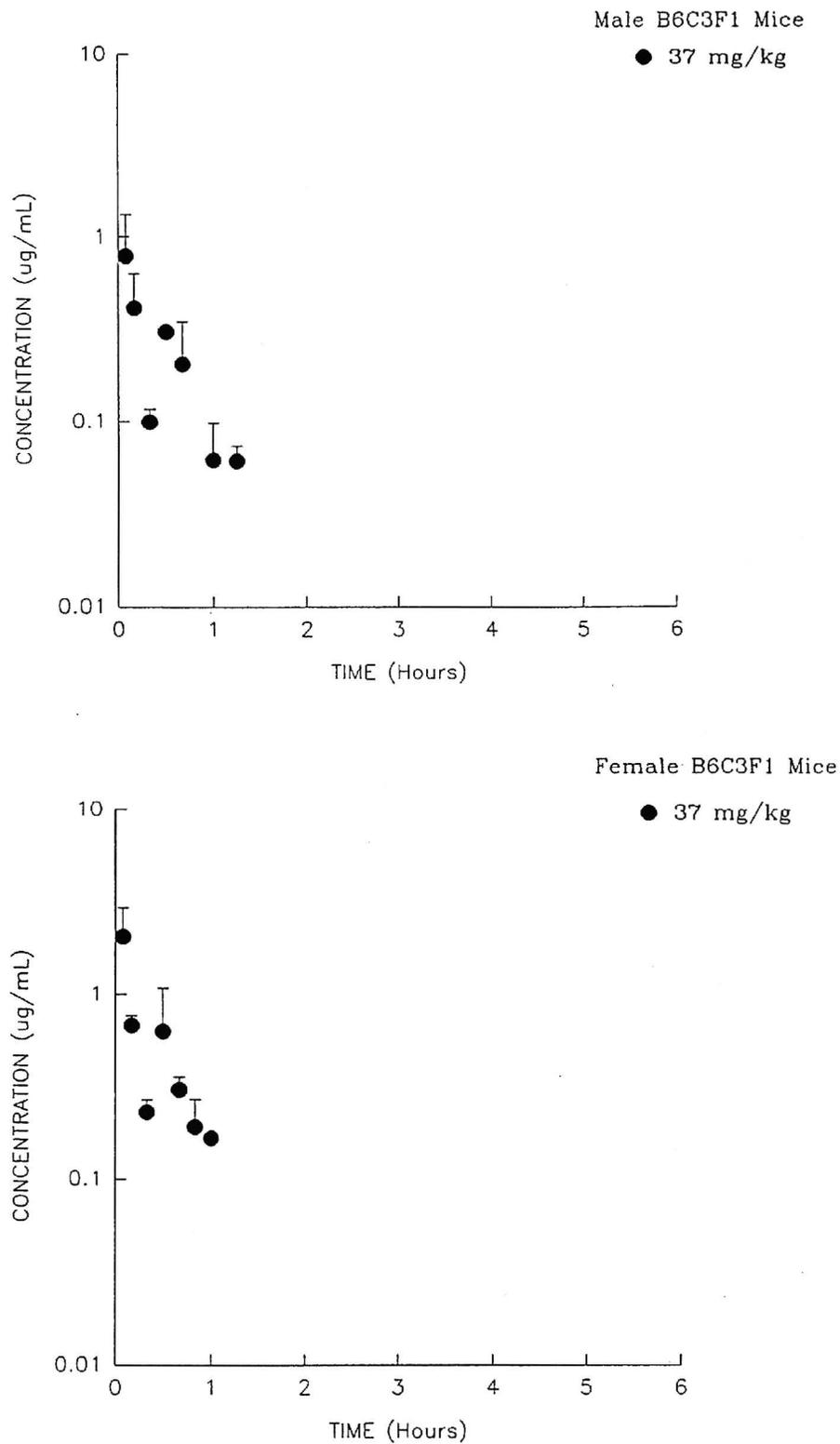
FIGURE I6
Plasma Concentrations of Methyleugenol in Mice
Administered 37 mg/kg Methyleugenol at the 12-Month Interim Evaluation
in the 2-Year Gavage Study of Methyleugenol
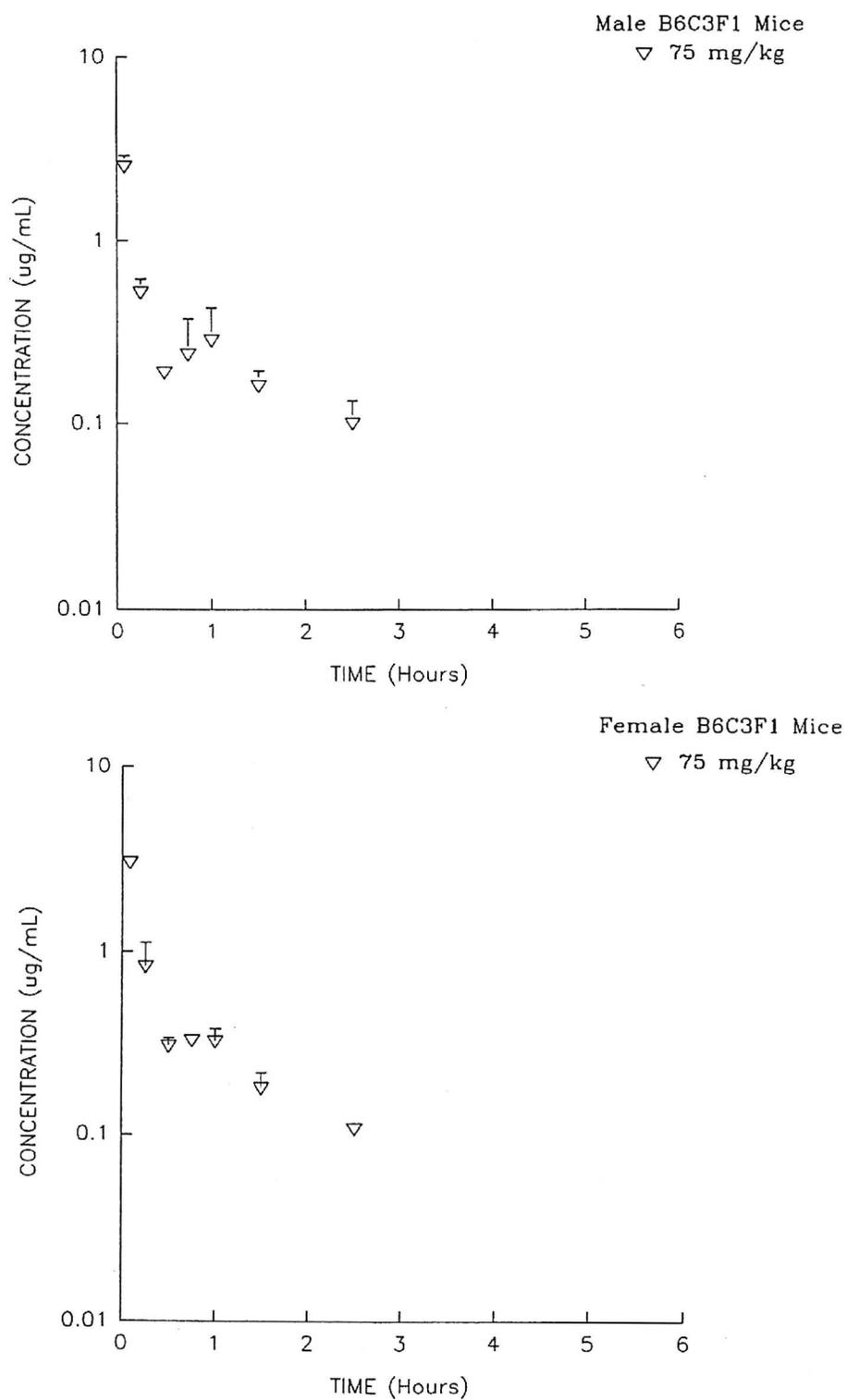
FIGURE I7
Plasma Concentrations of Methyleugenol in Mice
Administered 75 mg/kg Methyleugenol at the 12-Month Interim Evaluation
in the 2-Year Gavage Study of Methyleugenol
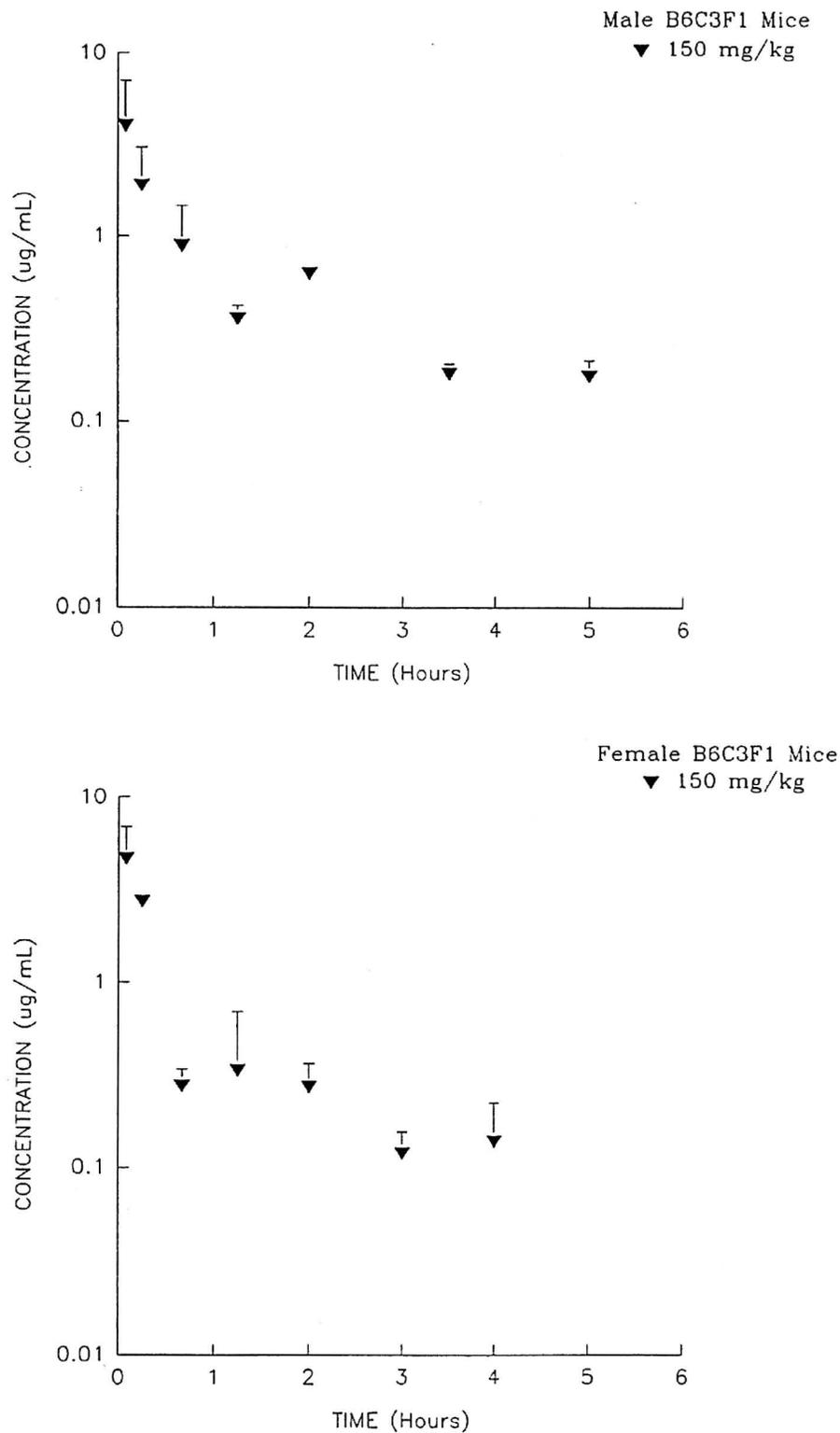
FIGURE I8
Plasma Concentrations of Methyleugenol in Mice
Administered 150 mg/kg Methyleugenol at the 12-Month Interim Evaluation
in the 2-Year Gavage Study of Methyleugenol
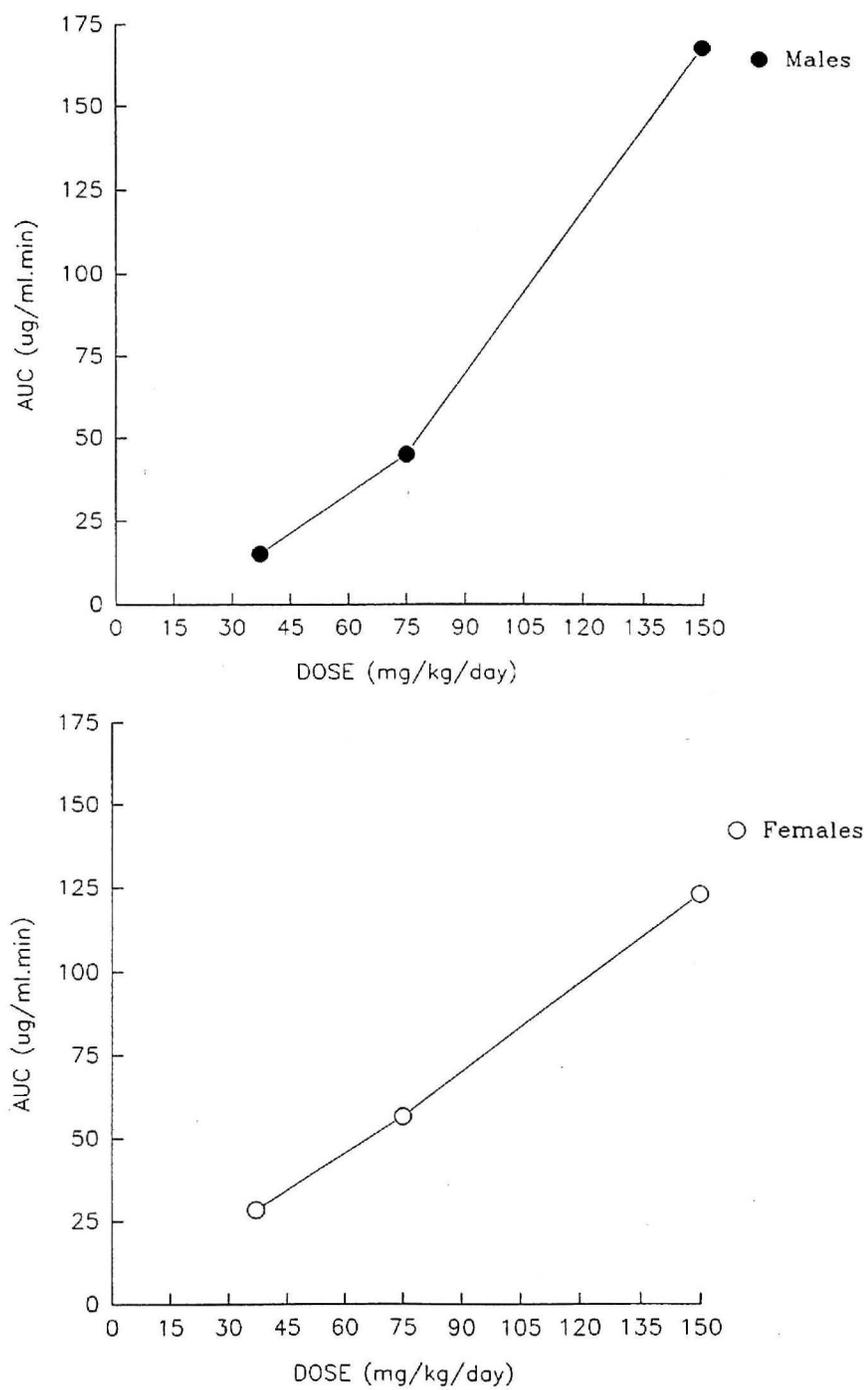
FIGURE I9
Plots of AUC Versus Dose for Methyleugenol in Mice
At the 12-Month Interim Evaluation in the 2-Year Gavage Study of Methyleugenol

TABLE I7
Plasma Concentrations of Methyleugenol in Aged Mice after a Single Gavage Dose
of 75 mg/kg Methyleugenol[a]

Time after Dosing (minutes)	Concentration[b] (μg/mL)
Male	
5	1.54 ± 0.89
20	0.821 ± 0.219[c]
50	0.279 ± 0.080[c]
90	0.101 ± 0.039[c]
150	0.067 ± 0.021
Female	
5	3.30 ± 0.83
20	1.527 ± 0.116
50	0.998 ± 0.414
90	0.355 ± 0.197[c]
150	0.137 ± 0.022

[a] Three animals were bled at each time point.
[b] Data are given in μg/mL as the mean $\pm$ standard error.
[c] Only two animals bled

TABLE I8
Toxicokinetic Parameters in Aged Mice after a Single Gavage Dose of 75 mg/kg Methyleugenol[a]

	C_{max} (μg/mL)	T_{max} (minutes)	$t_{1/2}$ (minutes)	AUC (μg/mL•minute)
Male				
	1.54	5	74.4	48.4
Female				
	3.30	5	76.3	119.4

[a] C_{max} = maximum mean concentration; T_{max} = time of maximum mean concentration; $t_{1/2}$ = elimination half-life; AUC = area under the curve
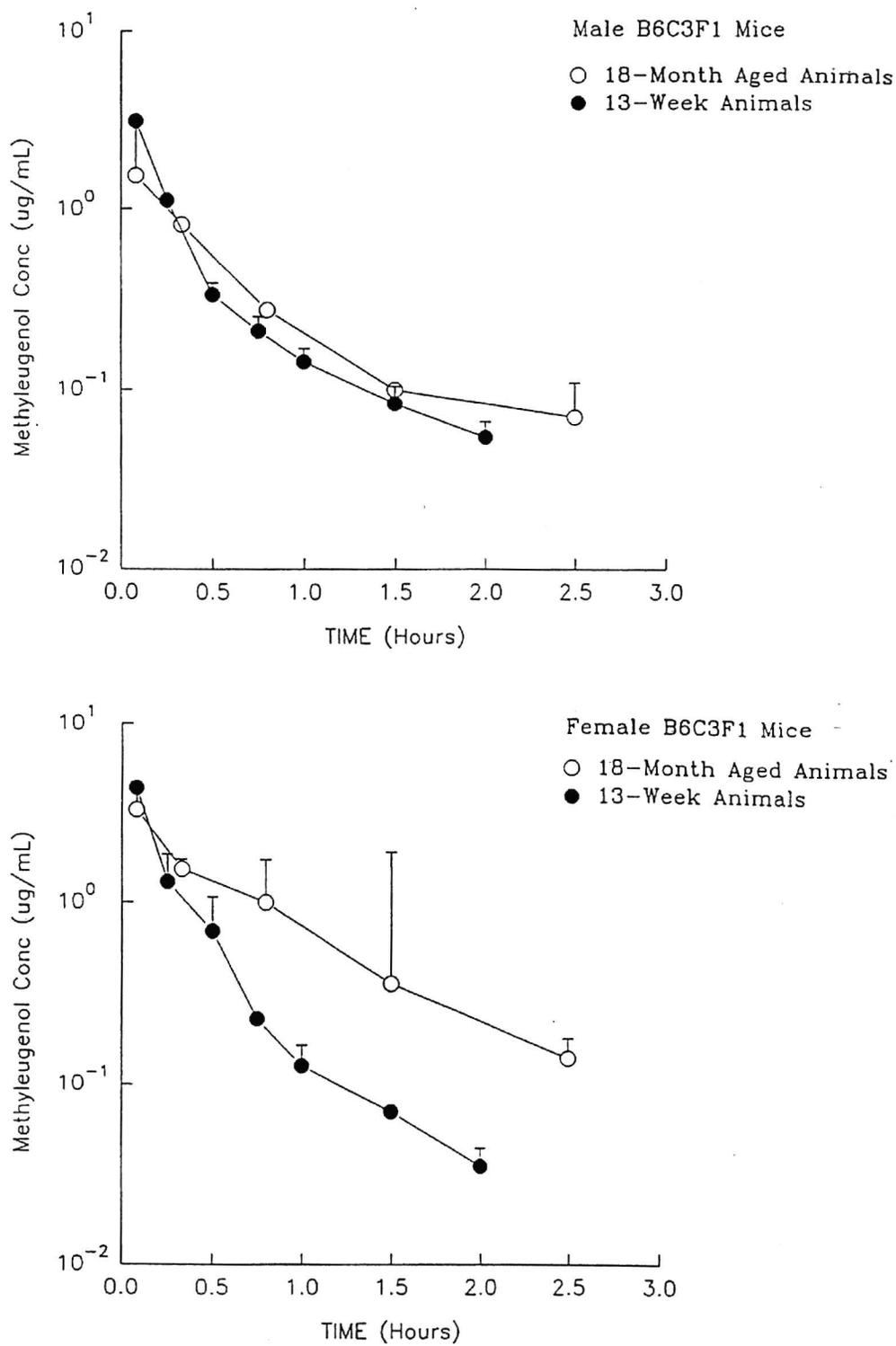
FIGURE I10
Plasma Concentrations of Methyleugenol after a Single Gavage Dose
of 75 mg/kg Methyleugenol in Aged and 13-Week-Old Mice

APPENDIX J
CHEMICAL CHARACTERIZATION
AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION
AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Methyleugenol

Methyleugenol was obtained from Elan Chemical Company (Newark, NJ) in two lots (8334801 and 9224705). Lot 8334801 was used during the 14-week studies, and lot 9224705 was used during the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and the study laboratory. Reports on analyses performed in support of the methyleugenol studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a clear, colorless, or pale yellow liquid, was identified as methyleugenol by infrared, ultraviolet/visible (lot 8334801), and nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure of methyleugenol and with the literature spectra (*Sadtler Standard Spectra*). The infrared and nuclear magnetic resonance spectra are presented in Figures J1 and J2. The density ($d_{22.6}^{24.6}$) of 1.030 ± 0.001 g/mL determined for lot 8334801 was consistent with a literature reference (Weast, 1982).

The purity of lot 8334801 was determined by elemental analyses, Karl Fisher water analysis, methoxy group determination, thin-layer chromatography (TLC), and gas chromatography. The purity of lot 9224705 was determined by high-performance liquid chromatography (HPLC). Methoxy group determination was performed by Galbraith Laboratories, Inc., using a gravimetric method in which the methyleugenol was boiled with hydroiodic acid and the vapor condensed into alcoholic silver nitrate. TLC was performed on Silica Gel 60 F-254 plates with two solvent systems: 1) ethyl acetate:methanol (80:20) and 2) chloroform (100%). Vanillin was used as a reference standard for both systems. Plates were examined under ultraviolet light (254 nm) and a spray of 0.2 mL of 37% formaldehyde in 10 mL concentrated sulfuric acid. Gas chromatography was performed with flame ionization detection and two systems:

> A) 1% SP-1000 on 100/120 Supelcoport glass column, a nitrogen carrier gas at a flow rate of 70 mL/minute, and an oven temperature program of 50°C with a 5-minute hold, then 50° to 250° C at 10° C per minute, and

> B) DB-5 capillary fused silica column, a helium carrier gas at a flow rate of 10 mL/minute, a nitrogen makeup gas at 25 mL/minute, and an oven temperature program of 50° C with a 5-minute hold, then 50° to 250°C at 10° C per minute.

The initial purity analysis of lot 9224705 was performed using HPLC with a Phenomenex Ultracarb 5 ODS (30) column using ultraviolet detection (280 nm) and a solvent system of: A) water:acetonitrile (1:1) and B) acetonitrile; the flow rate was 0.70 mL/min. The solvent program was 80:20 A:B (isocratic) for 30 minutes, then a linear gradient to 100% B in 5 minutes, with a 15-minute hold.

For lot 8334801, elemental analyses for carbon and hydrogen were in agreement with theoretical values for methyleugenol. Karl Fischer water analysis indicated $0.07\% \pm 0.01\%$ water. Methoxy group determination indicated a purity of $101.4\% \pm 0.3\%$. TLC indicated a major spot and a trace impurity by the first solvent system and a major spot, six trace impurities, and a slight trace impurity by the second system. Gas chromatography by each system resolved a major peak and impurities with cumulative areas of 0.38% (system A) and 0.47% (system B) relative to the major peak area. The overall purity of lot 8334801 was determined to be approximately 99%.

The purity analysis of lot 9224705 by HPLC showed one major peak and three impurities greater than 0.1% relative to the major peak area. The overall purity of this lot was determined to be approximately 99%.

Accelerated stability studies of the bulk chemical were performed by the analytical chemistry laboratory. Gas chromatography by system A was performed but with an isothermal oven temperature of 110° C. These studies indicated that methyleugenol is stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature (22° C, 14-week studies; 25° C, 2-year studies), protected from light in amber glass bottles with Teflon®-lined caps.

Stability of the bulk material was monitored during the 14-week studies with gas chromatography and during the 2-year studies using HPLC. No degradation of the bulk chemical was determined.

Methylcellulose
Methylcellulose (USP/FCC grade) was obtained from Fisher Scientific Company (St. Louis, MO, and Pittsburgh, PA) in three lots. Lot 874544 was used during the 14-week studies, and lots 876672 and 946150 were used during the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and the study laboratory.

Lot 876672 of the chemical, a white powder, was identified as methylcellulose by infrared, ultraviolet/ visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure of methylcellulose; the infrared spectrum was also consistent with a literature spectrum (*Sadtler Pharmaceuticals Grating Spectra,* 1974) of methylcellulose. No melting point was observed; the sample decomposed at 250° C to 300° C. The study laboratory confirmed the identity of lot 946150 with infrared spectroscopy.

The purity of lot 876672 was determined by elemental analyses, Karl Fischer water analysis, functional group titration, HPLC, and the complete battery of United States Pharmacopeia (USP) XXI analyses for the identification, apparent viscosity, weight loss on drying, residue on ignition, arsenic content, heavy metal content, and percent methoxy group content. Purity by functional group titration was performed by Galbraith Laboratories, Inc. (Knoxville, TN), using 0.01 N sodium thiosulfate to quantitate the methoxy group content. HPLC was performed with a Toyo Soda TSK G4000 SW column using refractive index detection and a solvent system of 0.005M sodium dodecyl sulfate in water; the flow rate was 1.0 mL/minute.

For lot 876672, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for methylcellulose, assuming 1.8° of substitution and corrected for 1.94% water; elemental analyses also indicated 0.06% sodium. Karl Fischer water analysis indicated 1.94% ± 0.03% water. Functional group titration indicated 30.62% ± 0.08% methoxy group content; this value is consistent with the theoretical value, assuming 1.8° of substitution. HPLC indicated one major peak and no impurities with areas of 0.1% or greater relative to the major peak area. Additional HPLC analyses using 100% water and with refractive index and low-wavelength ultraviolet detection indicated that the negative peak was an artifact of the sodium dodecylsulfate in the solvent system. The USP analyses for methylcellulose gave the following results: the sample remained stable when 1 N sodium hydroxide or hydrochloric acid was added, became cloudy upon heating and formed a flaky precipitate, which redissolved when the solution cooled, and formed a thin film when dried on a glass plate; the viscosity was 3,749 to 4,060 cP; weight loss on drying was 1.9% ± 0.3%; residue on ignition was less than 0.3%; absorbance between 535 and 540 nm did not exceed the absorbance of 3.0 mL of a standard arsenic preparation; the color of a sample, after ignition and then digestion with hydrochloric acid, was not darker than that of a standard preparation, indicating no excessive heavy metal impurities; and methoxy content was 30.3% ± 0.2%. These results indicated that lot 876672 met the USP specifications for methylcellulose.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory. Gas chromatography was performed using a 10% SP-2100 on 100/120 Chromosorb WHP glass column and a 5% OV-101 on 100/120 Chromosorb GHP stainless steel reference column with a thermal conductivity detector, a helium carrier gas at a flow rate of 20 mL/minute, and an isothermal oven temperature of $100°$ C. These studies indicated that methylcellulose was stable as a bulk chemical for 3 weeks when stored protected from light at temperatures up to $60°$ C. To ensure stability, the bulk chemical was stored at approximately $25°$ C in sealed containers, protected from light. Stability was monitored during the 2-year studies at approximately 4-month intervals by functional group titration for the methoxy group. No significant degradation for either lot was observed during the 2-year studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing methyleugenol with 0.5% aqueous methylcellulose to give the required concentrations (Table J1). The dose formulations were stored at room temperature in the dark in amber glass bottles sealed with Teflon®-lined caps and containing magnetic stir bars (2-year studies only) for up to 18 days for the 14-week studies and up to 35 days for the 2-year studies.

Homogeneity analyses of a 200 mg/g formulation were performed by the study laboratory using HPLC with a Brownlee RP-18 column using ultraviolet detection (280 nm) and a solvent system of water:acetonitrile (50:50); the flow rate was 1.0 mL/minute. Stability studies of a 1.0 mg/g formulation were performed using the same method. Homogeneity was confirmed, and the stability of the 1.0 mg/g formulation was confirmed for 3 weeks when formulations were stored at room temperature, protected from light. Formulations stored open to air and light showed an approximate 3% loss.

The homogeneity of 0.8 and 60 mg/mL formulations and stability of a 0.8 mg/mL formulation were analyzed by the study laboratory using HPLC with a Phenomenex Ultracarb 5 ODS (30) column using ultraviolet detection (280 nm) and a solvent system of water:acetonitrile (25:75); the flow rate was 0.70 mL/minute. Homogeneity was confirmed; stability was confirmed for 35 days when formulations were stored in sealed containers with minimal headspace, protected from light, at room temperature. During the studies, formulations were stored at room temperature in amber glass containers with Teflon®-lined lids.

