NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF ISOPRENE

(CAS NO. 78-79-5)

IN F344/N RATS

(INHALATION STUDIES)

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

July 1999

NTP TR 486

NIH Publication No. 99-3976

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 available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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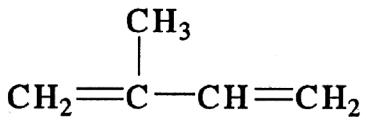
CONTENTS

ABSTRACT



ISOPRENE

CAS No. 78-79-5

Chemical Formula: C_5H_8 Molecular Weight: 68.1

Synonyms: Isopentadiene; β-methylbivinyl; 2-methyl-1,3-butadiene

Isoprene, the monomeric unit of natural rubber and naturally occurring terpenes and steroids, is primarily obtained as a by-product of naphtha cracking for ethylene production. It is emitted from plants and trees, has been detected in tobacco smoke and automobile exhaust, and was identified as a major endogenous hydrocarbon in human breath. Isoprene was selected for toxicologic evaluation because of its structural similarity to 1,3-butadiene, a potent, multi-organ, rodent carcinogen, and the potential for human exposure due to its large annual production volume. A previous 26-week inhalation study followed by a 26-week recovery period provided clear evidence of carcinogenic activity of isoprene in male B6C3F$_1$ mice. A similar study in male F344/N rats was inconclusive. Male and female F344/N rats were exposed to isoprene (99% pure) by whole body inhalation for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, mouse bone marrow and peripheral blood cells, and rat lung fibroblasts.

2-Year Study in Rats

Groups of 50 male and 50 female F344/N rats were exposed to 220, 700, or 7,000 ppm isoprene by inhalation, 6 hours per day, 5 days per week, for 105 weeks.

Survival and Body Weights

Survival rates and mean body weights of exposed male and female rats were similar to those of the chamber controls throughout the study.

Urinary Vinyl Lactic Acid Biomarker of Exposure

At 3, 6, 12, and 18 months, the concentrations of vinyl lactic acid normalized to creatinine in the urineincreased with increasing exposure concentration in all exposed groups of male and female rats; however, these increases were not proportional to isoprene exposure concentrations, indicating nonlinear metabolism over this range of exposure concentrations.

Pathology Findings

Exposure-related increases in the incidences of mammary gland fibroadenoma were observed in male rats in all groups. The incidences of fibroadenoma in 7,000 ppm males and in all groups of exposed females were significantly greater than those in the chamber control groups. The incidences of fibroadenoma in all exposed groups of males and females and of multiple fibroadenoma in 7,000 ppm males and in all groups of exposed females exceeded the historical control ranges. In addition, the finding of mammary gland carcinoma in exposed male rats was noteworthy

because this neoplasm rarely occurs in control male rats.

The incidences of renal tubule adenoma in 700 and 7,000 ppm males and of renal tubule hyperplasia in 7,000 ppm males were significantly greater than those in the chamber controls. The severity of kidney nephropathy was slightly increased in 7,000 ppm males when compared to chamber controls.

An exposure-related increase in the incidences of interstitial cell adenoma of the testis was observed in male rats. The incidences of bilateral interstitial cell adenoma and of unilateral and bilateral interstitial cell adenoma (combined) of the testis in 700 and 7,000 ppm males were significantly greater than those in the chamber controls. The incidences of interstitial cell adenoma in 700 and 7,000 ppm males exceeded the historical control range.

Several rare neoplasms including benign astrocytoma, malignant glioma, malignant medulloblastoma, benign meningeal granular cell tumor, and meningeal sarcoma were observed in the brain of exposed female rats. These neoplasms have seldom or never occurred in historical chamber controls.

The incidences of splenic fibrosis in 700 and 7,000 ppm males were significantly greater than that in the chamber control group.

GENETIC TOXICOLOGY

Isoprene was not mutagenic in *S. typhimurium* and did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells with or without exogenous metabolic activation; however, in mice, isoprene induced increases in the frequency of sister chromatid exchanges in bone marrow cells and in the frequency of micronucleated erythrocytes in peripheral blood. The cell cycle duration of proliferating bone marrow cells of mice exposed to 7,000 ppm isoprene was significantly lengthened. No increases in the frequency of chromosomal aberrations were observed in bone marrow cells of male mice after 12 days of exposure to isoprene, and lung fibroblasts of male and female rats exposed to isoprene for 4 weeks showed no increase in the frequency of micronuclei.

CONCLUSIONS

Under the conditions of this 2-year inhalation study, there was *clear evidence of carcinogenic activity** of isoprene in male F344/N rats based on increased incidences of mammary gland fibroadenoma and carcinoma, renal tubule adenoma, and testicular interstitial cell adenoma. There was *some evidence of carcinogenic activity* of isoprene in female F344/N rats based on increased incidences and multiplicity of mammary gland fibroadenoma. A low incidence of rare brain neoplasms in exposed female rats may have been due to exposure to isoprene.

Exposure to isoprene by inhalation for 2 years resulted in increased incidences of renal tubule hyperplasia and splenic fibrosis in male rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

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

	Male F344/N Rats	Female F344/N Rats
Concentrations in air	0, 220, 700, or 7,000 ppm	0, 220, 700, or 7,000 ppm
Body weights	Exposed groups similar to chamber control group	Exposed groups similar to chamber control group
Survival rates	18/50, 16/50, 15/50, 15/50	29/50, 30/50, 28/50, 27/50
Nonneoplastic effects	Kidney: renal tubule hyperplasia (standard evaluation - 0/50, 2/50, 6/50, 8/50; standard and extended evaluations combined - 7/50, 6/50, 13/50, 18/50) Spleen: fibrosis (11/50, 14/50, 24/50, 22/50)	None
Neoplastic effects	Mammary gland: fibroadenoma, multiple (1/50, 1/50, 0/50, 7/50); fibroadenoma, including multiple (2/50, 4/50, 6/50, 21/50); carcinoma (0/50, 1/50, 1/50, 2/50) Kidney: renal tubule adenoma (standard evaluation - 0/50, 2/50, 2/50, 6/50; standard and extended evaluations combined - 2/50, 4/50, 8/50, 15/50) Testis: interstitial cell adenoma, bilateral (20/50, 29/50, 37/50, 48/50); interstitial cell adenoma, including bilateral (33/50, 37/50, 44/50, 48/50)	Mammary gland: fibroadenoma, multiple (7/50, 12/50, 19/50, 17/50); fibroadenoma, including multiple (19/50, 35/50, 32/50, 32/50)
Uncertain findings	None	Brain: benign astrocytoma (0/50, 0/50, 1/50, 0/50); malignant glioma (0/50, 0/50, 0/50, 1/50); malignant medulloblastoma (0/50, 0/50, 0/50, 1/50); meninges, benign granular cell tumor (0/50, 1/50, 0/50, 1/50); meninges, sarcoma (0/50, 1/50, 0/50, 1/50)
Level of evidence of carcinogenic activity	Clear evidence	Some evidence

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*:	Negative with and without S9
Mouse bone marrow *in vivo*:	Positive
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Mouse bone marrow *in vivo*:	Negative
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo*:	Positive
Lung fibroblasts:	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 isoprene on 10 December 1997 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 10 December 1997, the draft Technical Report on the toxicology and carcinogenesis studies of isoprene 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. R.L. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of isoprene by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats. A previous NTP 26-week inhalation study followed by a 26-week recovery period had provided clear evidence of the multiple-site carcinogenicity of isoprene in male B6C3F$_1$ mice. The proposed conclusions for the 2-year study were *clear evidence of carcinogenic activity* in male F344/N rats and *some evidence of carcinogenic activity* in female F344/N rats.

Dr. Melnick discussed the metabolism of isoprene and compared the neoplasm responses and metabolism of isoprene with two close structural analogues, 1,3-butadiene and chloroprene, from previous 2-year NTP studies. Dr. Melnick then described a physiologically based pharmacokinetic model that was developed for isoprene and how it was used to evaluate dose-response relationships for neoplasm formation at the different sites.

Dr. Belinsky, a principal reviewer, agreed with the proposed conclusions.

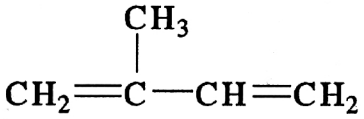
Dr. Medinsky, the second principal reviewer, agreed with the proposed conclusions. She said the structure-activity comparisons made with the analogues maximized the usefulness of the data collected on the three chemicals. Dr. Medinsky noted the use of pharmacokinetic data to obtain a more refined dose metric and a more refined demonstration of the changes in neoplasm response with respect to dose. She asked why the authors believed the parent substance, isoprene, might be involved directly in neoplasm formation. Dr. Melnick said the relationship in the kidney seems to be driven by the mono- or diepoxide intermediate, but in the mammary gland of male rats, there may be some contribution from the parent because of the greater response at the highest exposure concentration.

Dr. Cullen, the third principal reviewer, agreed with the proposed conclusions.

Dr. A.P. Leber, Goodyear Tire and Rubber Company, representing the International Institute for Synthetic Rubber Producers, said that he had specific comments on the neoplasms reported. With regard to rat renal tubule neoplasms, he said that the dimer of isoprene is limonene, which is known to be associated with α2u-microglobulin accumulation and renal neoplasms in rats. [Ed. Note: protein droplet accumulation was not observed in the male rat kidney in the current study]. In his view, the low incidence of carcinoma and lack of indication of a progression of male mammary gland neoplasms to malignancy supported only *some evidence*, as would also be the case with the testicular neoplasms. For the mammary gland neoplasms in female rats, he said that *equivocal evidence* would be appropriate.

Dr. Belinsky moved that the Technical Report on isoprene be accepted with revisions discussed and the conclusions as written for male rats, *clear evidence of carcinogenic activity*, and for female rats, *some evidence of carcinogenic activity*. Dr. Medinsky seconded the motion, which was accepted by six yes votes to one no vote (Dr. Goldsworthy) with one abstention because of company affiliation (Dr. Bus).

INTRODUCTION



ISOPRENE

CAS No. 78-79-5

Chemical Formula: C_5H_8 Molecular Weight: 68.1

Synonyms: Isopentadiene; β-methylbivinyl; 2-methyl-1,3-butadiene

CHEMICAL AND PHYSICAL PROPERTIES

Isoprene is a colorless, volatile, flammable liquid with a boiling point of 34.1 C and a vapor pressure of 493 mm Hg at 20¡ C (*Kirk-Othmer*, 1981; USEPA, 1984). The conversion factor for 1 ppm isoprene at 25¡ C and 760 mm Hg is 2.79 mg/m^3.

PRODUCTION, USE, AND HUMAN EXPOSURE

Isoprene is primarily obtained as a by-product of naphtha cracking for ethylene production. Synthetic routes of isoprene production include dehydrogenation of isopentane, dehydrogenation of tertiary amylenes, dimerization of propylene, and condensation of isobutene with formaldehyde (*Kirk-Othmer*, 1981). The annual production volume of isoprene in the United States in 1992 was approximately 480 million pounds (USITC, 1994). The worldwide production volume of isoprene is estimated to be 2.9 billion pounds (Taalman, 1996).

Isoprene is the monomeric unit of natural rubber and naturally occurring terpenes and steroids. It is emitted from plants and trees and has been detected in tobacco smoke and automobile exhaust (Taalman, 1996). The average yield of isoprene from cigarette smoke was estimated to be 3.1 mg/cigarette (IARC, 1994). Isoprene is highly reactive, with addition reactions similar to those of 1,3-butadiene. Approximately 95% of industrial isoprene is used in the preparation of *cis*-1,4-polyisoprene elastomers (*Kirk-Othmer*, 1981; Taalman, 1996). Isoprene is also used as a comonomer with isobutene in the production of butyl rubber. Polyisoprene elastomers are used in the manufacture of rubber tires, automotive parts, gaskets, footwear, paint resins, adhesives, and flooring (*Kirk-Othmer*, 1981; Taalman, 1996).

The National Occupational Exposure Survey estimated that between 1981 and 1983 approximately 3,700 workers were potentially exposed to isoprene (NIOSH, 1990). Most of these exposures involved residual monomeric isoprene in polyisoprene products. No information is available on consumer exposure or on residual concentrations of isoprene in polymeric elastomers. No regulatory standard has been established for isoprene in the United States.

Isoprene is formed endogenously in humans; concentrations in blood range from 15 to 70 nmol/L (Cailleux *et al.*, 1992). Isoprene was identified as the major endogenous hydrocarbon in human breath (DeMaster and Nagasawa, 1978; Gelmont *et al.*, 1981); exhalation of isoprene by human volunteers was estimated to be 2 to 4 mg per day (Gelmont *et al.*, 1981). Isoprene was also reported to be

produced endogenously by rats and mice at rates of 1.9 and 0.4 μmol/hr per kg body weight, respectively (Peter *et al.*, 1987). The availability and distribution of endogenous isoprene is partially controlled by isopentenyl pyrophosphate, the activated precursor for the synthesis of biomolecules that contain isoprene units.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The biotransformation of isoprene is similar to that of 1,3-butadiene, which involves initial oxidation to 1,2-epoxy-3-butene followed by hydrolysis to 3-butene-1,2-diol, conjugation with glutathione, or further oxidation to diepoxybutane (Malvoisin *et al.*, 1979; Malvoisin and Roberfroid, 1982; Csanády *et al.*, 1992). Cytochrome P_{450} 2E1 (CYP2E1) is the major enzyme involved in the hepatic metabolism of both isoprene and 1,3-butadiene.

Isoprene is metabolized to 3,4-epoxy-3-methyl-1-butene (EPOX-I) and 3,4-epoxy-2-methyl-1-butene (EPOX-II) by liver microsomal cytochrome P_{450}-dependent monooxygenases obtained from Wistar rats, Swiss mice, New Zealand rabbits, Syrian golden hamsters, and human cell lines (Figure 1; Del Monte *et al.*, 1985; Longo *et al.*, 1985; Gervasi and Longo, 1990; Bogaards *et al.*, 1996). EPOX-II is produced at about 20% to 25% the rate of EPOX-I formation. CYP2E1 elicits the highest rate of formation of isoprene monoepoxides, followed by CYP2B6 in human liver samples (Bogaards *et al.*, 1996). The epoxide intermediates of isoprene biotransformation may undergo hydrolysis to form vicinal diols (catalyzed by epoxide hydrolase), may be conjugated with glutathione (catalyzed by glutathione-S-transferase), or may be further oxidized to isoprene diepoxide (2-methyl-1,2:3,4-diepoxybutane) (Wistuba *et al.*, 1994). Both isoprene monoepoxides are further oxidized to isoprene diepoxide by rat, mouse, and human liver microsomes (Wistuba *et al.*, 1994; Bogaards *et al.*, 1996). The theoretical maximum rate (V_{max}) for isoprene oxidation to EPOX-I in mice is about seven times greater than that in rats, whereas the apparent K_m values are similar in rats and mice. Inhibition of epoxide hydrolase activity results in similar rates of monoepoxide formation in rat, mouse, and human liver microsomes (Bogaards *et al.*, 1996). Vinyl lactic acid (2-hydroxy-2-methyl-3-butenoic acid), an oxidation product of DIOL-I, was identified as the major urinary metabolite of isoprene metabolism in F344/N rats (Buckley *et al.*, 1999).

Peter *et al.* (1987) investigated the inhalation pharmacokinetics of isoprene in male Wistar rats and in male $B6C3F_1$ mice in closed desiccator jars equipped with an oxygen supply and soda lime for carbon dioxide absorption. Metabolism of isoprene was linear in rats and mice up to atmospheric concentrations of about 300 ppm. Saturation of isoprene metabolism was nearly complete at about 1,000 ppm in rats and at about 2,000 ppm in mice (Peter *et al.*, 1990). The maximal metabolic elimination rate of inhaled isoprene in mice (400 μmol/hr per kg) was about three times greater than that in rats (130 μmol/hr per kg). The whole body half-life of isoprene was 6.8 minutes in rats and 4.4 minutes in mice.

Metabolites of isoprene were detected in the blood, nose, lungs, liver, kidneys, and fat of male F344/N rats exposed to 1,480 ppm ^{14}C-labeled isoprene (Dahl *et al.*, 1987); however, the methodology used was inadequate to quantify tissue concentrations of specific intermediates. The diols formed by hydrolysis of the two monoepoxide intermediates of isoprene biotransformation, DIOL-I and DIOL-II, and isoprene diepoxide were not analyzed separately. In a species comparison on the disposition of inhaled isoprene, the percentage of inhaled isoprene metabolized in $B6C3F_1$ mice was two- to fivefold less than the percentage of inhaled isoprene metabolized in F344/N rats (Bond *et al.*, 1991). Isoprene-derived hemoglobin adducts were detected in the blood of Sprague-Dawley rats and $B6C3F_1$ mice exposed to [^{14}C]-isoprene by intraperitoneal injection or by inhalation (Sun *et al.*, 1989; Bond *et al.*, 1991).

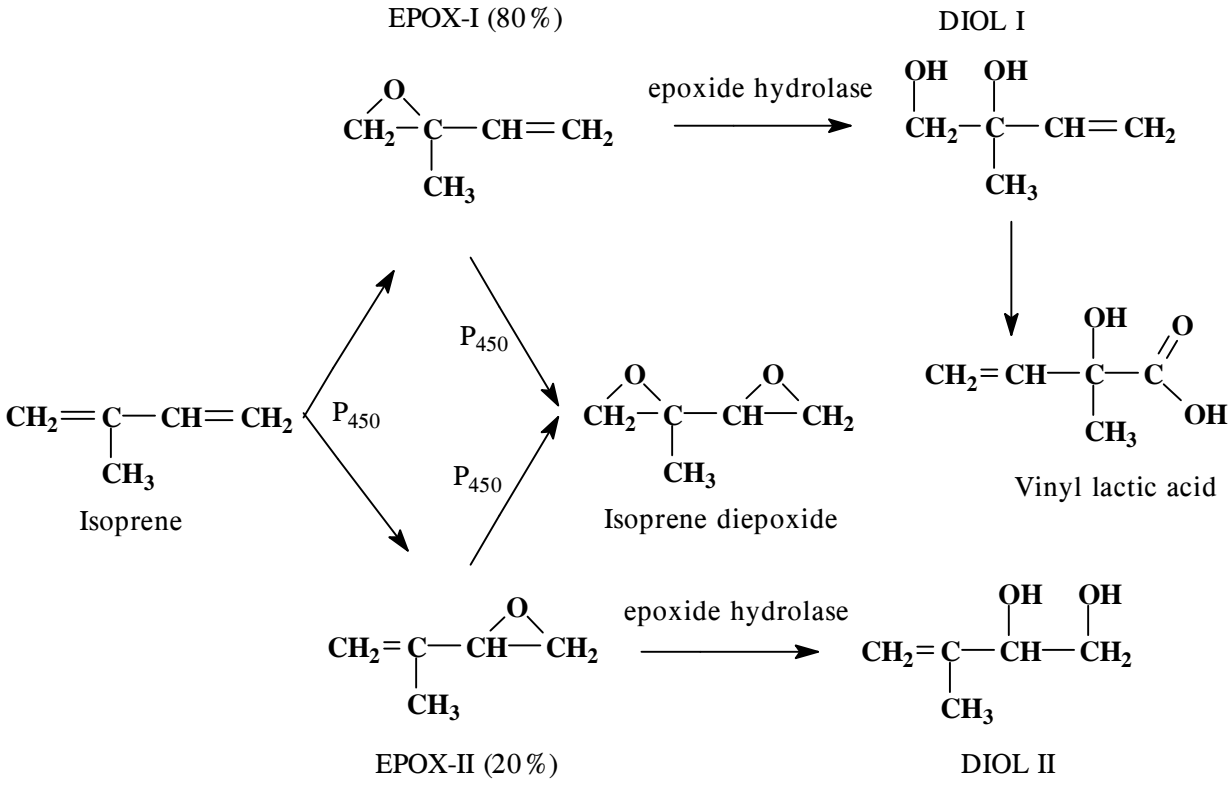


FIGURE 1
Microsomal Metabolic Pathways of Isoprene (Gervasi and Longo, 1990).
EPOX-I=3,4-epoxy-3-methyl-1-butene; EPOX-II=3,4-epoxy-2-methyl-1-butene

TOXICITY

Experimental Animals

The LC_{50} value for isoprene was reported to be 180 mg/L (about 64,500 ppm) in rats after 4 hours of exposure and 157 mg/L (about 56,300 ppm) in mice after 2 hours of exposure (Shugaev, 1969). No toxicologic changes were observed in rats (two per sex per group) after 15 daily inhalation exposures of 6 hours each to 1,670 ppm isoprene or after six exposures of 6 hours each to 6,000 ppm (Gage, 1970). There were no body weight effects in rats, mice, or rabbits exposed to 790 to 1,750 ppm isoprene 4 hours per day for 4 to 5 months (*Patty's*, 1981).