Periodic analyses of the dose formulations of methyleugenol were conducted at the study laboratory using HPLC with a Brownlee RP-18 column, ultraviolet detection (280 nm), and a solvent system of water:acetonitrile (50:50) at an isocratic flow rate of 1 mL/minute (14-week studies) or quantitation of ultraviolet absorbance in the range from 200 to 350 nm (2-year studies). Dose formulations were analyzed at the beginning, midpoint, and end of the 14-week studies; animal room samples of these dose formulations were also analyzed. All dose formulations and animal room samples were within 10% of the target concentrations (Table J2). During the 2-year studies, the dose formulations were analyzed approximately every 8 weeks; 96% (47/49) of the dose formulations for rats and 96% (43/45) of the dose formulations for mice were within 10% of the target concentrations; all animal room samples for rats and mice were also within 10% of the target concentrations. With the exception of the 7.5 mg/mL dose formulation mixed for mice on 14 December 1993, the dose formulations that were not within 10% of the target concentrations were remixed; the remixes were analyzed and were found to be within 10% of the target concentrations (Table J3). The 7.5 mg/mL dose formulation mixed for mice in December 1993 was within 11% of the target concentration and was considered adequate for use in the study. Results of periodic referee analyses performed by the analytical chemistry laboratory using HPLC/UV during the 14-week studies agreed with the results obtained by the study laboratory (Table J4).
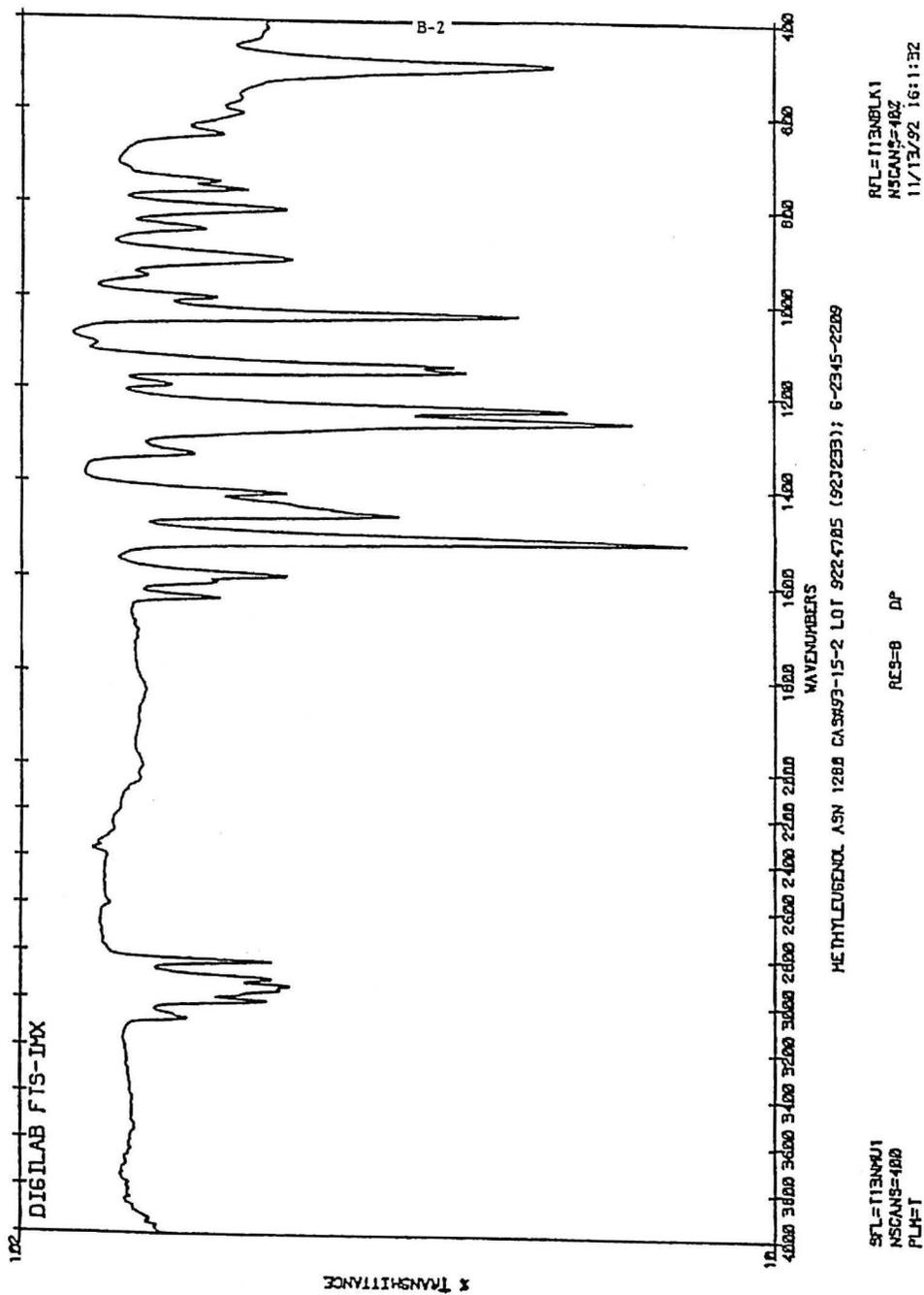
FIGURE J1
Infrared Absorption Spectrum of Methyleugenol
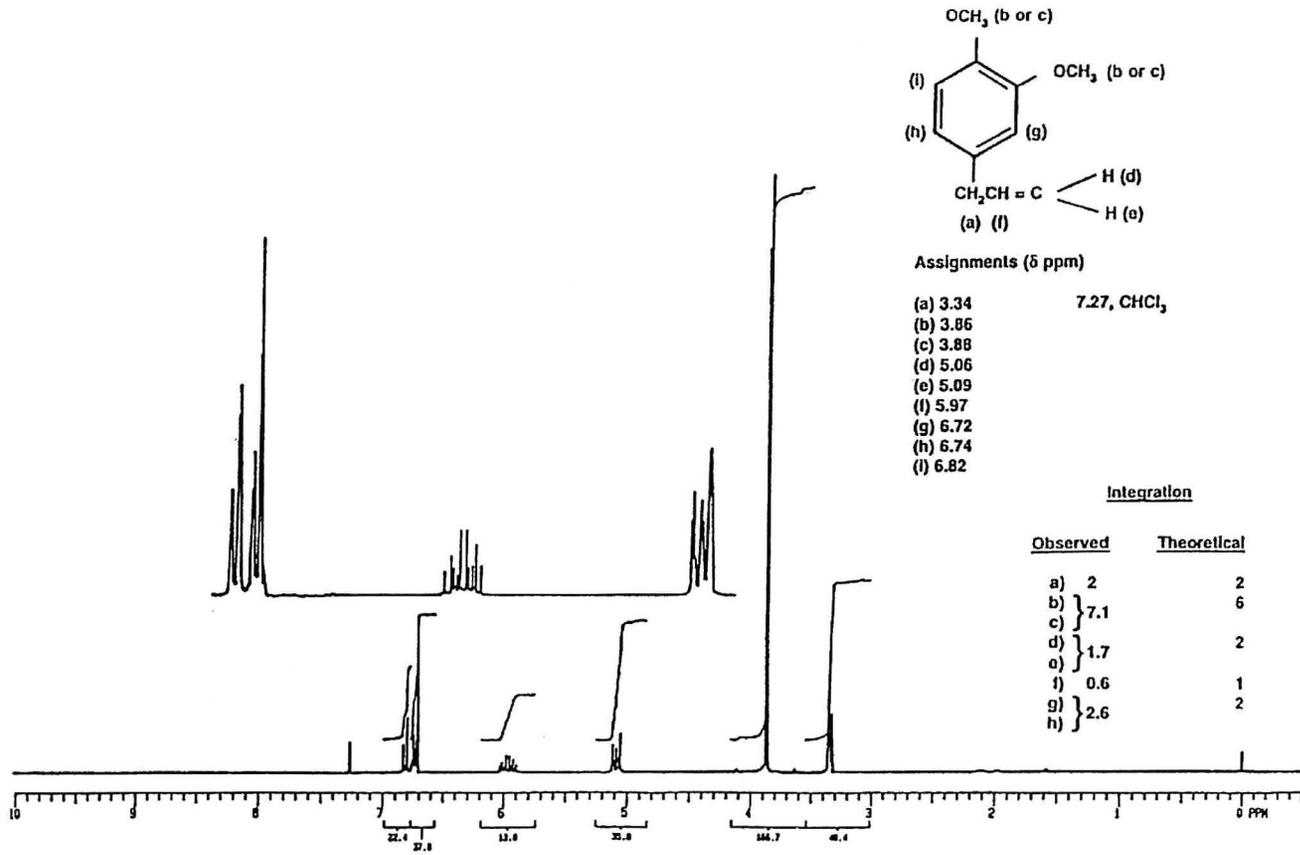
FIGURE J2
Nuclear Magnetic Resonance Spectrum of Methyleugenol

TABLE J1
Preparation and Storage of Dose Formulations in the Gavage Studies of Methyleugenol

14-Week Studies	2-Year Studies

Preparation

The vehicle was prepared by mixing methylcellulose and heated, deionized water with a magnetic stirrer to form a 0.5% solution, which was then cooled. Methyleugenol was slowly added to the 0.5% methylcellulose and mixed for 2 minutes using a homogenizer fitted with an anaerobic generator. The anaerobic generator was removed and the mixture was stirred with a magnetic stirrer for 1 hour.

The vehicle was prepared as for the 14-week studies. Methyleugenol was slowly added to 0.5% methylcellulose and then diluted to the required volume with additional 0.5% methylcellulose while being stirred continuously.

Chemical Lot Number

8334801

9224705

Maximum Storage Time

18 days

35 days

Storage Conditions

Stored in amber glass bottles sealed with a Teflon®-lined cap at room temperature in the dark

Same as 14-week studies; bottles contained magnetic stir bars

Study Laboratory

Southern Research Institute
(Birmingham, AL)

Battelle Columbus Laboratories
(Columbus, OH)

Referee Laboratory

Midwest Research Institute
(Kansas City, MO)

None

TABLE J2
**Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies
of Methyleugenol**

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration[a] (mg/g)	Difference from Target (%)
Rats				
13 June 1988[b]	14–15 June 1988	200	206	+3
		200	205	+3
		200	202	+1
20 June 1988	21 June 1988	2	2.02	+1
		6	5.58	−7
		20	20.2	+1
		60	61.8	+3
		200	205	+3
	5–6 July 1988[c]	2	2.02	+1
		6	6.04	+1
		20	20.6	+3
		60	58.2	−3
		200	196	−2
8 August 1988	9–12 August 1988	2	1.96	−2
		6	5.92	−1
		20	17.9	−10
		60	58.0	−3
		200	200	0
	30 August – 1 September 1988[c]	2	2.10	+5
		6	6.25	+4
		20	21.8	+9
		60	61.0	+2
		200	198	−1
16 September 1988	19–21 September 1988	2	1.98	−1
		6	6.00	0
		20	20.5	+3
		60	62.0	+3
		200	204	+2
	10–12 October 1988[c]	2	1.96	−2
		6	5.96	−1
		20	19.8	−1
		60	60.6	+1
		200	200	0

TABLE J2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Gavage Studies of Methyleugenol

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice				
13 June 1988[b]	14–15 June 1988	1	1.00	0
		1	1.02	+2
		1	0.968	−3
5 July 1988	5–6 July 1988	1	1.10	+10
		3	3.04	+1
		10	10.4	+4
		30	31.4	+5
		100	102	+2
	18–22 July 1988[c]	1	1.10	+10
		3	3.02	+1
		10	10.2	+2
		30	30.8	+3
		100	102	+2
8 August 1988	9–12 August 1988	1	1.02	+2
		3	3.02	+1
		10	10.0	0
		30	30.4	+1
		100	102	+2
	30 August– 1 September 1988[c]	1	1.03	+3
		3	2.99	0
		10	9.77	−2
		30	30.0	0
		100	102	+2
19 September 1988	19–21 September 1988	1	0.950	−5
		3	2.93	−2
		10	10.2	+2
		30	30.8	+3
		100	103	+3
19 September 1988	10–12 October 1988[c]	1	0.970	−3
		3	2.92	−3
		10	9.88	−1
		30	29.9	0
		100	97.6	−2

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg; 2 mg/g=10 mg/kg, 6 mg/g=30 mg/kg, 20 mg/g=100 mg/kg, 60 mg/g=300 mg/kg, 200 mg/g=1,000 mg/kg. For mice, dosing volume=10 mL/kg; 1 mg/g=10 mg/kg, 3 mg/g=30 mg/kg, 10 mg/g=100 mg/kg, 30 mg/g=300 mg/kg, 100 mg/g=1,000 mg/kg

[b] Homogeneity analyses; not used for dosing

[c] Animal room samples

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Methyleugenol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
1 February 1994	2 February 1994	7.5	6.900	−8
		15	14.27	−5
		30	41.22[b]	+37
		60	57.38	−4
3 February 1994	3 February 1994	30	30.47[c]	+2
1 & 3 February 1994[d]	23 March 1994	7.5	6.980	−7
		15	14.25	−5
		30	30.55	+2
		60	57.88	−4
28 March 1994	30 March 1994	7.5	7.077	−6
		15	15.38	+3
		30	29.54	−2
		60	59.91	0
28 March 1994[d]	5 May 1994	7.5	7.120	−5
		15	15.42	+3
		30	29.64	−1
		60	61.66	+3
23 May 1994	24 May 1994	7.5	7.082	−6
		15	15.53	+4
		30	30.20	+1
		60	61.14	+2
18 July 1994	18 & 20 July 1994	7.5	7.851	+5
		15	14.72	−2
		30	27.83	−7
		60	59.99	0
12 September 1994	13 September 1994	7.5	6.896	−8
		15	15.25	+2
		30	30.44	+1
		60	61.04	+2
12 September 1994[d]	18 October 1994	7.5	6.723[b]	−10
		15	14.29	−5
		30	30.49	+2
		60	61.15	+2
7 & 11 November 1994	10 & 11 November 1994	7.5	7.267	−3
		15	14.53	−3
		30	30.58	+2
		60	57.68	−4
3 January 1995	5 January 1995	7.5	7.722	+3
		15	15.46	+3
		30	32.15	+7
		60	62.96	+5

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Methyleugenol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
27 February 1995	27 February 1995	7.5	7.660	+2
		15	15.62	+4
		30	29.14	−3
27 February 1995[d]	5 April 1995	7.5	7.393	−1
		15	14.64	−2
		30	28.31	−6
24 April 1995	24 April 1995	7.5	7.737	+3
		15	15.44	+3
		30	29.18	−3
22 May 1995	24 May 1995	7.5	7.434	−1
		15	14.97	0
		30	30.55	+2
17 July 1995	18 July 1995	7.5	7.421	−1
		15	14.86	−1
		30	30.30	+1
9 October 1995	11 October 1995	7.5	7.435	−1
		15	15.02	0
		30	31.26	+4
9 October 1995[d]	16 November 1995	7.5	7.173	−4
		15	15.11	+1
		30	31.26	+4
4 December 1995	5 December 1995	7.5	6.539[b]	−13
		15	14.87	−1
		30	30.13	0
11 December 1995	11 December 1995	7.5	8.082[c]	+8
2 January 1996	3 January 1996	7.5	7.892	+5
		15	14.80	−1
		30	30.25	+1
Mice				
19 October 1993	20 October 1993	3.7	3.377	−9
		7.5	7.204	−4
		15	11.02[b]	−27
21 October 1993	21 October 1993	15	15.41[c]	+3
19 & 21 October 1993[d]	23 November 1993	3.7	3.367	−9
		7.5	7.382	−2
		15	15.27	+2

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Methyleugenol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
16 November 1993	17 November 1993	3.7	3.661	−1
		7.5	7.484	0
		15	14.46	−4
14 December 1993	15 December 1993	3.7	3.514	−5
		7.5	6.698	−11
		15	15.51	+3
1 February 1994	2 February 1994	3.7	3.532	−5
		7.5	6.900	−8
		15	14.27	−5
28 March 1994	30 March 1994	3.7	3.430	−7
		7.5	7.077	−6
		15	15.38	+3
28 March 1994[d]	5 May 1994	3.7	3.394	−8
		7.5	7.352	−2
		15	15.35	+2
23 May 1994	24 May 1994	3.7	3.538	−4
		7.5	7.082	−6
		15	15.53	+4
18 July 1994	19 & 20 July 1994	3.7	3.465	−6
		7.5	7.851	+5
		15	14.72	−2
12 September 1994	13 September 1994	3.7	3.629	−2
		7.5	6.896	−8
		15	15.25	+2
12 September 1994[d]	18 October 1994	3.7	3.891	+5
		7.5	6.723	−10
		15	14.29	−5
7 & 11 November 1994	10 & 11 November 1994	3.7	3.970	+7
		7.5	7.267	−3
		15	14.53	−3
3 January 1995	5 January 1995	3.7	3.436	−7
		7.5	7.722	+3
		15	15.46	+3

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Methyleugenol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
27 February 1995	27 February 1995	3.7	3.407	−8
		7.5	7.660	+2
		15	15.62	+4
27 February 1995[d]	5 April 1995	3.7	3.427	−7
		7.5	7.193	−4
		15	14.07	−6
24 April 1995	24 April 1995	3.7	3.824	+3
		7.5	7.737	+3
		15	15.44	+3
22 May 1995	24 May 1995	3.7	3.613	−2
		7.5	7.434	−1
		15	14.97	0
17 July 1995	18 July 1995	3.7	3.546	−4
		7.5	7.421	−1
		15	14.86	−1
9 October 1995	11 October 1995	3.7	3.529	−5
		7.5	7.435	−1
		15	15.02	0

[a] Results of duplicate analyses. For rats, dosing volume=5 mL/kg (rats); 7.5 mg/mL=37 mg/kg, 15 mg/mL=75 mg/kg, 30 mg/mL=150 mg/kg, 60 mg/mL=300 mg/kg. For mice, dosing volume=10 mL/kg (mice); 3.7 mg/mL=37 mg/kg; 7.5 mg/mL=75 mg/kg; 15 mg/mL=150 mg/kg
[b] Remixed; not used in study
[c] Results of remix
[d] Animal room samples

TABLE J4
**Results of Referee Analyses of Dose Formulations Administered to Rats and Mice
in the 14-Week Gavage Studies of Methyleugenol**

Date Prepared	Target Concentration (mg/g)	Determined Concentration (mg/g)	
		Study Laboratory[a]	Referee Laboratory[b]
Rats			
20 June 1988	60	61.8	60.5 ± 0.2
Mice			
8 August 1988	3	3.02	2.96 ± 0.03

[a] Results of duplicate analyses
[b] Results of triplicate analyses (mean ± standard error)

APPENDIX K
INGREDIENTS, NUTRIENT COMPOSITION,
AND CONTAMINANT LEVELS
IN NIH-07 RAT AND MOUSE RATION

TABLE K1
Ingredients of NIH-07 Rat and Mouse Ration[a]

Ingredients[b]	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

[a] NCI, 1976; NIH, 1978
[b] Ingredients were ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE K2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	d-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	d-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE K3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.90 ± 0.47	22.1 – 23.6	27
Crude fat (% by weight)	5.38 ± 0.21	5.00 – 5.80	27
Crude fiber (% by weight)	3.31 ± 0.36	2.80 – 4.30	27
Ash (% by weight)	6.29 ± 0.21	5.72 – 6.82	27
Amino Acids (% of total diet)			
Arginine	1.272 ± 0.083	1.100 – 1.390	12
Cystine	0.307 ± 0.068	0.181 – 0.400	12
Glycine	1.152 ± 0.051	1.060 – 1.220	12
Histidine	0.581 ± 0.029	0.531 – 0.630	12
Isoleucine	0.913 ± 0.034	0.867 – 0.965	12
Leucine	1.969 ± 0.053	1.850 – 2.040	12
Lysine	1.269 ± 0.050	1.200 – 1.370	12
Methionine	0.436 ± 0.104	0.306 – 0.699	12
Phenylalanine	0.999 ± 0.114	0.665 – 1.110	12
Threonine	0.899 ± 0.059	0.824 – 0.985	12
Tryptophan	0.216 ± 0.146	0.107 – 0.671	12
Tyrosine	0.690 ± 0.091	0.564 – 0.794	12
Valine	1.079 ± 0.057	0.962 – 1.170	12
Essential Fatty Acids (% of total diet)			
Linoleic	2.389 ± 0.223	1.830 – 2.570	11
Linolenic	0.257 ± 0.062	0.100 – 0.320	11
Vitamins			
Vitamin A (IU/kg)	6,494 ± 490	5,500 – 7,260	27
Vitamin D (IU/kg)	4,450 ± 1,382	3,000 – 6,300	4
α-Tocopherol (ppm)	35.24 ± 8.58	22.5 – 48.9	12
Thiamine (ppm)	18.38 ± 3.68	14.0 – 26.0	26
Riboflavin (ppm)	7.78 ± 0.899	6.10 – 9.00	12
Niacin (ppm)	98.73 ± 23.21	65.0 – 150.0	12
Pantothenic acid (ppm)	32.94 ± 8.92	23.0 – 59.2	12
Pyridoxine (ppm)	9.28 ± 2.49	5.60 – 14.0	12
Folic acid (ppm)	2.56 ± 0.70	1.80 – 3.70	12
Biotin (ppm)	0.265 ± 0.046	0.190 – 0.354	12
Vitamin B_{12} (ppb)	41.6 ± 18.6	10.6 – 65.0	12
Choline (ppm)	2,955 ± 382	2,300 – 3,430	11
Minerals			
Calcium (%)	1.17 ± 0.07	1.03 – 1.33	27
Phosphorus (%)	0.91 ± 0.04	0.860 – 1.000	27
Potassium (%)	0.886 ± 0.059	0.772 – 0.971	10
Chloride (%)	0.531 ± 0.082	0.380 – 0.635	10
Sodium (%)	0.316 ± 0.031	0.258 – 0.371	12
Magnesium (%)	0.165 ± 0.010	0.148 – 0.181	12
Sulfur (%)	0.266 ± 0.060	0.208 – 0.420	11
Iron (ppm)	348.0 ± 83.7	255.0 – 523.0	12
Manganese (ppm)	93.27 ± 5.62	81.7 – 102.0	12
Zinc (ppm)	59.42 ± 9.7	46.1 – 81.6	12
Copper (ppm)	11.63 ± 2.46	8.09 – 15.4	12
Iodine (ppm)	3.49 ± 1.14	1.52 – 5.83	11
Chromium (ppm)	1.57 ± 0.53	0.60 – 2.09	12
Cobalt (ppm)	0.81 ± 0.27	0.49 – 1.23	8

TABLE K4
Contaminant Levels in NIH-07 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.56 ± 0.17	0.10 – 0.80	27
Cadmium (ppm)	0.05 ± 0.02	0.04 – 0.13	27
Lead (ppm)	0.26 ± 0.10	0.20 – 0.50	27
Mercury (ppm)	<0.02		27
Selenium (ppm)	0.34 ± 0.10	0.10 – 0.50	27
Aflatoxins (ppb)	<5.0		27
Nitrate nitrogen (ppm)[c]	6.86 ± 2.18	2.90 – 11.0	27
Nitrite nitrogen (ppm)[c]	1.25 ± 0.87	0.30 – 3.50	27
BHA (ppm)[d]	1.04 ± 1.01	0.01 – 5.0	27
BHT (ppm)[d]	1.56 ± 1.16	0.10 – 5.00	27
Aerobic plate count (CFU/g)	155,778 ± 147,267	11,000 – 460,000	27
Coliform (MPN/g)	143 ± 536	3 – 2,800	27
Escherichia coli (MPN/g)	8 ± 3	3 – 10	27
Salmonella (MPN/g)	Negative		27
Total nitrosoamines (ppb)[e]	10.93 ± 2.59	4.0 – 14.7	27
N-Nitrosodimethylamine (ppb)[e]	9.17 ± 2.47	3.0 – 13.00	27
N-Nitrosopyrrolidine (ppb)[e]	1.76 ± 0.62	1.0 – 3.3	27
Pesticides (ppm)			
α-BHC	<0.01		
β-BHC	<0.02		27
γ-BHC	<0.01		27
δ-BHC	<0.01		27
Heptachlor	<0.01		27
Aldrin	<0.01		27
Heptachlor epoxide	<0.01		27
DDE	<0.01		27
DDD	<0.01		27
DDT	<0.01		27
HCB	<0.01		27
Mirex	<0.01		27
Methoxychlor	<0.05		27
Dieldrin	<0.01		27
Endrin	<0.01		27
Telodrin	<0.01		27
Chlordane	<0.05		27
Toxaphene	<0.10		27
Estimated PCBs	<0.20		27
Ronnel	<0.01		27
Ethion	<0.02		27
Trithion	<0.05		27
Diazinon	<0.10		27
Methyl parathion	<0.02		27
Ethyl parathion	<0.02		27
Malathion	0.14 ± 0.20	0.02 – 0.83	27
Endosulfan I	<0.01		27
Endosulfan II	<0.01		27
Endosulfan sulfate	<0.03		27

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

APPENDIX L
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from randomly selected rats and mice during the 14-week and 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to Microbiological Associates, Inc. (Bethesda, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
14-Week Study	
ELISA	
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/ sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	Study termination
KRV (Kilham rat virus)	Study termination
2-Year Study	
ELISA	
Mycoplasma arthriditis	Study termination
Mycoplasma pulmonis	Study termination
PVM	Study initiation, 1, 6, 12, and 18 months, study termination
RCV/SDA	Study initiation, 1, 6, 12, and 18 months, study termination
Sendai	Study initiation, 1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
M. arthriditis	Study termination
Hemagglutination Inhibition	
H-1	Study initiation, 1, 6, 12, and 18 months, study termination
KRV	Study initiation, 1, 6, 12, and 18 months, study termination

MICE

14-Week Study

ELISA

Ectromelia virus	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
MVM (minute virus of mice)	Study termination
Mouse adenoma virus	Study termination
MHV (mouse hepatitis virus)	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination

Immunofluorescence Assay

EDIM (epizootic diarrhea of infant mice)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination

Hemagglutination Inhibition

K (papovavirus)	Study termination
Polyoma virus	Study termination

2-Year Study

ELISA

Ectromelia virus	Study initiation, 1, 6, 12, and 18 months, study termination
EDIM	Study initiation, 1, 6, 12, and 18 months, study termination
GDVII	Study initiation, 1, 6, 12, and 18 months, study termination
LCM	Study initiation, 1, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	Study initiation, 1, 6, 12, and 18 months, study termination
MHV	Study initiation, 1, 6, 12, and 18 months, study termination
M. arthriditis	Study termination
M. pulmonis	Study termination
PVM	Study initiation, 1, 6, 12, and 18 months, study termination
Reovirus 3	Study initiation, 1, 6, 12, and 18 months, study termination
Sendai	Study initiation, 1, 6, 12, and 18 months, study termination

Immunofluorescence Assay

GDVII	Study termination
LCM	Study termination
MCMV (mouse cytomegalovirus)	Study termination
Reovirus 3	Study termination

MICE (continued)
2-Year Study (continued)
Hemagglutination Inhibition

K	Study initiation, 1, 6, 12, and 18 months, study termination
MVM	Study initiation, 1, 6, 12, and 18 months, study termination
Polyoma virus	Study initiation, 1, 6, 12, and 18 months, study termination

RESULTS

All test results were negative.

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

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

INTRODUCTION

Methyleugenol is used commercially as a flavoring agent in jellies, baked goods, nonalcoholic beverages, chewing gum, candy, puddings, relish, and ice cream, and as a fragrance in perfumes, creams, lotions, soaps, and detergents (*Fenaroli's*, 1975; Opdyke, 1979). Combined with pesticides, methyleugenol is used as an attractant for male fruit flies in control and eradication programs (Hays and Laws, 1991). Methyleugenol is also an active ingredient in traditional herbal medicines. Single-dose intravenous and oral gavage toxicokinetic studies of methyleugenol in male and female F344/N rats and B6C3F$_1$ mice were conducted by Battelle Columbus Laboratories (Columbus, OH).

MATERIALS AND METHODS

Methyleugenol was obtained from Elan Chemical Company (Newark, NJ) in one lot (9224705), which was also used in the 2-year studies conducted at Battelle Columbus Laboratories. Results of identity, purity, and stability analyses of lot 9224705 are presented in Appendix J. Methylcellulose for the gavage vehicle was obtained from Fisher Scientific Company (Pittsburgh, PA) in one lot (876672), which was also used in the 14-week and 2-year studies. Dose formulations for gavage administration were prepared in 0.5% aqueous methylcellulose. Dose formulations for intravenous injection were prepared by mixing methyleugenol with water:emulphor:ethanol (8:1:1). Analyses by the study laboratory using high-performance liquid chromatography with ultraviolet detection indicated that the dose formulations were stable for up to 35 days when stored in sealed containers at room temperature.

Male and female F344/N rats were obtained from Taconic Laboratories (Germantown, NY); male and female mice were obtained from Charles River Laboratories (Portage, MI). Animals were acclimated for 14 days prior to being assigned to the study. Rats and mice were housed individually in polycarbonate cages containing hardwood bedding (Sani-Chips®, P.J. Murphy Forest Products Corp., Montville, NJ). Room environmental conditions included a temperature range of 21° to 24° C, relative humidity of 40% to 65%, 12:12 hour light/dark cycle, and a minimum of 10 fresh air changes per hour. Animals received NIH-07 open formula diet and water *ad libitum*.

Groups of 12 male and 12 female rats were administered a single intravenous injection of 37 mg methyleugenol/kg body weight; or a single dose of 37, 75, or 150 mg methyleugenol/kg body weight by gavage. The dosing volumes were 2 mL/kg body weight by intravenous injection and 5 mL/kg by gavage. Groups of 24 male and 24 female mice were administered a single intravenous injection of 25 mg methyleugenol/kg body weight or a single dose of 25, 50, or 75 mg methyleugenol/kg body weight by gavage. The dosing volumes were 4 mL/kg body weight by intravenous injection and 10 mL/kg body weight by gavage. The animals were anesthetized with a mixture of carbon dioxide and oxygen, and blood samples were collected from the retroorbital sinus (rats and mice) or by cardiac puncture (mice).

In the rat intravenous injection study, blood was collected from three males and three females per time point at 2, 5, 15, 30, 45, 90, 180, and 360 minutes after methyleugenol administration. In the rat gavage study, blood was collected from three males and three females per time point at 5, 15, 30, 60, 90, 120, 240, and 360 minutes after methyleugenol administration. In the mouse intravenous injection study, blood was collected from two to four mice per time point at 2, 5, 15, 30, 45, 60, 180, and 300 minutes after methyleugenol administration. In the mouse gavage study, blood was collected from three males and three

females per time point at 5, 15, 30, 45, 60, 90, 120, and 240 minutes after methyleugenol administration. Each rat was bled twice and each mouse was bled once. The samples were collected into tubes containing EDTA as an anticoagulant; the plasma was separated by centrifugation and stored at approximately –20° C until analysis.

All animals were observed twice daily for signs of morbidity and mortality. Individual body weights were recorded at randomization and on the day each animal was dosed (study day 1). Body weights from study day 1 were used for the calculation of dosing volumes.

Plasma samples were analyzed in the Bioanalytical Chemistry Laboratory using the techniques described in the Materials and Methods section for the determination of methyleugenol in plasma.

Individual replicate values were recorded and summarized as the mean $\pm$ standard error of the mean. The limit of quantitation (LOQ) was 0.050 μg/mL. Concentrations below the LOQ were evaluated, and a high degree of precision and accuracy was found in the values, down to a concentration of 0.025 μg/mL. Values below the LOQ but above 0.025 μg/mL were used to calculate the mean. However, if a value was less than 0.025 μg/mL, then a value of 0.0125 μg/mL (midpoint between 0 and 0.025 μg/mL) was used to calculate the mean.

Plasma concentration values were recorded for individual animals, and the mean $\pm$ standard error was calculated by sex, dose group, and time point using tables and graphic illustrations. Graphic illustrations include semilog plots of concentration versus time and area under the curve (AUC) versus dose. Values for AUC were calculated for each concentration-versus-time profile using the trapezoidal method. A software program (Sigma Plot, Version 5.0) was used to calculate the AUC values. Reported toxicokinetic parameters, i.e., C_{max}, T_{max}, and $t_{1/2}$, are observed values only.