No toxicologic effects were observed in male or female F344/N rats exposed to isoprene vapors at concentrations ranging up to 7,000 ppm (6 hours per day, 5 days per week) for 2 weeks (Melnick *et al.*, 1990a; NTP, 1995) or 13 weeks (Melnick *et al.*, 1994; NTP, 1995). An increase in the incidence and severity of interstitial cell hyperplasia of the testis was observed in male rats exposed to 7,000 ppm isoprene by inhalation for 26 weeks (Melnick *et al.*, 1994; NTP, 1995).

Exposure of male and female $B6C3F_1$ mice to isoprene vapor (438 to 7,000 ppm, 6 hours per day, 5 days per week) for 2 weeks produced several toxicologic effects, including hematologic changes (decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts), testicular atrophy, thymic atrophy, olfactory epithelial degeneration, and forestomach epithelial hyperplasia (Melnick *et al.*, 1990a; NTP, 1995). Thirteen-week or 26-week inhalation exposures at concentrations ranging from 70 to 7,000 ppm isoprene also caused anemia in $B6C3F_1$ mice as well as spinal cord degeneration,

forestomach epithelial hyperplasia, olfactory epithelial degeneration, cytoplasmic vacuolization of hepatocytes, and testicular atrophy (Melnick *et al.*, 1994; NTP, 1995). Hindlimb paralysis and reduced hindlimb grip strength were also observed in isoprene-exposed mice.

Humans

Human volunteers experienced irritation in the upper respiratory tract after exposure to isoprene at concentrations of approximately 60 ppm (*Patty's*, 1981).

REPRODUCTIVE TOXICITY

Results of inhalation teratology studies did not show maternal or developmental toxicity in Sprague-Dawley rats exposed to isoprene vapor (days 6 to 19 of gestation) at concentrations up to 7,000 ppm. Exposure of Swiss (CD-1®) mice to 280, 1,400, or 7,000 ppm isoprene during days 6 to 17 of gestation resulted in decreased fetal weights and a greater percentage of fetuses per litter with supernumerary ribs, but no increase in the incidence of fetal malformations (NTP, 1995).

CARCINOGENICITY

Experimental Animals

Marginal increases in the incidences of interstitial cell adenoma of the testis occurred in male F344/N rats exposed to 700, 2,200, or 7,000 ppm isoprene vapor (6 hours per day, 5 days per week) for 26 weeks and then allowed to recover for an additional 26 weeks without exposure to isoprene (Melnick *et al.*, 1994; NTP, 1995). Increased incidences of neoplasms of the forestomach, harderian gland, liver, and lung occurred in male B6C3F$_1$ mice exposed by inhalation to 700 ppm isoprene or greater (Melnick *et al.*, 1994; NTP, 1995). Based on the results of this study, an International Agency for Research on Cancer (1994) panel concluded that there is sufficient evidence of carcinogenicity of isoprene in experimental animals and, consequently, isoprene is considered to be possibly carcinogenic to humans. Exposure-related increases in the incidences of forestomach, harderian gland, liver, and lung neoplasms also occurred in male B6C3F$_1$ mice exposed to isoprene vapor at concentrations ranging from 10 to 2,200 ppm for 4 or 8 hours per day, 5 days per week, for 20, 40, or 80 weeks, followed by a holding period until week 105 (Placke *et al.*, 1996). Increased incidences of hemangiosarcomas in the spleen and heart and increased incidences of histiocytic sarcomas were also detected in isoprene-exposed mice in this study.

Humans

No information on the carcinogenicity of isoprene in humans was reported in the literature.

GENETIC TOXICITY

Isoprene was not mutagenic in any of several strains of *Salmonella typhimurium* in the presence or absence of Aroclor-induced rat or hamster liver S9 (de Meester *et al.*, 1981; Mortelmans *et al.*, 1986). In addition, results of mutagenicity tests of EPOX-I and EPOX-II in *S. typhimurium* strains TA98 and TA100 were negative (Gervasi *et al.*, 1985). However, 2-methyl-1,2:3,4-diepoxybutane, which may be generated by further oxidation of the monoepoxides, was a potent mutagen in *S. typhimurium* strain TA100 (Gervasi *et al.*, 1985). The lack of mutagenicity of EPOX-I was attributed to steric and electronic effects by the methyl group on the oxirane ring, shifting its substitution towards an S_N1 mechanism with high reactivity towards water. Indeed, the half-life for spontaneous hydrolysis of this epoxide is only 1.25 hours, whereas the half-life of EPOX-II is 73 hours and the half-life of 2-methyl-1,2:3,4-diepoxybutane is 46 hours. However, Bleasdale *et al.* (1996) found high reactivity of EPOX-I with amino and thiolate nucleophiles by an S_N2 mechanism. Hence, the lack of mutagenicity of EPOX-I in *S. typhimurium* may actually be an artifact of the incubation protocol and the high spontaneous hydrolysis rate of this reactive compound. In addition, the possibility must be considered that the standard *S. typhimurium* preincubation protocol may not be optimal for detecting the mutagenicity of isoprene, a volatile chemical that requires multistep biotransformation to produce a mutagenic product.

Inhalation exposure of male B6C3F$_1$ mice to isoprene (6 hours per day for 12 days) at concentrations

ranging from 70 to 7,000 ppm produced significant increases in sister chromatid exchanges in bone marrow cells and in the frequencies of micronucleated polychromatic and normochromatic erythrocytes in peripheral blood (Tice *et al.*, 1988; Shelby, 1990); however, no increase in chromosomal aberrations was observed in the bone marrow cells of these mice. In addition, bone marrow cytotoxicity was evidenced by an increase in the average generation time of dividing bone marrow cells and a decrease in the percentage of circulating polychromatic erythrocytes. 1,3-Butadiene also induced increases in the frequency of sister chromatid exchanges in bone marrow cells and increased the frequency of micronucleated erythrocytes in peripheral blood of exposed mice (Tice *et al.*, 1987); however, unlike isoprene, 1,3-butadiene did induce chromosomal aberrations in mouse bone marrow cells. Isoprene did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells with or without S9 metabolic activation (NTP, 1995).

STUDY RATIONALE

Isoprene was selected for toxicologic evaluation because of its structural similarity to 1,3-butadiene, a potent, multiple-organ, rodent carcinogen, and the potential for human exposure due to its large annual production volume. Long-term inhalation studies have demonstrated that 1,3-butadiene is a multiple-organ carcinogen in Sprague-Dawley rats (Owen *et al.*, 1987) and in B6C3F_1 mice (Huff *et al.*, 1985; Melnick *et al.*, 1990b; NTP, 1984, 1993). The 26-week inhalation exposure plus 26-week recovery study provided clear evidence of the multiple-site carcinogenicity of isoprene in male B6C3F_1 mice (Melnick *et al.*, 1994; NTP, 1995). A similar inhalation study in rats was inadequate for assessment of the carcinogenicity of isoprene (IARC, 1994). Hence, a 2-year inhalation exposure study was performed in F344/N rats to provide an adequate database to evaluate the potential carcinogenicity of isoprene in a second species.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF ISOPRENE

Isoprene was obtained from the Goodyear Tire and Rubber Company (Akron, OH) in three lots (BC21028-F1-161-1, BC31019-F3010H-1, and BC40827-F603-1), which were used during the 2-year study. Identity and purity analyses were conducted by the study laboratory. Reports on analyses performed in support of the isoprene studies are on file at the National Institute of Environmental Health Sciences. The methods and results of these studies are detailed in Appendix E. All lots of the chemical, a clear, colorless liquid, were identified as isoprene by infrared and nuclear magnetic resonance spectroscopy (proton and C^{13}) and by two gas chromatography/mass spectrometry systems.

The purity of lot BC21028-F1-161-1 was determined by gas chromatography, gas chromatography/mass spectrometry analysis for *t*-butylcatechol and dimers, potentiometric titration for peroxides, and determination of polymer content. Gas chromatography was performed using two systems.

The total concentration of isoprene dimers ranged from 0.11% to 0.13%, well below the rejection limit of 0.3%. No other impurities with areas greater than 0.1% were detected. Concentrations of the inhibitor *t*-butylcatechol ranged from 185 to 246 ppm. Potentiometric titration was performed on the barrel of lot BC21028-F1-161-1 that gas chromatography/mass spectrometry indicated contained the lowest concentration of inhibitor; titration indicated a peroxide concentration of approximately 0.02 mEq/kg. Headspace samples taken from the storage barrels were analyzed with gas chromatography by one system; results indicated that isoprene accounted for greater than 99% of the peak area, and the concentrations of volatile impurities were not elevated. The polymer content of 0.025% to 0.070% was well below the rejection limit of 0.5%. The overall purity of greater than 99% for lot BC21028-F1-161-1 was in agreement with data provided by the manufacturer. Additional analyses of lot BC21028-F1-161-1 were performed with gas chromatography/mass spectrometry by a second system. No impurities were conclusively identified; however, one minor peak eluting just before the major peak had a retention time similar to that of 1,4-pentadiene or a pentadiene isomer.

The manufacturer indicated that lots BC31019-F3010H-1 and BC40827-F603-1 contained 99.8% and 99.7% isoprene, respectively. Lot BC31019-F3010H-1 contained 0.124% *cis*- and 0.032% *trans*-2-pentene, less than 0.1 ppm cyclopentadiene, 1 ppm alpha acetylenes, 217 ppm isoprene dimers, 213 ppm *t*-butylcatechol, and 16 ppm total sulfur. Lot BC40827-F603-1 contained 0.175% *cis*- and 0.054% *trans*-2-pentene, 2.44 ppm cyclopentadiene, 5 ppm alpha acetylenes, 189 ppm isoprene dimers, 130 ppm *t*-butylcatechol, and less than 1 ppm total sulfur.

Throughout the 2-year study, total isoprene dimer concentrations and *t*-butylcatechol concentrations were monitored with gas chromatography; peroxides and polymers were also monitored. The bulk chemical was initially stored in the original shipping containers at approximately 22° C. Because the rate of dimer formation was increased by approximately 1% per 361 days during the first four analyses, the bulk chemical was subsequently stored at 16° C to limit dimer formation to a maximum total concentration of 2%.

VAPOR GENERATION AND EXPOSURE SYSTEM

Isoprene vapor was generated with a rotary evaporation system. Isoprene was pumped from the bulk reservoir by a liquid micrometering pump into a rotating flask partially immersed in a hot water bath. Isoprene vapor passed from the flask into a chilled water condenser in which much of the vapor

condensed and returned to the evaporator flask. Uncondensed vapor was carried to the top of the condenser column by a metered stream of nitrogen that entered the bottom of the condenser column. Vapor temperature was monitored at the top of the condenser column by a temperature sensor. The total output of the generator was calculated from the metered nitrogen flow and an estimate of the isoprene vapor pressure at the exit temperature. Detailed descriptions of the inhalation chambers and the vapor generation system are provided in Appendix E.

After exiting the condenser column, the isoprene vapor was further diluted with nitrogen. The vapor then entered a short distribution manifold from which individual delivery lines carried metered amounts of vapor to each exposure chamber. Flow to each chamber was regulated by vacuum pumps located at the chamber end of each delivery line. A three-way valve, mounted between the distribution manifold and each chamber, directed vapor to the exposure chamber exhaust until a stable concentration of isoprene vapor was built up in the distribution line. At each chamber, the vapor was further diluted with charcoal- and HEPA-filtered air to the appropriate isoprene concentration.

The study laboratory designed the inhalation exposure chamber (Hazleton 2000, Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. A small particle detector (Type CN, Gardners Associates, Schenectady, NY) was used with and without animals in the exposure chambers to ensure that isoprene vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

VAPOR CONCENTRATION MONITORING

The isoprene concentrations in the exposure chambers were monitored by an on-line gas chromatograph. Samples were drawn from each exposure chamber, an on-line standard, and a charcoal-filtered air blank approximately every 15 minutes using a 12-port stream select valve. The gas chromatograph was checked against an on-line standard of isoprene in nitrogen throughout the day for instrument drift and was calibrated monthly against gravimetrically prepared standards. Additionally, the gas chromatograph was calibrated by a comparison of chamber concentration data to data from grab samples, which were collected with charcoal sampling tubes, extracted with toluene containing heptane as an internal standard, and analyzed by an off-line gas chromatograph. The volumes of gas were sampled at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standards of isoprene in toluene.

CHAMBER ATMOSPHERE CHARACTERIZATION

The times for the exposure concentrations to build up to 90% of the final exposure concentration (T_{90}) and the decay to 10% of the exposure concentration (T_{10}) were measured with and without animals present. Actual T_{90} values were 8 or 9 minutes without animals and 10 or 11 minutes with animals in the chambers. T_{10} values were 9 or 10 minutes without animals and ranged from 9 to 12 minutes with animals in the chambers. A T_{90} value of 12 minutes was selected for the 2-year study.

Studies of isoprene degradation and monitoring for impurities were conducted throughout the study. No significant degradation of isoprene was observed during the study.

2-YEAR STUDY

Study Design

The highest exposure level for toxicologic studies on isoprene was limited by the lower flammable limit value of 1.5% isoprene in air. The highest exposure concentration selected for this study was 7,000 ppm (less than 50% of the lower flammable limit value) because this concentration of isoprene did not produce any exposure-related toxicologic effects in male or female rats in the 13-week inhalation study (NTP, 1995). Because that concentration is greater than the concentration at which isoprene metabolism in rats is saturated (Peter *et al.*, 1990), lower exposure concentrations (220 and 700 ppm) were selected that were in the range of linear or near-linear pharmacokinetics.

Groups of 50 male and 50 female rats were exposed to isoprene at concentrations of 0, 220, 700, or 7,000 ppm by inhalation 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 105 weeks. Additional

groups of 10 male and 10 female rats were exposed to the same concentrations of isoprene for 4 weeks for a lung fibroblast assay (Appendix C).

Source and Specification of Animals

Male and female F344/N rats were obtained from Taconic Farms (Germantown, NY) for use in the 2-year study. Rats were quarantined for 14 days before the beginning of the study. Five male and five female rats were randomly selected for parasite evaluation and gross observation of disease. Rats were approximately 6 weeks old at the beginning of the study. The health of the animals was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix G).

Animal Maintenance

Rats were housed individually. Feed was available *ad libitum* except during exposure and urine collection periods; water was available *ad libitum*. Cages and racks were rotated within the inhalation chambers weekly. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix F.

Clinical Examinations and Pathology

All animals were observed twice daily. Body weights were recorded initially, monthly through week 89, at week 92, and then every 2 weeks until the end of the study, and clinical findings were recorded initially, monthly through week 89, and then every 2 weeks until the end of the study.

Urine samples were collected at 3, 6, 12, and 18 months from 10 male and 10 female rats from each group and analyzed for urine weight and the concentrations of creatinine and vinyl lactic acid (VLA), a metabolite of isoprene. Rats were placed in metabolism cages for 16 hours after exposure while urine was collected over ice. Water was provided during urine collection periods. All urine samples were analyzed for creatinine using a Cobas Fara chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ) and commercially available reagents. VLA content was measured by a coupled liquid chromatography/mass spectrometry system. VLA was separated from the matrix components using an Inertsil ODS2 high-performance liquid chromatography (HPLC) column (C_{18} bonded silica, MegaChem Technologies, Inc., Redondo Beach, CA) on a Hewlett-Packard Model 1050 HPLC analyzer (Hewlett-Packard, Palo Alto, CA). The mobile phase for this separation was 85 mM acetic acid in 50:50 methanol:water. The eluent was diverted into a Finnigan Model TSQ 7000 mass spectrometer (Finnigan Corp., San Jose, CA) and analyzed using negative ion electrospray ionization with selected reaction monitoring.

A complete necropsy and microscopic examination were performed on all core study rats. 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. For extended evaluation of renal proliferative lesions in male rats, kidneys were step sectioned at 1-mm intervals, and four additional sections were obtained from each kidney. 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 study, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the brain (females), kidney, liver (males), lung, mammary gland, nose, testes, and urinary bladder (females).
The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were

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

TABLE 1
Experimental Design and Materials and Methods in the 2-Year Inhalation Study of Isoprene

Study Laboratory
Battelle Pacific Northwest Laboratories (Richland, WA)

Strain and Species
F344/N rats

Animal Source
Taconic Farms (Germantown, NY)

Time Held Before Study
14 days

Average Age When Study Began
6 weeks

Date of First Exposure
24 June 1993

Duration of Exposure
6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 105 weeks

Date of Last Exposure
23 June 1995

Necropsy Dates
26-29 June 1995

Average Age at Necropsy
111 weeks

Size of Study Groups
50 males and 50 females

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

Animals per Cage
1

Method of Animal Identification
Tail tattoo

TABLE 1
Experimental Design and Materials and Methods in the 2-Year Inhalation Study of Isoprene

Diet
NIH-07 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, except during exposure and urine collection periods, changed weekly

Water
Softened tap water (Richland municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*

Cages
Stainless-steel wire-bottom (Hazleton Systems, Inc., Aberdeen, MD), rotated weekly

Cage Board
Bunzl (Cincinnati Paper Co., Cincinnati, OH) until November 1994 then Techsorb® (Shepherd Specialty Papers, Kalamazoo, MI) thereafter, changed daily

Chamber Air Supply Filters
Single HEPA (Flanders Filters, Inc., San Rafael, CA) and charcoal (RSE, Inc., New Baltimore, MI), changed as needed

Chambers
Stainless steel (Lab Products, Inc., Harford System Division, Aberdeen, MD), rotated weekly

Chamber Environment
Temperature: 21.5° to 27.6° C
Relative humidity: 28% to 84%
Room fluorescent light: 12 hours/day
Chamber air changes: 15/hour

Exposure Concentrations
0, 220, 700, or 7,000 ppm

Type and Frequency of Observation
Observed twice daily; animals were weighed initially, monthly through week 89, at week 92, and then every 2 weeks until the end of the study. Clinical findings were recorded initially, monthly through week 89, and then every 2 weeks until the end of the study.

Method of Sacrifice
70% CO_2 asphyxiation

Necropsy
Necropsy was performed on all animals.

Histopathology
Complete histopathology was performed on all core study rats. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone, brain, clitoral gland, esophagus, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (bronchial, mandibular, mesenteric, mediastinal), 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.

Urinary Vinyl Lactic Acid) Biomarker of Exposure
Urine was collected for 16 hours after exposure from 10 males and 10 females from each group at 3, 6, 12, and 18 months. Parameters evaluated included urine excretion, creatinine, and VLA.

***In vivo* Lung Fibroblast Study**
Supplemental groups of 10 male and 10 female rats were evaluated after 4 weeks of exposure for the frequency of micronuclei in lung fibroblasts.

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 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, and B5 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 and B3) 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 and B3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, to animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

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

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

Tests of significance included pairwise comparisons of each exposed group with the controls and a test for an overall exposure-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).

Determining Dose-Response Shape and ED_{10} Values for Isoprene

For those neoplasms showing chemical-related effects, the shape of the dose-response curve was estimated by fitting the following modified Weibull model (Portier *et al.*, 1986) to the data:

$$P(dose) = 1 - e^{-(intercept + scale \cdot dose^{shape})}$$

where *P(dose)* is the probability of a neoplasm for animals administered *dose* of isoprene. The

parameters *intercept, scale*, and *shape* are estimated via maximum likelihood estimation using the likelihood

$$L = \sum_{i=0}^{3} x_i \log[P(d_i)] + (n_i - x_i)\log[1-P(d_i)]$$

where x_i is the number of animals with neoplasm in dose group d_i, and n_i is the Poly-3 adjusted number of animals at risk in dose group d_i , i=0,1,2,3. A likelihood ratio test is used to test the hypothesis that the shape parameter equals 1. The test statistic is given as −2 times the differences in the log likelihoods. A one-sided test was used so that the critical values are 2.706 for P=0.05 and 5.410 for P=0.01 (these are the squares of the critical regions from standard normal distribution). The shape parameter was restricted to be less than or equal to 10.

If the estimated shape parameter is greater than 1, the resulting dose response has more curvature than a linear model and exhibits "threshold-like" behavior. If the estimated shape parameter is less than 1, then the dose-response curve is very steep in the low-dose region. The ED_{10} values obtained from these dose-response curves represent the exposure concentration associated with an excess cancer risk of 10%.

Analysis of Continuous Variables

Urinalysis 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 (Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (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).

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 2-year study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year study were submitted to the NTP Archives, this study was 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 isoprene 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 mouse bone marrow cells, and increases in the frequency of micronuclei in mouse peripheral blood erythrocytes and in rat lung fibroblasts. The protocols for these studies and the results are given in Appendix C.

The genetic toxicity studies of isoprene are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the molecular structure and the effects of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemical-induced DNA damage and to predict carcinogenicity in animals, based on the electrophilicity theory of chemical mutagenesis and the somatic mutation theory of cancer (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA

reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests correlate less well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. 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. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone.

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. But, 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

2-YEAR STUDY

Survival

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

Body Weights and Clinical Findings

The mean body weights of all groups of exposed males and females were similar to those of the chamber controls throughout the study (Figure 3; Tables 3 and 4). No chemical-related clinical findings were observed.

TABLE 2
Survival of Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	28	29	30	30
Natural deaths	4	5	5	5
Animals surviving to study termination	18	16	15[d]	15
Percent probability of survival at end of study[a]	36	32	30	30
Mean survival (days)[b]	643	654	652	642
Survival analysis[c]	P=0.944	P=0.847	P=0.726	P=0.807
Female				
Animals initially in study	50	50	50	50
Moribund	16	15	18	23
Natural deaths	5	5	4	0
Animals surviving to study termination	29	30	28	27
Percent probability of survival at end of study	58	60	56	54
Mean survival (days)	686	696	676	667
Survival analysis	P=0.589	P=0.889N	P=0.930	P=0.736

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

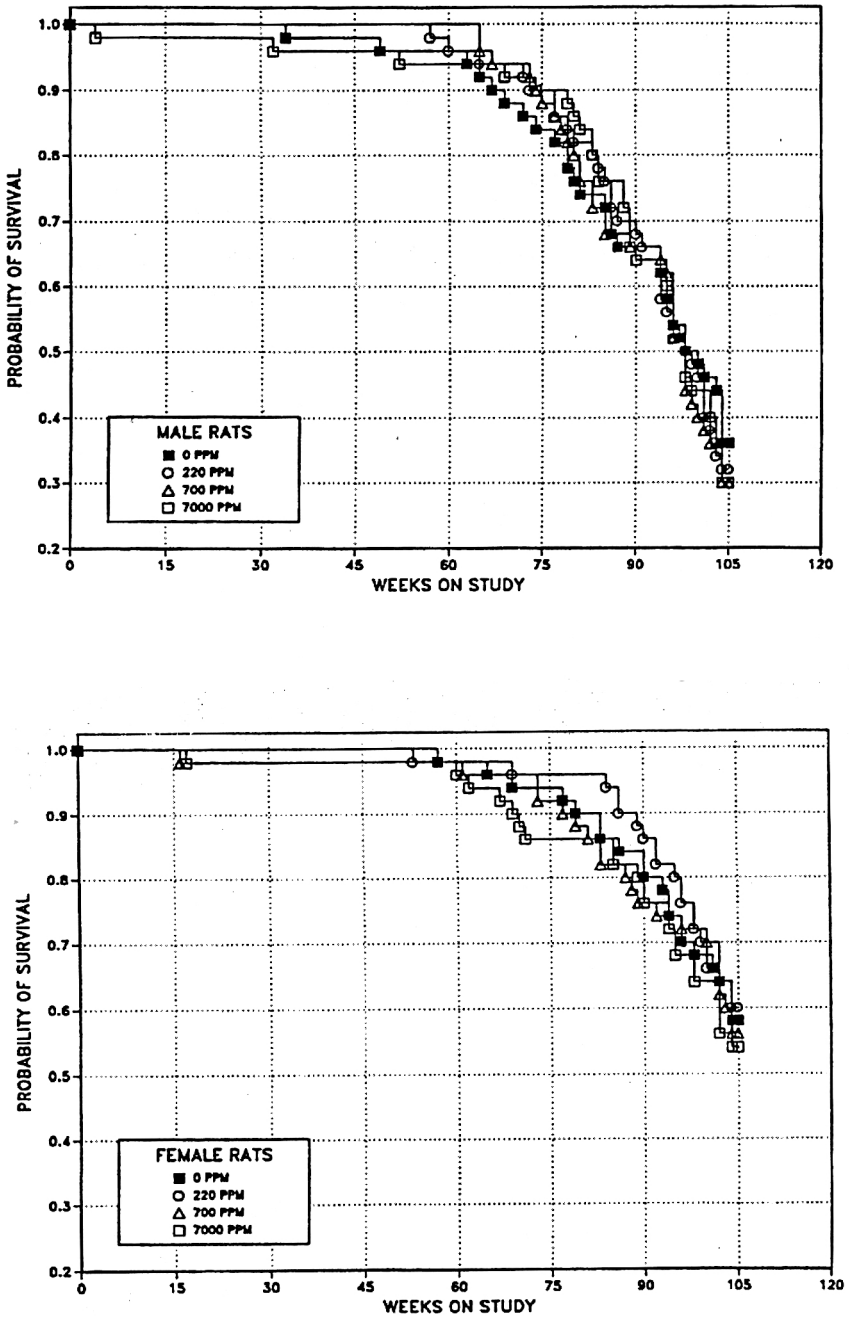


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Isoprene by Inhalation for 2 Years

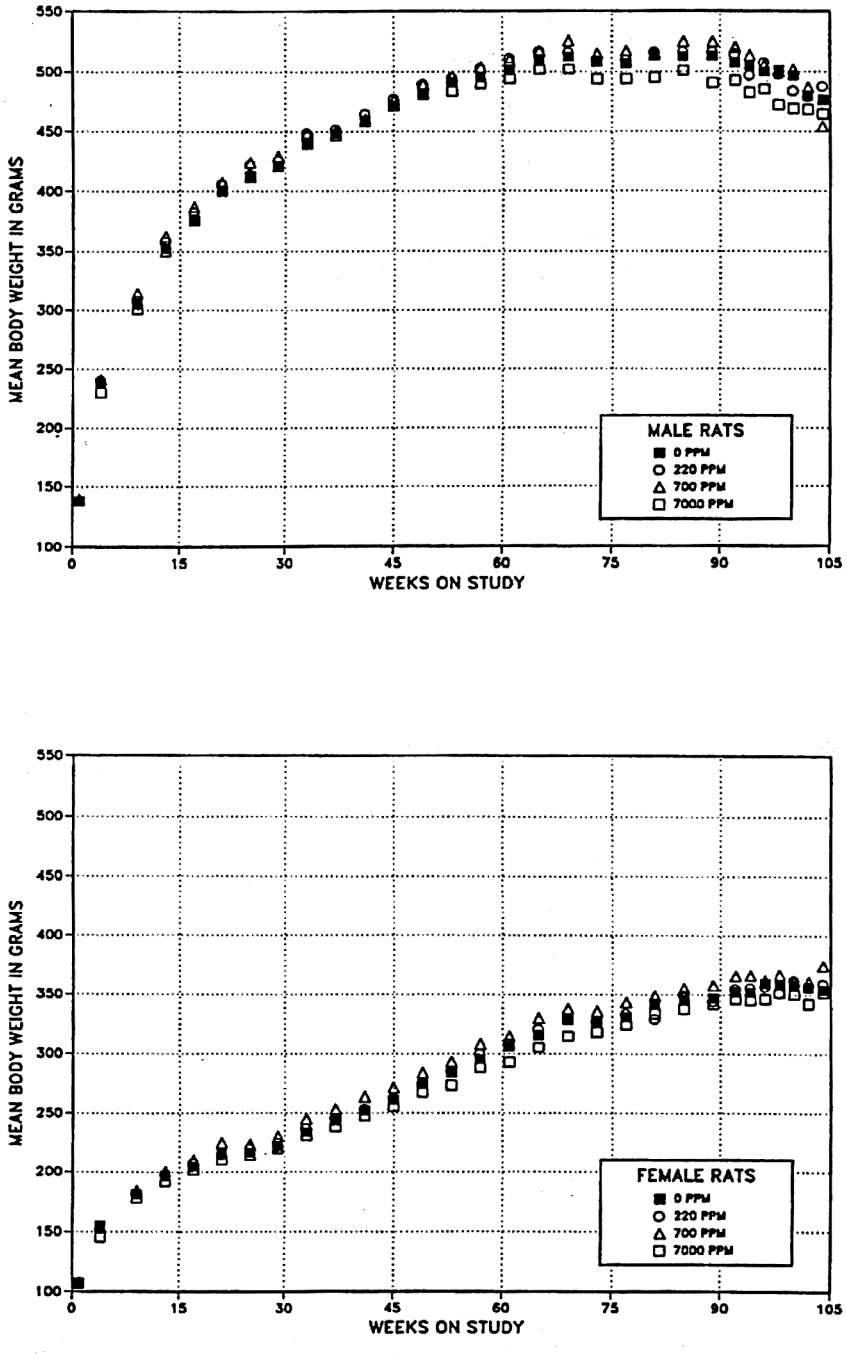


FIGURE 3
Growth Curves for Male and Female Rats
Exposed to Isoprene by Inhalation for 2 Years

TABLE 3
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Isoprene

Weeks on Study	Chamber Control		220 ppm			700 ppm			7,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	138	50	138	100	50	140	101	50	138	100	50
4	238	50	241	101	50	242	101	50	230	97	49
9	305	50	308	101	50	315	103	50	301	99	49
13	353	50	358	101	50	363	103	50	350	99	49
17	377	50	383	102	50	388	103	50	376	100	49
21	401	50	406	101	50	407	102	50	401	100	49
25	413	50	422	102	50	424	103	50	412	100	49
29	420	50	426	101	50	429	102	50	421	100	49
33	439	50	448	102	50	448	102	50	445	102	48
37	448	49	452	101	50	451	101	50	447	100	48
41	458	49	464	102	50	461	101	50	459	100	48
45	471	49	477	101	50	476	101	50	472	100	48
49	481	49	489	102	50	490	102	50	481	100	48
53	491	48	495	101	50	496	101	50	483	99	47
57	495	48	503	102	50	504	102	50	490	99	47
61	501	48	511	102	48	510	102	50	494	99	47
65	510	47	517	101	48	517	101	50	502	98	47
69	512	45	516	101	47	526	103	47	502	98	46
73	508	43	509	100	46	515	101	47	494	97	46
77	507	42	510	101	44	517	102	43	494	98	45
81	513	37	516	101	41	516	101	40	495	97	43
85	513	36	517	101	38	525	102	35	501	98	38
89	513	33	517	101	35	525	102	33	490	96	34
92	508	33	515	102	33	521	103	33	493	97	32
94	505	31	497	98	32	514	102	32	482	96	32
96	500	29	507	102	27	505	101	27	485	97	27
98	501	26	498	99	25	500	100	23	472	94	25
100	497	24	484	97	23	502	101	20	469	95	22
102	480	23	479	100	20	487	102	18	468	98	20
104	476	19	488	102	16	454	95	17	465	98	17
Mean for weeks											
1-13	259		261	101		265	102		255	99	
14-52	434		441	102		442	102		435	100	
53-104	502		505	101		508	101		487	97	

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

Weeks on Study	Chamber Control		220 ppm			700 ppm			7,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	107	50	107	100	50	108	101	50	107	100	50
4	155	50	153	99	50	154	100	50	145	94	50
9	181	50	182	101	50	184	102	50	179	99	50
13	197	50	197	100	50	200	102	50	192	98	50
17	204	50	207	101	50	210	103	49	202	99	49
21	215	50	214	99	50	225	104	49	210	98	49
25	216	50	219	102	50	224	104	49	214	99	49
29	221	50	224	101	50	230	104	49	220	99	49
33	234	50	237	101	50	245	105	49	231	99	49
37	244	50	246	101	50	254	104	49	238	98	49
41	252	50	254	101	50	264	105	49	248	98	49
45	261	50	263	101	50	272	104	49	256	98	49
49	275	50	275	100	50	284	104	49	268	98	49
53	284	50	286	101	50	293	103	49	274	96	49
57	295	50	300	102	49	308	104	49	288	98	49
61	306	49	309	101	49	315	103	49	293	96	48
65	315	49	320	102	49	330	105	48	306	97	47
69	329	48	330	100	49	338	103	48	315	96	46
73	327	47	328	100	48	336	103	47	318	97	43
77	331	47	333	101	48	343	104	46	325	98	43
81	343	45	329	96	48	349	102	44	334	98	43
85	346	43	349	101	47	356	103	41	338	98	43
89	347	42	345	99	45	358	103	38	342	99	40
92	352	40	355	101	42	366	104	37	347	98	38
94	352	39	356	101	41	366	104	37	346	98	36
96	359	36	357	99	38	362	101	37	347	96	34
98	359	34	351	98	37	367	102	36	352	98	32
100	358	34	362	101	33	362	101	36	350	98	32
102	356	33	356	100	33	361	102	32	343	96	31
104	354	32	359	101	32	375	106	29	352	99	28
Mean for weeks											
1-13	160		160	100		162	101		156	98	
14-52	236		238	101		245	104		232	98	
53-104	336		337	100		346	103		328	97	

Urinary Vinyl Lactic Acid — Biomarker of Exposure

Vinyl lactic acid (VLA) is the major urinary metabolite of isoprene in F344/N rats. At 3, 6, 12, and 18 months, the concentrations of VLA normalized to urine creatinine of all exposed groups of male and female rats increased with increasing exposure concentration (Figures 4 and 5). However, the increases in VLA normalized to urine creatinine were not proportional to isoprene exposure concentrations (Figures 6 and 7), indicating nonlinear metabolism over this range of exposure concentrations. This was true at all four collection intervals for males and females.

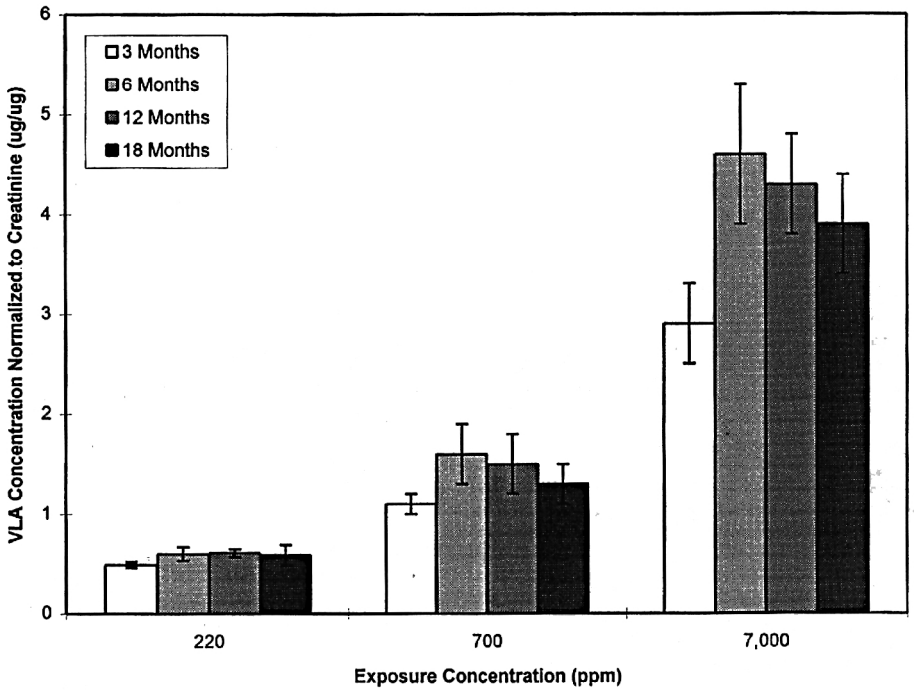


FIGURE 4
Mean (± Standard Deviation) Vinyl Lactic Acid Concentration Normalized to Creatinine for Male Rats in the 2-Year Inhalation Study of Isoprene

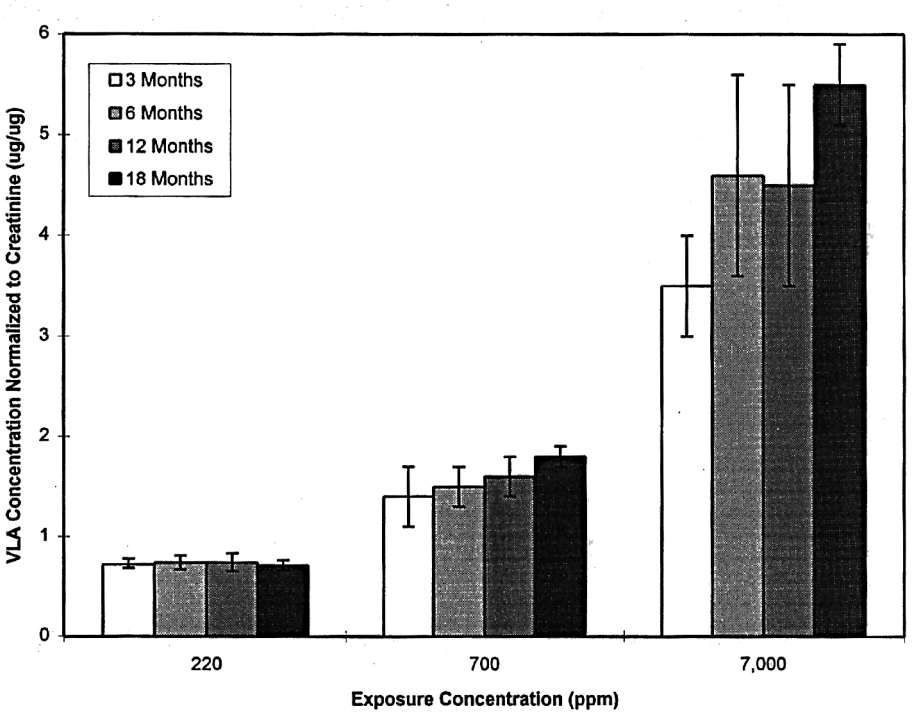


FIGURE 5
Mean (± Standard Deviation) Vinyl Lactic Acid Concentration Normalized to Creatinine for Female Rats in the 2-Year Inhalation Study of Isoprene