RESULTS

Rats

The toxicokinetic parameters are observed values taken from the actual plasma concentration-time profiles. No attempt was made to model the plasma concentration-time profile to obtain a best-fit curve. Semilogarithmic plasma concentration-versus-time graphs are shown in Figure M1 (intravenous) and Figures M2 and M3 (oral). Observed toxicokinetic parameters are summarized in Table M5.

Intravenous Administration

For the intravenous plasma concentration-time profile, a biphasic curve appears to be the best fit for the data points (Figure M1). A biphasic curve would suggest that the fate of methyleugenol is best described by a two-compartment open model. This model includes an initial tissue distribution phase (the initial portion of the biphasic curve) and an elimination phase (the terminal linear portion of the biphasic curve). The intravenous plasma concentration-time profile has well defined distribution and elimination phases.

Observed toxicokinetic parameters obtained following the single intravenous bolus injection included a maximum methyleugenol plasma concentration (C_{max}) of approximately 44.3 μg/mL (males) or 47.1 μg/mL (females) at 2 minutes after dosing (T_{max}) (Table M5). The $t_{1/2}$, which was estimated by visual inspection of the semilogarithmic plasma concentration-time profile, was determined to be approximately 75 minutes for both sexes. The AUC, calculated using the trapezoidal rule, was 581.4 μg/mL•min for males and 495.4 μg/mL•min for females.

Gavage Administration

The plasma concentration-time profiles for methyleugenol following a single oral gavage administration appeared biphasic (Figures M2 and M3). No initial upward phase was observed for characterizing the absorption phase. The biphasic curve was characterized by an initial rapidly decreasing phase, followed by a later slowly decreasing phase. The rapidly decreasing phase describes the distribution phase, and the slowly decreasing phase, or terminal linear portion, describes the elimination phase. These results are characteristic of a two-compartment open model with first-order absorption and elimination.

The observed C_{max} values were dose dependent and increased with increasing dose (Table M5). The increase in C_{max} values was within acceptable limits to be considered proportional with dose. The observed T_{max} values were independent of increasing dose. The T_{max} values were observed at the 5-minute time point for both sexes and all dose groups. The observed $t_{1/2}$ values were similar for both male and female rats and all dose groups. Even though the observed $t_{1/2}$ values for the male low- and mid-dose groups appear slightly (one- to twofold) lower, the disparity is attributed to the "eyeball" fit of the terminal linear phase and cursory extrapolation of the $t_{1/2}$ value from the curve. The area under the plasma concentration-time profile (AUC) increased linearly with increasing dose for both males and females. The absolute bioavailability percentages increased with increasing dose. Bioavailability values for methyleugenol in a methylcellulose vehicle and following a single oral gavage administration were considered to be low to moderate, with values ranging from approximately 6% to 20%.

Mice
Semilogarithmic plasma concentration-versus-time graphs are shown in Figure M4 (intravenous administration) and Figures M5 and M6 (oral administration). Observed toxicokinetic parameters are summarized in Table M6.

Intravenous Administration

The intravenous plasma concentration-time profile appears to be a biphasic curve, with a bend occurring at approximately 5 minutes (Figure M4). This profile suggests that these data are best characterized by a two-compartment open model, with an initial tissue distribution phase and a terminal linear elimination phase. Upon inspection of the raw data used to generate this curve (see Table M3), it was apparent that data points obtained at the 180-minute time point and beyond are based on plasma concentration values that are close to or below the limits of detection and/or quantitation. Therefore, the 180- and 300-minute values were not helpful in characterizing the terminal elimination phase of the curve and were not used to estimate the $t_{1/2}$ of methyleugenol in mice.

Observed toxicokinetic parameters obtained following the single intravenous bolus injection included a C_{max} of approximately 18.2 µg/mL (males) or 9.34 µg/mL (females) at 2 minutes after dosing (T_{max}) (Table M6). The $t_{1/2}$, which was estimated by visual inspection of the semilogarithmic plasma concentration-time profile, was determined to be approximately 15 minutes for males and females. The AUC, calculated by the trapezoidal rule from 0 to 180 minutes, was 116.4 µg/mL●min for males and 106.5 µg/mL●min for females.

Gavage Administration

Plasma concentration-time profiles following a single oral gavage administration were well characterized by the time points and doses selected for both sexes and all three dose groups. The profiles were biphasic, with an initial rapidly decreasing phase followed by a terminal slower decreasing phase (Figures M5 and M6). The initial phase indicates that methyleugenol undergoes distribution to peripheral compartment(s). The terminal linear phase was sufficiently characterized to determine the elimination kinetics of methyleugenol. No absorption phase could be characterized despite taking samples as early as 5 minutes

after dosing. Two female dose groups, 25 and 50 mg/kg, had one time point that may have occurred before the distribution/elimination rates exceeded the absorption rate, but no definitive characterization of absorption could be determined from such scant data.

The observed C_{max} values were dose dependent and increased with increasing dose (Table M6). The increase in C_{max} was proportional with dose for the males. For the females, C_{max} increased more than proportionally with dose. The observed T_{max} values occurred at 5 minutes for all groups except the female 25 mg/kg dose group, which had a T_{max} value of 15 minutes. However, the 5- and 15-minute plasma concentration values were virtually the same, making it difficult to know for certain whether the T_{max} is 5 or 15 minutes. Regardless, the rate of absorption of methyleugenol was very rapid. The observed $t_{1/2}$ was 30 minutes for all dose groups and both sexes. Thus, elimination was rapid, and there was no evidence of saturation of elimination for methyleugenol between doses of 25 to 75 mg/kg for males and females. The area under the plasma concentration-time profile (AUC) increased with increasing dose. Percent absolute bioavailability values increased with increasing dose. Bioavailability values for methyleugenol in a methylcellulose vehicle and following a single oral gavage administration were considered to be low to moderate, with values ranging from approximately 4% to 14% for the males and 3% to 19% for the females.

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TABLE M1
**Plasma Concentrations of Methyleugenol in F344/N Rats after a Single Intravenous Injection
of 37 mg/kg Methyleugenol**[a]

	Concentration[b] (μg/mL)
Male	
Time after Dosing (minutes)	
2	44.3 ± 13.7[c]
5	26.3 ± 0.3
15	8.25 ± 0.64
30	3.30 ± 0.46
45	2.775 ± 0.391
90	0.719 ± 0.081
180	0.240 ± 0.011
360	0.144 ± 0.030[c]
Female	
Time after Dosing (minutes)	
2	47.1 ± 2.4
5	27.1 ± 0.1[c]
15	8.97 ± 0.53
30	1.77 ± 0.35
45	0.802 ± 0.165
90	0.666 ± 0.048
180	0.186 ± 0.028[c]
360	0.055 ± 0.007

[a]   Three animals were bled at each time point.
[b]   Data are given in μg/mL as the mean ± standard error for three values.
[c]   Two values were used to calculate the concentration.

Males

Females
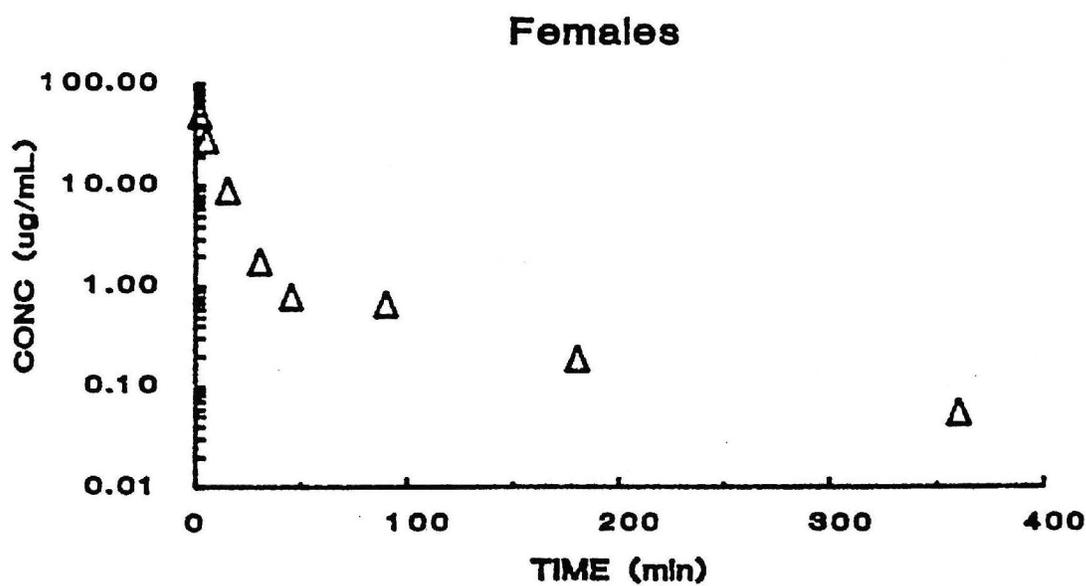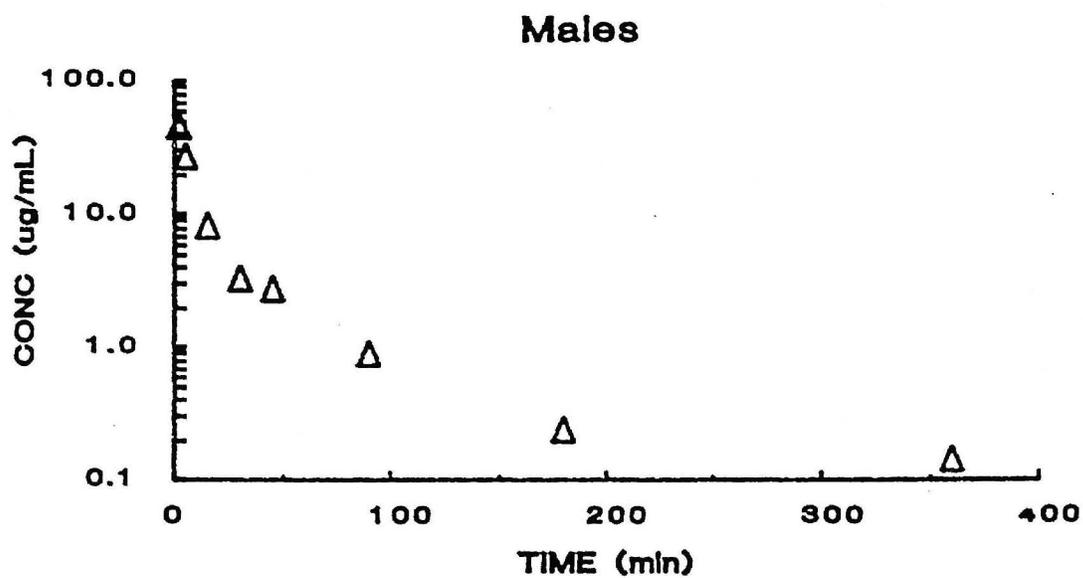
FIGURE M1
Plasma Concentrations of Methyleugenol in F344/N Rats
after a Single Intravenous Injection of 37 mg/kg Methyleugenol

TABLE M2
Plasma Concentrations of Methyleugenol in F344/N Rats after a Single Gavage Dose of Methyleugenol[a]

	Dose		
	37 mg/kg	**75 mg/kg**	**150 mg/kg**

Male

Time after Dosing (minutes)

	37 mg/kg	**75 mg/kg**	**150 mg/kg**
5	0.656 ± 0.083	1.517 ± 0.184	3.835 ± 1.002
15	0.481 ± 0.033	1.333 ± 0.258	1.974 ± 0.328
30	0.290 ± 0.021	1.170 ± 0.169	2.171 ± 0.431
60	0.186 ± 0.038	1.210 ± 0.320	1.975 ± 0.432
90	0.107 ± 0.030	0.645 ± 0.102	1.070 ± 0.061
120	0.054 ± 0.015[b]	0.400 ± 0.055	0.700 ± 0.126
240	0.034 ± 0.011[b]	0.124 ± 0.064[b]	1.548 ± 0.155
360	0.013 ± 0.000[b]	0.076 ± 0.016	0.614 ± 0.097

Female

Time after Dosing (minutes)

	37 mg/kg	**75 mg/kg**	**150 mg/kg**
5	1.141 ± 0.283	3.217 ± 0.727	8.297 ± 4.144
15	0.526 ± 0.082	1.165 ± 0.244	2.261 ± 0.722
30	0.199 ± 0.059	1.400 ± 0.263	3.000 ± 1.037
60	0.123 ± 0.055[b]	1.017 ± 0.042	1.256 ± 0.321
90	0.040 ± 0.004[b]	0.331 ± 0.067	0.769 ± 0.194
120	0.042 ± 0.016[b]	0.225 ± 0.029	0.486 ± 0.058
240	0.013 ± 0.000[b]	0.094 ± 0.032	0.531 ± 0.183
360	0.013 ± 0.000[b]	0.037 ± 0.013[b]	0.164 ± 0.054

[a] Three animals were bled at each time point. Data are given in μg/mL as the mean ± standard error for three values.
[b] Mean calculated using at least one value below the limit of quantitation (LOQ = 0.05 μg/mL). If the value was between the level of detection (LOD = 0.025 μg/mL) and the LOQ, then the measured value was used for mean and standard error calculation. However, if the value was below the LOD, 0.0125 μg/mL was used.
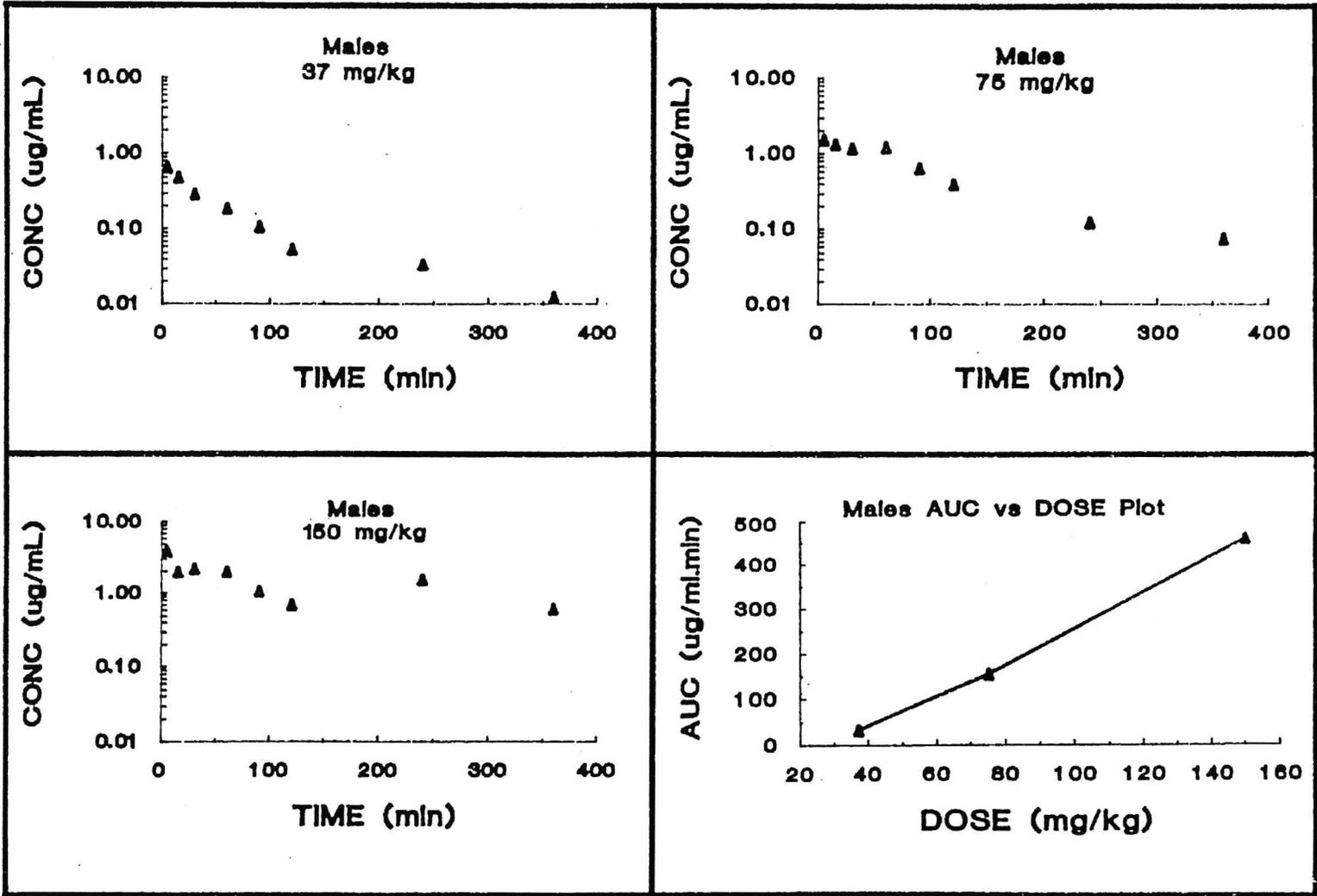
FIGURE M2
Plasma Concentrations of Methyleugenol in Male F344/N Rats
after a Single Gavage Dose of Methyleugenol and a Plot
of AUC versus Dose
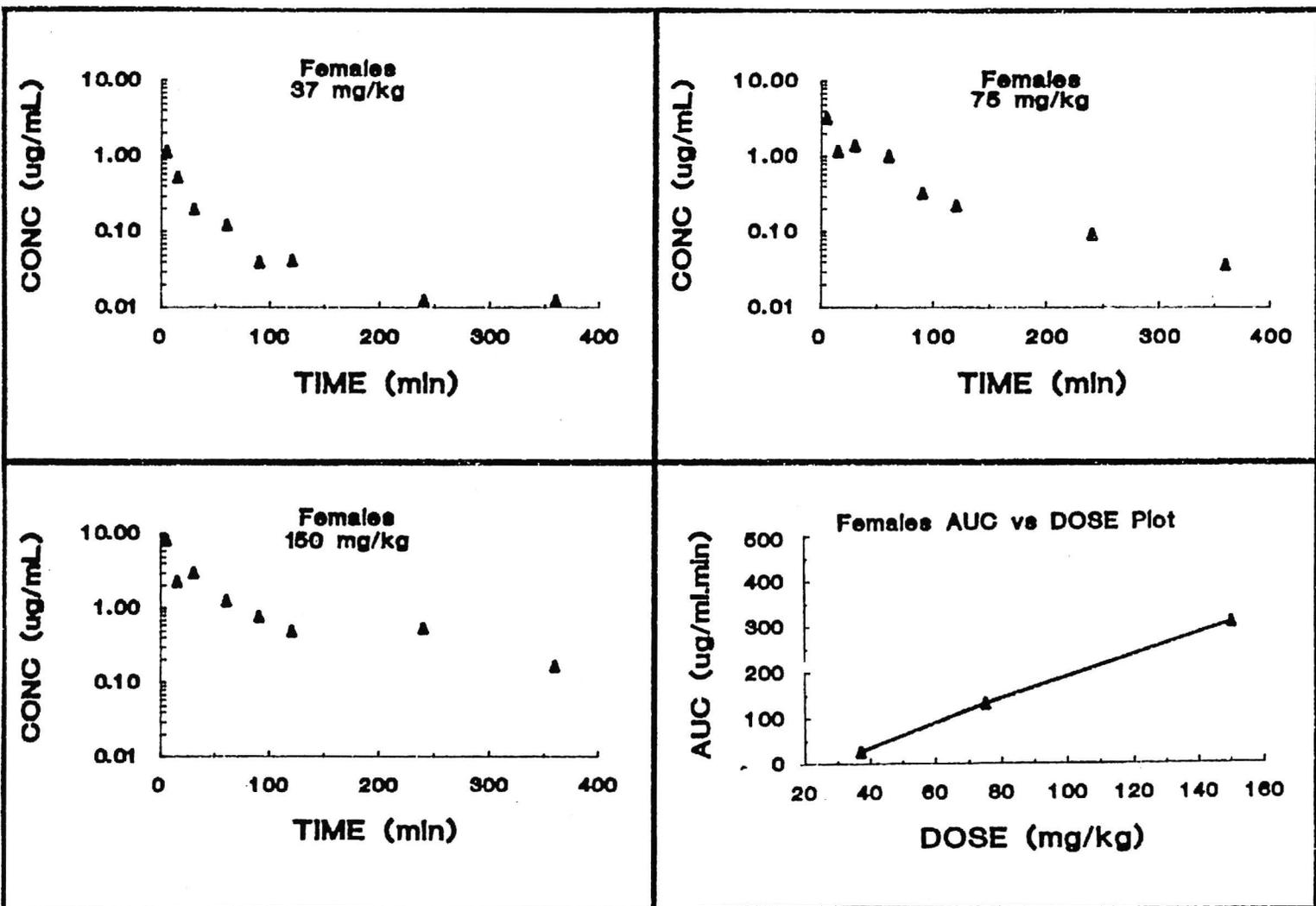
FIGURE M3
Plasma Concentrations of Methyleugenol in Female F344/N Rats
after a Single Gavage Dose of Methyleugenol and a Plot
of AUC versus Dose

TABLE M3
Plasma Concentrations of Methyleugenol in B6C3F$_1$ Mice after a Single Intravenous Injection of 25 mg/kg Methyleugenol[a]

	Concentration[b] (μg/mL)
Male	
Time after Dosing (minutes)	
2	18.20 ± 6.60[c]
5	7.01 ± 1.18[d]
15	1.75 ± 0.31
30	0.628 ± 0.227
45	0.270 ± 0.036
60	0.103 ± 0.018
180	0.020 ± 0.007[e]
300	0.030 ± 0.009[e]
Female	
Time after Dosing (minutes)	
2	9.34 ± 2.63
5	5.10 ± 1.91
15	2.36 ± 0.49
30	0.718 ± 0.227
45	0.237 ± 0.068
60	0.218 ± 0.112
180	0.013 ± 0.000[e]
300	0.013 ± 0.000[e]

[a]   Three animals were bled at each time point.
[b]   Data are given in μg/mL as the mean $\pm$ standard error for three values.
[c]   Two values were used to calculate the concentration.
[d]   Four animals bled
[e]   Mean calculated using at least one value below the limit of quantitation (LOQ = 0.05 μg/mL). If the value was between the level of detection (LOD = 0.025 μg/mL) and the LOQ, then the measured value was used for mean and standard error calculation. However, if the value was below the LOD, 0.0125 μg/mL was used.

Males

Females
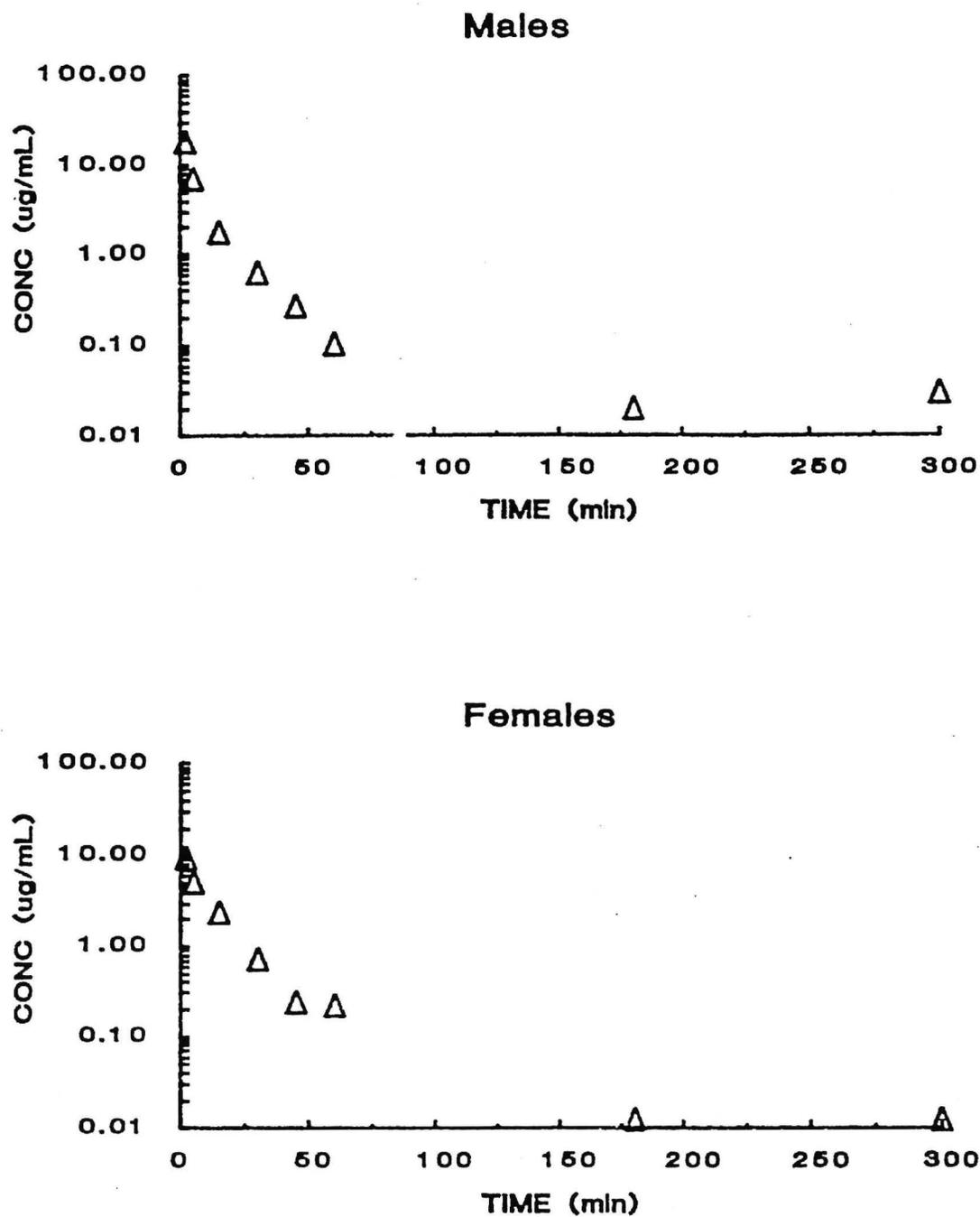
FIGURE M4
Plasma Concentrations of Methyleugenol in B6C3F$_1$ Rats
after a Single Intravenous Injection of 37 mg/kg Methyleugenol

TABLE M4
Plasma Concentrations of Methyleugenol in B6C3F$_1$ Mice after a Single Gavage Dose of Methyleugenol[a]

	Dose		
	25 mg/kg	**50 mg/kg**	**75 mg/kg**
Male			
Time after Dosing (minutes)			
5	0.382 ± 0.155	1.400 ± 0.157	3.100 ± 0.241
15	0.117 ± 0.010	0.713 ± 0.183	1.121 ± 0.190
30	0.044 ± 0.002[b]	0.237 ± 0.009	0.339 ± 0.032
45	0.042 ± 0.001[b]	0.143 ± 0.024	0.214 ± 0.025
60	0.033 ± 0.003[b]	0.067 ± 0.007	0.144 ± 0.016
90	0.013 ± 0.000[b]	0.041 ± 0.005[b]	0.084 ± 0.012
120	0.013 ± 0.000[b]	0.030 ± 0.001[b]	0.054 ± 0.007[b]
240	0.013 ± 0.000[b]	0.013 ± 0.000[b]	0.013 ± 0.000[b]
Female			
Time after Dosing (minutes)			
5	0.112 ± 0.005	1.008 ± 0.373	4.385 ± 0.125[c]
15	0.123 ± 0.027	0.879 ± 0.141	1.300 ± 0.316
30	0.042 ± 0.010[b]	0.202 ± 0.029	0.700 ± 0.216
45	0.030 ± 0.017[b]	0.127 ± 0.008	0.231 ± 0.008
60	0.013 ± 0.000[b]	0.054 ± 0.013[b]	0.127 ± 0.022
90	0.013 ± 0.000[b]	0.025 ± 0.006[b]	0.070 ± 0.003
120	0.013 ± 0.000[b]	0.018 ± 0.006[b]	0.035 ± 0.005[b]
240	0.013 ± 0.000[b]	0.013 ± 0.000[b]	0.013 ± 0.000[b]

[a]   Three animals were bled at each time point. Data are given in μg/mL as the mean ± standard error for three values.
[b]   Mean calculated using at least one value below the limit of quantitation (LOQ = 0.05 μg/mL). If the value was between the level of detection (LOD = 0.025 μg/mL) and the LOQ, then the measured value was used for mean and standard error calculation. However, if the value was below the LOD, 0.0125 μg/mL was used.
[c]   Mean is based on two values.
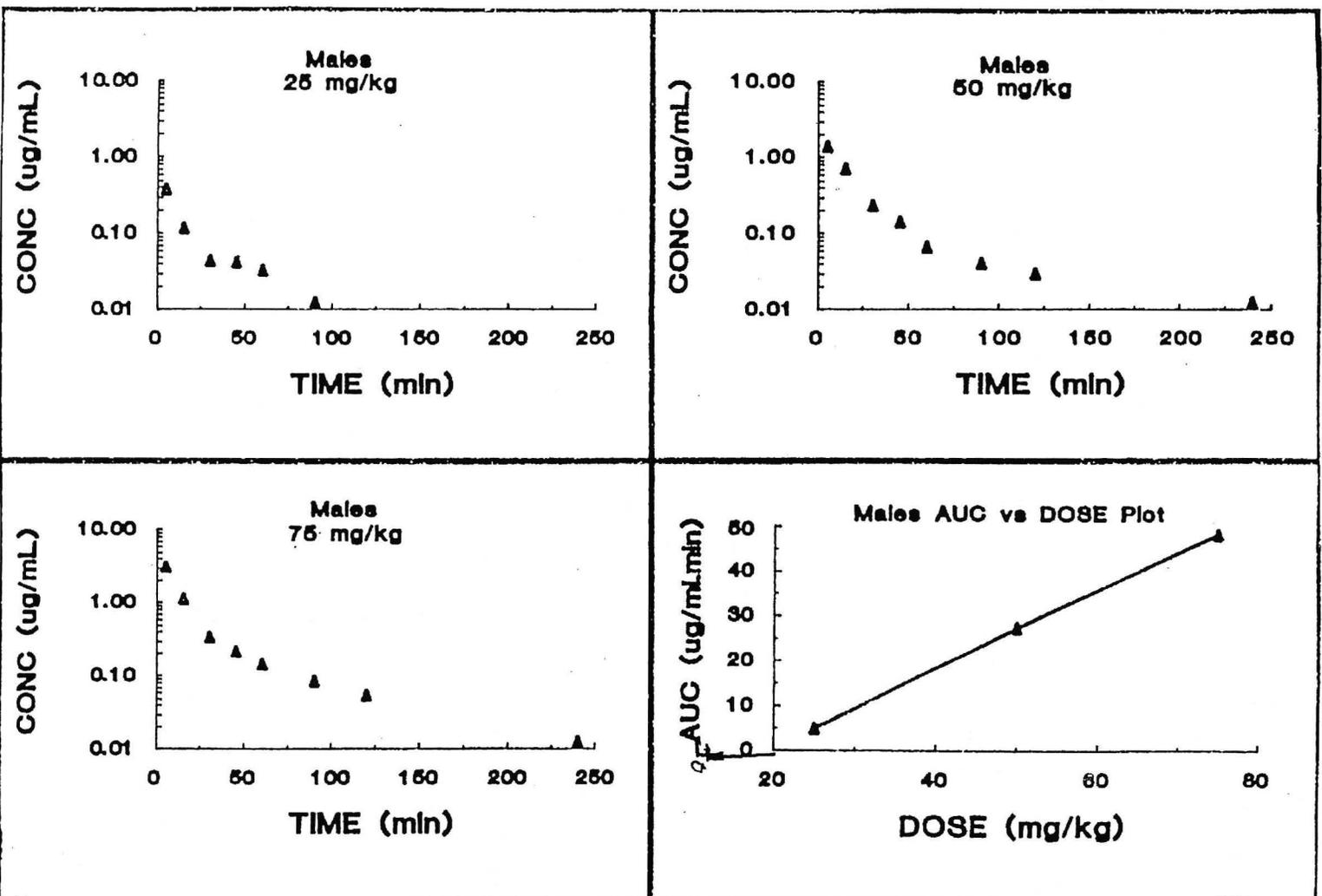
FIGURE M5
Plasma Concentrations of Methyleugenol in Male in B6C3F$_1$ Mice
after a Single Gavage Dose of Methyleugenol and a Plot
of AUC versus Dose
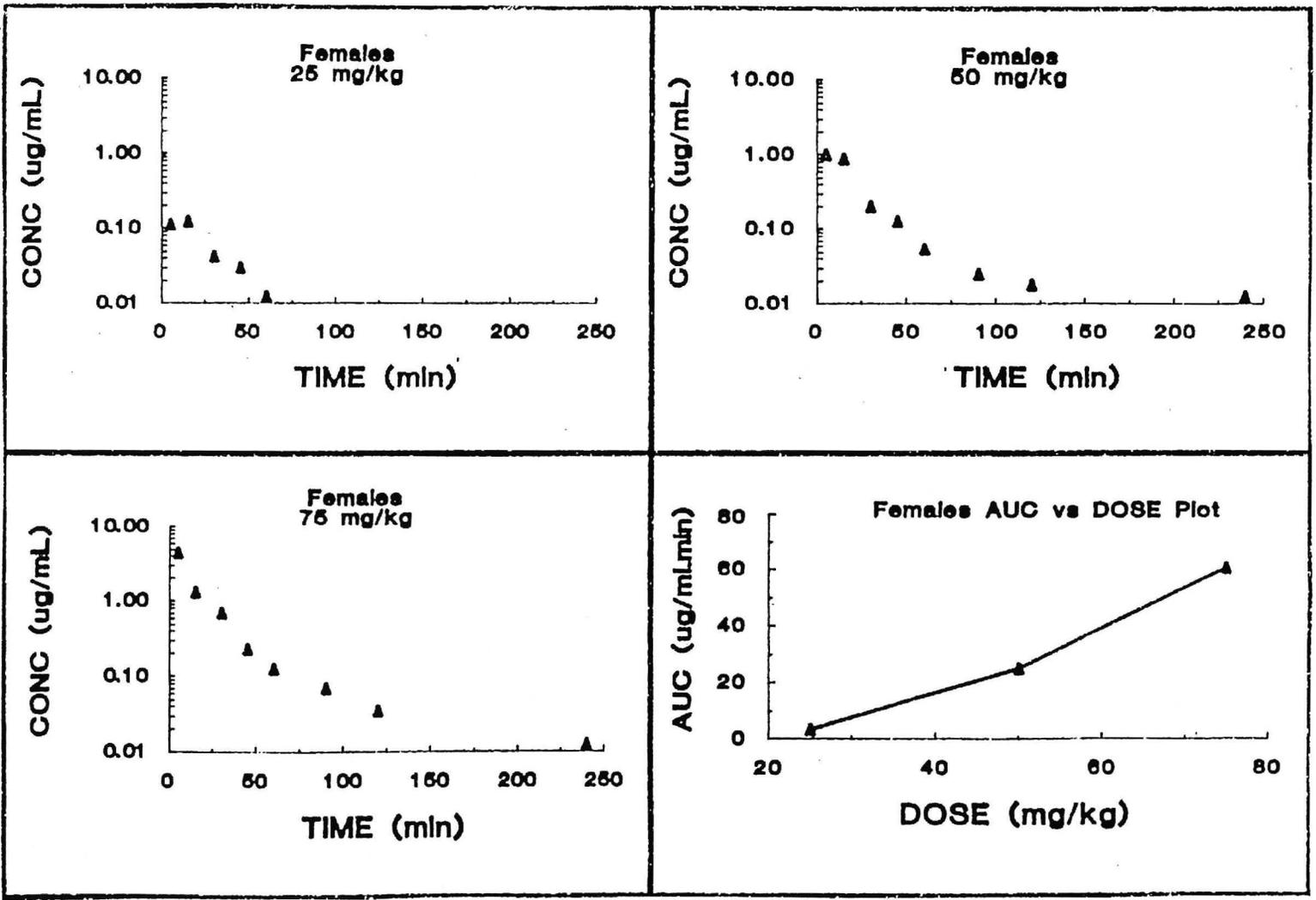
FIGURE M6
Plasma Concentrations of Methyleugenol in Female in B6C3F$_1$ Mice
after a Single Gavage Dose of Methyleugenol and a Plot
of AUC versus Dose

TABLE M5
Summary of Toxicokinetic Data from a Single-Dose Intravenous and Oral Gavage Methyleugenol Study in F344/N Rats[a]

Route	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (minutes)	$t_{\frac{1}{2}}$ (minutes)	AUC (μg/mL•min)	Absolute Bioavailability[b] (%)
Male						
Intravenous injection	37	44.3	2	75	581.4	—[c]
Gavage	37	0.656	5	60	33.5	5.8
Gavage	75	1.52	5	75	155.6	13.2
Gavage	150	3.84	5	115	459.5	19.5
Female						
Intravenous injection	37	47.1	2	75	495.4	—
Gavage	37	1.14	5	95	27.0	5.5
Gavage	75	3.22	5	80	133.1	13.3
Gavage	150	8.30	5	105	307.9	15.3

[a] C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration; $t_{\frac{1}{2}}$=elimination half-life; AUC=area under the curve calculated using the trapezoidal rule
[b] Calculated as $AUC_{oral}/AUC_{IV} \times Dose_{IV}/Dose_{oral} \times 100$
[c] Not applicable to intravenous dosing

TABLE M6
Summary of Toxicokinetic Data from a Single-Dose Intravenous and Oral Gavage Methyleugenol Study in B6C3F$_1$ Mice[a]

Route	Dose (mg/kg)	C$_{max}$ (μg/mL)	T$_{max}$ (minutes)	t$_{1/2}$ (minutes)	AUC (μg/mL•min)	Absolute Bioavailability[b] (%)
Male						
Intravenous injection	25	18.2	2	15	116.4	—[c]
Gavage	25	0.382	5	30	4.91	4.2
Gavage	50	1.40	5	30	27.4	11.8
Gavage	75	3.10	5	30	48.4	13.9
Female						
Intravenous injection	25	9.34	2	15	106.5	—
Gavage	25	0.123	15	30	3.27	3.1
Gavage	50	1.01	5	30	25.0	11.7
Gavage	75	4.39	5	30	60.5	18.9

[a] C$_{max}$=maximum mean concentration; T$_{max}$=time of maximum mean concentration; t$_{1/2}$=elimination half-life; AUC=area under the curve calculated using the trapezoidal rule from 0 to 180 minutes
[b] Calculated as AUC$_{oral}$/AUC$_{IV}$ × Dose$_{IV}$/Dose$_{oral}$ × 100
[c] Not applicable to intravenous dosing

APPENDIX N
IN VIVO AND *IN VITRO* METABOLISM, DISPOSITION, AND COVALENT BINDING OF METHYLEUGENOL

**Conducted at the University of Arizona,
I. Glenn Sipes, Principal Investigator, under NIEHS Contract A01-ES-35367**

IN VIVO AND *IN VITRO* METABOLISM, DISPOSITION, AND COVALENT BINDING OF METHYLEUGENOL

INTRODUCTION

The primary objective of these studies was to determine the absorption, distribution, metabolism, and excretion of methyleugenol following oral and intravenous exposure in male Fischer 344 rats and B6C3F$_1$ mice. Major metabolites eliminated in the urine following oral administration were determined. Bioavailability studies, including intravenous administration, were performed to determine blood levels of parent and major metabolites in the Fischer 344 rat. Tissue levels of radiolabeled compound following oral administration were determined. In addition, this study demonstrated that methyleugenol causes macromolecular binding (to DNA, protein, and lipid) in Fischer 344 rats, B6C3F$_1$ mice, and humans.

MATERIALS AND METHODS

Chemical Purity

The vendor (NEN; Boston, MA) determined the radiochemical purity of the methyleugenol used in these studies to be 99%. The study laboratory determined the purity of the methyleugenol to be 99% by both reverse phase and normal phase high performance liquid chromatography (HPLC).

After initiation of the *in vitro* studies, radiopurity checks were performed on [^{14}C]-methyleugenol using a reverse phase system. Radiochemical purity of previously unopened stock ampules of [^{14}C]-methyleugenol ranged between 93.9% and 96.3%. These data suggest that degradation of methyleugenol occurs over time. The degradation products elute in the region of microsomal metabolites of methyleugenol.

In vivo Studies with Methyleugenol

Oral Studies of Methyleugenol in the Male Fischer 344 Rat

A single dose of [^{14}C]-methyleugenol (118 mg/kg, 50 μCi/kg) in corn oil (5 mL/kg) was administered orally to three male Fischer 344 rats. Rats were housed in glass metabolism cages throughout the study. Urine (6, 12, 24, 48, and 72 hr) and feces (24, 48, and 72 hr) were collected and measured for radioactivity. Expired CO$_2$ and organics were also analyzed for the presence of [^{14}C]-equivalents over the 72-hour time course. At study termination the rats were killed by CO$_2$ asphyxiation. Various tissues were collected and then stored at -80° C until analysis. Blood, feces, and tissue samples were analyzed for total radioactivity using liquid scintillation counting of oxidized samples. Urine was also analyzed for the presence of parent and metabolites by HPLC.

Intravenous Disposition and Metabolism of Methyleugenol in the Male Fischer 344 Rat

A single dose of [^{14}C]-methyleugenol (11.8 mg/kg, 120 μCi/kg) in ethanol:Emulphor:saline (10:10:80, 2 mL/kg) was administered intravenously to three male Fischer 344 rats via an indwelling jugular vein cannula. Rats were housed in Nalgene™ metabolism cages throughout the study. Blood samples were collected via the jugular cannula at selected time points (0, 1, 4, 8, 12, 15, 20, 30, 40, and 50 min, and 1, 6, 12, 24, 48, and 72 hr). The samples were either counted for radioactivity or extracted with ethyl acetate and immediately analyzed by HPLC. At study termination the rats were killed by CO$_2$ asphyxiation. Blood was collected immediately from the posterior vena cava into a heparinized syringe and stored at -80° C until analysis. Blood and feces were analyzed for total radioactivity by scintillation counting of oxidized samples. Urine was also analyzed for the presence of parent and metabolites by HPLC.

Oral Study of Methyleugenol in the Female B6C3F$_1$ Mouse
A single dose of [^{14}C]-methyleugenol (118 mg/kg, 50 μCi/kg) in corn oil (5 mL/kg) was administered orally to three female B6C3F$_1$ mice. Mice were housed in glass metabolism cages throughout the study. Urine (6, 12, 24, 48, and 72 hr) and feces (24, 48, and 72 hr) were collected and measured for radioactivity. Expired CO$_2$ and organics were also analyzed for the presence of [^{14}C]-equivalents over the 72-hour time course. At study termination the mice were killed by CO$_2$ asphyxiation. Various tissues were collected and then stored at -80° C until analysis. Blood, feces, and tissue samples were analyzed for total radioactivity using liquid scintillation counting of oxidized samples. Urine was also analyzed for the presence of parent and metabolites by HPLC.

Urinary Metabolic Profiles for Methyleugenol
A single dose of [^{14}C]-methyleugenol was administered either orally (118 mg/kg, 50 μCi/kg) in corn oil (5 mL/kg) to three male Fischer 344 rats and three female B6C3F$_1$ mice or intravenously (11.8 mg/kg, 120 μCi/kg) in ethanol:Emulphor:saline (10:10:80, 2 mL/kg) to three male Fischer 344 rats. Animals were housed in glass metabolism cages throughout the 72-hour study. Urine was collected at 6, 12, 24, 48, and 72 hours. Samples from individual animals were pooled for each time point and then analyzed by reverse phase HPLC.

Methyleugenol Metabolism: Urinary Metabolic Profiles for Metabolite Identification in Rats and Mice
A single dose of [^{14}C]-methyleugenol was administered either orally (118 mg/kg, 50 μCi/kg) in corn oil (5 mL/kg) to three male Fischer 344 rats and three female B6C3F$_1$ mice or intravenously (11.8 mg/kg, 120 μCi/kg) in ethanol:Emulphor:saline (10:10:80, 2 mL/kg) to three male Fischer 344 rats. Animals were housed in glass metabolism cages throughout the study. Urine was collected at 6, 12, 24, 48, and 72 hours. Samples from individual animals from both 6 and 12 hours were pooled and then analyzed by HPLC using a Phenomenex Prodigy 5μ column. Fractions corresponding to the major metabolites were then collected, lyophilized, and subjected to LC-MS analysis. Putative structures were then assigned based on mass-spectral data for each metabolite, when possible.

In vitro Studies with Methyleugenol
Toxicity of Methyleugenol to Isolated Hepatocytes
Hepatocytes from male Fischer 344 rats, female B6C3F$_1$ mice, and two human donors were isolated and then incubated with various concentrations of methyleugenol for 18 hours. Toxicity to hepatocytes was estimated by the percent of LDH released into the medium relative to the total LDH present (in medium and cells).

Metabolic Profiles of Methyleugenol in Hepatocytes
Metabolic Profiles of Methyleugenol in Hepatocytes from Rats and Mice: Hepatocytes isolated from rats and mice were incubated with methyleugenol (250 μM) for 6 hours and the media were harvested for HPLC analysis. Major metabolites generated from both rat and mouse hepatocytes were collected for LC-MS analysis. To avoid the potential loss of metabolites in the collection and concentration steps, on column mass spectral identification was attempted using the Alphabond column. The on column approach centered upon identifying which metabolites were present in the media, even if quantification could not be determined. Since the on column identifications of the metabolites did not allow for the assignment of identities to individual peaks on a radiochromatogram (and thus quantitation), the collection of peaks from the Alphabond system was necessary to identify all the metabolites. Media from rat hepatocyte incubations was used to generate a chromatogram to assign numerical identities to the peaks visible on the UV trace. The peaks were then collected from HPLC runs, neutralized by adding ammonium hydroxide, concentrated by centrifugation, and subjected to LC-MS analysis. Putative structures for the peaks were assigned based

on mass-spectral data for each metabolite when possible. Putative identities for the peaks found in mouse and human hepatocytes were assigned by co-elution.

Identification of Metabolite Peaks on Original (Partisil) Gradient by Co-elution after Identification on Mass Spectrometer and Final Gradient Modification: Peaks collected and identified using the Alphabond system were reinjected on the Partisil gradient to assign identities to the peaks for the rat. By co-elution, these peak identities were established for the mouse and human hepatocyte incubations. The hydroxyl metabolite was also injected onto this gradient and eluted at 55 minutes.

Binding to DNA
In vitro Binding of Methyleugenol to Purified DNA: Methyleugenol (80 nmol) was incubated for 1 hour at 37° C with 1 mg of calf thymus DNA, with or without a rat hepatic S9 fraction, and an NADPH recycling system. Following incubation, the aqueous phase was washed first with ethyl acetate and then DNA was extracted using the classic phenol procedure. DNA was precipitated using cold ethanol and then resuspended in Tris/magnesium chloride buffer. Samples were analyzed by spectrophotometry for purity and concentration and the radioactivity counted using a scintillation counter. Similar experiments were performed to determine if methyleugenol showed similar DNA binding in the presence of S9 derived from rats, mice, and humans.

Unscheduled DNA Synthesis in Rat, Mouse, and Human Hepatocytes: Hepatocytes from three male Fischer 344 rats, three female B6C3F$_1$ mice, and three human donors were incubated with various concentrations of methyleugenol for 18 hours. Unscheduled DNA synthesis (UDS) in hepatocytes was estimated by the mean number of silver grains in the nucleus relative to grains in the cytosol.

UDS Modulation in Rat and Mouse Hepatocytes: To establish if the sulfation or epoxidation pathways of activation were involved in the genotoxicity of methyleugenol, the UDS assay was repeated at the most genotoxic dose (10 μM) of methyleugenol in male Fischer 344 rats and three female B6C3F$_1$ mice. Cells were concurrently exposed to either 15 μM PCP or 2,000 μM CHO.

Protein Binding in Rat Liver and Glandular Stomach by Methyleugenol: Nine male Fischer 344 rats received a single oral dose of 118 mg/kg of [^{14}C]-methyleugenol (50 μCi/kg) in corn oil (5 mL/kg). Three of the animals were killed by carbon dioxide asphyxiation at 1, 3.5, and 24 hours after dosing. The liver (at 1, 3.5, and 24 hours) and glandular stomach (at 1 and 3.5 hours) were removed and frozen at -80° C until processing. In processing the organs, tissue was removed from the freezer, frozen in liquid nitrogen, and then pulverized. Protein was extracted using trichloroacetic acid; extractions were repeated until no radioactivity could be detected in the wash. The protein was then washed with ethanol to remove any non-covalently bound methyleugenol. Radioactivity associated with the protein was determined by scintillation counting. Binding to protein was also determined for three rats 12 hours following a 11.8 mg/kg intravenous dose of methyleugenol.

RESULTS
In vivo Studies with Methyleugenol
Oral Studies of Methyleugenol in the Male Fischer 344 Rat
Approximately 72% of the total administered radioactivity was excreted in the urine by 72 hours (Figure N1). Approximately 13% was recovered in feces, and less than 0.1% was recovered as [^{14}C]-CO$_2$ or expired [^{14}C]-organics. [^{14}C]-Equivalents determined in tissues accounted for less than 0.4% of the administered dose. The amount of [^{14}C]-equivalents in specific tissues and the tissue to blood ratios are

presented in Table N1. Blood collected at 72 hours and oxidized contained approximately 0.1% of the administered dose. Less than 0.6% of the administered dose was accounted for at any time point in blood samples counted for radioactivity. Neither methyleugenol nor its metabolites were detected in the extracted blood samples analyzed by HPLC.

Intravenous Disposition and Metabolism of Methyleugenol in the Male Fischer 344 Rat
Approximately 86% of the total administered radioactivity was excreted in the urine by 72 hours. Approximately 9% was recovered in feces. [^{14}C]-Equivalents determined in tissues accounted for less than 0.4% of the administered dose (Table N1). Blood collected at 72 hours contained approximately 0.1% of the administered dose. After 12 hours, radioactivity was still present in the blood for up to 48 hours, but at very low levels (200 to 400 dpm total). Parent methyleugenol was present in blood samples counted for radioactivity for the first six hours and not thereafter. During these 6 hours, the curves for total radioactivity and methyleugenol in blood were parallel (Figure N2). Pharmacokinetic parameters are presented in Table N2. Possible metabolites were detected in the blood through 50 minutes but never exceeded 2.5% of the administered dose at any of the time points analyzed. Maximum metabolite recovery occurred at 8 minutes.

Oral Study of Methyleugenol in the Female B6C3F$_1$ Mouse
Approximately 85% of the total administered radioactivity was excreted in the urine by 72 hours (Figure N3). Approximately 6% was recovered in feces, and less than 0.1% was recovered as [^{14}C]-CO$_2$ or expired [^{14}C]-organics. [^{14}C]-Equivalents determined in tissues accounted for less than 0.3% of the administered dose (Table N3). Blood collected at 72 hours contained less than 0.02% of the administered dose.

Urinary Metabolic Profiles for Methyleugenol
With either route of administration, the majority of the metabolites were excreted in the urine within 24 hours of dosing.

Methyleugenol Metabolism: Urinary Metabolic Profiles for Metabolite Identification in Rats and Mice
Due to low amounts of radioactivity in the urine beginning at 24 hours, samples from 24 hours and later were not analyzed by HPLC. Putative identities of the peaks from the urinary metabolic profiles are shown in Table N4. The metabolites in rats and mice suggest that methyleugenol can undergo demethylation, ring or side chain hydroxylation, and that these hydroxylated metabolites are subjected to sulfation or glucuronidation.

In vitro Studies with Methyleugenol
Toxicity of Methyleugenol to Isolated Hepatocytes
Methyleugenol required millimolar concentrations to elicit toxicity in hepatocytes isolated from male rats, female mice, and human donors, but the hepatotoxic concentrations in human cells were significantly less than those needed for rat and mouse hepatocytes. The cytotoxicity of methyleugenol to human hepatocytes is similar to that in rodents. Although human hepatocytes do appear to be slightly more susceptible than rat and mouse hepatocytes, human hepatocytes had higher basal LDH release levels and more variability between donors.

Metabolic Profiles of Methyleugenol in Hepatocytes

Metabolic Profiles of Methyleugenol in Hepatocytes from Rats and Mice: Media from rat and mouse hepatocytes incubated for 6 hours eluted major peaks at 52 to 53 minutes. No definitive structures can be assigned at this time.

In all, five metabolites were identified in the rat, mouse and human hepatocyte media. All three species produce a metabolite with a molecular weight of 239 with a fragmentation pattern consistent with a glycine conjugate. All three species also produced a metabolite with fragmentation patterns consistent with a demethylated and sulfated metabolite (molecular weight of 243), as well as a metabolite fragmentation pattern consistent with a hydroxylated and sulfated metabolite (molecular weight of 273). A metabolite with fragmentation patterns consistent with a sulfated diol metabolite (molecular weight of 291) was seen in mouse and human, but not in rat hepatocyte media. This may be because the rat does not produce this metabolite, or that it was undetectable. The fifth metabolite with fragmentation patterns consistent with a hydroxylated glucuronide (molecular weight of 369) was seen in rat and mouse but not human hepatocyte media.

Peaks identified included two glycine conjugates produced after some intermediate metabolism: a smaller conjugate with a molecular weight of 239 and a larger conjugate with less intermediate metabolism prior to conjugation. A hydroxylated and glucuronide conjugated metabolite was also seen, as was a glutathione conjugate. This metabolite would not be seen in urine as it would most likely be further metabolized to a mercapturic acid in the kidney. An hydroxylated and sulfated metabolite was also seen. For these last three metabolites mass spectroscopy is not sufficiently definitive to establish the site of the hydroxylation/conjugation. As a result, the initial modification of the molecule could take place on one of several places in the ring of methyleugenol or on the side chain, including the 1-C of the chain. Other metabolites included the sulfated diol and the diol itself resulting from the addition of oxygen across the double bond to form an epoxide which is subsequently opened to a diol. Finally, a metabolite with a fragmentation pattern consistent with hydroxylation of the parent molecule was identified. The fragmentation pattern is not definitive enough to establish the location of the hydroxylation; the hydroxyl group could be on the ring or on the chain, including the 1-OH position.

Identification of Metabolite Peaks on Original (Partisil) Gradient by Co-elution after Identification on Mass Spectrometer and Final Gradient Modification: In human hepatocytes, only 25% of the radioactive peak areas was converted to metabolites, while 75% remained as methyleugenol (Figure N4). One metabolite comprising 4% was consistent with a glycine conjugate with a molecular weight of 239. A peak consisting of 6% contained a glycine conjugate with a molecular weight of 279 and a demethylated and sulfated metabolite (glucuronide was not seen in the on column injections for human). The peak consistent with a diol was 14%. The remaining 1% of the metabolites was not identified.

Binding to DNA

In vitro Binding of Methyleugenol to Purified DNA: The binding of methyleugenol to calf thymus DNA was dependent on hepatic biotransforming enzymes (Table N5). Incubations that did not contain S9 did not elicit binding to DNA. The binding of methyleugenol to DNA was dependent on hepatic biotransforming enzymes. Incubations that did not contain S9 did not elicit binding to DNA. When S9 was obtained from the livers of rats and mice pretreated with Arochlor 1254, methyleugenol binding to DNA occurred at higher levels than in the presence of S9 from non-induced animals, significantly so in the mouse. There were no major differences between rat, mouse, and human DNA binding of methyleugenol in the presence of non-induced S9. Human S9 tended to cause less binding of methyleugenol overall as compared to S9 from rats and mice.

Unscheduled DNA Synthesis in Rat, Mouse, and Human Hepatocytes: Methyleugenol showed levels of UDS that were approximately half the value of the positive control, and the level of UDS was similar in hepatocytes from rats and mice. In both species, the dose response curve for methyleugenol was atypical; DNA damage appeared to be greater at lower methyleugenol concentrations and appeared to return to control levels at higher concentrations. Often, an inverse dose response relationship is observed for cytotoxic chemicals; however, methyleugenol did not cause extensive cytotoxicity at these concentration ranges. In human hepatocytes, methyleugenol caused significantly less UDS than that seen in rats and mice.

UDS Modulation in Rat and Mouse Hepatocytes: UDS was similarly affected by modulators in hepatocytes from rats and mice. The addition of cyclohexane oxide did not significantly alter the level of genotoxicity thereby suggesting that the metabolic pathway does not involve epoxidation (Figure N5). The elevated UDS levels seen with methyleugenol were decreased significantly in the presence of pentachlorophenol, suggesting the sulfation pathway may play a part in the genotoxicity of methyleugenol.

Protein Binding in Rat Liver and Glandular Stomach by Methyleugenol: Binding to protein in liver and stomach was similar, with slightly more binding seen in the stomach than in the liver following oral dosing and more in liver than stomach following intravenous dosing. Protein binding by methyleugenol after oral dosing in both liver and stomach was at the same level regardless of time after dosing.
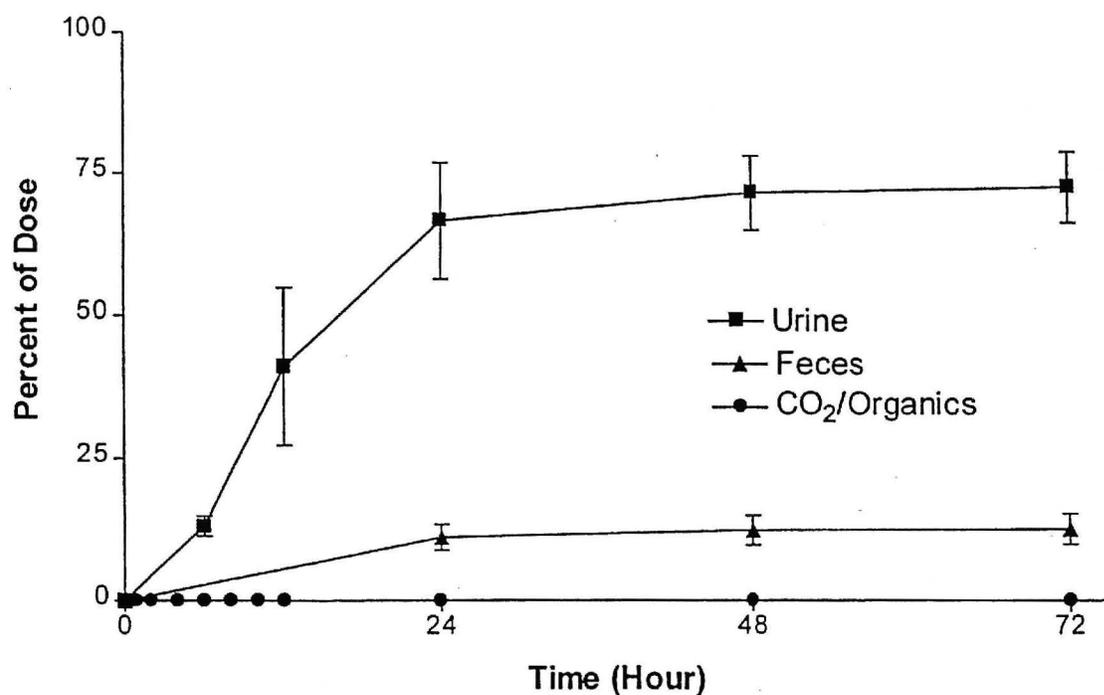
FIGURE N1
Cumulative Excretion of Total Radioactivity in Urine, Feces, and Expired Carbon Dioxide and Organics after Oral Administration of [14C]-Methyleugenol to Male Fischer 344 Rats. Rats (n=3) were administered a single oral dose of [14C]-methyleugenol (118 mg/kg, 50 µCi/kg) and then monitored for excretion of [14C]-equivalents for 72 hours.