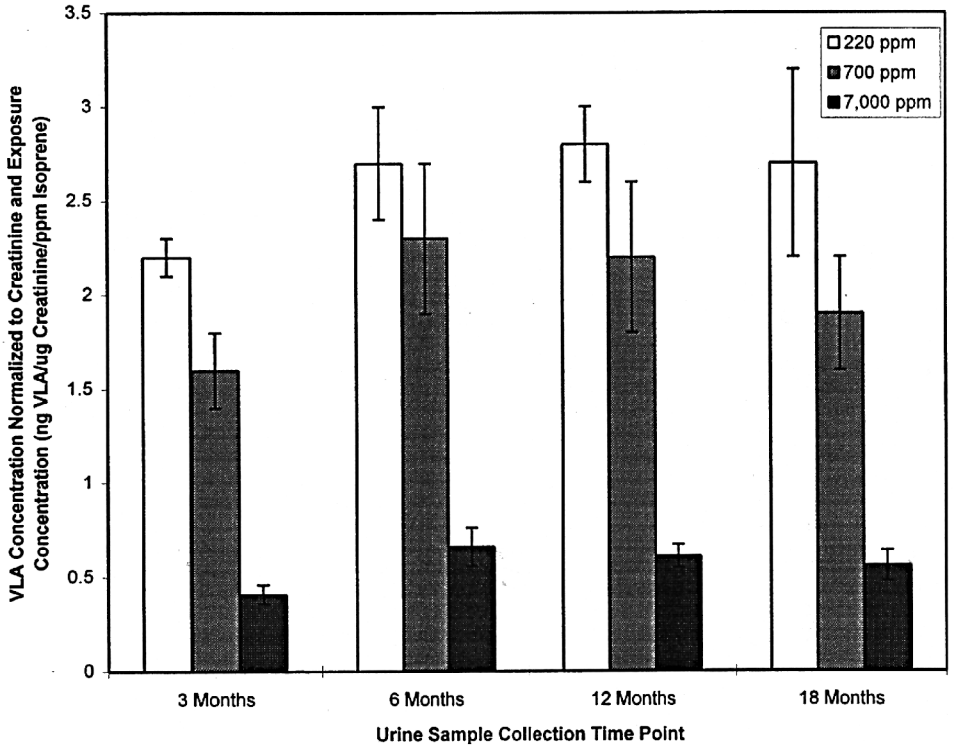


FIGURE 6
Mean (± Standard Deviation) Vinyl Lactic Acid Excretion Normalized to Creatinine and Exposure Concentration for Male Rats in the 2-Year Inhalation Study of Isoprene

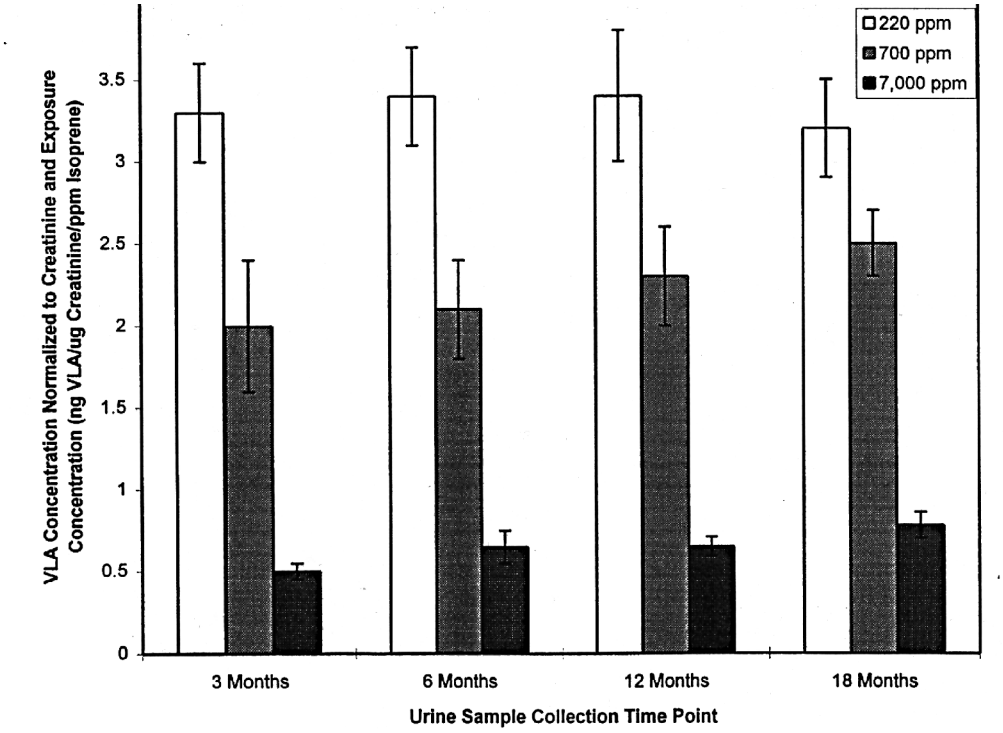


FIGURE 7
Mean (± Standard Deviation) Vinyl Lactic Acid Excretion Normalized to Creatinine and Exposure Concentration for Female Rats in the 2-Year Inhalation Study of Isoprene

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the mammary gland, kidney, testis, brain, spleen, and parathyroid gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analysis 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.

Mammary Gland: Exposure-related increases in the incidences of fibroadenoma occurred in male rats (Tables 5 and A3). The incidences of fibroadenoma in 7,000 ppm male rats and in all exposed groups of female rats were significantly greater than those in the chamber control groups (Tables 5 and B3). The incidences of fibroadenoma in all exposed groups of males and females exceeded the historical control ranges (Tables 5, A4a, and B4a). Similarly, the incidences of multiple fibroadenoma in 7,000 ppm male rats and in all exposed groups of female rats were greater than in the chamber control groups. The incidences of multiple fibroadenoma in 7,000 ppm males and in all exposed groups of females exceeded the historical control ranges. Multiple fibroadenoma was not reported in 12 previous inhalation studies in male F344/N rats conducted at this laboratory. In addition, the finding of mammary gland carcinoma in exposed male rats was particularly noteworthy because this neoplasm rarely occurs in control male rats (Tables 5 and A4a). Single mammary gland carcinomas were observed in a 220 ppm male, a 700 ppm male, and two 7,000 ppm males. Fibroadenomas were characterized by collections of glandular epithelium arranged in acini and ducts and surrounded by fibrous connective tissue (Plate 1). The relative amounts of glandular and fibrous elements varied greatly among neoplasms. Epithelial cells were well differentiated and arranged in a single layer of cuboidal epithelium which was often vacuolated. Carcinomas had solid, papillary, and acinar patterns formed by cuboidal to columnar epithelium and fine fibrovascular stroma.

TABLE 5
Incidences of Neoplasms of the Mammary Gland in Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Male				
Number Necropsied	50	50	50	50
Fibroadenoma, Multiple[a]	1	1	0	7*
Fibroadenoma (Includes Multiple)[b]				
Overall rate[c]	2/50 (4%)	4/50 (8%)	6/50 (12%)	21/50 (42%)
Adjusted rate[d]	5.4%	10.5%	15.8%	54.2%
Terminal rate[e]	1/18 (6%)	2/16 (13%)	1/15 (7%)	10/15 (67%)
First incidence (days)	724	629	591	664
Poly-3 test[f]	P<0.001	P=0.353	P=0.140	P<0.001
Carcinoma[g]	0	1	1	2
Fibroadenoma or Carcinoma[h]				
Overall rate	2/50 (4%)	5/50 (10%)	7/50 (14%)	21/50 (42%)
Adjusted rate	5.4%	13.1%	18.4%	54.2%
Terminal rate	1/18 (6%)	2/16 (13%)	2/15 (13%)	10/15 (67%)
First incidence (days)	724	629	591	664
Poly-3 test	P<0.001	P=0.229	P=0.083	P<0.001
Female				
Number Necropsied	50	50	50	50
Fibroadenoma, Multiple[i]	7	12	19**	17**
Fibroadenoma (Includes Multiple)[j]				
Overall rate	19/50 (38%)	35/50 (70%)	32/50 (64%)	32/50 (64%)
Adjusted rate	42.9%	74.3%	73.7%	73.2%
Terminal rate	12/29 (41%)	23/30 (77%)	22/28 (79%)	20/27 (74%)
First incidence (days)	596	482	539	432
Poly-3 test	P=0.105	P<0.001	P=0.002	P=0.002
Carcinoma	4	2	1	3
Fibroadenoma or Carcinoma[k]				
Overall rate	20/50 (40%)	35/50 (70%)	32/50 (64%)	32/50 (64%)
Adjusted rate	45.2%	74.3%	73.7%	73.2%
Terminal rate	13/29 (45%)	23/30 (77%)	22/28 (79%)	20/27 (74%)
First incidence (days)	596	482	539	432
Poly-3 test	P=0.127	P=0.003	P=0.004	P=0.004

* Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with neoplasm
[b] Historical incidence for 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 17/905 (1.9% ± 2.0%); range, 0%-6%
[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 in animals surviving until the end of the study
[f] Beneath the chamber control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[g] Historical incidence: 1/905 (0.1% ± 0.5%); range, 0%-2%
[h] Historical incidence: 19/905 (2.1% ± 1.9%); range, 0%-6%; includes one adenoma
[i] Historical incidence at Battelle Pacific Northwest Laboratories: 46/598 (7.7% ± 6.1%); range, 0%-18%
[j] Historical incidence: 315/903 (34.9% ± 9.9%); range, 20%-54%
[k] Historical incidence: 305/903 (38.8% ± 10.5%); range, 22%-54%; includes adenomas

Kidney: Initially, a single hematoxylin- and eosin-stained section of each kidney was prepared. Because of a slightly positive trend in the incidences of proliferative lesions in the standard evaluation in male rats, additional step sections of kidney were prepared from the remaining formalin-fixed tissues. Eight additional kidney sections taken at 1-mm intervals were prepared for each male. Additional males with renal tubule adenoma or focal hyperplasia were identified. The incidences of these proliferative lesions in standard and extended evaluations are presented in Table 6.

Multiple renal tubule adenomas were detected in a 220 ppm male, a 700 ppm male, and a 7,000 ppm male following the step-section evaluation (Table 6). The single-section, step-section, and combined single- and step-section incidences of renal tubule adenoma and renal tubule hyperplasia in 7,000 ppm males were significantly greater than those in the chamber controls (Tables 6 and A3). The incidence of renal tubule adenoma was also increased in 700 ppm males compared to the chamber controls when the single-section and step-section data were combined. The trend test showed a significant exposure-related increase in the incidence of renal tubule adenoma. The severity of nephropathy was slightly increased in 7,000 ppm males when compared to the chamber controls.

Renal tubule hyperplasia, as defined in the present study, was distinguished from regenerative epithelial changes commonly seen as a part of nephropathy and was considered to be a preneoplastic lesion. Renal tubule hyperplasia, adenoma, and carcinoma constitute a morphologic continuum. Hyperplasia was generally a focal, minimal to mild lesion consisting of tubules that were dilated 1.5 to 2 times the normal diameter and lined by increased numbers of tubule epithelial cells, which partially or totally filled the tubule lumen. Cells within hyperplastic lesions varied slightly in size and sometimes stained more basophilic than normal cells, but otherwise they appeared similar to normal tubule epithelial cells. Renal tubule adenomas were larger, discrete lesions, ranging from greater than five tubule diameters to 1 mm or more in size. Cells within adenomas were mildly to moderately pleomorphic, sometimes had vacuolated cytoplasm, and tended to form complex patterns, particularly microtubular structures. The renal tubule carcinoma was well differentiated from adenomas in that it was larger and less discrete and had more anaplasia and cellular atypia (Plate 2). The renal tubule carcinoma was characterized by neoplastic cells that had vesiculate nuclei with prominent nucleoli and increased numbers of mitotic figures. Histopathologic changes typically associated with renal α2u-globulin accumulation (e.g., protein droplet accumulation) were not observed in male rats exposed to isoprene.

Oncocytic hyperplasia was identified in step-section evaluation in one 220 ppm male and three 7,000 ppm males. This lesion was characterized by individual tubules or small clusters of tubules that were somewhat dilated and totally filled by large polygonal cells with abundant, brightly eosinophilic, granular cytoplasm and small, centrally located, basophilic nuclei (oncocytes). These lesions are thought to arise from the distal tubule epithelium. No oncocytic neoplasms were observed in males. A mesenchymal neoplasm of the kidney was observed in a 7,000 ppm male and a 220 ppm female. These neoplasms were small and characterized by poorly circumscribed cortical accumulations of dense fibrous and myxomatous connective tissue with scattered trapped cortical tubules.

TABLE 6
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Single Sections (Standard Evaluation)				
Number Examined Microscopically	50	50	50	50
Nephropathy[a]	50 (2.8)[b]	50 (2.7)	50 (2.9)	49 (3.2)
Renal Tubule, Hyperplasia	0	2 (3.5)	6* (2.3)	8** (3.0)
Renal Tubule Adenoma	0	2	2	6*
Renal Tubule Carcinoma	0	0	1	0
Renal Tubule Adenoma or Carcinoma[c]				
Overall rate[d]	0/50 (0%)	2/50 (4%)	2/50 (4%)	6/50 (12%)
Adjusted rate[e]	0.0%	5.3%	5.3%	15.8%
Terminal rate[f]	0/18 (0%)	0/16 (0%)	0/15 (0%)	1/15 (7%)
First incidence (days)	—[h]	559	566	630
Poly-3 test[g]	P=0.009	P=0.244	P=0.242	P=0.016
Step Sections (Extended Evaluation)				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia	7 (2.0)	5 (2.6)	9 (2.1)	15* (2.2)
Renal Tubule Adenoma, Multiple	0	1	1	1
Renal Tubule Adenoma (includes Multiple)	2	2	6	10*
Single Sections and Step Sections (Combined)				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia	7 (2.0)	6 (2.8)	13 (2.3)	18** (2.6)
Renal Tubule Adenoma, Multiple	0	1	1	1
Renal Tubule Adenoma (includes Multiple)	2	4	8*	15**
Renal Tubule Carcinoma	0	0	1	0
Renal Tubule Adenoma or Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	8/50 (16%)	15/50 (30%)
Adjusted rate	5.4%	10.5%	21.0%	38.5%
Terminal rate	0/18 (0%)	1/16 (6%)	4/15 (27%)	5/15 (33%)
First incidence (days)	704	559	566	622
Poly-3 test	P<0.001	P=0.352	P=0.047	P<0.001

* Significantly different (P≤0.05) from the chamber 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 NTP inhalation studies with chamber control groups (mean ± standard deviation): 10/902 (1.1% ± 1.2%); range, 0%-4%
[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 in animals surviving until the end of the study
[g] Beneath the chamber control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Not applicable; no neoplasms in animal group

Testis: An exposure-related increase in the incidence of interstitial cell adenoma of the testis occurred in male rats. The incidences of bilateral interstitial cell adenoma and of combined unilateral and bilateral interstitial cell adenoma in 700 and 7,000 ppm males were significantly greater than those in the chamber controls (Tables 7 and A1). The incidences of interstitial cell adenoma in 700 and 7,000 ppm males exceeded the historical control range (Tables 7 and A4c). Interstitial cell adenomas were equal in size to, or larger than, a seminiferous tubule (Plates 3 and 4). For any testis in which an adenoma was present along with hyperplasia of the interstitial cells, only the adenoma was diagnosed; however, when one testis had an adenoma and the contralateral testis had foci of hyperplasia, both diagnoses were recorded. Because of the exposure-related increase in the incidences of bilateral interstitial cell adenoma, the incidences of interstitial cell hyperplasia (15/50, 13/50, 8/50, 0/50; Table A5) are not well reflected in this data set.

TABLE 7
Incidences of Neoplasms and Nonneoplastic Lesions of the Testis in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Number Examined Microscopically	50	50	50	50
Interstitial Cell Adenoma, Bilateral [a]	20	29	37**	48**
Interstitial Cell Adenoma (includes bilateral)[b]				
Overall rate[c]	33/50 (66%)	37/50 (74%)	44/50 (88%)	48/50 (96%)
Adjusted rate[d]	78.4%	85.3%	94.4%	99.9%
Terminal rate[e]	16/18 (89%)	16/16 (100%)	15/15 (100%)	15/15 (100%)
First incidence (days)	454	398	454	361
Poly-3 test[f]	P<0.001	P=0.259	P=0.010	P<0.001

** Significantly different (P≤0.01) from the chamber control group by the Poly-3 test
[a] Number of animals with lesion
[b] Historical incidence for 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 628/905 (69.4% ± 9.7%); range, 46%-83%
[c] Number of animals with neoplasm per number of animals with testis examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence in animals surviving until the end of the study
[f] Beneath the chamber control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to the pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

Brain: In the central nervous system, two neoplasms were observed in exposed males, seven in exposed females, and none in the chamber controls (Table 8). A malignant astrocytoma (neuroglial cell origin) was observed in a 7,000 ppm male, and a benign granular cell tumor (meningeal cell origin) was observed in a 700 ppm male. Malignant astrocytomas rarely occur in chamber control males (Tables 8 and A4d), and granular cell neoplasms have not been detected in chamber control males. A benign astrocytoma (neuroglial cell origin) was observed in a 700 ppm female, a malignant glioma (neuroglial cell origin) was observed in a 7,000 ppm female, a malignant medulloblastoma (undifferentiated cell origin) was observed in a 7,000 ppm female, single granular cell tumors (meningeal cell origin) were present in a 220 ppm female and a 7,000 ppm female, and single meningeal sarcomas (meningeal cell origin) were present in a 220 ppm female and a 7,000 ppm female. Medulloblastomas have not been detected in chamber control females, and both malignant gliomas and benign astrocytomas occur rarely in chamber control females (Tables 8 and B4b). Meningeal cell sarcomas and granular cell neoplasms are rarely observed in chamber control females.