TABLE N1
Tissue Distribution of Dosed Radioactivity in Male Fischer 344 Rats
72 Hours after a Single Oral Dose or a Single Intravenous Dose of [^{14}C]-Methyleugenol

Tissue	Oral Dose (118 mg/kg)		Intravenous Dose (11.8 mg/kg)	
	Recovery of Dose[a] (%)	Tissue:Blood Ratio[b]	Recovery of Dose[a] (%)	Tissue:Blood Ratio[b]
Blood	0.068 ± 0.01	1.00	0.078 ± 0.01	1.00
Brain	0.001 ± 0.00	0.08	0.001 ± 0.00	0.09
Fat	0.049 ± 0.01	0.49	0.054 ± 0.00	0.50
Heart	0.001 ± 0.00	0.30	0.001 ± 0.00	0.27
Kidneys	0.007 ± 0.00	0.95	0.012 ± 0.00	1.38
Large intestine	0.005 ± 0.00	0.66	0.005 ± 0.00	0.66
Liver	0.104 ± 0.00	2.54	0.089 ± 0.02	2.18
Lungs	0.003 ± 0.00	0.68	0.004 ± 0.00	0.78
Muscle	0.073 ± 0.02	0.17	0.081 ± 0.01	0.17
Skin	0.064 ± 0.01	0.47	0.064 ± 0.01	0.42
Small intestine	0.005 ± 0.00	0.38	0.005 ± 0.00	0.41
Spleen	0.001 ± 0.00	0.36	0.001 ± 0.00	0.33
Stomach, glandular	0.000 ± 0.00	0.29	0.000 ± 0.00	0.41
Stomach, muscular	0.001 ± 0.00	0.51	0.002 ± 0.00	0.46
Testes	0.002 ± 0.00	0.22	0.002 ± 0.00	0.14

[a] Mean ± standard deviation; n=3
[b] Mean ratio of [^{14}C]-methyleugenol equivalents in tissue to [^{14}C]-methyleugenol in blood; calculated from dpm per gram of tissue divided by dpm per gram of blood
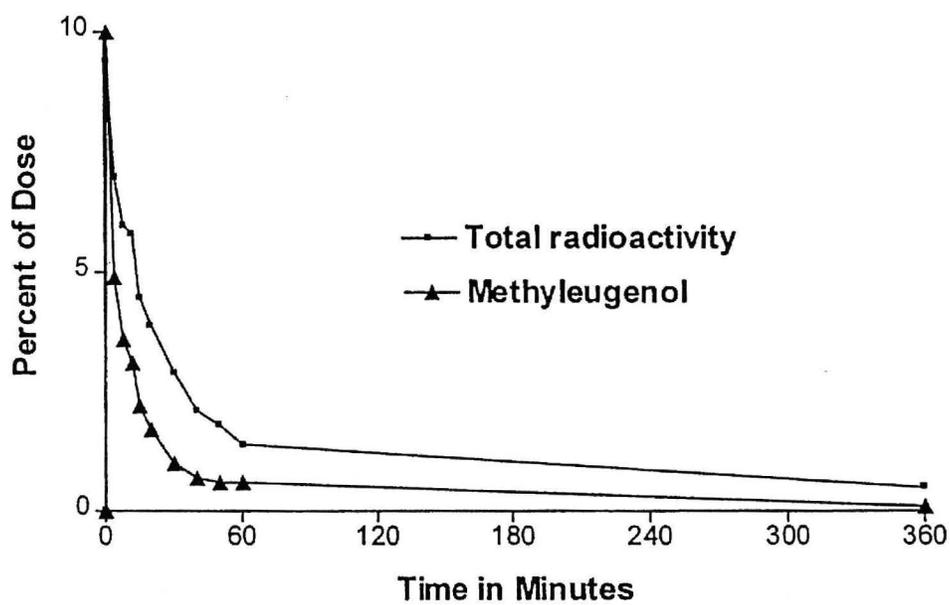
FIGURE N2
Percent Total Radioactivity and Percent Methyleugenol in the Blood
after an Intravenouse Dose of Methyleugenol. Rats (n=3) were administered a single intravenouse dose of
[14C]-methyleugenol (11.8 mg/kg, 120 µCi/kg) and then monitored for [14C]-equivalents and parent in blood for
360 minutes.

TABLE N2
Pharmacokinetic Parameters of Intravenously Administered Methyleugenol[a]

V_{ss} (L/kg)	$t_{1/2}$ (min)	MRT (min)	V_D (L/kg)	AUC (μg/min/mL)	CL_s (L/min/kg)
9.02 ± 1.36	51.45 ± 5.18	61.76 ± 7.95	2.73 ± 0.42	81.83 ± 5.65	0.15 ± 0.01

[a] Mean $\pm$ standard error; V_{ss}=steady-state apparent volume of distribution; $t_{1/2}$=terminal half-life; MRT=mean residence time; V_D=volume of distribution; AUC=area under the curve; CL_s=systemic body clearance
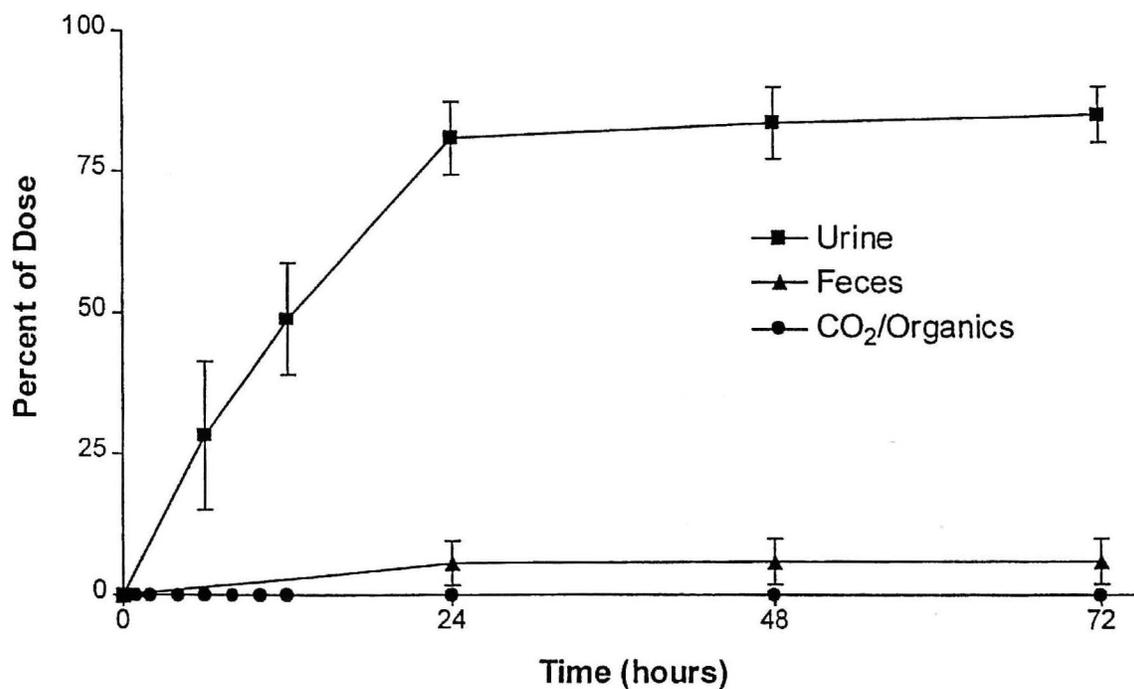
FIGURE N3
Cumulative Excretion of Total Radioactivity in Urine, Feces, and Expired Carbon Dioxide and Organics after Oral Administration of [14C]-Methyleugenol to Female Fischer 344 Rats. Rats (n=3) were administered a single oral dose of [14C]-methyleugenol (118 mg/kg, 50 µCi/kg) and then monitored for excretion of [14C]-equivalents for 72 hours.

TABLE N3
Tissue Distribution of Dosed Radioactivity in Female B6C3F$_1$ Mice 72 Hours after a Single Oral Dose
of [^{14}C]-Methyleugenol

Tissue	Oral Dose (118 mg/kg)	
	% Recovery of Dose[a]	Tissue:Blood Ratio[b]
Blood	0.013 ± 0.01	1.00
Brain	0.003 ± 0.00	0.89
Fat	0.093 ± 0.02	6.65
Heart	0.001 ± 0.00	0.98
Kidneys	0.005 ± 0.00	2.36
Large intestine	0.003 ± 0.00	1.71
Liver	0.050 ± 0.01	5.07
Lungs	0.004 ± 0.00	3.42
Muscle	0.051 ± 0.07	0.96
Ovaries	0.003 ± 0.00	100.15
Skin	0.046 ± 0.02	1.77
Small intestine	0.006 ± 0.00	1.17
Spleen	0.002 ± 0.00	6.77
Stomach, glandular	0.002 ± 0.00	8.60
Stomach, muscular	0.003 ± 0.00	5.21

[a] Mean ± standard deviation; n=3
[b] Mean ratio of [^{14}C]-methyleugenol equivalents in tissue to [^{14}C]-methyleugenol in blood; calculated from dpm per gram of tissue divided by dpm per gram of blood.

TABLE N4
Putative Identities of Peaks from Urinary Metabolic Profile for Methyleugenol
after Oral Administration to Male Fischer 344 Rats, Intravenous Administration
to Male Fischer 344 Rats, and Oral Administration to Female B6C3F$_1$ Mice[a]

Peak Label and Proposed Identity	Rat Oral Dose (118 mg/kg)		Rat Intravenous Dose (11.8 mg/kg)		Mouse Oral Dose (118 mg/kg)	
	Present	%	Present	%	Present	%
A Unknown	Yes	25	Yes	22	Yes	4
B Hydroxylated methyleugenol	Yes	13	Yes[b]	9	Yes[b]	13
C Hydroxylated and sulfated methyleugenol	Yes	10	Yes[b]	6	Yes[b]	4
D Unknown	No	0	Yes	9	No	0
E Unknown	No	0	No	0	Yes	23
F Glucuronide	Yes	20	Yes[b]	11	Yes[b]	6
G Unknown	Yes	15	Yes	16	Yes	13
H Mercapturic acid	Yes	8	Yes[b]	9	Yes	6
I Mixture of sulfates	Yes	9	Yes[b]	14	Yes[b]	4
Minor metabolites not labeled		0		4		27
Total:		100		100		100

[a] Percent of radioactivity in sample
[b] Identification based on co-elution with rat oral dose metabolite
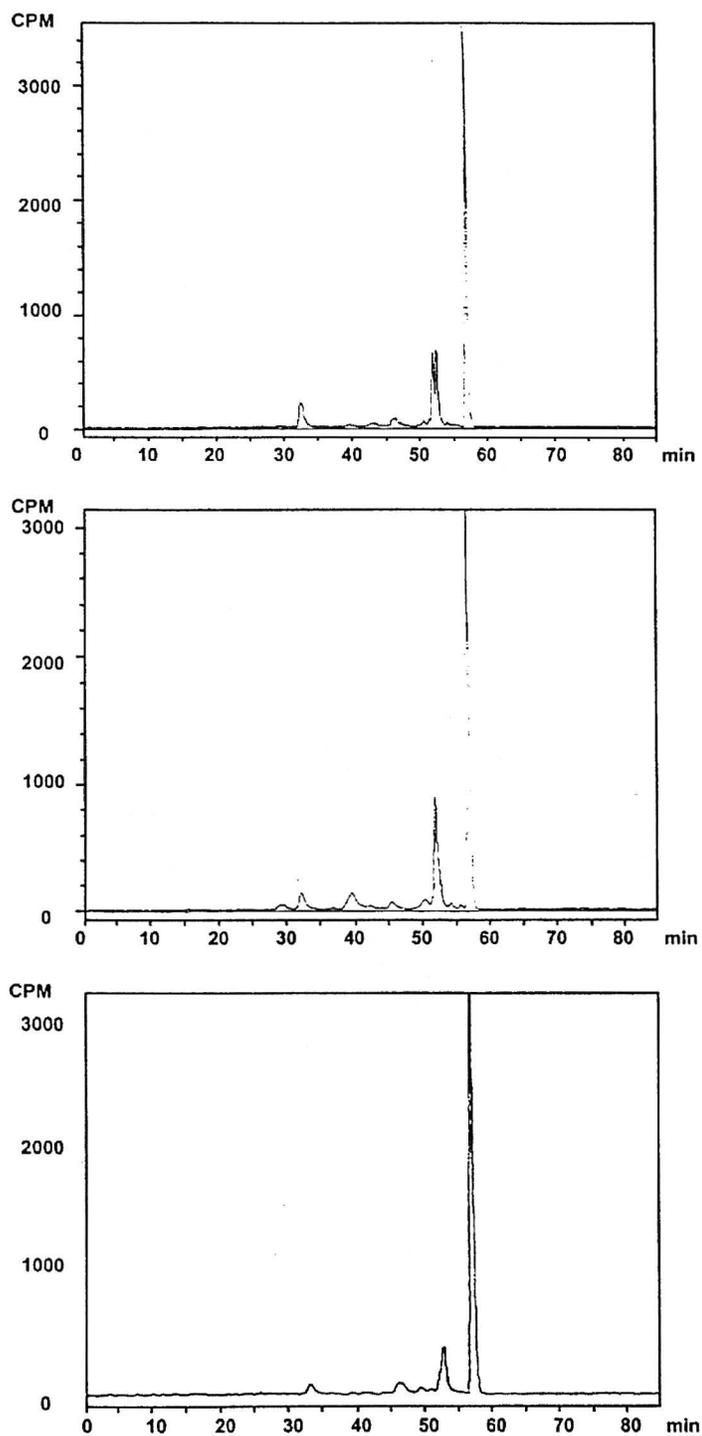
FIGURE N4
Representative Radiochromatograms of Media Taken from Hepatocytes Isolated
from Male Fischer 344 Rats, Female B6C3F$_1$ Mice, and Human Donors Incubated with Methyleugenol
for 6 hours. Hepatocytes were incubated with [^{14}C]-methyleugenolfor 6 hours at a concentration of 250 µM.
Media were harvested, the pH adjusted to 4.0, and analyzed by HPLC. Retention time of methyleugenol is
apprroximately 57 minutes.

TABLE N5
***In vitro* DNA Binding Activity of Methyleugenol with and without S9 Liver Preparations from Male Fischer 344 Rats, Female B6C3F$_1$ Mice, and Human Donors**[a]

Species	Without S9	With Arochlor-induced S9	With Non-induced S9
Rat	4.8 ± 0.8	130 ± 40.1	53 ± 31.9
Mouse	—[b]	376 ± 155	54 ± 6.2
Human	—	—	24 ± 15.9

[a] pmol/mg DNA; mean $\pm$ standard deviation; n=3 or 4 separate experiments
[b] Not done
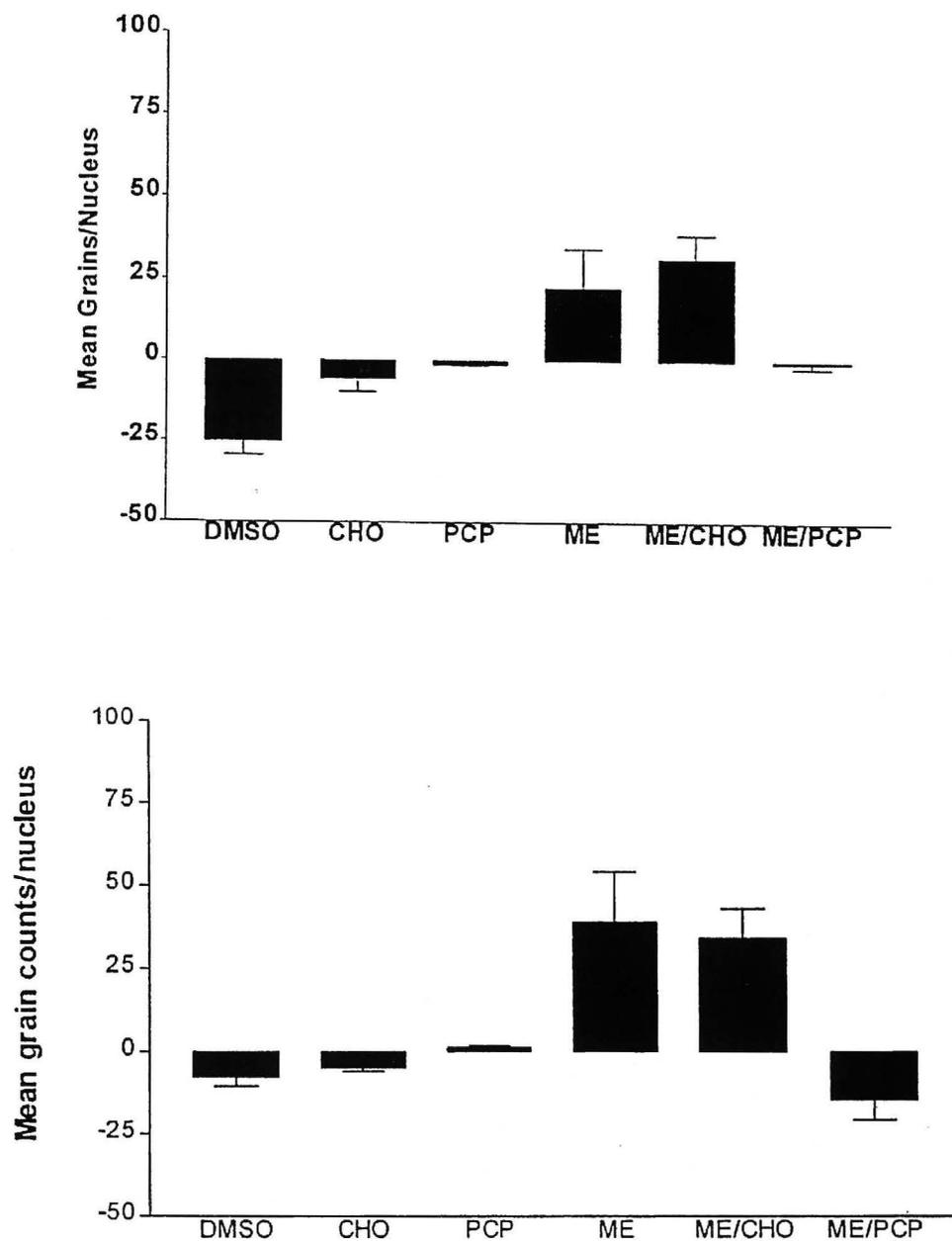
FIGURE N5
UDS in Isolated Hepatocytes of Male Fischer 344 Rats and Female B6C3F$_1$ Mice
Caused by 18-Hour Incubation with Methyleugenol in the Presence of Modulators. USD in hepatocytes was estimated by the mean number of silver grains in the nucleus relative to grains in the cytosol. Data are expressed as mean ± standard error (n=3). DMSO=dimethylsulfoxide; CHO=cyclohexane oxide; PCP=pentachlorophenol; ME=methyleugenol.

APPENDIX O
PHARMACOKINETIC MODEL

PHARMACOKINETIC MODEL

INTRODUCTION

A physiologically based pharmacokinetic model mathematically representing the absorption, distribution, metabolism, and elimination of methyleugenol in rats and mice was developed to describe the processes involved in methyleugenol toxicokinetics, help identify dose metrics for analyzing dose-response relationships, and provide a basis for describing methyleugenol toxicokinetics in humans. The differential equations used in this model to describe mass transfer of methyleugenol between tissue compartments have parameters representing physiological quantities (body weight, blood flow rates, tissue volumes, hematocrit) and chemical-specific parameters (partition coefficients, absorption rates, metabolism rates, and permeability constants). The physiological parameters were chosen from literature values for male and female rats and mice. The chemical-specific parameters were estimated from the plasma concentration time course data following intravenous and gavage administration of methyleugenol to rats and mice (Appendix M). Absorption rates, metabolism rates, and permeability constants were estimated separately for rats and mice.

In the process of developing the model, several alternative models were developed including 1) a model with detailed gastrointestinal absorption, 2) a model with defined capillary permeability, and 3) alternative models describing the metabolism of methyleugenol. Extrahepatic metabolism to describe the disappearance of methyleugenol from the bloodstream was also assessed.

The model was then used to predict maximum hepatic methyleugenol concentrations, average daily hepatic methyleugenol concentrations, and 24-hour integrated rates of hepatic metabolism. These predictions were then compared to the adjusted rates of liver neoplasms from the 2-year studies in rats and mice.

MODEL DEVELOPMENT

The model (Figure O1) contains compartments for arterial and venous blood and tissue and capillary spaces for gastrointestinal tract, liver, kidney, fat, slowly perfused tissues (for example, skin, muscle, and bone) and rapidly perfused tissues (for example, heart, lungs, brain, and viscera). There was also a compartment for gastrointestinal tract lumenal space. The primary site of metabolism was assumed to be the liver. Kidney was included as a potential site of metabolism and elimination for methyleugenol. The other compartments were included due to their role in the absorption and distribution kinetics. Organ perfusion was represented as blood flow from the arterial space, through the capillary space, and exiting to the venous space. The exception is that portal effluent from the gastrointestinal tract was passed from the gastrointestinal blood to the liver blood. Consequently, 20% of the hepatic perfusion is via the hepatic artery, and the remainder from portal flow. Table O1 gives the volume of each of these organs/groupings/tissues along with overall body weight and other physiological parameters for the species being modeled.

Methyleugenol was administered either by intravenous injection or by gavage. Plasma concentrations were assumed to be identical with total blood concentrations.

Methyleugenol has been shown to be metabolized in both liver slices and cultured hepatocytes from rats and mice (Appendix O). Information on the rate of metabolism in the liver or in any other tissue was not available. Several urinary metabolites from oral or intravenous administration of methyleugenol to rats and mice have been identified (Appendix O). These metabolites were products of oxidation on the ring (including displacement of methoxy groups) or on the propenyl side chain. Oxidative products are

eliminated as their glucuronide, sulfate, or glycine conjugates (Appendix O). This suggests that oxidative metabolism is a primary step in the metabolism of methyleugenol. No information was available on the rate of production, extent of distribution, or rate of elimination of individual metabolites. Therefore, the model did not include estimates of the concentrations of individual metabolic products in any tissue, and no conclusions can be reached about which metabolite(s) may be responsible for methyleugenol toxicity.

Absorption, permeability, and metabolic parameter values for male and female animals were estimated simultaneously, with different parameters representing body weight, tissue and capillary volumes, blood flows, and chyme flows. The absorption of methyleugenol was modeled as occurring from the gastrointestinal lumen compartment into the gastrointestinal capillary space, equilibrating with gastrointestinal tissues, and then entering the liver capillary space via the portal blood flow. The distribution of methyleugenol between each tissue and its capillary bed was explicitly modeled with the tissue permeability as an adjustable parameter. The following equations were used to represent oral absorption of methyleugenol:

$$\frac{dA_{lumen}}{dt} = -k_a \bullet A_{lumen} - A_{chyme} \bullet A_{lumen}$$

$$\frac{dA_{GIcap}}{dt} = Q_{GItissue}\left(\frac{A_{blood}}{Vol_{blood}} - \frac{A_{GIcap}}{Vol_{GIcap}}\right) - \frac{dA_{tissue}}{dt} + k_a \bullet A_{lumen}$$

$$\frac{dA_{livercap}}{dt} = Q_{livertissue}\left(\frac{A_{blood}}{Vol_{blood}} - \frac{A_{livercap}}{Vol_{livercap}}\right) + Q_{GItissue}\left(\frac{A_{blood}}{Vol_{blood}} - \frac{A_{GIcap}}{Vol_{GIcap}}\right) - \frac{dA_{tissue}}{dt} + k_a \bullet A_{lumen}$$

where A = amount of methyleugenol in tissue or lumen (mmol), k_a = first-order rate constant for absorption of methyleugenol from gastrointestinal (GI) tract lumen into portal blood (hr^{-1}), Q = flow rate for blood or gastrointestinal tract lumen contents (L/hr), and Vol = volume of tissue or lumen (L).

The following equations were used to represent distribution of methyleugenol to non-metabolizing tissues:

$$\frac{dA_{tissue}}{dt} = Q_{tissue}Perm\left(\frac{A_{cap}}{Vol_{cap}} - \frac{A_{tissue}}{Vol_{tissue}PC_{tissue}}\right)$$

$$\frac{dA_{cap}}{dt} = Q_{tissue}\left(\frac{A_{blood}}{Vol_{blood}} - \frac{A_{cap}}{Vol_{cap}}\right) - \frac{dA_{tissue}}{dt}$$

where Perm = capillary permeability (unitless) and PC = tissue:blood partition coefficient (unitless).

The octanol-water partition coefficient (K_{ow}) was determined to be 800 (Battelle, 1998). From this value, tissue:blood partition coefficients were estimated using published methods (Fisherova-Bergerova *et al.*, 1984; Abraham *et al.*, 1985; Lyman *et al.*, 1990). The values used were: fat 82.7, gastrointestinal tract 3.0, kidney 2.4, liver 4.1, slowly perfused tissues 3.0, and rapidly perfused tissues 3.6.

The metabolism of methyleugenol in rats was assumed to be confined to the liver and represented as a Michaelis-Menten process. The metabolism of methyleugenol in mice was assumed to be confined to the liver and kidney and represented as a Michaelis-Menten process. The same K_m was used for both liver and

kidney in mice, but different values were estimated for V_{max}. The following equations were used to represent distribution and metabolism of methyleugenol in metabolizing tissues:

$$\frac{dA_{tissue}}{dt} = Q_{tissue}Perm\left(\frac{A_{cap}}{Vol_{cap}} - \frac{A_{tissue}}{Vol_{tissue}PC_{tissue}}\right) - \frac{V_{max} \bullet A_{tissue}}{k_m + \frac{A_{tissue}}{Vol_{tissue}}}$$

$$\frac{dA_{cap}}{dt} = Q_{tissue}\left(\frac{A_{blood}}{Vol_{blood}} - \frac{A_{cap}}{Vol_{cap}}\right) - \frac{dA_{tissue}}{dt}$$

where V_{max}=Michaelis-Menten maximal rate of metabolism (mM/hr) and K_m=Michealis-Menten concentration at half-maximal rate (mM).

Parameters describing absorption and metabolism were estimated from the plasma concentration time course data using log-transformed least squares methods (Bard, 1974; Bailer and Portier, 1990). Goodness of fit was evaluated using both graphical and statistical methods (Bard, 1974). In addition, the contributions of parameters to the overall fit of the model were assessed statistically by the use of a likelihood-ratio test (Bard, 1974). The MATLAB program package with Simulink, Optimization Toolbox, and Statistics Toolbox (The MathWorks, Inc., Natick, MA) was used for both optimization and final simulation. The computer programs used are available from the National Toxicology Program.

RESULTS

Graphs of the fits of the model to male and female rat data are shown in Figures O2 and O3, respectively. Graphs of the fits to male and female mouse data are shown in Figures O4 and O5, respectively. The model presented in Figure O1 and Table O2 for methyleugenol disposition in rats and mice was the best fitting product after testing of multiple alternative hypotheses. Subdividing the gastrointestinal compartment into stomach, intestine, and colon did not improve the fit to the data over a unitary compartment in either rats or mice (likelihood ratio test $P > 0.10$). The absorption of methyleugenol in rats and mice was so rapid as to approximate instantaneous absorption, and was estimated using very fast linear kinetics (k_a set to 10^6 hr^{-1}). The use of a linear model for metabolism in rats resulted in systematic overprediction at the low oral dose (37 mg/kg). This systematic error was eliminated by the use of a Michaelis-Menten model for metabolism, although it provided no statistical improvement in goodness of fit compared to the linear model ($P > 0.5$). The addition of extrahepatic metabolism did not improve the fit of the model to the rat data. In mice, the use of a Michaelis-Menten model for metabolism provided a much better fit to the data than a linear model ($P < 0.005$). Confining methyleugenol metabolism to the liver in the mouse resulted in systematic overprediction at the intravenous dose (37 mg/kg). The addition of extrahepatic metabolism in mice, modeled as occurring in the kidney, eliminated this systematic error, although it provided no statistically significant improvement in fit compared to a linear model.

The rat and mouse models were used to predict various dose metrics from single oral doses of 37, 75, and 150 mg methyleugenol per kg body weight, doses used in the 2-year gavage studies. The maximum concentration (C_{max}) and the integrated concentration of methyleugenol in the liver (AUC_{liver}) over the 24 hours following the doses were estimated, as was the integrated rate of hepatic metabolism (AUC_{met}) over the same 24 hours. The resulting values are listed in Table O3, along with the adjusted rates of primary liver neoplasms from the 2-year studies. With every doubling of administered dose in male and female rats, the C_{max} increased 3- to 7-fold and AUC_{liver} increased 6- to 12-fold. With every doubling of dose in male and female mice, the C_{max} increased approximately 3-fold and AUC_{liver} increased four- to five-fold. These results suggest that metabolism of methyleugenol is saturated at these doses. AUC_{met} increased linearly with applied dose in both rats and mice, which is to be expected when both the absorption and

metabolism of methyleugenol are rapid and complete. The adjusted hepatic tumor response did not increase in the same pattern as C_{max} or AUC_{liver}, lending support to the hypothesis that methyleugenol metabolites may play a role in methyleugenol-induced carcinogenicity. The tumor incidence in mice plateaued at approximately 100% at doses above 37 mg/kg, indicating that the entire dose-response trend occurs at lower doses in mice.

DISCUSSION

Several alternative models were tested in the course of producing this model structure. In the gastrointestinal absorption submodel, three different mathematical representations of the rate of absorption were tested: a linear model, a saturable Michaelis-Menten model, and a Hill model capable of displaying negative and positive cooperativity in transport. Neither the Michaelis-Menten model nor the Hill model significantly improved model fit. This is very likely due to the speed of absorption and the dearth of data at extremely early time points, preventing these models from being distinguished. More complex, multi-compartmental gastrointestinal tract models were also investigated, but all served to delay uptake and detract from the fit to the data. The simplifying step of setting capillary permeability to an arbitrarily high value, approximating a "flow-limited" distribution to tissues, did not significantly reduce the fit of the data to the model. A need for an extrahepatic site for metabolism was indicated for mice but not for rats. The lack of a need for extrahepatic metabolism in the rat model does not lead to the conclusion that extrahepatic metabolism of methyleugenol is not important, only that the blood flow and metabolic capacity modeled for rats was sufficient to act as a surrogate for all methyleugenol metabolism. Extrahepatic metabolism in the mouse model was included in the kidney as a representative organ with significant phase 1 metabolic capacity.

The primary conclusions that can be reached from the physiologically based pharmacokinetic model are: 1) absorption of oral doses of methyleugenol in rats and mice is rapid and complete, 2) distribution of methyleugenol to tissues is not hampered by capillary permeability, and 3) metabolism of methyleugenol is saturable and must have some extrahepatic component in the mouse.

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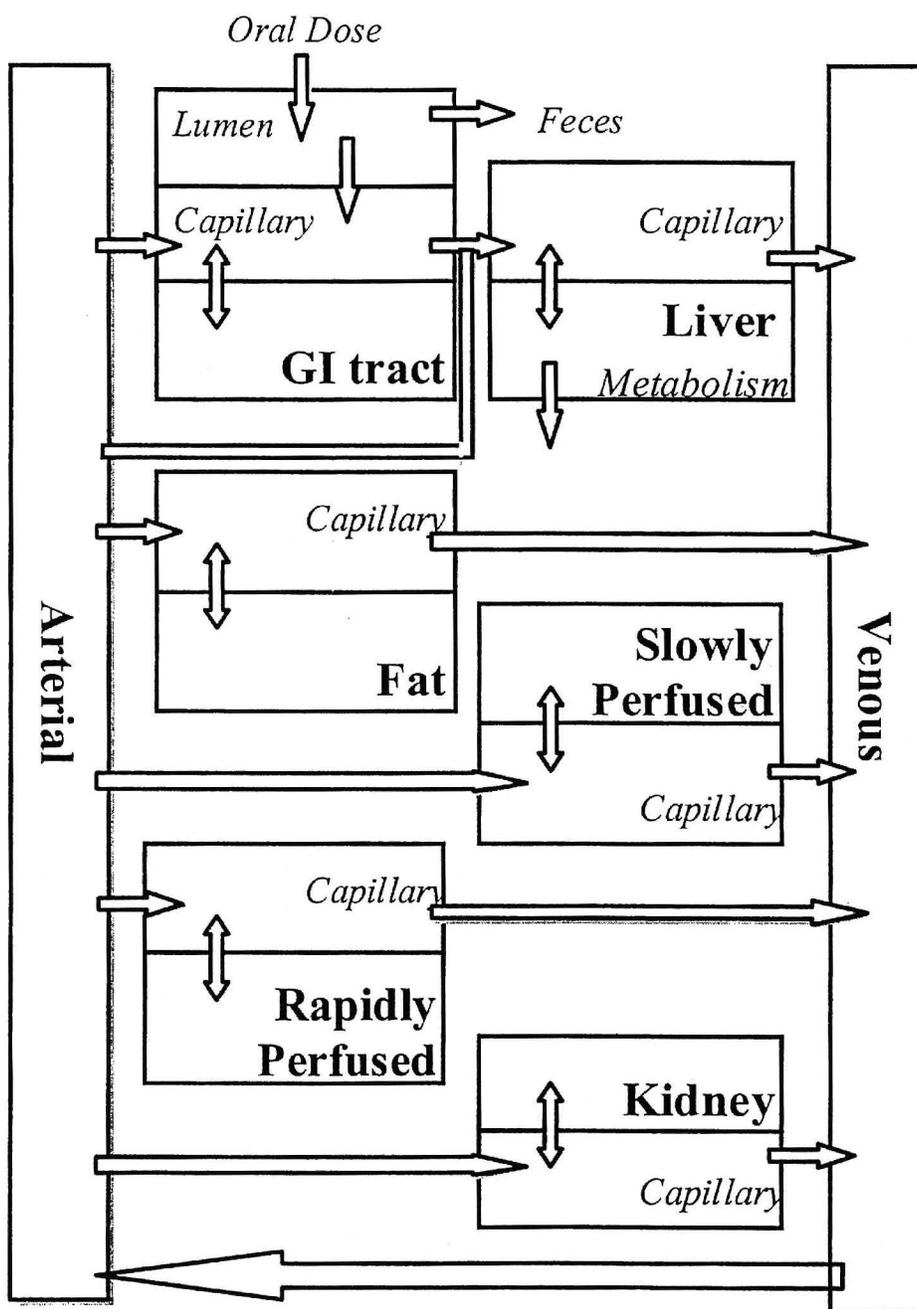
FIGURE O1
Schematic Representation of the Physiologically Based Pharmacokinetic Model
for Methyleugenol in Rats and Mice

TABLE O1
Physiological Parameters for Rats and Mice

	Male Rats	Female Rats	Male Mice	Female Mice
Cardiac Output (L/hr/kg$^{0.7}$)[a]	14.7	14.7	15.3	15.3
Body Weight (kg)[b]	0.287	0.170	0.029	0.024
Chyme Flow Rate (mL/hr)[c]	1.5	0.88	0.5	0.5
Tissue Volumes (% of body weights)				
Arterial Blood[d]	0.466	0.43	0.32	0.32
Venous Blood[d]	1.362	1.29	0.95	0.95
Fat[e]	7	7	4	4
Slowly perfused[f]	54.2	56	52.9	52.9
Richly perfused[g]	19.45	26.66	14.99	14.99
Kidney[h]	1.48	0.85	1.67	1.67
Liver[i]	3.7	4.5	5.49	5.49
Stomach[j]	0.486	0.63	0.72	0.72
Stomach lumen[j]	0.91	0.91	1.30	1.30
Intestine[j]	1.58	2.05	2.33	2.33
Duodenum lumen[j]	3.1	3.1	0.48	0.48
Jejunum lumen[j]	3.1	3.1	3.89	3.89
Colon[j]	0.795	1.03	1.179	1.179
Colon lumen[j]	1.6	1.6	2.34	2.34
Tissue Capillary Volumes (% of tissue volume)				
Fat[k]	2	2	3	3
Slowly perfused[k]	2	2	2	2
Richly perfused[l]	10	10	10	10
Kidney[k]	16	16	18.3	18.3
Liver[k]	13.8	13.8	13.8	13.8
Stomach[m]	4.11	4.11	4.11	4.11
Intestine[m]	2.65	2.65	2.65	2.65
Colon[m]	2.33	2.33	2.33	2.33

TABLE O1
Physiological Parameters for Rats and Mice

	Male Rats	Female Rats	Male Mice	Female Mice
Tissue Blood Flow (% of cardiac output)				
Fat[n]	6.5	6.5	1.88	1.88
Slowly perfused[o]	33.4	33.4	39.6	39.6
Richly perfused[g]	27.4	27.4	24.4	24.4
Kidney[p]	13.3	13.3	16.3	16.3
Liver (hepatic)[q]	3.9	3.9	3.7	3.7
Stomach[r]	1.2	1.2	1.1	1.1
Intestine[r]	11.6	11.6	10.5	10.5
Colon[r]	2.7	2.7	2.5	2.5

[a] Averages of literature values
[b] Averages of subject values from toxicokinetic studies
[c] Calculated from gastrointestinal lumen volumes and transit times (Altman and Ditmer, 1971)
[d] For rats, blood volume (Davies and Morris, 1993) after subtracting capillary volumes, with 75% venous and 25% arterial; for mice, blood volume (average of literature values) after subtracting capillary volumes, with 75% venous and 25% arterial
[e] For rats, values from Delp *et al.* (1991); for mice, values are averages of literature values
[f] Averages of literature values from muscle and skin
[g] Calculated residual values
[h] For male rats, value from Delp *et al.* (1991); for female rats, value from Buelke-Sam *et al.* (1982); for mice, values from ILSI (1994)
[i] For male rats and male and female mice, values from ILSI (1994); for female rats, value from Buelke-Sam *et al.* (1982)
[j] Values from Roth *et al.* (1993)
[k] Values from ILSI (1994)
[l] Averages of remaining tissues
[m] For rats, values from Altman and Ditmer (1971); for mice, values derived by analogy to rats
[n] For rats, values from Delp *et al.* (1991); for mice, values from King *et al.* (1983)
[o] For rats, values from Delp *et al.* (1991); for mice, values from ILSI (1994)
[p] For rats, values from Delp *et al.* (1991); for mice, values from Davies and Morris (1993)
[q] For rats, values are averages of literature values; for mice, values from ILSI (1994)
[r] For rats, values from Roth *et al.* (1993); for mice, total gastrointestinal tract blood flow (Altman and Ditmer, 1971) apportioned between tissues as in rats
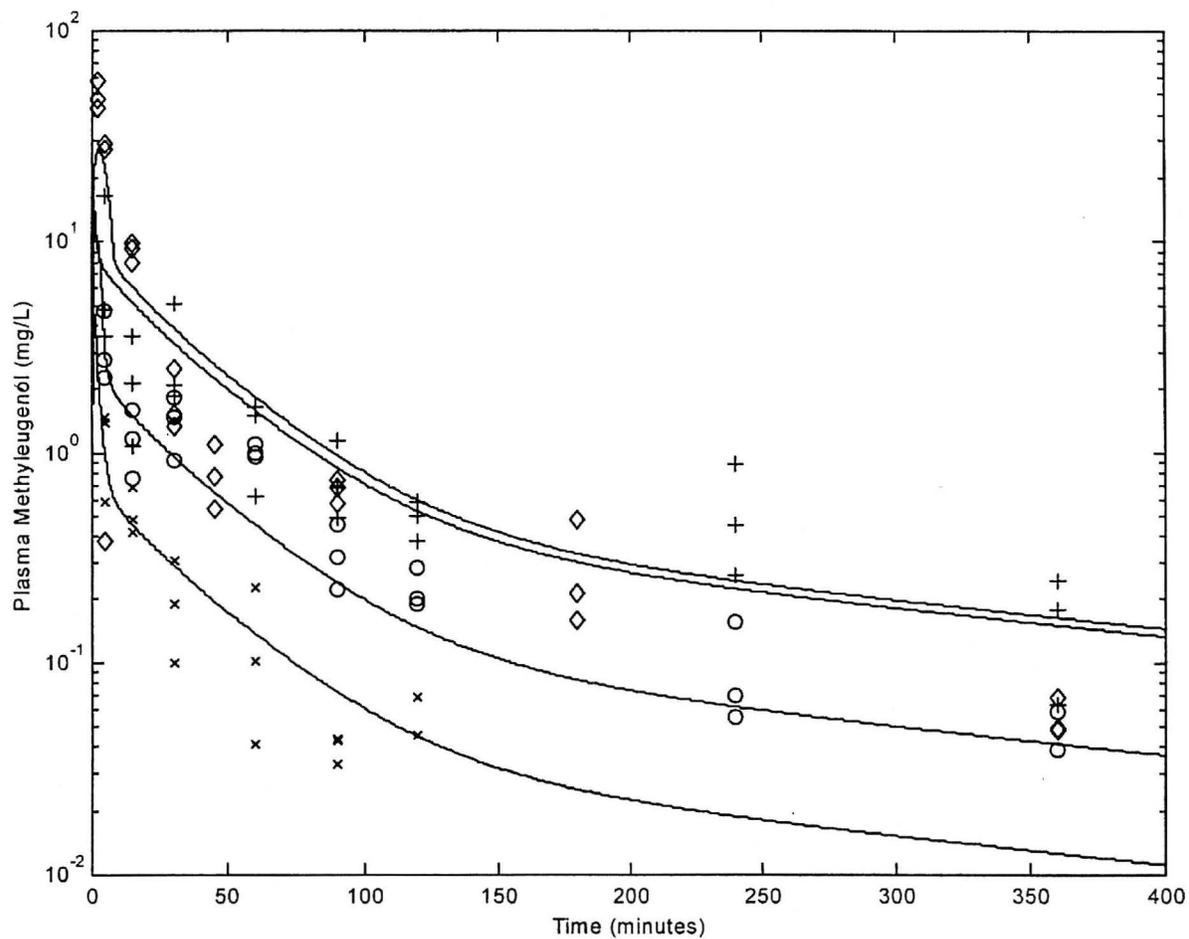
FIGURE O2
Plasma Methyleugenol Concentrations in Male Rats after a Single Intravenous or Gavage Dose. The solid
lines represent the fit of these data to the physiologically based pharmacokinetic model ($\times$ = 37 mg/kg
gavage; o = 75 mg/kg gavage; + = 150 mg/kg gavage; $\diamond$ = 37 mg/kg intravenous).
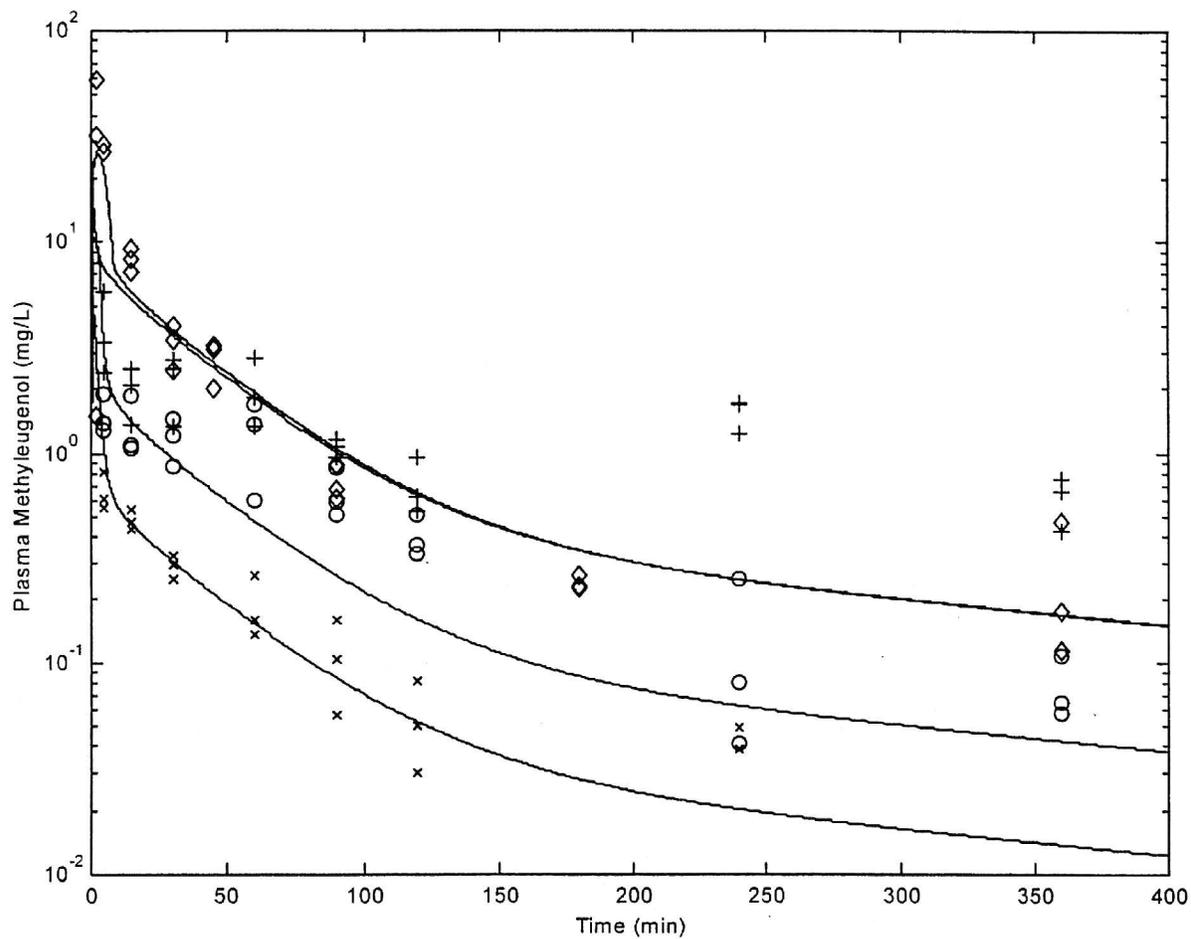
FIGURE O3
Plasma Methyleugenol Concentrations in Female Rats after a Single Intravenous or Gavage Dose. The solid lines represent the fit of these data to the physiologically based pharmacokinetic model ($\times$ = 37 mg/kg gavage; o = 75 mg/kg gavage; + = 150 mg/kg gavage; $\lozenge$ = 37 mg/kg intravenous).
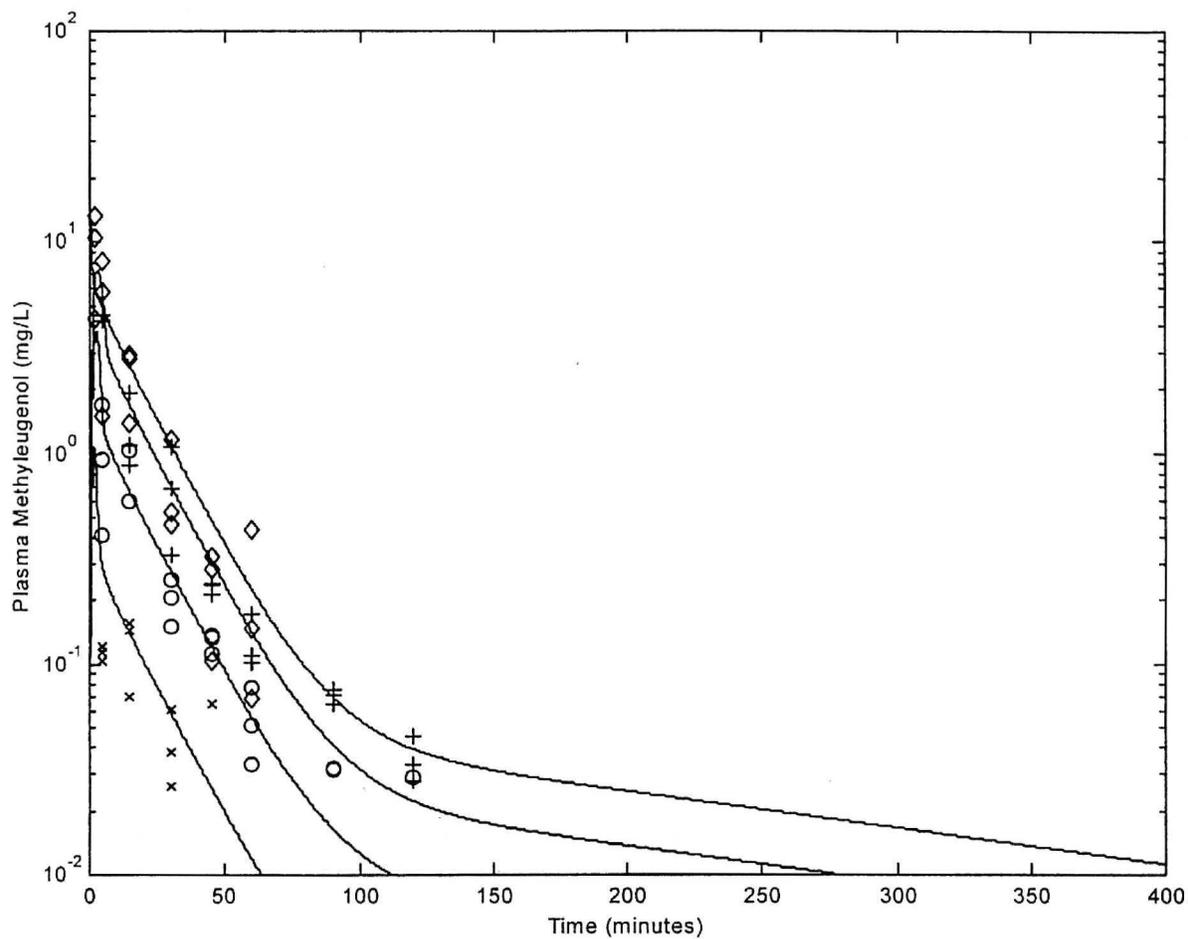
FIGURE O4
Plasma Methyleugenol Concentrations in Male Rats after a Single Intravenous or Gavage Dose. The solid
lines represent the fit of these data to the physiologically based pharmacokinetic model ($\times$ = 25 mg/kg
gavage; o = 50 mg/kg gavage; + = 75 mg/kg gavage; ◊ = 25 mg/kg intravenous).
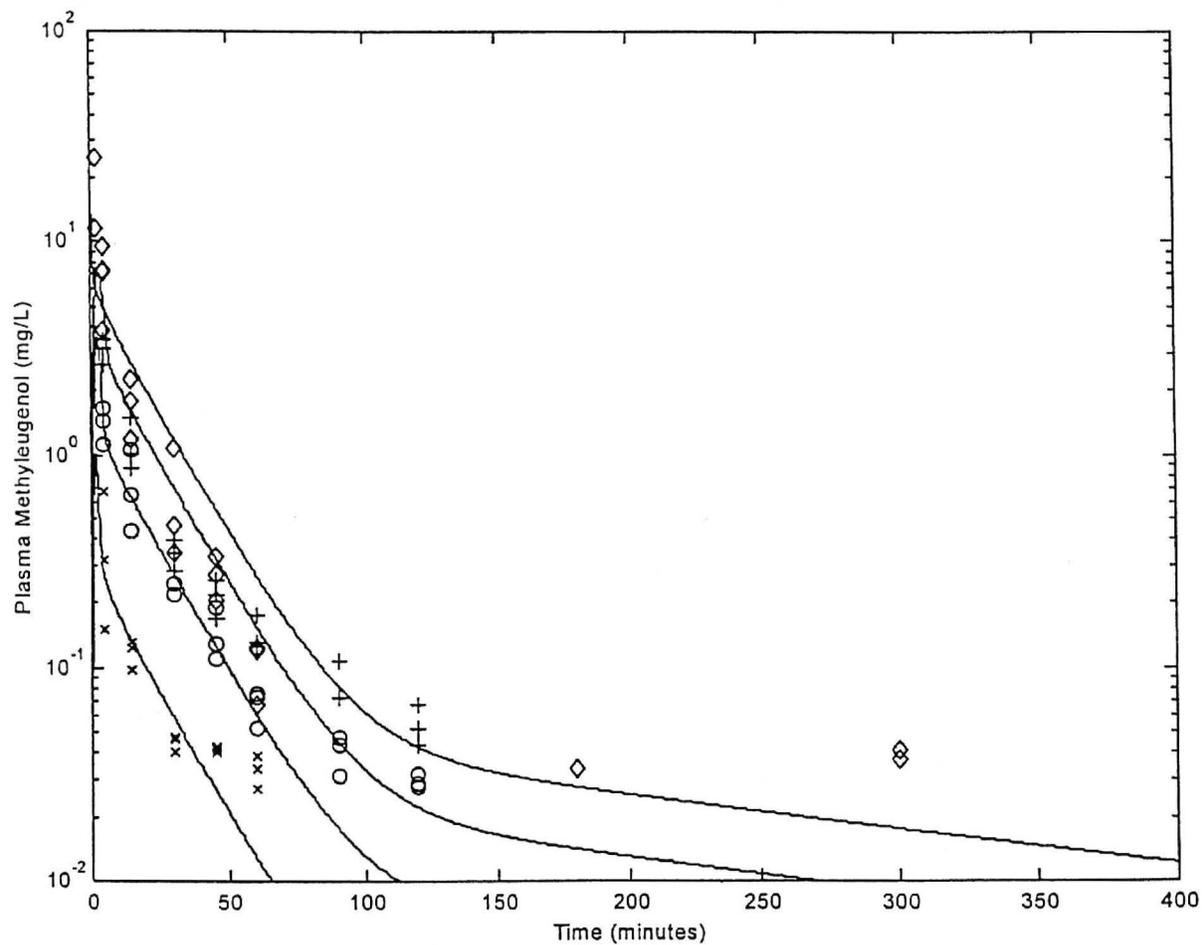
FIGURE O5
Plasma Methyleugenol Concentrations in Female Mice after a Single Intravenous or Gavage Dose. The solid lines represent the fit of these data to the physiologically based pharmacokinetic model ($\times$ = 25 mg/kg gavage; o = 50 mg/kg gavage; + = 75 mg/kg gavage; $\Diamond$ = 25 mg/kg intravenous).

TABLE O2
Estimated Pharmacokinetic Parameters of Methyleugenol for Rats and Mice[a]

	Rats	Mice
k_a (min^{-1})	set to 10^6	set to 10^6
Perm	set to 10^6	set to 10^6
$V_{maxliver}$ (mM/min)	140 (110-170)	74 (60-91)
$V_{maxkidney}$ (mM/min)	Not used	15 (6-35)
K_m (mM)	0.074 (0.026-0.210)	0.21 (0.11-0.41)

[a]   95% confidence intervals in parentheses. k_a=first-order rate constant for absorption of methyleugenol from gastrointestinal tract lumen into portal blood; Perm=capillary permeability; V_{max}=Michaelis-Menten maximal rate of metabolism; K_m=Michealis-Menten concentration at half-maximal rate

TABLE O3
Dose Metrics Calculated from Simulated Single Gavage Doses of Methyleugenol

Dose (mg/kg)	Peak Liver Concentration (C_{max}; mM)	24-Hour Liver AUC (AUC_{liver}; mM)	24-Hour Hepatic Metabolism (AUC_{met}; mmol)	Adjusted Rate of Primary Liver Neoplasms (%)
Male Rats				
0	—	—	—	2.5
37	0.38	0.01	0.05	19.6
75	2.47	0.11	0.11	33.4
150	8.95	0.73	0.22	76.6
Female Rats				
0	—	—	—	16.6
37	0.46	0.01	0.04	34.4
75	2.76	0.12	0.08	64.4
150	9.44	0.75	0.15	94.0
Male Mice				
0	—	—	—	65.4
37	0.95	0.04	0.006	97.4
75	3.05	0.19	0.012	96.4
150	8.05	0.76	0.023	86.7
Female Mice				
0	—	—	—	54.8
37	1.00	0.04	0.005	100.0
75	3.15	0.19	0.009	100.0
150	8.18	0.75	0.018	97.7

APPENDIX P
SERUM GASTRIN
AND GLANDULAR STOMACH pH LEVELS

Conducted at The National Institute of Environmental Health Sciences

SERUM GASTRIN
AND GLANDULAR STOMACH pH LEVELS

INTRODUCTION

In the 14-week and 2-year gavage studies of methyleugenol, increased incidences of lesions of the glandular stomach were observed in dosed rats and mice. Therefore, the NIEHS conducted additional gavage studies to examine the effects of methyleugenol on serum gastrin and glandular stomach pH levels in rats and mice.

MATERIALS AND METHODS

Suspensions of methyleugenol in 0.5% aqueous methylcellulose were obtained from Battelle Columbus Laboratories (Columbus, OH) at concentrations of 0, 15, 30, and 100 mg/kg. These suspensions were mixed with additional methylcellulose to achieve the desired dose formulations. Rats received 0, 37, 75, 150, 300, or 1,000 mg methyleugenol/kg body weight, and mice received 0, 9, 18.5, 37, 75, 150, or 300 mg/kg once daily, 5 days per week for 30 or 90 days. The dose formulations were stored in bottles with Teflon®-lined lids at room temperature, protected from light, for 2 weeks.

Female F344/N rats (5 weeks old) and male B6C3F$_1$ mice (4 weeks old) were obtained from Taconic Laboratory Animals and Services (Germantown, NY) and acclimated to laboratory conditions for 14 days. Rats were housed two per cage and mice were housed 10 per cage. Rats and mice were kept in individual Illinois® chambers where the temperature was 21° to 23° C, the humidity was 50% $\pm$ 10%, and the room air was changed 10 to 12 times per hour. Fluorescent light was provided on a 12-hour light-dark cycle. NIH-07 rat and mouse feed and water were available *ad libitum*. Cages were changed twice weekly. Doses were based on individual animal weights; animals were weighed every tenth dose, and the doses were adjusted accordingly. For each exposure period, groups of 10 female rats and 10 male rats were used in each dose group.

After 30 or 90 days, the animals were fasted for 24 hours and given free access to water. Animals were anesthetized by CO_2. A midline abdominal incision was made, and blood was drawn from the dorsal aorta (rats) or by cardiac puncture (mice) using a 22-gauge needle and a 10 mL (rats) or 5 mL (mice) syringe. Individual blood samples were centrifuged at 1,500 rpm for 30 minutes using a Sorvall GLC-1 centrifuge. Sera were collected in 1.5 mL Nalgene® tubes, and the tubes placed on dry ice. Serum gastrin levels were determined by radioimmunoassay at Analytics Incorporated, Gaithersburg, MD.

After blood collection, animals were euthanized by CO_2, and the stomach removed. The duodenum distal to the pylorus was incised and an Accumet Microprobe Combination Electrode (Fisher Scientific, Pittsburgh, PA) inserted. The pH reading was recorded from an Accumet Basic pH meter (Fisher Scientific).

RESULTS

All animals survived to study termination. Methyleugenol produced glandular stomach atrophy localized in chief cells. These cells are responsible for secretion of hydrochloric acid into the lumen of the stomach. Elevated gastric pH was observed in 1,000 mg/kg per day female rats treated for 30 days (Table P1). This response was sustained and was observed in 300 and 1,000 mg/kg female rats at 90 days. Serum gastrin levels increased in response to higher gastric pH levels due to the absence of the acid feedback repression mechanism; serum gastrin levels were significantly increased in female rats given 300 or 1,000 mg/kg for 30 days and in those given 150 mg/kg or greater for 90 days (Table P1). Male mice appeared to be less sensitive to both the increase in glandular stomach pH and increased serum gastrin production (Table P2).

TABLE P1
Serum Gastrin and Glandular Stomach pH Levels
in Female F344/N Rats Treated with Methyleugenol by Gavage[a]

Dose (mg/kg)	Serum Gastrin (pg/mL)		Glandular Stomach pH	
	30 Days	90 Days	30 Days	90 Days
0	62 ± 7	41 ± 3	2.2 ± 0.1	2.2 ± 0.3
37	$25 \pm 4^{*}$	46 ± 3	2.4 ± 0.3	1.8 ± 0.1
75	$22 \pm 2^{*}$	60 ± 6	1.7 ± 0.1	2.2 ± 0.2
150	57 ± 14	$88 \pm 11^{***}$	1.9 ± 0.2	2.0 ± 0.2
300	$127 \pm 23^{*}$	$409 \pm 68^{***}$	$1.6 \pm 0.2^{*}$	$3.1 \pm 0.5^{*}$
1,000	$604 \pm 20^{***}$	$506 \pm 192^{**}$	$6.4 \pm 0.1^{***}$	$3.8 \pm 0.3^{***}$

* $P \leq 0.05$
** $P \leq 0.002$
*** $P \leq 0.001$
[a]   Data are presented as mean $\pm$ standard error; n=10

TABLE P2
Serum Gastrin and Glandular Stomach pH Levels
in Male B6C3F$_1$ Mice Treated with Methyleugenol by Gavage[a]

Dose (mg/kg)	Serum Gastrin (pg/mL)		Glandular Stomach pH	
	30 Days	90 Days	30 Days	90 Days
0	3.5 ± 0.8	3.1 ± 0.7	2.1 ± 0.1	2.8 ± 0.3
9	2.9 ± 1.0	3.5 ± 0.8	2.3 ± 0.1	2.6 ± 0.3
18.5	3.5 ± 0.6	1.9 ± 0.4	2.2 ± 0.1	2.3 ± 0.1
37	3.8 ± 1.0	2.6 ± 0.8	2.5 ± 0.1	3.0 ± 0.4
75	4.8 ± 1.6	3.0 ± 0.6	$2.7 \pm 0.2^{*}$	2.7 ± 0.3
150	$15 \pm 2.0^{**}$	6.9 ± 2.2	2.4 ± 0.1	2.0 ± 0.1
300	$11 \pm 2.0^{*}$	7.5 ± 2.3	2.4 ± 0.2	2.0 ± 0.1

* $P \leq 0.05$
** $P \leq 0.001$
[a]   Data are presented as mean $\pm$ standard error; n=10

APPENDIX Q
CELL PROLIFERATION
IN THE LIVER AND STOMACH
OF F344/N RATS AND B6C3F$_1$ MICE

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CELL PROLIFERATION
IN THE LIVER AND STOMACH
OF F344/N RATS AND B6C3F$_1$ MICE

INTRODUCTION

The objective of this study was to examine cell proliferation in the stomach and liver of rats and mice administered methyleugenol for 30 or 90 days.

MATERIALS AND METHODS

F344/N female rats (5 weeks old) and B6C3F1 male mice (4 weeks old) purchased from Taconic Farms were acclimated to laboratory conditions for 14 days. Rats were housed two to a cage while mice were housed ten to a cage. Rats received 0, 37, 75, 150, 300 or 1000 mg/kg/day 5 days per week for 30 days or 90 days. Mice received 0, 9, 18.5, 37, 75, 150 and 300 mg/kg/day on the same dosing schedule.

Two hours prior to termination, rats and mice were given an intraperitoneal injection of 100 mg/kg 5-bromo-2′-deoxyuridine (BrdU; Sigma Chemical Co., St. Louis, MO) in 0.01 N sodium hydroxide. The animals were anesthetized with carbon dioxide and exsanguinated, and livers were blotted, weighed, and fixed in neutral buffered formalin. A midlobe radial section of the right anterior lobe was fixed in neutral buffered formalin for 24 hours. A cross section of duodenum was also fixed as a positive control for the proper operation for the minipump used in the staining technique. Tissues were embedded in paraffin, and serial sections were mounted onto Superfrost Plus® slides (Fisher Scientific, Pittsburgh, PA). Following deparaffination and rehydration, one set of slides was stained with hematoxylin and eosin for histopathologic evaluation and another set was stained immunohistochemically for BrdU incorporation by a variation on the method of Sugihara *et al.* (1986), as described by Cunningham and Matthews (1991) and Cunningham *et al.* (1991).