TABLE 8
Incidences of Neoplasms of the Brain in Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Malignant Astrocytoma[a,b]	0	0	0	1
Meninges, Benign Granular Cell Tumor[c]	0	0	1	0
Female				
Number Examined Microscopically	50	50	50	50
Benign Astrocytoma[d]	0	0	1	0
Malignant Glioma[d]	0	0	0	1
Malignant Medulloblastoma[e]	0	0	0	1
Meninges, Benign Granular Cell Tumor[f]	0	1	0	1
Meninges, Sarcoma[e]	0	1	0	1

[a] Number of animals with lesion
[b] Historical incidence for 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 1/904 (0.1% ± 0.5%); range, 0%-2%
[c] Historical incidence: 0/904
[d] Historical incidence: 1/899 (0.1% ± 0.5%); range, 0%-2%
[e] Historical incidence: 0/899
[f] Historical incidence (all sites): 2/899 (0.2% ± 0.7%); range, 0%-2%

Astrocytomas consisted of poorly circumscribed aggregates of loosely to densely packed, small, astrocytic-like cells with inconspicuous vascularization. The margins of the neoplasm were not well defined, and neoplastic cells extended into the adjacent brain parenchyma (Plates 5, 6, and 7). The medulloblastoma consisted of primitive undifferentiated cells and was highly cellular; the neoplastic cells were often arranged in rows or in densely packed clusters (Plate 8). The malignant glioma consisted of a compressed nodular mass composed of sheets and clusters of neoplastic cells (pleomorphic cells with hyperchromatic to vesicular nuclei) separated by variable amounts of eosinophilic matrix (Plates 9 and 10). Meningeal sarcomas were small, poorly circumscribed masses composed of interlacing bundles and streams of spindle cells interspersed with large, pleomorphic cells with indistinct borders and prominent vesicular nuclei. Granular cell neoplasms were small discrete nodules of closely packed, large, polygonal cells with abundant, finely granular, eosinophilic cytoplasm.

Brain neoplasms may have been related to isoprene exposure in female rats because of the increases in the incidences of rare brain neoplasms, some of which were malignant, and because there were no brain neoplasms in the chamber controls. However, a number of characteristics associated with exposure to neurocarcinogenic agents were not observed. There were no consistent decreases in the age at which neoplasms appeared (latency) or in survival, no exposure-response relationship for brain neoplasms, and no predominance in any specific type (origin) of brain neoplasm. These factors contributed to the conclusion that the marginal increase in brain neoplasms in females could not be related with certainty to the administration of isoprene.

Spleen: The incidences of splenic fibrosis (chamber control, 11/50; 220 ppm, 14/50; 700 ppm, 24/50; 7,000 ppm, 22/50; Table A5) in 700 and 7,000 ppm males were significantly greater than that in the chamber control group. Severities of fibrosis (1.9, 1.9., 2.0, 2.3) were also slightly increased. Splenic fibrosis consisted of irregular, variably sized areas of condensed collagenous stroma in the subcapsular red pulp areas that partly or completely replaced any cellular constituents.

Parathyroid Gland: There was a significant increase in the incidence of parathyroid gland hyperplasia in 7,000 ppm males compared to the chamber controls (5/48, 6/46, 8/45, 13/47; Table A5). This lesion is commonly observed in male rats with nephropathy.

GENETIC TOXICOLOGY

In vitro mutagenicity tests with isoprene yielded negative results. No increase in mutations was noted in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537 treated with isoprene (100 to 10,000 μg/plate), with or without induced rat or hamster liver S9 metabolic activation enzymes (Mortelmans *et al.*, 1986; Table C1). Also, no increases in sister chromatid exchanges or chromosomal aberrations were noted in cultured Chinese hamster ovary cells treated with isoprene, with or without S9 (Tables C2 and C3). Concentrations of isoprene may have been reduced by evaporation during incubation in these *in vitro* tests.

In contrast to the negative results seen in these *in vitro* experiments, *in vivo* tests for chromosomal effects in mice yielded mostly positive results. Bone marrow cells from male mice exposed to isoprene for 12 days via inhalation showed significantly increased levels of sister chromatid exchanges; however, the incidence of bone marrow cells with chromosomal aberrations in male mice treated for 12 days with isoprene was not increased (Tice *et al.*, 1988; Shelby, 1990; Table C4). The frequencies of micronucleated normochromatic erythrocytes and polychromatic erythrocytes were also significantly elevated in peripheral blood samples obtained from the male mice exposed for 12 days to isoprene (Tice *et al.*, 1988; Table C5). The negative bone marrow chromosomal aberration test results and the positive peripheral blood micronuclei results were from the same animals. These apparently conflicting results may imply that chromosomal damage, not detected by traditional chromosomal aberration tests, was induced by isoprene, or, perhaps, that the chromosomal aberration test was not sufficiently sensitive to detect a rather low level of induced structural damage. Further evidence of the ability of isoprene to increase the frequency of micronucleated erythrocytes in mice comes from results of a 13-week study in which males and females showed significantly elevated frequencies of micronucleated normochromatic erythrocytes and polychromatic erythrocytes after inhalation of isoprene (Table C6). Analysis of average generation time and mitotic index data from mice exposed for 12 days to isoprene indicates no change in the percentage of bone marrow cells engaged in division but a significant lengthening, at the high dose of 7,000 ppm isoprene, of the cell cycle duration of proliferating cells (Tice *et al.*, 1988; Table C5). The percentage of circulating polychromatic erythrocytes decreased in an exposure-related fashion in the 12-day study. However, in the mice exposed for 13 weeks, the percentage of polychromatic erythrocytes among erythrocytes in peripheral blood was increased (Table C6). This may represent an adaptation to cytotoxicity with chronic exposure and result from increased erythropoiesis to compensate for depletion of the erythrocyte population.

In male and female rats exposed to isoprene for 4 weeks, no significant increase in the frequency of micronucleated lung fibroblasts was observed (Table C7).

DISCUSSION AND CONCLUSIONS

Isoprene (2-methyl-1,3-butadiene) was selected for toxicologic and carcinogenic evaluations because of its structural similarity to 1,3-butadiene, a known human carcinogen and a potent, multiple-organ, rodent carcinogen (NTP, 1993), and because of the potential for human exposure to isoprene due to its large annual production volume. 1,3-Butadiene induced benign and malignant neoplasms at multiple organ sites in experimental animals at exposure concentrations as low as 6.25 ppm. Studies in humans have consistently found excess mortality from lymphatic and hematopoietic cancers associated with occupational exposure to 1,3-butadiene. Inhalation toxicology and carcinogenesis studies on chloroprene, the 2-chloro analogue of 1,3-butadiene, have been reported (NTP, 1998). Chloroprene was also carcinogenic at multiple sites in rats and mice at exposure concentrations ranging from 12.8 to 80 ppm.

Numerous similarities were observed in the laboratory studies of isoprene and of 1,3-butadiene. A 14-day inhalation study of isoprene in B6C3F$_1$ mice identified several toxic effects that were similar to those produced from exposure to 1,3-butadiene, including a mild nonresponsive anemia, testicular atrophy, forestomach epithelial hyperplasia, and olfactory epithelial degeneration (Melnick *et al.*, 1990a; NTP, 1995). A 14-day inhalation study of isoprene in F344/N rats showed that, as with 1,3-butadiene, this species is less sensitive than mice to equivalent exposures of isoprene; no clinical or histopathologic effects were observed in rats exposed to isoprene concentrations up to 7,000 ppm. Isoprene and 1,3-butadiene are metabolized to mono- and diepoxide intermediates by microsomal cytochrome P_{450}-dependent monooxygenases (CYP2E1) (Malvoisin *et al.*, 1979; Malvoisin and Roberfroid, 1982; Del Monte *et al.*, 1985; Longo *et al.*, 1985; Bogaards *et al.*, 1996). The diepoxide intermediates of both compounds are mutagenic in *Salmonella typhimurium* (unlike isoprene, the monoepoxide intermediate of butadiene biotransformation is also mutagenic in *S. typhimurium*) (Gervasi *et al.*, 1985). Both compounds increased sister chromatid exchanges in bone marrow cells and increased the frequency of micronucleated erythrocytes in mouse peripheral blood (Tice *et al.*, 1987, 1988). Unlike isoprene, 1,3-butadiene also induced chromosomal aberrations in mouse bone marrow cells.

As a consequence of the numerous similar qualitative effects of isoprene and 1,3-butadiene (hematology, histopathology, metabolism, and genotoxicity), 13-week inhalation studies of isoprene were expanded to include a 26-week exposure followed by a 26-week recovery period to determine if isoprene was carcinogenic like 1,3-butadiene (Melnick *et al.*, 1994; NTP, 1995). The extended exposure was conducted in male F344/N rats and male B6C3F$_1$ mice at concentrations ranging from 70 to 7,000 ppm isoprene. Because high incidences of lethal thymic lymphoma occurred as early as week 23 in mice exposed to 625 ppm 1,3-butadiene (Melnick *et al.*, 1990a), it was expected that the extended exposure design would reveal exposure-related lymphomas if isoprene acted similarly.

The 13-week and/or 26-week inhalation exposures of mice to isoprene caused a mild, normochromic, nonresponsive anemia, spinal cord degeneration, testicular atrophy, degeneration of the olfactory epithelium, and epithelial hyperplasia of the forestomach (Melnick *et al.*, 1994; NTP, 1995). Following the 26-week recovery period, incidences of benign and malignant neoplasms in the forestomach, harderian gland, liver, and lung were significantly increased. Neoplasms were also induced in each of these organs in mice exposed to 1,3-butadiene (NTP, 1984, 1993; Huff *et al.*, 1985, Melnick *et al.*, 1990b). However, unlike 1,3-butadiene, exposure to isoprene did not induce lymphoma or hemangiosarcoma of the heart. The same neoplastic effects identified in the 26-week inhalation study were observed in a 2-year inhalation study of isoprene in B6C3F$_1$ mice; in addition, increased incidences of hemangiosarcoma in the spleen and heart and increased incidences of histiocytic sarcoma were detected (Placke *et al.*, 1996). Hence, the carcinogenicity of isoprene in mice has been well established, and an International Agency for Research

on Cancer (1994) panel concluded that there is sufficient evidence for the carcinogenicity of isoprene in experimental animals.

The 13-week inhalation study of isoprene produced no exposure-related toxicologic effects in male or female F344/N rats (Melnick *et al.*, 1994; NTP, 1995). After 26 weeks of exposure, male rats in the 7,000 ppm group had an increased incidence and severity of interstitial cell hyperplasia of the testis. Following the 26-week recovery period, the only effect in rats was a marginal exposure-related increase in the incidence of testicular interstitial cell adenoma. Because 2-year exposures were necessary to demonstrate the carcinogenicity of 1,3-butadiene in rats (Owen *et al.*, 1987), the 26-week exposure study in male rats was not considered to be adequate for an assessment of the carcinogenic potential of isoprene in this species.

The present 2-year inhalation studies were designed to evaluate and characterize the potential carcinogenicity of isoprene in rats. The highest exposure concentration selected for this study was 7,000 ppm because results of the 13-week inhalation study indicated that this exposure was well tolerated by male and female rats. Because the 7,000 ppm concentration is greater than the concentration at which isoprene metabolism in rats is saturated (Peter *et al.*, 1990), lower exposure concentrations (220 and 700 ppm) in the range of linear or near-linear pharmacokinetics were selected.

There were no differences in survival, mean body weights, or clinical findings in male and female F344/N rats exposed to 220, 700, or 7,000 ppm isoprene for 2 years (6 hours per day, 5 days per week) relative to the chamber controls. Three sites of neoplasm induction by isoprene in rats were observed in the current study: the kidney and testis in males and the mammary gland in males and females. In addition, the observation of several rare brain neoplasms in exposed female rats raised the possibility that this may be a site of neoplasm induction by isoprene.

In male rats, an exposure-related increase in the incidences of mammary gland fibroadenoma was observed, and the incidences of single and multiple fibroadenoma were significantly increased in the 7,000 ppm group compared to the chamber control group. The incidences of fibroadenoma in the 220 and 700 ppm groups were not significantly greater than in the chamber control group; however, they exceeded the historical control range. In addition, four mammary gland carcinomas, which rarely occur in chamber control male rats, were observed only in groups exposed to isoprene.

The incidences of mammary gland fibroadenoma in the 220, 700, and 7,000 ppm groups of female rats were significantly greater than the incidence in the chamber control group and exceeded the historical control range. The incidences of multiple fibroadenoma were also significantly increased in the 700 and 7,000 ppm groups of female rats compared to the chamber controls. In all exposed groups, the incidences of multiple fibroadenoma exceeded the historical control range. These findings clearly demonstrate that isoprene affects neoplasm multiplicity in the mammary gland. The incidences of mammary gland carcinoma were not increased in isoprene-exposed female rats.

Increases in the incidences and multiplicities of mammary gland fibroadenoma were also observed in female Sprague-Dawley rats exposed to 1,3-butadiene (Owen *et al.*, 1987; Melnick and Huff, 1992) and in female F344/N rats exposed to chloroprene (NTP, 1998). Hence, the female rat mammary gland is a common target of carcinogenesis for this group of chemicals. The finding that exposure to isoprene caused mammary gland carcinomas and single and multiple fibroadenomas in male rats was unexpected. Mammary gland neoplasms were not induced in male rats exposed to 1,3-butadiene or chloroprene (Owen *et al.*, 1987; NTP, 1998).

Exposure-related increases in the incidences of renal tubule adenoma and renal tubule hyperplasia were observed in male rats. The incidences of renal tubule adenoma in the 7,000 ppm group and of renal tubule hyperplasia in the 700 and 7,000 ppm groups were significantly greater than in the chamber controls. Renal tubule hyperplasia is thought to represent an early stage in the morphologic continuum of proliferative kidney lesions leading to renal tubule adenoma and carcinoma. A renal tubule carcinoma was seen in one male rat in the 700 ppm group. Renal tubule hyperplasia observed in this study was distinguishable from regenerative epithelial changes commonly seen as part of the spontaneous nephropathy in aging F344/N rats. Because renal tubule

neoplasms are uncommon in chamber control F344/N rats, additional kidney sections were examined from chamber control and exposed male rats to provide a clearer indication of the potential exposure-related effects of isoprene in this organ. The incidences of renal tubule adenoma were significantly increased in the 700 and 7,000 ppm groups compared to chamber controls based on analysis of the combined original and step-section data and were equivalent to or exceeded the highest control incidence reported from 13 other studies that also included step sectioning (Eustis *et al.*, 1994). Renal tubule neoplasms were also induced in male F344/N rats exposed to chloroprene (NTP, 1998) but not in male Sprague-Dawley rats exposed to 1,3-butadiene (Owen *et al.*, 1987). These different responses may be due to strain differences in susceptibility or to differences in tissue distribution and reactivity of carcinogenic intermediates.

Although the spontaneous incidence of interstitial cell adenoma of the testis is high in F344/N rats, an exposure-related increase in the incidences of testicular adenoma was observed in the present study. The incidences of this lesion in the 700 and 7,000 ppm groups were significantly greater than in the chamber controls and exceeded the historical control range. The finding that the incidences of bilateral interstitial cell adenoma were also increased in an exposure-related fashion further supports the contention that the increased incidences of these neoplasms were due to exposure to isoprene. The previous 26-week exposure/26-week recovery study of isoprene had identified the testis as a target site of isoprene-induced interstitial cell proliferative lesions in male rats (Melnick *et al.*, 1994; NTP, 1995). The increased incidences and bilateral response observed in the present study confirm and strengthen the previous indication of an increase in incidences of interstitial cell adenoma of the testis in male rats exposed to isoprene. The incidences of interstitial cell adenoma of the testis were also increased in Sprague-Dawley rats exposed to 1,3-butadiene (Owen *et al.*, 1987) but not in F344/N rats exposed to chloroprene (NTP, 1998).

Several rare brain neoplasms were observed in female rats exposed to isoprene, whereas none were observed in the chamber controls. An astrocytoma and glioma of neuroglial origin were observed in a 700 and a 7,000 ppm female, respectively. A neoplasm of undifferentiated cell origin (medulloblastoma) was observed in a 7,000 ppm female. Neoplasms of meningeal cell origin (granular cell tumors and sarcomas) were observed in 220 and 7,000 ppm females. In NTP F344/N rat studies, neoplasms of neuroglial origin (astrocytoma, oligodendroglioma, and glioma) generally are associated with chemical exposure, and for the most part, the incidences of these neoplasms are marginally increased. Astrocytomas and oligodendrogliomas are diagnosed based on the predominant cell type. However, when a glial neoplasm consists of more than 20% of a second cell type, it is often diagnosed as a glioma (cell type not specified). Statistical evaluations are generally strengthened if neoplasm types with similar cellular origin are combined; however, this may be lessened if neoplasm types with different histogenesis such as gliomas and granular cell tumors are combined. Based on the above considerations and the fact that chemically induced brain neoplasms are generally of neuroglial cell origin, it was not considered appropriate to combine the various neoplasm types in this study. In contrast to the isoprene study, glial cell neoplasms were observed in male Sprague-Dawley rats exposed to 1,3-butadiene and were considered as a possible exposure-related effect (Melnick and Huff, 1992). While the pattern of brain neoplasms observed in the isoprene study is not necessarily consistent with that expected with chemical induction, the occurrence of seven brain neoplasms in exposed females must be considered to be at least potentially related to isoprene exposure. Spontaneous primary brain neoplasms are relatively uncommon in the F344/N rat, and the incidences are generally slightly greater in males than in females.

A summary of the sites with increased incidences of neoplasms in inhalation carcinogenicity studies of isoprene, 1,3-butadiene, and chloroprene is provided in Table 9. There are remarkable similarities in sites affected within a given species. Also, different responses between rats and mice are noted for the three chemicals.

TABLE 9
Summary of Sites of Increased Incidences of Neoplasms in 2-Year Inhalation Studies of Isoprene, 1,3-Butadiene, and Chloroprene in Male and Female Rats and Mice

	Isoprene[a]	1,3-Butadiene[b]	Chloroprene[c]
Rats			
	Kidney (M)	Brain (M)	Kidney (M,F)
	Mammary Gland (M,F)	Mammary Gland (F)	Lung (M)
	Testis (M)	Pancreas (M)	Mammary Gland (F)
		Testis (M)	Oral Cavity (M,F)
		Thyroid Gland (F)	Thyroid Gland (M,F)
		Uterus (F)	
		Zymbal's Gland (F)	
Mice			
	Circulatory System (Heart and Spleen Hemangiosarcoma) (M,F)	Circulatory System (Heart and Spleen Hemangiosarcoma) (M,F)	Circulatory System (M,F)
	Forestomach (M)	Forestomach (M)	Forestomch (M,F)
	Harderian Gland (M,F)	Harderian Gland (M,F)	Harderian Gland (M,F)
	Hematopoietic System (M)	Hematopoietic System (M,F)	Kidney (M,F)
	Liver (M)	Liver (M)	Liver (F)
	Lung (M)	Lung (M,F)	Lung (M,F)
	Pituitary Gland (F)	Kidney (M)	Mammary Gland (F)
		Mammary Gland (F)	Mesentery (F)
		Ovary (F)	Skin (F)
		Preputial Gland (M)	Zymbal's Gland (F)

[a] Current study in F344/N rats and studies in male B6C3F$_1$ mice exposed to 700 to 7,000 ppm (Melnick *et al.*, 1994; NTP, 1995) or exposed to 70 to 2,200 ppm (Placke *et al.*, 1996). The only study in female mice used 70 ppm as the highest exposure concentration.
[b] Study in Sprague-Dawley rats exposed to 1,000 and 8,000 ppm (Owen *et al.*, 1987) and study in B6C3F$_1$ mice exposed to 6.25 to 625 ppm (Melnick *et al.*, 1990a; NTP, 1993)
[c] Studies in F344/N rats and B6C3F$_1$ mice exposed to 12.8 to 80 ppm (NTP, 1998)

The mammary gland was the most common target site for carcinogenicity of isoprene, 1,3-butadiene, and chloroprene in rats and mice (Owen *et al.*, 1987; Melnick *et al.*, 1990a; NTP, 1993, 1995, 1998; Melnick *et al.*, 1994; Placke *et al.*, 1996). In female rats, the incidences and multiplicities of fibroadenoma were increased at all exposure concentrations of isoprene, 1,3-butadiene, and chloroprene. With each of these chemicals, the incidence in the highest exposure group was not very different from the incidence in the lowest exposure group. Although isoprene produced significantly increased incidences of fibroadenoma at concentrations of 220 ppm or greater and 1,3-butadiene produced significantly increased incidences of fibroadenoma at concentrations of 1,000 ppm or greater, it is difficult to conclude that isoprene is more active than 1,3-butadiene because lower exposure concentrations of the latter chemical were not tested. In any case, isoprene appears to be at least as active as 1,3-butadiene in inducing mammary gland neoplasms in female rats.

Unlike 1,3-butadiene, isoprene also induced exposure-related increases in the incidences of mammary gland neoplasms and renal neoplasms in male rats. In these respects, isoprene elicits greater carcinogenic activity than 1,3-butadiene. Chemically induced neoplasms in the mammary gland of male rats are uncommon, occurring in only four of nearly 500 studies conducted by the NTP. Glycidol, an epoxide alkylating agent, also induced mammary gland neoplasms in male and female rats (NTP, 1990), and similarly, the carcinogenic effects of isoprene may be mediated by its epoxide intermediates.

The testis was also a common site of carcinogenesis in rats exposed to isoprene or 1,3-butadiene. Although strain differences in the spontaneous incidences of interstitial cell adenoma of the testis in F344/N rats and Sprague-Dawley rats complicate comparisons, a greater increase in incidence was observed at 700 ppm isoprene than at 1,000 ppm 1,3-butadiene, and isoprene induced exposure-related increases in the incidences of bilateral interstitial cell adenoma. Hence, isoprene appears to be more active than 1,3-butadiene at inducing testicular neoplasms.

Comparisons of results of carcinogenicity studies of isoprene with those of 1,3-butadiene in male B6C3F$_1$ mice reveal several common sites of neoplasia (Melnick *et al.*, 1996; Placke *et al.*, 1996). A comprehensive comparison of the carcinogenic effects of these two chemicals in female mice is not possible because the only reported study of isoprene carcinogenicity in female mice used 70 ppm as the highest exposure concentration (Placke *et al.*, 1996). In that study, incidences of splenic hemangiosarcoma, harderian gland adenoma, and pituitary gland adenoma were increased in the 70 ppm group compared to the chamber controls. However, Placke *et al.* (1996) did not detect increases in incidences of neoplasms of the lung, liver, mammary gland, or ovary as had been observed in female mice exposed to 62.5 ppm or lower concentrations of 1,3-butadiene for 2 years (Melnick *et al.*, 1990a; NTP, 1993). Studies with higher exposure concentrations of isoprene in female mice would be necessary to conclude whether isoprene is also carcinogenic at these sites. In male mice, 1,3-butadiene appears to be more active than isoprene at inducing neoplasms of the forestomach, heart (hemangiosarcoma), hematopoietic system, and lung when comparisons are based on exposure concentrations that produced significant increases in neoplasm incidence.

The mechanisms of carcinogenesis of 1,3-butadiene, isoprene, and chloroprene have not been fully determined. As the parent compounds are not mutagenic *per se*, it is likely that metabolism to reactive alkylating intermediates is involved in their carcinogenicity. The monoepoxide intermediates of isoprene metabolism were not mutagenic in *Salmonella typhimurium*, but the diepoxide intermediate was as mutagenic as diepoxybutane (Gervasi *et al.*, 1985). However, based on the greater incidences of neoplasms of the mammary gland, kidney, and testis in male rats exposed to 7,000 ppm compared to 700 ppm isoprene, potential involvement of the parent compound cannot be discounted. Dose response analyses, as described below, can help clarify the issue of possible involvement of the parent compound in the carcinogenic process resulting from exposure to isoprene. Differences in stability, distribution, and reactivity of the various metabolic intermediates, as well as differences in DNA repair, may account for species- and site-specific differences in the carcinogenic effects of 1,3-butadiene and its congeners. In addition, the carcinogenic pattern in rats exposed to isoprene (kidney in males but not in females, mammary gland in males and females, and neoplasms of the testis) suggests a possible hormonal involvement.

If the carcinogenic effects resulting from exposure to isoprene are similarly mediated by the epoxide intermediates rather than the parent compound, then estimating tissue concentrations of the isoprene epoxides using biologically based models should provide a stronger scientific basis for species- and dose-response extrapolations. A physiologically based pharmacokinetic model for isoprene was developed to examine dose-response relationships associated with exposure to isoprene (Appendix H). The pharmacokinetic model permits estimation of dose measures (e.g., tissue levels of epoxide intermediates) that could serve as indices of risk in evaluations of dose-response relationships.

Though several assumptions were used to develop the isoprene pharmacokinetic model (e.g., biochemical parameters for isoprene metabolism that have not been reported in the literature were assumed to be equivalent to those for 1,3-butadiene metabolism), and tissue time-course data for isoprene and its epoxide metabolites are not available, the model reproduced diverse sets of experimental data related to the toxicokinetics of isoprene in rats (e.g., isoprene uptake in closed chambers, urinary excretion of vinyl lactic acid after inhalation exposure, and disposition of intraperitoneally administered isoprene). Consequently, the model was used to predict cumulative concentrations of isoprene and isoprene monoepoxides in the blood, kidney, liver, and lung of rats exposed to

isoprene by inhalation for 1 week (5 exposure days followed by 2 nonexposure days) at the same concentrations used in the 2-year carcinogenicity study. Because calculated tissue concentrations of isoprene monoepoxides return to their control values during the 2 days of nonexposure, cumulative tissue doses over 2 years of exposure are proportional to the 1-week values. The cumulative concentrations of isoprene and isoprene monoepoxides in blood were used in the dose-response analyses because changes in the other tissues with increasing isoprene exposure were similar to those in blood.

The shapes of the dose-response curves and the ED_{10} values for carcinogenic effects of isoprene in rats were estimated by fitting a modified Weibull model (Portier *et al.*, 1986) to the Poly-3 survival-adjusted neoplasm rates presented in Table 10. Three dose metrics were included in these analyses: isoprene exposure, cumulative blood isoprene concentrations, and cumulative isoprene monoepoxide concentrations. If the estimated shape parameter is significantly greater than one, the resulting dose response has more curvature than a linear model (shape parameter equal to one) and exhibits a sublinear "threshold-like" behavior. If the estimated shape parameter is significantly less than one, the dose response is very steep (supralinear) in the low-dose region. The ED_{10} values represent the exposure concentration associated with an excess cancer risk of 10% at each site.

Neither the cumulative blood concentrations of isoprene nor isoprene monoepoxide provided a better fit with the neoplasm data than did external isoprene exposure. For most of the isoprene-induced neoplastic effects, the dose-responses were consistent with a linear model. The dose response for mammary gland neoplasms in female rats had an estimated shape parameter of zero with confidence bounds that did not include one (indicating a supralinear response). The incidences of mammary gland neoplasms in all exposed groups of female rats were greater than those in the chamber control group and were nearly equal at each of the three concentrations studied. These data do not provide sufficient information to characterize the dose response at exposures lower than those used in the present studies.

Several shape parameter values that appeared to deviate from unity were not significant because large confidence limits surrounded these values. The large confidence limits were due in part to the small number of exposure groups used in the carcinogenicity studies. The shape parameter value for mammary gland neoplasms in male rats nearly achieved significance, suggesting a sublinear response, when the dose was expressed as cumulative blood isoprene monoepoxide concentration. The shape parameter values for the three male rat neoplasm responses were numerically greater than one (not significant) when the dose metric was cumulative blood isoprene monoepoxide concentration and numerically less than one (not significant) when isoprene exposure or cumulative blood isoprene concentration was used as the dose metric. The latter two measures of dose are interchangeable because cumulative blood isoprene concentrations were proportional to isoprene exposure.

To better visualize the impact of the different dose metrics (isoprene exposure and cumulative blood isoprene epoxide concentration) on the dose-response relationships, dose response curves for the four sites of isoprene-induced neoplasia in F344/N rats are shown in Figures 8a and 8b. A saturable response is evident at each site when the neoplasm response is plotted against the isoprene exposure concentration (Figure 8a); however, when the neoplasm response is plotted against the cumulative blood isoprene monoepoxide concentration, the increased incidences of renal and testicular neoplasms in male rats appear to be proportional to the increase in dose (Figure 8b). The twofold increase in renal neoplasm response between 700 and 7,000 ppm is nearly the same as the increase in blood concentration of isoprene monoepoxides. These findings support the view that the carcinogenic effects of isoprene in the kidney are mediated by the epoxide intermediates and that the less-than-proportional response between 700 and 7,000 ppm is due to saturation of the cytochrome P_{450}-mediated oxidation of isoprene.

TABLE 10
Dose Response Parameters (Shape and ED_{10}) for Isoprene-Induced Neoplasms in F344/N Rats Based on Isoprene Exposures or 1-Week Cumulative Blood Levels of Isoprene or Isoprene Monoepoxide

Neoplasm	Dose Metric					
	Isoprene Exposure[a] (ppm)		Blood Isoprene[b] (μmol/L·7 days)		Blood Isoprene Monoepoxide[c] (μmol/L·7 days)	
	Shape (LCL/UCL)[d]	ED_{10} (LCL/UCL)	Shape (LCL/UCL)	ED_{10} (LCL/UCL)	Shape (LCL/UCL)	ED_{10} (LCL/UCL)
Male						
Mammary Gland Neoplasms	0.659 (0.328/>10)	378 (48/6,870)	0.565 (0.279/>10)	838 (66/25,730)	2.37 (0.947/>10)	7,860 (3,100/17,100)
Renal Tubule Adenoma	0.480 (0.148/2.08)	350 (6.5/3,880)	0.348 (0.169/1.66)	312 (23/12,400)	1.54 (0.403/5.96)	6,910 (1,010/14,100)
Testicular Adenoma	0.729 (0.221/1.72)	23 (<0.01/283)	0.604 (0.111/1.57)	29 (<0.01/774)	2.11 (0.482/6.42)	2,760 (21/7,400)
Female						
Mammary Gland Neoplasms	0 (0.0/0.27)	<0.1 (<0.01/3.6)	0.108 (0.03/0.467)	<0.1 (<0.01/625)	0.222 (0.08/1.01)	0.1 (<0.01/2,270)

[a] Isoprene exposures are 0, 220, 700, and 7,000 ppm.
[b] Cumulative blood isoprene concentrations are 38.7, 584, 2,160, and 26,200 μmol/L·7 days (Table H8).
[c] Cumulative blood isoprene monoepoxide concentrations are 426, 4,920, 9,620, and 17,400 μmol/L·7 days (Table H8).
[d] LCL=95% lower confidence limit; UCL=95% upper confidence limit

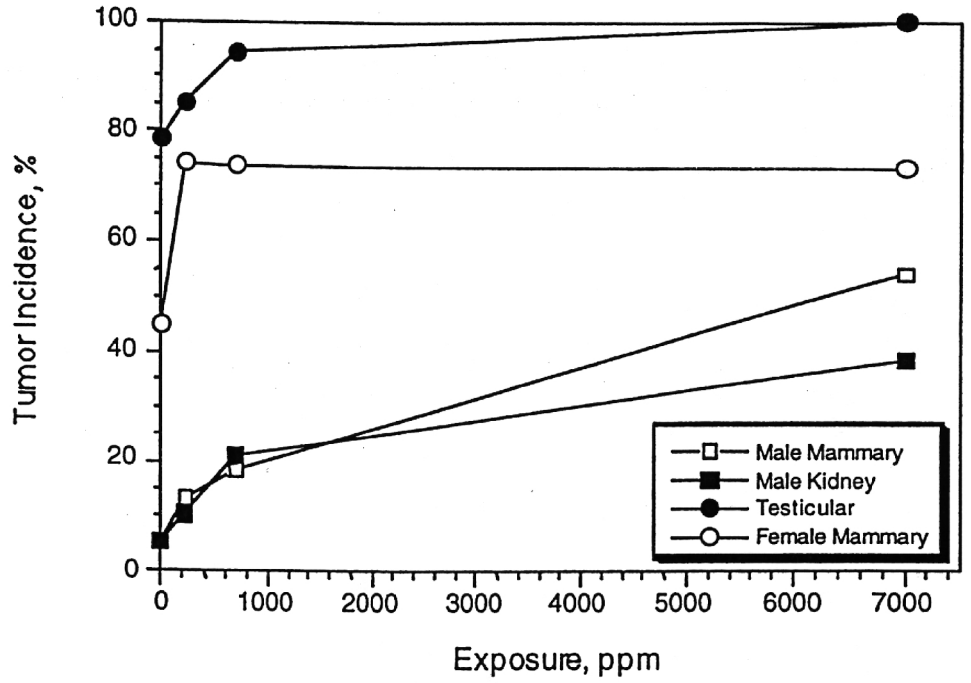


FIGURE 8a
Dose Response for Neoplasms Induced in F344/N Rats Exposed to Isoprene by Inhalation for 2 Years [Dose is expressed as external isoprene exposure (ppm)]

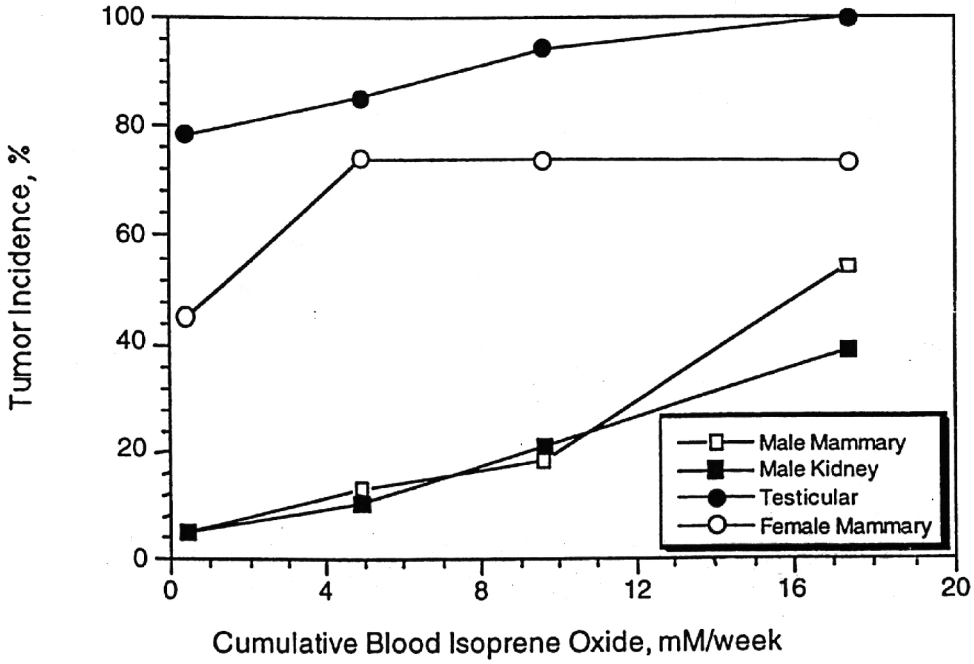


FIGURE 8b
Dose Response for Neoplasms Induced in F344/N Rats Exposed to Isoprene by Inhalation for 2 Years (Dose is expressed as cumulative blood concentration of isoprene oxide)

As noted previously, the carcinogenic effects of isoprene in the male mammary gland nearly achieved significance for a sublinear dose-response when dose was expressed as the cumulative blood concentration of isoprene epoxides. The greater response at 7,000 ppm suggests that other factors may be contributing to this effect, e.g., a combination of effects from isoprene epoxides plus the parent compound. Extrapolations of the male rat mammary gland neoplasm response (linear versus sublinear) to doses lower than those used in this study will require more information on the actual causal agent(s) (isoprene epoxides and/or isoprene).

ED_{10} values derived from dose-response curves may vary depending on the dose metric that is used, as well as the extrapolation model. Isoprene exposures that are associated with the ED_{10} values shown in Table 10 when cumulative blood isoprene monoepoxide concentration was used as the dose metric in male rats were approximately 390 ppm for mammary gland neoplasms, 320 ppm for renal tubule adenoma, and 100 ppm for testicular adenoma. Only the latter value appeared to differ from ED_{10} values obtained with isoprene exposure as the dose meteric. Because of the small number of exposure groups used in the carcinogenicity studies, the confidence intervals surrounding the estimated ED_{10} values were large and it was not possible to distinguish definitively differences in these values with the three different dose metrics. Greater credibility is given to those values that incorporate biologically based estimates of dose.

CONCLUSIONS

Under the conditions of this 2-year inhalation study, there was *clear evidence of carcinogenic activity** of isoprene in male F344/N rats based on increased incidences of mammary gland fibroadenoma and carcinoma, renal tubule adenoma, and testicular interstitial cell adenoma. There was *some evidence of carcinogenic activity* of isoprene in female F344/N rats based on increased incidences and multiplicity of mammary gland fibroadenoma. A low incidence of rare brain neoplasms in exposed female rats may have been due to exposure to isoprene.