Immunohistochemistry: Serial sections of paraffin-embedded tissues were cut at 5 μm and placed on positively charged slides (Superfrost Plus®) to ensure adhesion during processing for BrdU. Standard immunohistochemical methods were used to stain tissues for BrdU.

Cell Proliferation Measurements: Systemic delivery of BrdU was confirmed for each study animal by positive staining in the crypts of the villi within the duodenum. BrdU incorporation was quantified in the epithelium of the forestomach, gastric pits, and fundic glands of the glandular stomach, pyloric region, and liver using light microscopy. In the forestomach, the unit length labeling index was scored in four 0.25-mm^2, randomly selected fields as the number of BrdU-labeled epithelial cells/mm^2 muscularis mucosa. For measuring cell proliferation in the gastric pits, fundic glands, and pyloric region, the unit area labeling index was assessed as the number of BrdU-labeled epithelial cells/mm^2 and scored in four 0.25-mm^2, randomly selected areas. The labeling index in the liver was assessed as the percentage of BrdU-labeled hepatocytes scored in 10 randomly selected fields; at least 3,000 hepatocytes were scored per animal.

Statistical Analysis: The Student's *t*-test (two-sided, unequal variance) was used to compare control and dosed groups at each time point. A P value of 0.05 was considered statistically significant.

RESULTS

Cell proliferation: For rats, group mean cell proliferation data are presented in Table Q1; fold increases in cell proliferation over controls are presented by time point and target tissue in Table Q2. Data for mice are given in Tables Q3 and Q4.

Treatment-related increases in cell proliferation were observed in the fundic glands of the glandular portion of the stomach of rats and mice and in the liver of rats. Although cell proliferation was determined to be significant in some cases of forestomach, gastric pits, and pyloric region, these minor increases in cell proliferation were not considered to be biologically significant.

In this study, biological significance was defined as a group mean labeling index at least twofold greater than the mean control values, with at least one animal in a dosed group having a cell proliferation value greater than the highest control value. Further support for biological significance came from demonstration of a dose-response relationship. Using these criteria, biologically significant increases in cell proliferation were observed at 30 days in the forestomach of rats receiving 1,000 mg/kg and in the fundic gland and liver of rats receiving 150, 300, or 1,000 mg/kg (Table Q2). At 90 days, biologically significant increases were observed in the fundic gland of rats receiving 37, 75, or 150 mg/kg and in the liver of rats receiving 150 mg/kg. In mice, biologically significant increases were observed in the fundic gland at 30 days in the 150 and 300 mg/kg groups and at 90 days in the 18.5, 37, and 75 mg/kg groups (Table Q4).

DISCUSSION

In this study, cell proliferation was measured within the squamous epithelium of the forestomach, the gastric pits and fundic glands of the glandular portion of the stomach, pyloric region, and the liver of rats and mice administered methyleugenol for 30 or 90 days. Treatment-related increases in cell proliferation were observed in the fundic gland of rats and mice and the liver of rats. Previous studies with methyleugenol revealed that the stomach and liver are target organs for carcinogenicity. The results of the present study support a role for cell proliferation in the pathogenesis of methyleugenol carcinogenicity.

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Sugihara, H., Hattori, T., and Fukuda, M. (1986). Immunohistochemical detection of bromodeoxyuridine in formalin-fixed tissues. *Histochemistry* **85**, 193-195.

TABLE Q1
Cell Proliferation in F344/N Rats Treated with Methyleugenol by Gavage for 30 or 90 Days[a]

Dose (mg/kg)	Cell Proliferation				
	Forestomach	Gastric Pits	Fundic Glands	Pyloric Region	Liver
Day 30					
0	1.0	1.0	1.0	1.0	1.0
37	2.0	1.1	2.0	1.5	2.4
75	1.6	1.3	1.0	1.4	1.0
150	1.5	1.6	11.0	1.3	3.0
300	1.7	1.7	54.0	1.2	11.2
1,000	3.9	1.7	61.0	1.2	18.8
Day 90					
0	1.0	1.0	1.0	1.0	1.0
37	1.1	1.2	2.0	0.9	4.2
75	1.5	0.9	2.0	0.9	2.4
150	1.1	1.0	6.3	1.0	4.8

[a] Data are given as the fold increase over the mean control value.

TABLE Q2
Mean Cell Proliferation Indices in F344/N Rats Treated with Methyleugenol by Gavage for 30 or 90 Days

Dose (mg/kg)	Labeling Index[a]				
	Forestomach	Gastric Pits	Fundic Glands	Pyloric Region	Liver
Day 30					
0	14	52	1	94	0.086
37	28	58	2*	143*	0.205
75	23*	67	1	135*	0.086
150	21	85*	11*[b]	121*	0.254*[b]
300	24*[b]	88*	54*[b]	113*	0.962*[b]
1,000	54*[b]	87*	61*[b]	116*	1.618*[b]
Day 90					
0	24	61	4	149	0.116
37	27	73	8[b]	130	0.482
75	37	55	8*[b]	137	0.284
150	27	59	25*[b]	152	0.553*[b]

* Significantly different ($P \leq 0.05$) from the control group by Student's *t*-test
[a] Labeling indices are given as percent for liver and as fold increase over control value for other tissues.
[b] Result is considered to be biologically significant due to the value being at least twofold greater than the mean control value and at least one animal having a value greater than the highest individual control value. Biological significance is also supported by demonstration of a dose response.

TABLE Q3
Cell Proliferation in B6C3F$_1$ Mice Treated with Methyleugenol by Gavage for 30 or 90 Days[a]

Dose (mg/kg)	Cell Proliferation				
	Forestomach	**Gastric Pits**	**Fundic Glands**	**Pyloric Region**	**Liver**
Day 30					
0	1.0	1.0	1.0	1.0	1.0
9	2.0	0.7	0.3	0.6	0.7
18.5	1.3	0.7	0.3	0.7	0.0
37	1.7	0.6	0.3	0.8	2.7
75	1.7	0.9	1.0	0.8	1.0
150	2.0	1.5	19.0	0.9	2.3
300	1.3	1.3	6.0	1.0	1.0
Day 90					
0	1.0	1.0	1.0	1.0	1.0
9	0.8	1.2	3.0	1.0	1.4
18.5	0.5	1.1	6.0	1.0	2.0
37	0.7	0.9	6.0	1.1	2.8
75	0.8	1.2	15.0	1.1	1.6

[a]   Data are given as the fold increase over the mean control value.

TABLE Q4
Mean Cell Proliferation Indices in B6C3F$_1$ Mice Treated with Methyleugenol by Gavage for 30 or 90 Days

Dose (mg/kg)	Labeling Index[a]				
	Forestomach	**Gastric Pits**	**Fundic Glands**	**Pyloric Region**	**Liver**
Day 30					
0	3	80	3	134	0.003
9	6*	57*	1	82*	0.002
18.5	4	57*	1	99*	0.000
37	5	51*	1	102*	0.008
75	5	71	3	113*	0.003
150	6	118*	57*[b]	117	0.007
300	4	100*	18*[b]	132	0.003
Day 90					
0	10	96	1	124	0.005
9	8	115	3*	130	0.007
18.5	5*	103	6*[b]	123	0.010
37	7	84	6*[b]	140	0.014
75	8	113	15*[b]	140	0.008

*   Significantly different (P≤0.05) from the control group by Student's *t*-test
[a]   Labeling indices are given as percent for liver and as fold increase over control value for other tissues.
[b]   Result is considered to be biologically significant due to the value being at least twofold greater than the mean control value and at least one animal having a value greater than the highest individual control value. Biological significance is also supported by demonstration of a dose response.

APPENDIX R
MUTATION OF β-CATENIN BUT NOT H-*ras*
IN HEPATOCELLULAR ADENOMAS
AND CARCINOMAS OF B6C3F₁ MICE TREATED
WITH METHYLEUGENOL FOR 2 YEARS

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MUTATION OF β-CATENIN BUT NOT H-*ras* IN HEPATOCELLULAR ADENOMAS AND CARCINOMAS OF B6C3F₁ MICE TREATED WITH METHYLEUGENOL FOR 2 YEARS

INTRODUCTION

In the 2-year methyleugenol mouse study, numerous neoplasms were diagnosed in various organs; several were treatment-specific. Increases in the incidences of hepatocellular adenomas, hepatocellular carcinomas, and hepatoblastomas were observed in all dosed groups of male and female mice.

In evaluating potential hazards of chemical exposure to humans, it is important to assess how the chemical acts at the molecular level, i.e., through a genotoxic mechanism or via promotion of spontaneous damage. In the past, the patterns of mutations in protooncogenes such as *ras* and in tumor suppressor genes such as p53 have been found to help in the understanding of tumorigenesis. For example, in some neoplasms the profiles of activating mutations in *ras* genes are specific for particular chemicals and differ from those detected in spontaneous neoplasms. In this study, hepatocellular adenomas and carcinomas from B6C3F₁ mice treated with methyleugenol were examined for genetic alterations in H-*ras*, p53, and β-catenin, genes that have been shown to be altered in human cancers. After β-catenin mutations were found in these neoplasms, the neoplasms were examined for overexpression of β-catenin protein by Western blot hybridization analysis and immunohistochemical methods (Devereux *et al.*, 1999).

MATERIALS AND METHODS

Hepatocellular Neoplasms: Similar numbers of hepatocellular neoplasms from three methyleugenol dose groups (37, 75, or 150 mg/kg) and from control male and female B6C3F₁ mice were used.

DNA Isolation: The DNA isolation procedure has been described previously (Marmur, 1961; Devereux *et al.*, 1993).

Mutation Identification: Single-strand conformation polymorphism (SSCP) analysis was carried out on polymerase chain reaction (PCR) products corresponding to exon 2 of H-*ras* and exon 2 of β-catenin (Devereux *et al.*, 1999).

Immunohistochemistry: Hepatocellular neoplasms were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin. Localization of p53 protein expression and β-catenin expression was investigated using a rabbit polyclonal anti-p53 antibody (CM5 from Vector Laboratories) and a polyclonal goat anti-β-catenin antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Nonimmune rabbit IgG (Jackson Immuno Research Laboratories, West Grove, PA) was used as the negative control at equivalent conditions in place of the primary antibody.

RESULTS

Twenty-nine methyleugenol-induced liver neoplasms from B6C3F₁ mice were examined for molecular alterations in exon 2 of the β-catenin gene (Devereux *et al.*, 1999), the region that contains potential phosphorylation sites for the glycogen-serine kinase-3β (GSK-3β) enzyme. Twenty (69%) of the neoplasms exhibited mutations consisting of 18 point mutations and 2 deletion mutations (Table R1). In contrast, of

22 spontaneous liver neoplasms, only two (9%) had mutations. All of the point mutations affected codons 32, 33, 34, or 41, sites that are targeted for phosphorylation by the GSK-3β kinase or that are involved in ubiquitination of the protein and are important in regulation of β-catenin turnover. Eleven different base substitutions were represented among the four mutant codons, and there was no preponderance of G to T or G to A mutations. The relationship between frequency of β-catenin mutations and methyleugenol dose in the liver neoplasms also was examined. Nine of 12 neoplasms (75%) from the 37 mg/kg dose group, six of nine (67%) from the 75 mg/kg dose group, and five of eight (63%) from the 150 mg/kg dose group had β-catenin mutations, indicating that there was no association between mutation frequency and dose level. Mutations of β-catenin were detected almost equally in adenomas and carcinomas, suggesting that mutation of this gene is an early event in hepatocellular tumorigenesis.

Some of the neoplasms also were evaluated for expression of the β-catenin protein. Four methyleugenol-induced hepatocellular neoplasms with mutations analyzed by Western blot hybridization showed accumulation of β-catenin protein, while two neoplasms without mutations had lower expression, similar to that observed for the normal liver control. In addition, 16 methyleugenol-induced liver neoplasms were examined by immunohistochemistry and eight of 10 neoplasms with mutations demonstrated some positive staining for the β-catenin protein. Strong positive staining of an altered hepatocellular focus from a methyleugenol-treated mouse provided further evidence that upregulation and accumulation of β-catenin occur early in tumorigenesis. Staining within the cells was localized to cell membranes, although some cytoplasmic staining was visible in some of the neoplasms.

These same methyleugenol-induced liver neoplasms were analyzed by immunohistochemistry for mutations in codon 61 of the H-*ras* protooncogene and altered p53 protein expression that would be indicative of a mutation in p53. However, no H-*ras* mutations were identified, and no p53 overexpression was detected in any of these neoplasms. These results indicate that H-*ras* protooncogene activation and p53 mutations are probably not involved in the liver neoplasm response to methyleugenol treatment in B6C3F$_1$ mice.

DISCUSSION

In this study, somatic mutations of β-catenin were identified in 69% of hepatocellular neoplasms from B6C3F$_1$ mice treated with methyleugenol but in only 9% of spontaneous liver neoplasms (Devereux *et al.*, 1999). These results are important because identical mutations have been found in human hepatocellular cancers (de la Coste *et al.*, 1998), suggesting similar pathways of carcinogenesis in both species. In colorectal carcinogenesis, mutations in either the adenomatous polyposis coli (APC) gene or β-catenin gene cause β-catenin protein accumulation and upregulation of the Wnt-signaling pathway, resulting ultimately in cell proliferation and decreased apoptosis (Morin *et al.*, 1997). Most colon neoplasms have APC mutations (Jen *et al.*, 1994; Kinzler and Vogelstein, 1996), and most of those colon neoplasms that lack APC mutations have β-catenin mutations (Morin *et al.*, 1997), indicating the importance of this pathway in colon carcinogenesis. The findings of β-catenin mutations in a majority of the methyleugenol-induced hepatocellular neoplasms and a good correlation between mutations and β-catenin protein accumulation suggest that mutation of APC did not play a role in tumorigenesis in the mice in this study.

The finding of mutations in both adenomas and carcinomas in addition to strong immunohistochemical staining for β-catenin in an altered hepatocellular focus indicates that this mutation is an important early event in chemical carcinogenesis in the B6C3F$_1$ mouse. A strong chemical-specific pattern of mutation was not observed for methyleugenol, suggesting that these genetic alterations were caused either by indirect DNA damage or by spontaneous mutations with clonal outgrowth of the lesions being promoted by the chemical.

The findings that no H-*ras* mutations occurred in the hepatocellular neoplasms from any of the three dosed groups and that there was no dose-response relationship of β-catenin mutations provide evidence that there

were few, if any, spontaneous neoplasms among those in the three dosed groups. Even at the lowest dose, there is an indication that methyleugenol caused alterations in the β-catenin gene, which contributes significantly to the development of hepatocellular neoplasms.

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TABLE R1
**Summary of β-Catenin Mutations in Hepatocellular Neoplasms
from B6C3F₁ Mice in the 2-Year Gavage Study of Methyleugenol**

Treatment Group	Frequency	Codon	Mutation	Bases	n[a]	Amino Acid[b]
Methyleugenol	20/29 (69%)	32	GAT to GTT	A to T	5	Asp to Val
(37, 75, or		32	GAT to TAT	G to T	1	Asp to Tyr
150 mg/kg)		32	GAT to GGT	A to G	3	Asp to Gly
		32	GAT to CAT	G to C	1	Asp to His
		33	TCT to TAT	C to A	1	Ser to Tyr
		33	TCT to TTT	C to T	1	Ser to Phe
		34	GGA to AGA	G to A	1	Gly to Arg
		34	GGA to CGA	G to C	1	Gly to Arg
		34	GGA to GTA	G to T	2	Gly to Val
		41	ACC to ATC	C to T	1	Thr to Ile
		41	ACC to GCC	A to G	1	Thr to Ala
		5-10	Deletions	N/A	2	
Control	2/22 (9%)	32	GAT to GCT	A to C	1	Asp to Ala
		33	TCT to TTT	C to T	1	Ser to Phe

[a] n=number of neoplasm samples with that mutation
[b] Asp=aspartate; Val=valine; Tyr=tyrosine; Gly=glycine; His=histidine; Ser=serine; Phe=phenylalanine; Arg=arginine;
   Thr=threonine; Ile=isoleucine; Ala=alanine

APPENDIX S
IMPACT OF *HELICOBACTER HEPATICUS* INFECTION IN B6C3F$_1$ MICE FROM 12 NTP 2-YEAR CARCINOGENESIS STUDIES

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IMPACT OF *HELICOBACTER HEPATICUS* INFECTION IN B6C3F₁ MICE FROM 12 NTP 2-YEAR CARCINOGENESIS STUDIES

ABSTRACT

Male and female B6C3F$_1$ mice from 12 NTP 2-year carcinogenesis studies were found to be infected with *Helicobacter hepaticus*. Many of the male mice from nine of these studies ("affected" studies) had an associated hepatitis. The current evaluations were performed in an attempt to determine if the data from the *H. hepaticus*-affected NTP B6C3F$_1$ mouse studies were compromised and unsuitable for cancer hazard identification. The incidences of neoplasms of the liver (both hepatocellular neoplasms and hemangiosarcoma), but not of other organs in control male B6C3F$_1$ mice, were found to be increased in affected studies compared to control males from unaffected studies. The increased incidence of hepatocellular neoplasms was observed in those males exhibiting *H. hepaticus*-associated hepatitis. Other observations further differentiated control male mice from affected and unaffected studies. H-*ras* codon 61 CAA-to-AAA mutations were less common in liver neoplasms in males from affected studies compared to historical and unaffected study controls. In addition, increases in cell proliferation rates and apoptosis were observed in the livers of male mice with *H. hepaticus*-associated hepatitis. These data support the hypothesis that the increased incidence of liver neoplasms is associated with *H. hepaticus* and that hepatitis may be important in the pathogenesis. Therefore, interpretation of carcinogenic effects in the liver of B6C3F$_1$ mice may be confounded if there is *H. hepaticus*-associated hepatitis.

INTRODUCTION

Helicobacter-Induced Diseases

Since the bacterium *H. pylori* was isolated from humans in 1983, numerous *Helicobacter* species have been identified in several laboratory and domestic animal species. Their pathogenicity varies, with some species inducing significant disease while others appear merely to colonize the gastrointestinal tract. *H. pylori* is known to cause chronic gastritis and peptic ulcers in humans (Marshall and Warren, 1984; Graham, 1989; Lee *et al.*, 1993) and, more recently, has been linked to adenocarcinoma and mucosa-associated lymphoma of the stomach (Fox *et al.*, 1989; Nomura *et al.*, 1991; Parsonnet *et al.*, 1991; Wotherspoon *et al.*, 1993). Based on epidemiological and pathology findings, the International Agency for Research on Cancer (1994) has classified *H. pylori* as a group 1 carcinogen in humans. *H. hepaticus* is associated with an increase in liver neoplasm incidences in A/JCr mice (Ward *et al.*, 1994a; Fox *et al.*, 1996).

H. hepaticus commonly colonizes the gastrointestinal tract of many strains of mice from many sources (Fox *et al.*, 1994; Ward *et al.*, 1994b; Shames *et al.*, 1995). It has been shown to be pathogenic, with hepatitis highly prevalent in some strains of mice (A/JCr, BALB/cAnNCr, C3H/HeNCr, SJL/NCr, and SCID/NCr) (Ward *et al.*, 1994b). Intestinal colonization does not necessarily result in subsequent hepatitis, and the conditions that lead to migration of the organism from the intestine to the liver have not been determined. *H. hepaticus* appears to reside primarily within the bile canaliculi. Male mice were reported to have a greater incidence and severity of hepatitis than female mice, and this finding occurred in NTP studies as well. The recently identified *H. bilis*, like *H. hepaticus*, colonizes the biliary tract, liver, and intestine of mice. While *H. bilis* has been identified in animals with chronic hepatitis, whether it caused the hepatitis is not known (Fox *et al.*, 1995).

The pathogenesis of *H. hepaticus*-induced disease has not been fully characterized. In susceptible strains of mice, *H. hepaticus* can cause acute, focal, nonsuppurative, necrotizing hepatitis, which progresses to chronic, active hepatitis characterized by minimal necrosis, hepatocytomegaly, oval cell hyperplasia, and cholangitis. *H. hepaticus* has been found to possess high levels of urease (Fox *et al.*, 1994). *H. hepaticus* is often isolated from the cecum and colon but is not necessarily isolated from the liver of A/JCr mice, even though these animals develop severe hepatitis. Culture supernatants from several strains of *H. hepaticus* and several other *Helicobacter* species were shown to cause cytopathic effects in a rodent hepatocyte cell line (Taylor *et al.*, 1995). Ward *et al.* (1996) suggested that autoimmunity may play a role in the progressive hepatitis and carcinogenesis in livers infected with *H. hepaticus*.

NTP Infectious Disease Surveillance
In 1993, during the histological evaluation of an NTP 2-year study, pathologists identified a constellation of liver lesions (hepatitis) in control and treated male mice that was consistent with what would later be described in mice infected with *H. hepaticus* (Ward *et al.*, 1993, 1994a; Fox *et al.*, 1994). Subsequently, pathology results from all mouse studies begun since 1984 (67 two-year studies) were reviewed for diagnoses of the characteristic hepatitis; the lesions were identified in nine studies (NTP, 1998a,b,c,d, 1999a,b). Silver stains revealed helical bacteria consistent with *Helicobacter* present in the liver of male mice in the nine studies.

Every reasonable measure is taken to prevent the occurrence of infectious diseases during NTP 2-year carcinogenicity studies. When infections occasionally occur, care is taken to identify the causal agent and its source, measures are taken to ensure that animals in later studies will not be infected, and the potential impact on biological parameters (primarily neoplastic endpoints) important in interpretation of the study is determined. To date, animals (control and treated) from a few studies have had a mild pulmonary inflammatory response presumed to be caused by an infectious agent. In other studies, there have been utero-ovarian infections with *Klebsiella* sp. (Rao *et al.*, 1987) and fungal infections of the nasal cavity. For scientifically valid reasons, interpretation of chemical-related effects was not considered significantly compromised in any of these studies. Unlike the previous infections, *H. hepaticus* involves the liver, the major metabolic organ, and has been associated with an increase in incidences of liver neoplasms in the A/JCr mouse (Ward *et al.*, 1994a). Therefore, when the contemporary epizootic of *H. hepaticus* infection in the United States affected several NTP studies, use of the data for hazard identification was questioned. The first step was to determine the extent of the infection within NTP studies and then evaluate the impact the infection had on biological parameters important in interpretation of the carcinogenic potential of test chemicals.

MATERIALS AND METHODS
Histologic Examination
Studies in which mice were potentially infected with *H. hepaticus* were identified by reviewing the summary pathology tables for characteristic diagnoses: oval and/or biliary epithelial hyperplasia, hepatocyte enlargement (often diagnosed as karyomegaly), chronic inflammation, and regenerative hyperplasia. All 13-week and 2-year studies begun by the NTP since 1984 and for which complete pathology data were available (67 two-year studies) were examined. Eight contemporary studies in which the characteristic lesions were not identified from pathology tables were randomly selected for histologic reevaluation. Slides containing sections of hematoxylin- and eosin-stained livers from 20 to 25 control and 20 to 25 high-dose male mice from each of seven 2-year studies and one 13-week study (10 animals from each group) were reexamined microscopically for the presence of hepatitis potentially related to

H. hepaticus infection. Hepatitis consistent with that observed with *H. hepaticus* infection was not observed in any of these studies.

Liver sections from five or more animals from each of nine 2-year studies in which hepatitis was observed were prepared using the Warthin-Starry silver stain or Steiner's modification to identify silver-positive helical bacteria.

PCR-RFLP Detection of *Helicobacter* DNA

Assays based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) were conducted at the NIEHS (Malarkey *et al.*, 1997) and the University of Missouri Research Animal Diagnostic and Investigative Laboratory (MU-RADIL) (Riley *et al.*, 1996) on liver tissue from approximately 20 animals from each of 32 NTP 2-year studies (including the nine affected studies) and three NTP 13-week studies. The majority of these studies were selected because they were begun at approximately the same time (1988-1990) as the nine affected studies. Also, two earlier studies (1984-1985; mouse life-span and *p*-nitroaniline studies) and one later study (1993; methyleugenol) were selected. The mouse life-span study was designed to evaluate the incidences of spontaneous changes associated with age; therefore, there is no NTP Technical Report. Frozen tissue was available from 22 of these studies, while only formalin-fixed tissue was available for the remaining ten 2-year studies and the three 13-week studies. Most of the assays were conducted by MU-RADIL, which used *Helicobacter* genus-specific primers; MU-RADIL used restriction endonucleases on a subset of positives to determine if the species was *H. hepaticus*. DNA was isolated from frozen liver samples with a QIAamp Tissue Kit (Qiagen Inc., Chatsworth, CA) according to the manufacturer's recommendations or routine phenol/chloroform extraction (Malarkey *et al.*, 1997). DNA content and purity were determined spectrophotometrically by measuring the A_{260}/A_{280} optical density ratio. To isolate DNA from paraffin-embedded samples, five 10-μm sections were washed twice with 1 mL xylene and twice with 500 μL ethanol. Tissues were then dried within a vacuum centrifuge prior to DNA isolation as described above. Routine measures were taken to avoid contamination at every step from tissue collection to PCR amplification, and concurrently run controls without DNA were consistently negative.

Statistical Analyses

Multiple regression procedures were used to compare control neoplasm rates in the nine affected studies with the 26 unaffected contemporary studies which had no histologic evidence of *H. hepaticus*-associated liver disease. While frozen liver tissue was unavailable from 13 of these 26 studies, none showed the hepatitis indicative of *H. hepaticus* and thus were assumed to be unaffected. Potential confounding factors such as body weight, date study was begun, route of administration, and animal supplier were included as covariables in the statistical analysis.

Analysis for H-*ras* Codon 61 CAA-to-AAA Mutations

For analyses of formalin-fixed tissue, three to five unstained serial sections (10 μm thick) were cut from paraffin blocks containing hepatocellular adenomas or carcinomas. Paraffin-embedded tissues were deparaffinized and rehydrated prior to being digested with proteinase k overnight at 55° C to isolate DNA. Frozen tissues were digested with 10 mg/mL pronase in 1% sodium dodecyl sulfate in TNE buffer (10 mM TRIS, 150 mM NaCl, and 2 mM EDTA; pH 7.5) overnight at 37° C; DNA was isolated by phenol chloroform extraction and precipitated with ethanol (Marmur, 1961; Sills *et al.*, 1995).

Nested primers were used for amplification of exon 2 of H-*ras* by PCR. The outer primers were 5'-CCA CTA AGC CTG TTG TGT TTT GCA G-3' (forward primer) and 5'-CTG TAC TGA TGG ATG TCC TCG AAG GA-3' (reverse primer). The inner primers (second round of amplification) were

5'-GAC ATC TTA GAC ACA GCA GTT-3' (forward primer) and 5'-GGT GTT GTT GAT GGC AAA TAC-3' (reverse primer). Although the normal sequence of codon 60 is GCT, the forward PCR primer is made with a T at the penultimate 3' base to create the restriction site for Mse1.

A nonradioactive RFLP method was employed to identify CAA-to-AAA mutations in the H-*ras* gene at codon 61 in liver neoplasms (Lee and Drinkwater, 1995). This was based on Mse1 enzyme restriction cutting only the sequence 5'-TTAA-3'. Thus, Mse1 will detect C→A conversion mutation at the first position of codon 61.

Analysis of PCNA and Apoptosis

Detailed methods are included in a report by Nyska *et al.* (1997). Cell proliferation was assessed in nonneoplastic areas of the liver, kidney, and lung by determining a PCNA S-phase labeling index (the percentage of cells in S phase). The identification of apoptotic cells was based on morphologic criteria (Garewal *et al.*, 1996; Goldsworthy *et al.*, 1996) and confirmed immunohistochemically by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) procedure (Gavrieli *et al.*, 1992).

RESULTS AND DISCUSSION

Identification of *H. hepaticus* Infection in NTP Studies

Determining the extent of *H. hepaticus* infection involved a three-pronged approach of histologic evaluation, silver stains, and PCR-RFLP based assays; all were necessary because of the limitations identified for each. In NTP studies, and as reported in other studies (Ward *et al.*, 1994b), there were no obvious clinical signs of infection, and the only significant histologic lesion (hepatitis) was observed in the liver, primarily in males. Therefore, summary pathology tables were reviewed to identify studies that may have been affected by *H. hepaticus*-associated hepatitis. Male mice from nine studies were identified (Table S1) as having the hepatitis. Eight of the nine studies were begun during a time span of about 6 months (July 1990 to January 1991), while the other study was begun much earlier (October 1988). The hepatitis was not observed in any 13-week studies. Use of histologic evaluation for identification of infected animals has limitations, however. It is somewhat insensitive, as *H. hepaticus* has been cultured and identified by PCR-RFLP methods within livers of animals with no histological evidence of infection (Fox *et al.*, 1999). This may be explained in part by the limited sampling (two liver sections) and the sometimes focal nature of *H. hepaticus*-associated hepatitis. Also, while in the more severely affected animals the hepatitis appears somewhat characteristic, component lesions of the hepatitis are not pathognomonic, and, when the hepatitis is subtle in 2-year old animals, it is more difficult to recognize or attribute to *H. hepaticus*.

Within affected studies, the incidences of the hepatitis in male mice varied from 16% to 78% (Table S1). While generally mild to moderate, the hepatitis varied in severity from barely detectable in some animals to extensive liver involvement and regeneration in others. Only a few females were identified as having the characteristic hepatitis (Table S1). In general, the incidences and severities of *H. hepaticus*-associated hepatitis were similar between control and treated groups. This constellation of nonneoplastic liver lesions, while not pathognomonic, was certainly suggestive of an *H. hepaticus* infection, particularly when observed in control animals. Characteristic lesions included proliferation of oval and/or biliary epithelial cells, hepatocyte enlargement (diagnosed as karyomegaly), and chronic inflammation. In many instances, areas of regenerative hyperplasia were identified within diseased liver.

Helicobacter spp. are not usually observed on routine histologic examination of hematoxylin and eosin-stained sections of liver. The methods for confirmation of infection with *Helicobacter* include Warthin-Starry silver stain or Steiner's modification (Garvey *et al.*, 1985) of this stain for direct microscopic observation of the organisms in tissue; however, this can be a relatively insensitive technique when few organisms are present. In most instances, histologic differentiation between *Helicobacter* species is not possible. Speciation can usually be accomplished with electron microscopy, but this technique is both time consuming and labor intensive. Microbiologic culture of feces, cecal smears, and fresh or frozen liver is also possible. Currently, assays involving amplification of the DNA of the organism using PCR are the most rapid and perhaps the most sensitive methods of detection, and the use of restriction endonucleases has allowed a determination of the species present. PCR-based methods also can be used on feces, cecal contents, or liver homogenates and are most sensitive when using fresh or frozen tissue (Riley *et al.*, 1996; Malarkey *et al.*, 1997).

Using Warthin-Starry silver stains or Steiner's modification on the livers of five or more animals per study, helical bacteria (*Helicobacter*) were identified in animals from the nine affected studies. In some animals, helical bacteria were numerous, suggesting a heavy bacterial burden in these infected animals. However, even in these animals with abundant organisms, few to none were observed in proliferative hepatic lesions such as foci and neoplasms. Helical bacteria were not identified in approximately 25% of males with moderate hepatitis and were rarely identified in males without hepatitis or in females. The absence of identification of helical organisms by silver stains does not preclude infection, nor does the presence of organisms confirm *H. hepaticus*. Based upon current knowledge, however, the characteristic liver lesions in B6C3F$_1$ mice, coupled with the presence of silver-positive helical organisms, are highly suggestive of *H. hepaticus* infection.

As the NTP evaluation evolved, PCR-based assays were developed that appeared more sensitive than histologic evaluation and silver stains for identification and speciation of *Helicobacter*. Therefore, PCR-RFLP-based assays were used to confirm the presence of pathogenic *Helicobacter* (primarily *H. hepaticus*) within the nine affected studies and to determine whether there was *H. hepaticus* infection in other NTP studies. Unfortunately, none of the PCR-based assays had been specifically developed for, or proven reliable for use with, formalin-fixed tissue. Frozen tissue was available from a limited number of animals from a limited number of NTP studies, including only three of the nine affected studies. Furthermore, available frozen liver was almost always limited to tissue from a neoplasm, and, based upon results obtained with silver stains, organisms are generally not readily observed within proliferative hepatic lesions, even when organisms are abundant in adjacent liver tissue. Because the availability of frozen tissue was limited, a PCR-RFLP-based assay was developed and evaluated (Malarkey *et al.*, 1997) for use with frozen or formalin-fixed tissue.

The NIEHS and MU-RADIL laboratories conducted PCR-RFLP-based assays on 32 NTP 2-year studies and three NTP 13-week studies (data not shown); frozen tissues from 22 of the 2-year studies were available. All three bioassays in which hepatitis was identified and for which frozen tissue was available were positive for *H. hepaticus* by the PCR-RFLP-based assays (Table S2). At a third laboratory, *H. hepaticus* was also cultured from the liver tissue of animals in one of these studies (Fox *et al.*, 1999). Formalin-fixed tissues from two of the three studies were evaluated and were also positive; these tissues had been fixed in formalin for less than 48 hours. In the other six affected studies, for which only formalin-fixed tissue was available, *H. hepaticus* was identified in only 1 of 120 animals (Table S2). This decreased sensitivity was considered to be related to the prolonged formalin fixation (Malarkey *et al.*, 1997) rather than proof of an absence of *H. hepaticus*. The presence or absence of *H. hepaticus* apparently cannot be confirmed with current PCR-RFLP-based assays in liver that has been fixed in formalin for long periods

(weeks or months). In the three 13-week studies with formalin-fixed tissue, only 1 of 30 animals was positive for *H. hepaticus*.

Within the three affected, PCR-RFLP-positive 2-year studies, *H. hepaticus* was often identified by PCR in frozen livers of mice that had no overt hepatitis. In fact, based upon the combined data from two studies (including PCR results from three laboratories), of 57 animals without characteristic liver lesions, 13 of 24 male mice (54%) and 17 of 33 female mice (52%) were positive for *H. hepaticus*. Furthermore, *H. hepaticus* was identified by PCR in frozen liver of several animals from three "unaffected" studies in which hepatitis typical of that associated with *H. hepaticus* was not observed (Table S2). Apparent variability occurs between various strains of mice and between individual mice from affected studies in developing hepatitis in response to *H. hepaticus* infection. One would assume that, within affected studies, most or all animals have been exposed to the organism, and even animals resistant to developing hepatitis may have organisms within the liver. This assumption is supported by the fact that animals without hepatitis are often positive with PCR-RFLP-based assays. Therefore, although alternative explanations are possible, the three PCR-RFLP-positive studies in which liver lesions are absent are assumed to be true positives. In fact, helical organisms were identified with a silver stain in one animal from one of these studies (Malarkey *et al.*, 1997). Therefore, in addition to assessing the affect of *H. hepaticus* in the nine affected 2-year studies, the significance of a positive PCR-RFLP assay for *H. hepaticus* in the absence of liver lesions is also an important question.

Inconsistent Results with PCR-Based Methods

As with any technique, the PCR-RFLP-based assays have limitations even when used to assay fresh and frozen tissue. One assessment of the variability in results of PCR and serologic analyses for *Helicobacter* among three commercial laboratories revealed significant inconsistencies (Dew *et al.*, 1997). Others (J.M. Ward and J. Thigpen, personal communications) have obtained similarly inconsistent results when sending replicate samples to different laboratories. Though the number of samples evaluated by both the NIEHS and MU-RADIL laboratories was limited, there was good, but not complete, correlation of PCR-RFLP results. Also, within the affected studies, the PCR assays were not positive in some animals with liver disease. This result may be explained, in part, by the fact that the only frozen tissues available were neoplasms; as described above, neoplasms are expected to have fewer organisms.

Analysis of *H. hepaticus*-Affected and Unaffected Studies
for Incidence of Common Neoplasms

To determine whether the incidences of various neoplasms were different between control groups from affected and unaffected studies, the nine affected studies were compared to 26 unaffected studies begun at relatively similar times (Table S3). There were no statistically significant differences in body weight or survival among the affected and unaffected studies. The neoplasms evaluated represent those that occurred at high enough incidences in various organs for statistically significant differences to be detected. Using multiple regression procedures, male mice in the nine affected studies were demonstrated to have a significantly ($P < 0.05$) increased incidence of only two neoplasm types, both of which were in the liver (hepatocellular neoplasms and hemangiosarcoma), when compared to the unaffected studies. Because of these differences, there was also a corresponding significant difference in the overall incidence of malignant neoplasms (all sites) as well as in the overall proportion of neoplasm-bearing animals. No other tissue site showed a significant difference in the incidence of neoplasms. For female mice, the slightly increased incidence of hepatocellular neoplasms observed in the affected studies was not statistically significant.

This seemingly simple analysis is complicated by several potential confounding variables. There have been coordinate, time-related increases in body weight and in the incidence of liver neoplasms in mice in NTP

studies (Haseman, 1992). Table S4 presents the liver neoplasm incidences in relation to the dates the studies began and clearly shows the increases in liver neoplasm incidences and body weights (Seilkop, 1995). In assessing differences in neoplasm incidences between *H. hepaticus*-affected and unaffected studies, the most relevant comparison would be between studies begun at approximately the same time. The starts of 20 of the 26 unaffected studies were clustered near the early part of the time frame (April 1988 to June 1990), while the starts of the affected studies were clustered toward the later end, with eight of the nine studies begun between July 1990 and January 1991; incidences of liver neoplasms in these later studies are expected to be higher based on trends in body weight alone. While the slightly increased incidences of liver neoplasms observed in female control mice in the nine affected studies is likely due to clustering in time, clearly, this alone cannot account for the increased liver neoplasm incidences observed in control male mice in the affected studies (Table S3).

Ideally, unaffected studies used in the above comparison should not only be free of histologic evidence of infection with *H. hepaticus* but should be confirmed as negative by PCR assays. Thirteen of these 26 studies could not be confirmed as negative by PCR because frozen tissue was not available; however, *H. hepaticus*-associated hepatitis was not present in any of the 26 studies. Because these and other data reported to date suggest that hepatitis is associated with neoplasm development in the liver, it seems reasonable to include those 13 studies, unconfirmed by PCR, in this analysis. The majority of the 13 studies confirmed as negative by PCR were begun much earlier than the clearly affected studies, and, therefore, comparing them alone to the nine affected studies is not reasonable. Although not presented here, a number of comparisons were made with various groupings of studies based on the degree of confidence in their infection status. Although the outcomes of the various comparisons varied somewhat, incidences of hepatocellular neoplasms and hemangiosarcomas of the liver were consistently increased in control male mice from affected studies compared to control males from unaffected studies. Significantly increased liver neoplasm incidences generally were not observed in females. Importantly, the following data corroborate the findings and association with *H. hepaticus* identified in these analyses.

Analysis of Hepatitis-Positive and Hepatitis-Negative Mice for Liver Neoplasm Incidence

Several infectious agents known to be associated with increased incidences of neoplasms cause chronic inflammation in the target tissue or organ. It is commonly hypothesized that this inflammatory process may cause or contribute to the development of neoplasms. One approach to address this was to stratify the mice from the affected studies according to the severity of hepatitis and examine liver neoplasm incidences in relation to these groupings. Thus, animals within the nine affected studies were placed into three groups: 1) animals with mild to moderate hepatitis considered related to *H. hepaticus* infection (+), 2) animals with minimal to mild hepatitis that may have been associated with *H. hepaticus* (±), and 3) animals with no hepatitis that was considered to be associated with *H. hepaticus* (−). Within these groupings, the incidence of liver neoplasms was significantly increased (P<0.05) in males with mild to moderate *H. hepaticus*-associated hepatitis (+) when compared to animals without such hepatitis (Table S5). The neoplasm incidence in animals with minimal lesions (±) was also increased. The liver neoplasm incidence in males without hepatitis (58%) was similar to the incidence (54.8%) in males from the 26 unaffected studies (Table S3). This analysis clearly suggests an association of *H. hepaticus*-associated hepatitis with increased liver neoplasm incidences. Females showed a similar trend, albeit not significant; however, these comparisons are weak because of the low numbers of females with hepatitis.

Analysis of H-*ras* Oncogene Mutations in Liver Neoplasms in Mice from Affected and Unaffected Studies

Liver neoplasms commonly occur in control $B6C3F_1$ mice in 2-year studies. In the historical database of 333 male and female mice with liver neoplasms, 106 (32%) had H-*ras* codon 61 CAA-to-AAA mutations (Maronpot *et al.*, 1995). This historical control database is composed primarily of male data; however, adequate numbers of females have been assayed, and there was no significant difference in the incidences of CAA-to-AAA mutations between males and females.

In an attempt to examine further whether *H. hepaticus* infection had an effect on the development of hepatocellular neoplasms, neoplasms from control male mice from selected affected (NTP, 1998a,b, 1999a) and unaffected (NTP, 1993, 1999c) studies were evaluated for H-*ras* codon 61 CAA-to-AAA mutations (Table S6). Only 6% (2/33) of the hepatocellular neoplasms from control males with hepatitis from three affected studies had this mutation. This percentage is significantly (P<0.01) less than the 32% (11/34) observed in males from the two unaffected studies and less than the 32% (106/333) that occurred in historical control animals. In addition, neoplasms from males without hepatitis from the affected, PCR-positive triethanolamine study (NTP, 1999a) and the unaffected, PCR-positive methyleugenol study (NTP, 2000) were evaluated; the incidences of mutations in those groups were 3/14 (21%) and 2/17 (12%), respectively.

Neoplasms from control female mice (none had hepatitis) from affected and unaffected studies were evaluated for the CAA-to-AAA mutation (Table S6). The mutation rate was low in both the affected studies (1/25; 4%) and the unaffected study (1/11; 9%) when compared to the 32% observed in the historical control groups.

The finding of a different H-*ras* mutation profile in neoplasms of male mice from affected studies tends to support the association of increased neoplasm incidences with *H. hepaticus,* although there is no mechanistic understanding behind this observation. In a study of *H. hepaticus*-infected A/JCr mice, *ras* mutations were not detected in the 25 hepatocellular neoplasms analyzed using a PCR/single-strand conformation polymorphism assay (Sipowicz *et al.*, 1997). Because of the low spontaneous rate of liver neoplasms in the A/JCr mouse, there are few or no conclusive data on *ras* mutations in uninfected animals, however. Point mutations at codons 12, 13, and 61 of the Ki-, Ha- and N-*ras* genes were not identified in 45 early gastric carcinomas in humans, whether or not *H. pylori* was present (Craanen *et al.*, 1995). If the increased incidence of hepatocellular neoplasms is associated with hepatitis, as many suspect, then one would expect the neoplasms from animals without hepatitis to have a similar mutational profile as that of the historical controls. The data do not provide a clear answer, because the hepatitis-free males from the affected triethanolamine study (NTP, 1999a) and the males from the methyleugenol study (NTP, 2000), which were positive by PCR but lacked hepatitis, had mutation frequencies between those of the unaffected controls and the hepatitis-positive mice. Furthermore, mutations in neoplasms from females, none of which had hepatitis, from two affected and one unaffected study were very low compared to the historical controls. These findings were unexpected, and their significance is not understood.

H. hepaticus-Associated Alterations in Cell Kinetics

Studies evaluating cell kinetics were completed to explore further the link between hepatitis and the increased incidence of liver neoplasms (Table S7; Nyska *et al.*, 1997). One of the major objectives was to determine whether there were differences between PCNA labeling indices in the livers of animals with hepatitis from three affected studies, cobalt sulfate heptahydrate, chloroprene, and triethanolamine (NTP, 1998a,b, 1999a), compared to animals without hepatitis, whether from the same three affected studies or from an unaffected study, 1-trans-delta[9]-tetrahydrocannabinol (NTP, 1996). Male mice with hepatitis from

the three affected studies had a significantly increased (P < 0.001) labeling index, with a 24-fold increase over males from the unaffected study and a sixfold increase over males without hepatitis from the same three affected studies (Table S7). The labeling index increase in these mice was substantial and was considered biologically significant. Male mice without hepatitis from the three affected studies had a significantly greater labeling index (increased fourfold) than male mice from the unaffected study (Table S7). The significance of this finding is uncertain, as differences of a similar magnitude were observed in other comparisons. For example, the labeling index of females from the unaffected 1-trans-delta^9-tetrahydrocannabinol study (Table S7; NTP, 1996) was increased fivefold over females from the PCR-positive, hepatitis-negative scopolamine hydrobromide trihydrate study (NTP, 1997). Such differences may be within the limits of normal variability for 2-year-old animals.

A second objective of the cell proliferation studies of the liver was to determine if labeling indices were increased in animals from the PCR-positive, hepatitis-negative methyleugenol (NTP, 2000), scopolamine hydrobromide trihydrate (NTP, 1997), and mouse life-span studies compared to an unaffected PCR-negative and hepatitis-negative 1-trans-delta^9-tetrahydrocannabinol study (NTP, 1996). The scopolamine hydrobromide trihydrate study was evaluated and included in the study by Nyska *et al.* (1997), while the methyleugenol and mouse life-span studies were completed later and are included in Table S7. The labeling indices of males from two of these three studies were almost identical to those of males from the unaffected study. However, the labeling index of males from the mouse life-span study is increased approximately fivefold over that of males from the unaffected study as well as fivefold over the labeling indices of males from the two like studies of scopolamine hydrobromide trihydrate and methyleugenol. This finding suggests that the increase observed in the mouse life-span study is not attributable to the presence of *H. hepaticus,* as two other studies also positive for *H. hepaticus* did not show a similar increase.

The cell proliferation data for the liver from NTP studies are consistent with data from a study by Fox *et al.* (1996) in which cell proliferation indices were evaluated at 8, 10, and 13 months in the A/JCr mouse, which is generally believed to be more susceptible to *H. hepaticus*-associated hepatitis than the B6C3F$_1$ mouse. In the study by Fox *et al.* (1996), cell proliferation rates were significantly increased at all time points in males. Some increases were observed in females in that study but did not reach statistical significance. An increased incidence of hepatocellular neoplasms was observed only in the males. Though liver lesions were observed in females in that study, they were less severe than those in males.

In addition to the liver, cell proliferation indices (PCNA) were evaluated in the kidneys and lungs of male and female mice in affected studies versus those in unaffected studies (Nyska *et al.*, 1997). No apparent effect of *H. hepaticus* infection or the presence of hepatitis on PCNA indices was observed for the kidneys or lungs.

Apoptosis (programmed cell death) is another important parameter in evaluations of cell kinetics. The apoptotic index in the liver of male mice with hepatitis from an affected study, cobalt sulfate heptahydrate (NTP, 1998a), was significantly (P < 0.01) greater than that observed in males from the unaffected 1-trans-delta^9-tetrahydrocannabinol study and the PCR-positive, hepatitis-negative scopolamine hydrobromide trihydrate study (Nyska *et al.*, 1997). For females, there were no significant differences among the three studies.

Two 13-week studies which were begun during the same time as the nine affected studies were randomly selected for evaluation of PCNA indices. *H. hepaticus* was not identified in either of the studies by PCR-RFLP; however, as with all NTP 13-week studies, only tissue fixed in formalin for an unspecified period was available. Because of this, no true negative control group was available; therefore, the labeling index of these 19- to 20-week-old animals was compared to values cited in the literature (Eldridge and

Goldsworthy, 1996) for 20-week-old B6C3F$_1$ mice. The labeling index in the NTP studies clearly was not increased (data not shown).

The Impact of *H. hepaticus* on the Interpretation of 2-Year Carcinogenesis Studies

Increases in the incidences of neoplasms are associated with a number of infectious agents. The chronic inflammation caused by these agents has been hypothesized to be important in the pathogenesis of the increased neoplasm incidences (e.g., gastric cancer associated with *H. pylori*). The increased incidences of liver neoplasms in male mice from the nine affected NTP studies were observed in the animals with *H. hepaticus*-associated hepatitis. Neoplasms from males with hepatitis tended to have an H-*ras* mutation profile different from that of animals from unaffected studies. Further, cell replication rates at 2 years were significantly higher in males with hepatitis compared to those in males without hepatitis. The data suggest that *H. hepaticus*-associated hepatitis is associated with the increased incidences of liver neoplasms in the male B6C3F$_1$ mouse. Therefore, the most important consideration in evaluating the impact of *H. hepaticus* infection on the interpretation of study results appears to be the presence or absence of significant hepatitis.

For any carcinogenicity study, data within and specific to the individual study provide the greatest basis for an accurate interpretation. However, it is prudent to consider and evaluate all data or information which may affect the interpretation. Based upon the data presented in this and other reports, general guidelines emerge that may be useful in interpreting potential chemical-associated carcinogenic effects in *H. hepaticus*-infected B6C3F$_1$ mice. In a study with sufficient evidence of *H. hepaticus*-associated hepatitis ($>10\%$ of the animals having the characteristic hepatitis may be a reasonable guideline), interpretation of increased incidences of liver neoplasms (hepatocellular neoplasms and hemangiosarcoma) of male mice is considered to be potentially confounded.

Altered chemical uptake and metabolism, due to the intestinal load of *H. hepaticus* and to *H. hepaticus*-associated liver disease, respectively, are possible reasons for considering that the male mouse response to chemical administration at sites other than the liver should also be considered confounded. Data do not currently exist that definitively answer this question. In this group of nine studies, however, there is no evidence to suggest that affected mice responded to chemical treatment in organs other than the liver in a manner different from mice in nonaffected studies. Within each study, there was excellent concordance in chemical-associated neoplasms between the male mice and the females, which had little or no hepatitis (Table S8). Furthermore, analyses indicate that *H. hepaticus* is not associated with neoplastic responses outside the liver; incidences of neoplasms at sites other than the liver were not different between control groups from affected and unaffected studies (Table S3). Cell replication rates in two major organs (lung and kidney) also were not increased in control groups from affected studies compared to those from unaffected studies.

One of the more difficult issues to address is whether interpretation of a treatment-related increase in liver neoplasm incidences in the female mouse is confounded when *H. hepaticus*-associated hepatitis is present within the male mice in the study. Most evidence to date links hepatitis with the increased liver neoplasm incidences observed in males, and female B6C3F$_1$ mice in affected studies do not have significant hepatitis at 2 years. The lack of hepatitis in females, however, is based on an analysis in which only late time points were evaluated histologically. Therefore, it is conceivable that hepatitis along with increased cell proliferation could have occurred earlier and resolved by 18 months to 2 years. Data collected to date, however, suggest that *H. hepaticus*-associated hepatitis is a late-developing and persistent disease in the B6C3F$_1$ mouse. *H. hepaticus*-associated hepatitis has never been observed in any NTP 13-week studies, including five begun during the same 6-month time span as eight of the nine affected 2-year studies. Also, within affected 2-year studies, more males (51%) that were 18 to 24 months of age had hepatitis than those

(34%) that were 12 to 18 months of age. This is consistent with a report by Ward *et al.* (1994b) that *H. hepaticus*-associated liver lesions are not observed at early time points in the B6C3F$_1$ mouse.

Nonetheless, within affected studies, female control mice did have a slightly elevated incidence of liver neoplasms when compared to control mice from unaffected studies, and the data derived from the H-*ras* mutation frequency analysis were inconclusive. The possibility that *H. hepaticus*-infected female mice from affected studies may respond differently to a liver carcinogen than mice from unaffected studies cannot be eliminated at this time. However, because within an affected study hepatitis is observed only rarely in females, until definitive data suggest otherwise, it is concluded that the interpretation of an apparent chemical-induced neoplastic effect in the liver of female mice is not confounded. To censor the few females with *H. hepaticus*-associated hepatitis from any statistical analyses of hepatocellular neoplasms would be prudent. Studies in the ostensibly more sensitive A/JCr mouse (Fox *et al.*, 1996) also showed significant increases in neoplasm incidences and cell proliferation rates in the liver of *H. hepaticus*-infected males, but not females.

Another concern is how to interpret possible chemical-related effects in a study in which the status of *H. hepaticus* infection cannot be determined by PCR-RFLP because only tissues fixed in formalin for more than 48 hours are available. While histologic evaluation is inadequate to identify infection, it appears adequate for identifying hepatitis severe enough to alter the outcome of the study. Therefore, in the absence of significant histologic evidence of *H. hepaticus*-associated hepatitis, the outcome of a 2-year study should not be considered potentially compromised.

The causality between *H. hepaticus* infection and neoplasia has not been proven in the B6C3F$_1$ mouse in these studies, nor has the mechanism of this association been determined; further studies are needed. However, sufficient information exists to make reasonable scientific judgments relative to the interpretation of data from the nine 2-year carcinogenicity studies in the B6C3F$_1$ mouse. Refinements to the above interpretive positions may occur if warranted by future information.

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TABLE S1

Incidence of *Helicobacter hepaticus*-Associated Hepatitis in Control B6C3F$_1$ Mice from Nine NTP 2-Year Studies[a]

	Incidence of Hepatitis (%)	
Study	**Males**	**Females**
Sodium xylenesulfonate	78	4
AZT/5,000 U α-interferon A/D	76	4
Cobalt sulfate heptahydrate	72	8
AZT/500 U α-interferon A/D	66	0
Chloroprene	54	0
Theophylline	32	0
α-Interferon A/D	22	4
Triethanolamine	20	0
AZT	16	2
Average	48	2

[a] Includes regeneration and mild to marked (excludes minimal) chronic inflammation, karyomegaly, oval cell hyperplasia, and bile duct hyperplasia. AZT=3′-azido-3′-deoxythymidine

TABLE S2

Identification of *Helicobacter hepaticus* with PCR-RFLP-Based Assays in Control B6C3F$_1$ Mice from 32 NTP 2-Year Studies and Three NTP 13-Week Studies[a]

		H. hepaticus-Positive Studies[b]	
Type of Sample	**Total Studies**	**Affected Studies**	**Unaffected Studies**
13-Week Studies			
Formalin-fixed liver	3	—	1/3[c]
2-Year Studies			
Frozen liver	22	3/3	3/19
Formalin-fixed liver	10	1/6[c]	0/4

[a] PCR-RFLP=polymerase chain reaction-restriction fragment length polymorphism
[b] Number of *H. hepaticus*-positive studies/number of affected or unaffected studies. Affected studies are those in which hepatitis typical of that associated with *H. hepaticus* infection occurred in many male mice.
[c] Only one animal in the positive study was positive for *H. hepaticus*.

TABLE S3
Comparison of Neoplasm Incidences in Control B6C3F₁ Mice
from *Helicobacter hepaticus*-Affected and Unaffected NTP 2-Year Studies

	Males		Females	
	Affected Studies[a]	**Unaffected Studies**	**Affected Studies**	**Unaffected Studies**
Number of studies	9	26	9	26
Survival (%)	64	71	68	68
12-Month body wt (g)	48.0	48.3	48.1	47.0
Neoplasm incidence (%)				
Liver	71.3*	54.8	50.3	40.5
Lung	26.6	23.2	7.6	10.3
Pituitary gland	0.4	0.8	14.7	14.3
Harderian gland	5.6	6.1	6.0	4.9
Lymphoma	6.9	6.3	16.2	15.5
Circulatory system	9.8	6.0	5.3	4.7
liver only	7.1*	2.5	—	—
All benign	61.8	57.2	59.1	54.6
All malignant	61.3*	40.9	50.0	44.2
All neoplasms	88.0*	77.4	82.7	75.4

*   Significantly different (P≤0.05) from the unaffected studies
[a]   Affected studies are those in which hepatitis typical of that associated with *H. hepaticus* infection occurred in many male mice.

TABLE S4
Liver Neoplasm Incidences and Body Weights of Control B6C3F$_1$ Mice
in Relation to Study Start Dates of *Helicobacter hepaticus*-Affected and Unaffected NTP 2-Year Studies[a]

Study Start Date	Liver Neoplasm Incidence (%)		Mean Body Weight (g)	
	Affected Studies[a]	Unaffected Studies	Affected Studies	Unaffected Studies
Male				
April to September 1988	—	43.8 (8)[b]	—	46.2 (8)
October 1988	62.0 (1)	—	48.3 (1)	—
November 1988 to September 1989	—	52.6 (7)	—	48.7 (7)
October 1989 to June 1990	—	61.2 (5)	—	48.9 (5)
July 1990 to January 1991	72.5 (8)	66.2 (4)	48.0 (8)	49.0 (4)
February 1991 to April 1992	—	68.0 (2)	—	52.8 (2)
Average	71.3	54.8	48.0	48.3
Female				
April to September 1988	—	31.1 (8)	—	44.8 (8)
October 1988	46.0 (1)	—	46.4 (1)	—
November 1988 to September 1989	—	39.9 (7)	—	47.2 (7)
October 1989 to June 1990	—	38.6 (5)	—	45.9 (5)
July 1990 to January 1991	50.9 (8)	54.2 (4)	48.3 (8)	48.0 (4)
February 1991 to April 1992	—	58.0 (2)	—	55.6 (2)
Average	50.3	40.5	48.1	47.0

[a] Includes nine affected studies (those in which hepatitis typical of that associated with *H. hepaticus* infection occurred in many male mice) and 26 unaffected studies
[b] Number of studies is given in parentheses.

TABLE S5
Association of Liver Neoplasm Incidence and Severity of *Helicobacter hepaticus*-Associated Hepatitis in Control B6C3F$_1$ Mice from Nine Affected NTP 2-Year Studies[a]

	Liver Neoplasm Incidence	
Severity of Hepatitis	Males	Females
Absent	101/175 (58%)	196/396 (49%)
Minimal	44/57 (77%)	23/42 (55%)
Mild/moderate	176/218 (81%)	7/11 (64%)
Significance of association	P < 0.05	NS[b]

[a]   Affected studies are those in which hepatitis typical of that associated with *H. hepaticus* infection occurred in many male mice.
[b]   NS = not significant

TABLE S6
H-*ras* Codon 61 AAA Mutations in Spontaneous Liver Neoplasms in Control B6C3F$_1$ Mice from *Helicobacter hepaticus*-Affected and Unaffected NTP 2-Year Studies

Study	Affected[a]	H-*ras* AAA Mutations
Male		
Cobalt sulfate heptahydrate	+	0/10 (0%)
Chloroprene	+	1/13 (8%)
Triethanolamine	+	1/10 (10%)
Oxazepam	—	7/18 (39%)
Diethanolamine	—	4/16 (25%)
Historical control database		106/333 (32%)
Female		
Chloroprene	+	0/10 (0%)
Triethanolamine	+	1/15 (7%)
Diethanolamine	—	1/11 (9%)
Historical control database		106/333 (32%)

[a]   + = affected; — = not affected. Affected studies are those in which hepatitis typical of that associated with *H. hepaticus* infection occurred in many male mice.

TABLE S7
Proliferating Cell Nuclear Antigen Labeling Indices in the Liver of Control B6C3F$_1$ Mice[a]

	Hepatitis	No. of Animals	PCNA Labeling Index[b]	Average PCNA Labeling Index[c]
Male				
Cobalt sulfate heptahydrate[d]	+	15	0.535 ± 0.129	
Chloroprene[d]	+	12	1.452 ± 0.386	
Triethanolamine[d]	+	9	1.215 ± 0.374	1.011
Cobalt sulfate heptahydrate	—	7	0.175 ± 0.117	
Chloroprene	—	10	0.296 ± 0.124	
Triethanolamine	—	12	0.100 ± 0.042	0.186
1-Trans-delta^9-tetrahydrocannabinol[e]	—	15	0.042 ± 0.011	
Scopolamine hydrobromide trihydrate[f]	—	14	0.043 ± 0.012	
Methyleugenol[f]	—	14	0.077 ± 0.020	
Mouse life-span study[f]	—	15	0.217 ± 0.880	
Female				
Cobalt sulfate heptahydrate	+	5	0.161 ± 0.062	
Cobalt sulfate heptahydrate	—	17	0.055 ± 0.015	
Chloroprene	—	12	0.154 ± 0.050	
Triethanolamine	—	12	0.138 ± 0.053	0.108
1-Trans-delta^9-tetrahydrocannabinol	—	13	0.156 ± 0.047	
Scopolamine hydrobromide trihydrate	—	15	0.032 ± 0.009	

[a]   A portion of these data are presented in Nyska *et al.* (1997).   + =hepatitis present; — =no hepatitis present
[b]   Mean ± standard error; PCNA=proliferating cell nuclear antigen
[c]   Average of the mean labeling indices for animals from all three studies
[d]   Affected study (one in which hepatitis typical of that associated with *H. hepaticus* occurred in many male mice)
[e]   Unaffected study (one in which the typical hepatitis did not occur in mice)
[f]   Unaffected study with no typical hepatitis, but positive for *H. hepaticus* by polymerase chain reaction-restriction fragment length polymorphism-based assay

TABLE S8
**Summary of Target Sites of Carcinogenicity in B6C3F₁ Mice from NTP 2-Year Studies
with *Helicobacter hepaticus*-Associated Hepatitis**

	Males	Females
Chloroprene	Lung Circulatory system[a] Harderian gland Forestomach Kidney	Lung Circulatory system Harderian gland Forestomach Liver Skin Mesentery Zymbal's gland Mammary gland
Cobalt sulfate heptahydrate[b]	Lung	Lung
Triethanolamine	Liver	Liver
AZT[c]	None	Vagina
Sodium xylenesulfonate	None	None
Theophylline	None	None

[a] Hemangioma and hemangiosarcoma of the liver were excluded from the analysis in males.
[b] An apparent treatment-related increase in the incidence of hemangiosarcoma of the liver was discounted in male mice because of the presence of *H. hepaticus*.
[c] AZT=3′-azido-3′-deoxythymidine. Includes four studies: AZT; α-interferon A/D; AZT/500 U α-interferon A/D; and AZT/5,000 U α-interferon A/D