Exposure to isoprene by inhalation for 2 years resulted in increased incidences of renal tubule hyperplasia and splenic fibrosis in male rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR INHALATION STUDY OF ISOPRENE

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

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	28	29	30	30
Natural deaths	4	5	5	5
Survivors				
Died last week of study			1	
Terminal sacrifice	18	16	14	15
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(49)	(49)	(47)	(50)
Intestine large, rectum	(47)	(49)	(46)	(50)
Anus, leiomyoma			1 (2%)	
Anus, squamous cell papilloma		1 (2%)		
Intestine large, cecum	(48)	(47)	(46)	(50)
Intestine small, jejunum	(46)	(46)	(47)	(47)
Carcinoma				1 (2%)
Intestine small, ileum	(46)	(47)	(47)	(49)
Liver	(50)	(50)	(50)	(50)
Hepatocellular carcinoma		1 (2%)		
Hepatocellular adenoma		2 (4%)		
Histiocytic sarcoma		1 (2%)		
Osteosarcoma, metastatic, bone				1 (2%)
Mesentery	(8)	(12)	(11)	(11)
Oral mucosa	(1)	(1)		
Gingival, sarcoma	1 (100%)			
Pancreas	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	1 (2%)	3 (6%)	
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Leiomyosarcoma			1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Carcinoid tumor benign		1 (2%)		
Tongue			(1)	(1)
Squamous cell papilloma				1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Carcinoma				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant	3 (6%)	1 (2%)	1 (2%)	
Pheochromocytoma benign	13 (26%)	11 (22%)	16 (32%)	15 (30%)
Bilateral, pheochromocytoma benign	5 (10%)	4 (8%)	4 (8%)	3 (6%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Endocrine System (continued)				
Islets, pancreatic	(50)	(50)	(50)	(49)
Adenoma	1 (2%)	2 (4%)	5 (10%)	1 (2%)
Carcinoma	2 (4%)		1 (2%)	1 (2%)
Parathyroid gland	(48)	(46)	(45)	(47)
Adenoma		1 (2%)		
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, adenoma	39 (78%)	40 (80%)	39 (78%)	31 (63%)
Thyroid gland	(49)	(50)	(49)	(50)
C-cell, adenoma	3 (6%)	4 (8%)	3 (6%)	3 (6%)
C-cell, carcinoma	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Follicular cell, adenoma	1 (2%)	1 (2%)	1 (2%)	
Follicular cell, carcinoma	1 (2%)	1 (2%)	1 (2%)	
General Body System				
Peritoneum	(2)		(1)	(3)
Tissue NOS	(1)	(2)		
Osteosarcoma		1 (50%)		
Sarcoma		1 (50%)		
Schwannoma malignant	1 (100%)			
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	1 (2%)	3 (6%)	3 (6%)
Carcinoma	3 (6%)	2 (4%)	2 (4%)	
Prostate	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma	1 (2%)			
Seminal vesicle	(47)	(48)	(47)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	20 (40%)	29 (58%)	37 (74%)	48 (96%)
Interstitial cell, adenoma	13 (26%)	8 (16%)	7 (14%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Lymph node	(6)	(7)	(7)	(9)
Lymph node, bronchial	(44)	(39)	(42)	(35)
Histiocytic sarcoma		1 (3%)		
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (3%)		
Lymph node, mandibular	(49)	(46)	(46)	(44)
Lymph node, mesenteric	(50)	(49)	(49)	(50)
Histiocytic sarcoma		1 (2%)		
Lymph node, mediastinal	(46)	(44)	(45)	(44)
Histiocytic sarcoma		1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Sarcoma		1 (2%)		
Thymus	(47)	(44)	(49)	(47)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Integumentary System				
Mammary gland	(42)	(43)	(47)	(44)
Carcinoma		1 (2%)	1 (2%)	2 (5%)
Fibroadenoma	1 (2%)	3 (7%)	6 (13%)	14 (32%)
Fibroadenoma, multiple	1 (2%)	1 (2%)		7 (16%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Keratoacanthoma	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Lipoma	1 (2%)			1 (2%)
Squamous cell carcinoma	1 (2%)		1 (2%)	1 (2%)
Squamous cell papilloma		1 (2%)		
Trichoepithelioma			1 (2%)	
Pinna, melanoma malignant				1 (2%)
Sebaceous gland, adenoma		1 (2%)		
Subcutaneous tissue, fibroma		4 (8%)	1 (2%)	2 (4%)
Subcutaneous tissue, fibrosarcoma			1 (2%)	
Subcutaneous tissue, lipoma		1 (2%)		
Subcutaneous tissue, schwannoma malignant			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteoma		1 (2%)		
Osteosarcoma				1 (2%)
Vertebra, chordoma		1 (2%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant				1 (2%)
Granular cell tumor benign			1 (2%)	
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma	1 (2%)			3 (6%)
Carcinoma, metastatic, thyroid gland				1 (2%)
Chordoma, metastatic, bone		1 (2%)		
Histiocytic sarcoma		1 (2%)		
Melanoma malignant, metastatic, skin				1 (2%)
Osteosarcoma, metastatic, bone				1 (2%)
Osteosarcoma, metastatic, uncertain primary site				1 (2%)
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		
Squamous cell carcinoma		1 (2%)		
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Nose	(50)	(50)	(49)	(50)
Special Senses System				
Zymbal's gland		(1)	(1)	(1)
Carcinoma		1 (100%)	1 (100%)	1 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Lipoma			1 (2%)	
Mesenchymal tumor benign				1 (2%)
Renal tubule, adenoma		2 (4%)	2 (4%)	6 (12%)
Renal tubule, carcinoma			1 (2%)	
Urinary bladder	(50)	(49)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Leukemia mononuclear	24 (48%)	26 (52%)	25 (50%)	25 (50%)
Mesothelioma benign	2 (4%)	2 (4%)	1 (2%)	
Mesothelioma malignant	3 (6%)	1 (2%)	1 (2%)	4 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	50	49	48
Total primary neoplasms	150	166	174	184
Total animals with benign neoplasms	49	50	49	48
Total benign neoplasms	108	125	135	139
Total animals with malignant neoplasms	34	33	30	34
Total malignant neoplasms	42	41	39	45
Total animals with metastatic neoplasms		2		5
Total metastatic neoplasms		3		6
Total animals with malignant neoplasms of uncertain primary site				1

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: Chamber Control

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

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: Chamber Control

Number of Days on Study	6 9 4	7 0 4	7 2 1	7 2 3	7 2 3	7 2 4	7 2 8	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	
Carcass ID Number	0 3 7	0 1 4	0 3 5	0 1 1	0 4 1	0 4 5	0 1 6	0 0 4	0 0 5	0 1 0	0 1 3	0 2 2	0 2 4	0 2 8	0 3 2	0 3 3	0 4 3	0 4 8	0 4 9	0 0 6	0 3 1	0 3 4	0 3 8	0 3 9	0 4 2	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery						+													+						+	8
Oral mucosa								+																		1
Gingival, sarcoma								X																		1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X															X									3
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant			X														X						X			3
Pheochromocytoma benign	X			X			X			X			X					X				X	X	X	X	13
Bilateral, pheochromocytoma benign											X			X	X											5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma														X												1
Carcinoma															X			X								2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X	X	X	X	39
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma															X											3
C-cell, carcinoma												X														1
Follicular cell, adenoma		X																								1
Follicular cell, carcinoma																									X	1
General Body System																										
Peritoneum											+															2
Tissue NOS																										1
Schwannoma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: Chamber Control

Number of Days on Study	2 3 5	3 4 2	4 4 0	4 5 4	4 6 8	4 8 2	5 0 2	5 1 8	5 3 9	5 4 7	5 5 1	5 5 5	5 6 4	5 9 2	5 9 7	6 0 0	6 0 4	6 5 2	6 5 2	6 5 9	6 6 4	6 7 1	6 7 2	6 7 5	6 8 6
Carcass ID Number	0 1 2	0 0 2	0 2 1	0 0 3	0 1 7	0 1 8	0 4 6	0 0 8	0 2 5	0 2 3	0 3 6	0 2 6	0 4 0	0 1 9	0 3 0	0 1 5	0 4 4	0 0 1	0 0 9	0 2 9	0 5 0	0 4 7	0 0 7	0 2 7	0 2 0
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X										
Carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma						X								X				X						X	
Interstitial cell, adenoma				X	X										X		X		X		X	X	X		
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+								+												
Lymph node, bronchial	+	M	M	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	M	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	M	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Fibroadenoma																									
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma																									
Lipoma																						X			
Squamous cell carcinoma				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: Chamber Control

Number of Days on Study	6 9 4	7 0 4	7 2 1	7 2 3	7 2 3	7 2 4	7 2 8	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 4 4	7 4 4	7 4 4	
Carcass ID Number	0 0 3 7	0 0 1 4	0 0 3 5	0 0 1 1	0 0 1 1	0 0 4 5	0 0 1 6	0 0 0 4	0 0 0 5	0 0 1 0	0 0 1 3	0 0 2 2	0 0 2 4	0 0 2 8	0 0 3 2	0 0 3 3	0 0 4 3	0 0 4 8	0 0 4 9	0 0 6	0 0 3 1	0 0 3 4	0 0 3 8	0 0 3 9	0 0 4 2	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma										X																2
Carcinoma											X		X									X				3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma														X												1
Seminal vesicle	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X		X			X	X	X	X	X	X	X		X		X	X	X	X	X	X					20
Interstitial cell, adenoma				X											X							X		X	X	13
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+						+						+		+											6
Lymph node, bronchial	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mediastinal	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	42
Fibroadenoma						X																				1
Fibroadenoma, multiple																								X		1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma													X													1
Keratoacanthoma																	X									1
Lipoma																										1
Squamous cell carcinoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma																					X					1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																						+				1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: Chamber Control

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: Chamber Control

Number of Days on Study	6 9 4	7 0 4	7 2 1	7 2 3	7 2 3	7 2 4	7 2 8	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 3 7	0 1 4	0 3 5	0 1 1	0 4 1	0 4 5	0 1 6	0 0 4	0 0 5	0 1 0	0 1 3	0 2 2	0 2 4	0 2 8	0 3 2	0 3 3	0 4 3	0 4 8	0 4 9	0 0 6	0 3 1	0 3 4	0 3 8	0 3 9	0 4 2	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X	X		X		X	X			X			X			X	X	X	X		X	X			24
Mesothelioma benign														X							X					2
Mesothelioma malignant											X															3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 220 ppm

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 220 ppm

Number of Days on Study	6 9 3	6 9 8	7 0 2	7 0 2	7 0 3	7 1 4	7 1 7	7 1 9	7 2 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	2 2 0 2 2	2 0 9	2 0 2 0	2 4 8	2 4 9	2 0 4	2 2 9	2 1 4	2 1 1	2 1 0 1	2 0 0 7	2 1 5	2 1 6	2 2 3	2 2 8	2 3 3	2 3 4	2 3 5	2 3 6	2 3 7	2 4 4	2 4 6	2 1 0	2 1 7	2 2 7	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Anus, squamous cell papilloma				X																						1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma					X																					1
Hepatocellular adenoma																						X				2
Histiocytic sarcoma																										1
Mesentery					+						+						+				+			+		12
Oral mucosa																								+		1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma										X																1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoid tumor benign		X																								1
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign										X	X		X			X										11
Bilateral, pheochromocytoma benign						X									X		X									4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																	X									2
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	46
Adenoma																								X		1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X		X	X	X	X	X	X			X	X	X		X	X	X	X	X		X	X	X	X	40
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma				X					X			X						X								4
C-cell, carcinoma																										1
Follicular cell, adenoma							X																			1
Follicular cell, carcinoma													X													1
General Body System																										
Tissue NOS																										2
Osteosarcoma																										1
Sarcoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 220 ppm

Number of Days on Study	3 9 8	4 2 0	4 5 5	4 9 9	5 1 0	5 3 5	5 3 8	5 4 9	5 5 9	5 8 1	5 8 8	5 9 2	5 9 7	6 0 2	6 0 4	6 2 9	6 3 5	6 5 2	6 5 7	6 5 7	6 5 7	6 6 4	6 7 0	6 7 1	6 8 2
Carcass ID Number	2 1 8	2 3 2	2 4 5	2 4 0	2 1 9	2 2 5	2 0 2	2 2 1	2 0 3	2 4 2	2 3 0	2 0 5	2 1 3	2 1 2	2 0 4	2 0 8	2 4 6	2 5 0	2 4 1	2 1 2	2 2 6	2 3 8	2 3 9	2 4 7	2 4 3	2 3 1
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma	X																								
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma							X				X	X		X			X		X	X	X			X	X
Interstitial cell, adenoma	X					X																			
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+	+							+						+					
Lymph node, bronchial	+	+	M	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+
Histiocytic sarcoma								X																	
Pheochromocytoma malignant, metastatic, adrenal medulla												X													
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Lymph node, mediastinal	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Histiocytic sarcoma								X																	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Sarcoma																									X
Thymus	M	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+
Carcinoma																									
Fibroadenoma																X	X								
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma																									
Squamous cell papilloma																									
Sebaceous gland, adenoma											X														
Subcutaneous tissue, fibroma							X												X						
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteoma																									
Vertebra, chordoma																					X				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 220 ppm

Number of Days on Study	6 9 3	6 9 8	7 0 2	7 0 2	7 0 3	7 1 4	7 1 7	7 1 9	7 2 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 4 4	7 4 4	7 3 4	
Carcass ID Number	2 2 2	2 0 9	2 0 2	2 4 0	2 4 9	2 0 4	2 2 9	2 1 4	2 1 1	2 0 1	2 0 7	2 1 5	2 1 6	2 2 3	2 2 8	2 3 3	2 3 4	2 3 5	2 3 6	2 3 7	2 4 4	2 4 6	2 0 0	2 1 7	2 2 7	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																					X					1
Carcinoma						X																				2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
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	29
Interstitial cell, adenoma	X				X				X					X				X				X				8
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node															+		+							+		7
Lymph node, bronchial	+	M	+	M	M	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	39
Histiocytic sarcoma																										1
Pheochromocytoma malignant, metastatic, adrenal medulla																										1
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	M	+	+	+	44
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	M	I	43
Carcinoma							X																			1
Fibroadenoma														X												3
Fibroadenoma, multiple																				X						1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma														X												1
Keratoacanthoma											X														X	2
Squamous cell papilloma														X												1
Sebaceous gland, adenoma																										1
Subcutaneous tissue, fibroma					X						X															4
Subcutaneous tissue, lipoma																						X				1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteoma																					X					1
Vertebra, chordoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 220 ppm

Number of Days on Study	3 9 8	4 2 0	4 5 5	4 9 9	5 1 0	5 3 5	5 3 8	5 4 9	5 5 9	5 8 1	5 8 8	5 9 2	5 9 7	6 0 2	6 0 4	6 2 9	6 3 5	6 5 2	6 5 7	6 5 7	6 5 7	6 6 4	6 7 0	6 7 1	6 8 2
Carcass ID Number	2 1 8	2 3 2	2 4 5	2 4 0	2 1 9	2 2 5	2 0 2	2 2 1	2 0 3	2 4 2	2 3 0	2 0 5	2 1 3	2 2 4	2 0 8	2 4 6	2 5 0	2 4 1	2 1 2	2 2 6	2 3 8	2 3 9	2 4 7	2 4 3	2 3 1
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chordoma, metastatic, bone																					X				
Histiocytic sarcoma								X																	
Pheochromocytoma malignant, metastatic, adrenal medulla												X													
Squamous cell carcinoma																					X				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye											+			+											
Zymbal's gland																									
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma									X																
Urinary bladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Leukemia mononuclear				X		X	X							X		X	X	X	X	X					X
Mesothelioma benign																									
Mesothelioma malignant																					X				

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 220 ppm

Number of Days on Study	6 9 3	6 9 8	7 0 2	7 0 2	7 0 3	7 1 4	7 1 7	7 1 9	7 2 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	2 2 2	2 0 9	2 2 0	2 4 8	2 4 9	2 0 4	2 2 9	2 1 4	2 1 1	2 0 1	2 0 7	2 1 5	2 1 6	2 2 3	2 2 8	2 3 3	2 3 4	2 3 5	2 3 6	2 3 7	2 4 4	2 4 6	2 0 0	2 1 7	2 1 7	2 2 7	Total Tissues/ Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Chordoma, metastatic, bone																										1
Histiocytic sarcoma																										1
Pheochromocytoma malignant, metastatic, adrenal medulla																										1
Squamous cell carcinoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye						+																				3
Zymbal's gland		+																								1
Carcinoma		X																								1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma								X																		2
Urinary Bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear	X	X	X			X				X	X		X		X	X	X	X	X			X	X	X	X	26
Mesothelioma benign							X						X													2
Mesothelioma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 700 ppm

Number of Days on Study	4 5 4	4 5 4	4 6 9	5 1 0	5 1 8	5 2 1	5 3 7	5 4 1	5 4 7	5 5 5	5 6 6	5 6 6	5 7 6	5 8 0	5 9 1	5 9 4	6 2 1	6 5 5	6 6 4	6 6 7	6 6 7	6 6 8	6 7 0	6 7 1	6 8 1
Carcass ID Number	4 2 2	4 4 0	4 2 0	4 2 3	4 2 4	4 2 1	4 3 5	4 3 8	4 0 7	4 4 7	4 1 2	4 3 9	4 0 2	4 3 4	4 1 8	4 4 8	4 4 1	4 0 1	4 0 5	4 0 8	4 2 6	4 2 7	4 5 0	4 1 7	4 3 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	I	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Anus, leiomyoma																								X	
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery					+	+		+		+	+														+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																					X				
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Cardiovascular System																									
Blood vessel											+														
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign				X					X	X												X			X
Bilateral, pheochromocytoma benign											X														
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X														
Carcinoma																		X							
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X	X	X	X	X		X			X		X	X	X	X	X		X	X	X	X	X	X	X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma			X																						
C-cell, carcinoma																									
Follicular cell, adenoma																									
Follicular cell, carcinoma																									
General Body System																									
Peritoneum												+													
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma	X								X																
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma							X	X	X		X	X		X		X	X		X	X	X	X	X	X	X
Interstitial cell, adenoma		X		X						X					X			X							

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 700 ppm

Number of Days on Study	6 8 3	6 8 4	6 8 6	6 9 2	6 9 5	7 0 6	7 1 2	7 2 2	7 2 7	7 2 8	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	4 3 0	4 4 6	4 1 1	4 0 9	4 4 2	4 2 5	4 0 4	4 1 3	4 1 6	4 3 1	4 0 3	4 1 9	4 2 8	4 2 9	4 3 3	4 3 6	4 3 7	4 4 3	4 4 4	4 4 5	4 4 9	4 0 6	4 1 0	4 1 4	4 1 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Anus, leiomyoma																										1
Intestine large, cecum	+	+	+	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery							+	+			+				+										+	11
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X												X											3
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue	+																									1
Cardiovascular System																										
Blood vessel					+																					2
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	X			X		16
Bilateral, pheochromocytoma benign					X					X					X											4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X			X											X	X			5
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X		X	X	X	X	X	X	X	X	X	X	X	X			X	X		X		X	X		X	39
Thyroid gland	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma														X					X							3
C-cell, carcinoma				X																						1
Follicular cell, adenoma																		X								1
Follicular cell, carcinoma														X												1
General Body System																										
Peritoneum																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma												X					X						X			3
Carcinoma																										2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																				X						1
Seminal vesicle	+	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
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	37
Interstitial cell, adenoma		X	X																							7

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 700 ppm

Number of Days on Study	4 5 4	4 5 4	4 6 9	5 1 0	5 1 8	5 2 1	5 3 7	5 4 1	5 4 7	5 5 5	5 6 6	5 6 6	5 7 6	5 8 0	5 9 1	5 9 4	6 2 1	6 5 5	6 6 4	6 6 7	6 6 7	6 6 8	6 7 0	7 7 1	6 8 1
Carcass ID Number	4 2 2	4 4 0	4 2 0	4 2 3	4 2 4	4 2 1	4 3 5	4 3 8	4 0 7	4 4 7	4 1 2	4 3 9	4 0 2	4 3 4	4 4 8	4 1 8	4 4 1	4 0 1	4 0 5	4 0 8	4 2 6	4 2 7	4 5 0	4 1 7	4 3 2
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Lymph node				+												+		+						+	
Lymph node, bronchial	M	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	M	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mediastinal	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma															X				X						X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma																									
Squamous cell carcinoma																									
Trichoepithelioma																									
Subcutaneous tissue, fibroma															X										
Subcutaneous tissue, fibrosarcoma														X											
Subcutaneous tissue, schwannoma malignant														X											
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor benign																									
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye													+												
Zymbal's gland		+																							
Carcinoma		X																							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																				X					
Renal tubule, adenoma												X													
Renal tubule, carcinoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X			X	X	X	X						X	X	X	X		X			X	
Mesothelioma benign																									
Mesothelioma malignant												X													

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 700 ppm

Number of Days on Study	6 8 3	6 8 4	6 8 6	6 9 2	6 9 5	7 0 6	7 1 2	7 2 2	7 2 7	7 2 8	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 4 4	7 4 4	7 3 4	
Carcass ID Number	4 3 0	4 4 6	4 1 1	4 0 9	4 4 2	4 2 5	4 0 4	4 1 3	4 1 6	4 3 1	4 0 3	4 1 9	4 2 8	4 2 9	4 3 3	4 3 6	4 3 7	4 4 3	4 4 4	4 4 5	4 4 9	4 0 6	4 1 0	4 1 4	4 1 5	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node													+		+						+					7
Lymph node, bronchial	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	42
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mediastinal	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	45
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Carcinoma												X														1
Fibroadenoma	X									X							X									6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma		X																								1
Keratoacanthoma																		X								1
Squamous cell carcinoma													X													1
Trichoepithelioma									X																	1
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granular cell tumor benign																		X								1
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																+							+			3
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lipoma																										1
Renal tubule, adenoma			X																							2
Renal tubule, carcinoma			X																							1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X	X		X		X		X		X	X	X	X			X			X	X	X			25
Mesothelioma benign															X											1
Mesothelioma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 7,000 ppm

Number of Days on Study	0262	2262	3619	4718	5117	5488	5606	5666	5811	5881	5888	6011	6112	6227	6218	6621	6722	6730	6964	6964	6967	6967	6968	6804	6881
Carcass ID Number	6005	6022	6029	6203	6040	6435	6348	6423	6437	6445	6501	6512	6528	6627	6717	6721	6808	6832	6909	6949	6942	6946	6938	6941	6918
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Carcinoma					X																				
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma, metastatic, bone																									
Mesentery								+														+		+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																+									
Squamous cell papilloma																X									
Cardiovascular System																									
Blood vessel																						+			
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma														X											
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign						X							X	X					X	X					
Bilateral, pheochromocytoma benign																						X		X	X
Islets, pancreatic	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																X									
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X		X	X	X		X		X	X	X	X	X			X	X	X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma									X																
C-cell, carcinoma													X												
General Body System																									
Peritoneum				+																	+				M
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 7,000 ppm

Number of Days on Study	6 8 5	6 8 6	6 9 1	7 0 8	7 0 9	7 2 1	7 2 1	7 2 3	7 2 7	7 2 7	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	6 1 1	6 3 3	6 5 0	6 3 0	6 1 9	6 0 2	6 4 7	6 1 6	6 1 5	6 4 3	6 0 4	6 0 6	6 1 3	6 2 0	6 2 4	6 2 5	6 2 6	6 3 1	6 3 4	6 3 6	6 4 4	6 4 7	6 0 7	6 1 0	6 1 4	6 1 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Carcinoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, bone										X																1
Mesentery				+		+			+	+		+					+	+								11
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell papilloma																										1
Cardiovascular System																										
Blood vessel				+													+									3
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign			X	X	X	X	X									X					X	X	X	X		15
Bilateral, pheochromocytoma benign																										3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Carcinoma																				X						1
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X		X			X	X	X			X	X		X	X	X		X	X	X	X	X	X	X	X	31
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																				X					X	3
C-cell, carcinoma														X										X		3
General Body System																										
Peritoneum									+																	3
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma										X	X			X												3
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	X	48

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 7,000 ppm

Number of Days on Study	0 2 3 4 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6
	2 2 6 7 1 4 6 6 8 8 8 8 1 1 1 2 2 2 3 6 6 6 6 6 8
	6 2 1 8 7 8 0 6 1 1 8 8 1 2 8 1 2 0 4 4 7 7 8 0 1
Carcass ID Number	6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
	0 2 2 0 4 3 4 2 3 4 0 1 2 2 1 2 0 3 0 4 4 4 4 3 4
	5 2 9 3 0 5 8 3 7 5 1 2 8 7 7 1 8 2 9 9 2 6 8 1 8
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ + + + + + + + +
Lymph node, bronchial	+ M + + + M + + + + + + + M + + + + + + + + M + M
Lymph node, mandibular	+ + + + + + + + + + + + + M + + + + + + + + + +
Lymph node, mesenteric	+ +
Lymph node, mediastinal	M + + + + + + + + + + + M + + + + + + M + + + + M
Spleen	+ +
Thymus	+ + + + + + + + + + + + + + + + + + + M + + + + +
Integumentary System	
Mammary gland	M + M + + + + + M + + + + + + + + + + + M + + + +
Carcinoma	
Fibroadenoma	X
Fibroadenoma, multiple	X X X
Skin	+ +
Basal cell adenoma	X
Keratoacanthoma	
Lipoma	
Squamous cell carcinoma	
Pinna, melanoma malignant	
Subcutaneous tissue, fibroma	X
Musculoskeletal System	
Bone	+ +
Osteosarcoma	
Nervous System	
Brain	+ +
Astrocytoma malignant	
Respiratory System	
Larynx	+ +
Lung	+ +
Alveolar/bronchiolar carcinoma	X X
Carcinoma, metastatic, thyroid gland	X
Melanoma malignant, metastatic, skin	
Osteosarcoma, metastatic, bone	
Osteosarcoma, metastatic, uncertain primary site	
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	
Nose	+ +
Trachea	+ +
Special Senses System	
Eye	+
Zymbal's gland	+
Carcinoma	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 7,000 ppm

Number of Days on Study	6 8 5	6 8 6	6 9 1	7 0 8	7 0 9	7 2 1	7 2 1	7 2 3	7 2 7	7 2 7	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	6 1 1	6 1 3	6 5 0	6 3 0	6 1 9	6 0 2	6 4 7	6 1 6	6 1 5	6 4 3	6 0 4	6 0 6	6 1 3	6 2 0	6 2 4	6 2 5	6 2 6	6 3 1	6 3 4	6 3 6	6 4 4	6 4 7	6 0 1	6 1 4	6 1 3	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node												+			+									+		9
Lymph node, bronchial	M	M	+	+	M	+	+	+	M	M	M	+	M	+	+	M	+	+	+	M	+	+	M	+	+	35
Lymph node, mandibular	M	+	M	+	+	+	+	+	M	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	44
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	44
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	44
Carcinoma						X											X									2
Fibroadenoma	X	X		X	X	X	X							X	X	X	X			X				X	X	14
Fibroadenoma, multiple										X									X		X	X				7
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																										1
Keratoacanthoma			X																				X			2
Lipoma										X																1
Squamous cell carcinoma		X																								1
Pinna, melanoma malignant				X																						1
Subcutaneous tissue, fibroma															X											2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma										X																1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant								X																		1
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma									X																	3
Carcinoma, metastatic, thyroid gland																										1
Melanoma malignant, metastatic, skin				X																						1
Osteosarcoma, metastatic, bone										X																1
Osteosarcoma, metastatic, uncertain primary site															X											1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung									X																	1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																				+						2
Zymbal's gland																										1
Carcinoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 7,000 ppm

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Inhalation Study of Isoprene: 7,000 ppm

Number of Days on Study	6 8 5	6 8 6	6 9 1	7 0 8	7 0 9	7 2 1	7 2 1	7 2 3	7 2 7	7 2 7	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	6 1 1	6 3 3	6 5 0	6 3 0	6 1 9	6 0 2	6 4 7	6 1 6	6 1 5	6 4 3	6 0 4	6 0 6	6 1 3	6 2 0	6 2 4	6 2 5	6 2 6	6 3 1	6 3 4	6 3 6	6 4 4	6 0 7	6 1 0	6 1 4	6 3 9	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesenchymal tumor benign																										1
Renal tubule, adenoma				X		X		X											X							6
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X		X	X	X		X		X		X	X		X	X	X			X				X	X	25
Mesothelioma malignant									X																	4

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	18/50 (36%)	15/50 (30%)	20/50 (40%)	18/50 (36%)
Adjusted rate[b]	46.3%	37.0%	49.7%	44.8%
Terminal rate[c]	10/18 (56%)	6/16 (38%)	9/15 (60%)	5/15 (33%)
First incidence (days)	539	538	510	548
Poly-3 test[d]	P=0.530	P=0.264N	P=0.469	P=0.535N
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	0/50 (0%)
Adjusted rate	8.2%	2.6%	2.7%	0.0%
Terminal rate	2/18 (11%)	0/16 (0%)	1/15 (7%)	0/15 (0%)
First incidence (days)	721	592	733 (T)	—[e]
Poly-3 test	P=0.182N	P=0.293N	P=0.302N	P=0.115N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	20/50 (40%)	16/50 (32%)	20/50 (40%)	18/50 (36%)
Adjusted rate	51.4%	39.0%	49.7%	44.8%
Terminal rate	11/18 (61%)	6/16 (38%)	9/15 (60%)	5/15 (33%)
First incidence (days)	539	538	510	548
Poly-3 test	P=0.496N	P=0.179N	P=0.530N	P=0.351N
Kidney (Renal Tubule): Adenoma (Single Sections)				
Overall rate	0/50 (0%)	2/50 (4%)	2/50 (4%)	6/50 (12%)
Adjusted rate	0.0%	5.3%	5.3%	15.8%
Terminal rate	0/18 (0%)	0/16 (0%)	0/15 (0%)	1/15 (7%)
First incidence (days)	—	559	566	630
Poly-3 test	P=0.009	P=0.244	P=0.242	P=0.016
Kidney (Renal Tubule): Adenoma (Step Sections)				
Overall rate	2/50 (4%)	2/50 (4%)	6/50 (12%)	10/50 (20%)
Adjusted rate	5.4%	5.3%	16.0%	26.1%
Terminal rate	0/18 (0%)	1/16 (6%)	4/15 (27%)	4/15 (27%)
First incidence (days)	704	702	566	622
Poly-3 test	P=0.004	P=0.688N	P=0.134	P=0.014
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	2/50 (4%)	4/50 (8%)	8/50 (16%)	15/50 (30%)
Adjusted rate	5.4%	10.5%	21.0%	38.5%
Terminal rate	0/18 (0%)	1/16 (6%)	4/15 (27%)	5/15 (33%)
First incidence (days)	704	559	566	622
Poly-3 test	P<0.001	P=0.352	P=0.047	P<0.001
Kidney (Renal Tubule): Adenoma or Carcinoma (Single Sections)				
Overall rate	0/50 (0%)	2/50 (4%)	2/50 (4%)	6/50 (12%)
Adjusted rate	0.0%	5.3%	5.3%	15.8%
Terminal rate	0/18 (0%)	0/16 (0%)	0/15 (0%)	1/15 (7%)
First incidence (days)	—	559	566	630
Poly-3 test	P=0.009	P=0.244	P=0.242	P=0.016
Kidney (Renal Tubule): Adenoma or Carcinoma (Single and Step Sections)				
Overall rate	2/50 (4%)	4/50 (8%)	8/50 (16%)	15/50 (30%)
Adjusted rate	5.4%	10.5%	21.0%	38.5%
Terminal rate	0/18 (0%)	1/16 (6%)	4/15 (27%)	5/15 (33%)
First incidence (days)	704	559	566	622
Poly-3 test	P<0.001	P=0.352	P=0.047	P<0.001

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	7.9%	0.0%	0.0%
Terminal rate	0/18 (0%)	1/16 (6%)	0/15 (0%)	0/15 (0%)
First incidence (days)	—	592	—	—
Poly-3 test	P=0.324N	P=0.124	—[f]	—
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	1/49 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.7%	0.0%	0.0%	7.9%
Terminal rate	1/18 (6%)	0/16 (0%)	0/15 (0%)	0/15 (0%)
First incidence (days)	733 (T)	—	—	630
Poly-3 test	P=0.043	P=0.495N	P=0.498N	P=0.317
Mammary Gland: Fibroadenoma				
Overall rate	2/50 (4%)	4/50 (8%)	6/50 (12%)	21/50 (42%)
Adjusted rate	5.4%	10.5%	15.8%	54.2%
Terminal rate	1/18 (6%)	2/16 (13%)	1/15 (7%)	10/15 (67%)
First incidence (days)	724	629	591	664
Poly-3 test	P<0.001	P=0.353	P=0.140	P<0.001
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	7/50 (14%)	21/50 (42%)
Adjusted rate	5.4%	13.1%	18.4%	54.2%
Terminal rate	1/18 (6%)	2/16 (13%)	2/15 (13%)	10/15 (67%)
First incidence (days)	724	629	591	664
Poly-3 test	P<0.001	P=0.229	P=0.083	P<0.001
Pancreas: Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	8.1%	2.7%	8.0%	0.0%
Terminal rate	1/18 (6%)	1/16 (6%)	1/15 (7%)	0/15 (0%)
First incidence (days)	600	733 (T)	667	—
Poly-3 test	P=0.132N	P=0.302N	P=0.663N	P=0.117N
Pancreatic Islets: Adenoma				
Overall rate	1/50 (2%)	2/50 (4%)	5/50 (10%)	1/49 (2%)
Adjusted rate	2.7%	5.3%	13.3%	2.7%
Terminal rate	1/18 (6%)	1/16 (6%)	3/15 (20%)	0/15 (0%)
First incidence (days)	733 (T)	592	566	621
Poly-3 test	P=0.323N	P=0.511	P=0.103	P=0.757N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	6/50 (12%)	2/49 (4%)
Adjusted rate	8.2%	5.3%	15.9%	5.3%
Terminal rate	3/18 (17%)	1/16 (6%)	3/15 (20%)	1/15 (7%)
First incidence (days)	733 (T)	592	566	621
Poly-3 test	P=0.343N	P=0.485N	P=0.252	P=0.490N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	39/50 (78%)	40/50 (80%)	39/50 (78%)	31/49 (63%)
Adjusted rate	86.0%	84.4%	82.9%	73.5%
Terminal rate	16/18 (89%)	12/16 (75%)	10/15 (67%)	13/15 (87%)
First incidence (days)	342	420	454	560
Poly-3 test	P=0.066N	P=0.529N	P=0.444N	P=0.095N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Preputial Gland: Adenoma				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	5.4%	2.7%	8.1%	8.1%
Terminal rate	1/18 (6%)	1/16 (6%)	3/15 (20%)	2/15 (13%)
First incidence (days)	597	733 (T)	733 (T)	727
Poly-3 test	P=0.390	P=0.497N	P=0.497	P=0.501
Preputial Gland: Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	0/50 (0%)
Adjusted rate	8.2%	5.2%	5.2%	0.0%
Terminal rate	3/18 (17%)	0/16 (0%)	0/15 (0%)	0/15 (0%)
First incidence (days)	733 (T)	398	454	—
Poly-3 test	P=0.123N	P=0.480N	P=0.481N	P=0.115N
Preputial Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	3/50 (6%)	5/50 (10%)	3/50 (6%)
Adjusted rate	13.5%	7.8%	13.1%	8.1%
Terminal rate	4/18 (22%)	1/16 (6%)	3/15 (20%)	2/15 (13%)
First incidence (days)	597	398	454	727
Poly-3 test	P=0.393N	P=0.336N	P=0.614N	P=0.353N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	2.7%	8.0%	2.7%	5.4%
Terminal rate	1/18 (6%)	3/16 (19%)	1/15 (7%)	1/15 (7%)
First incidence (days)	733 (T)	733 (T)	733 (T)	691
Poly-3 test	P=0.593	P=0.311	P=0.760N	P=0.506
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rate	5.3%	8.0%	5.4%	8.0%
Terminal rate	1/18 (6%)	3/16 (19%)	2/15 (13%)	1/15 (7%)
First incidence (days)	454	733 (T)	733 (T)	686
Poly-3 test	P=0.499	P=0.500	P=0.690	P=0.501
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Squamous Cell Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted rate	8.0%	8.0%	10.8%	10.5%
Terminal rate	2/18 (11%)	3/16 (19%)	2/15 (13%)	1/15 (7%)
First incidence (days)	454	733 (T)	684	622
Poly-3 test	P=0.487	P=0.664	P=0.495	P=0.507
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	0/50 (0%)	4/50 (8%)	1/50 (2%)	2/50 (4%)
Adjusted rate	0.0%	10.4%	2.7%	5.3%
Terminal rate	0/18 (0%)	1/16 (6%)	0/15 (0%)	1/15 (7%)
First incidence (days)	—	538	591	588
Poly-3 test	P=0.587	P=0.065	P=0.504	P=0.242
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	0/50 (0%)	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rate	0.0%	10.4%	5.3%	5.3%
Terminal rate	0/18 (0%)	1/16 (6%)	0/15 (0%)	1/15 (7%)
First incidence (days)	—	538	580	588
Poly-3 test	P=0.642	P=0.065	P=0.244	P=0.242

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Testes: Adenoma				
Overall rate	33/50 (66%)	37/50 (74%)	44/50 (88%)	48/50 (96%)
Adjusted rate	78.4%	85.3%	94.4%	99.9%
Terminal rate	16/18 (89%)	16/16 (100%)	15/15 (100%)	15/15 (100%)
First incidence (days)	454	398	454	361
Poly-3 test	P<0.001	P=0.259	P=0.010	P<0.001
Thyroid Gland (C-cell): Adenoma				
Overall rate	3/49 (6%)	4/50 (8%)	3/49 (6%)	3/50 (6%)
Adjusted rate	8.1%	10.6%	8.2%	8.0%
Terminal rate	1/18 (6%)	2/16 (13%)	2/15 (13%)	2/15 (13%)
First incidence (days)	482	702	469	581
Poly-3 test	P=0.546N	P=0.506	P=0.659	P=0.657N
Thyroid Gland (C-cell): Carcinoma				
Overall rate	1/49 (2%)	1/50 (2%)	1/49 (2%)	3/50 (6%)
Adjusted rate	2.8%	2.7%	2.8%	8.0%
Terminal rate	1/18 (6%)	0/16 (0%)	0/15 (0%)	2/15 (13%)
First incidence (days)	733 (T)	657	692	611
Poly-3 test	P=0.171	P=0.750N	P=0.760N	P=0.321
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	4/49 (8%)	5/50 (10%)	4/49 (8%)	6/50 (12%)
Adjusted rate	10.8%	13.2%	10.9%	15.7%
Terminal rate	2/18 (11%)	2/16 (13%)	2/15 (13%)	4/15 (27%)
First incidence (days)	482	657	469	581
Poly-3 test	P=0.348	P=0.512	P=0.641	P=0.383
All Organs: Mononuclear Cell Leukemia				
Overall rate	24/50 (48%)	26/50 (52%)	25/50 (50%)	25/50 (50%)
Adjusted rate	57.5%	61.9%	58.8%	59.3%
Terminal rate	9/18 (50%)	12/16 (75%)	8/15 (53%)	8/15 (53%)
First incidence (days)	468	499	510	361
Poly-3 test	P=0.565N	P=0.424	P=0.542	P=0.525
All Organs: Benign or Malignant Mesothelioma				
Overall rate	5/50 (10%)	3/50 (6%)	2/50 (4%)	4/50 (8%)
Adjusted rate	13.3%	7.9%	5.3%	10.4%
Terminal rate	3/18 (17%)	1/16 (6%)	1/15 (7%)	0/15 (0%)
First incidence (days)	539	657	566	478
Poly-3 test	P=0.544	P=0.353N	P=0.216N	P=0.489N
All Organs: Benign Neoplasms				
Overall rate	49/50 (98%)	50/50 (100%)	49/50 (98%)	48/50 (96%)
Adjusted rate	99.9%	100.0%	99.3%	99.9%
Terminal rate	18/18 (100%)	16/16 (100%)	15/15 (100%)	15/15 (100%)
First incidence (days)	342	398	454	361
Poly-3 test	P=0.996	P=1.000	P=0.968N	P=1.000
All Organs: Malignant Neoplasms				
Overall rate	34/50 (68%)	33/50 (66%)	30/50 (60%)	34/50 (68%)
Adjusted rate	76.4%	73.5%	66.6%	75.0%
Terminal rate	15/18 (83%)	12/16 (75%)	9/15 (60%)	9/15 (60%)
First incidence (days)	235	398	454	361
Poly-3 test	P=0.456	P=0.470N	P=0.197N	P=0.539N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	49/50 (98%)	48/50 (96%)
Adjusted rate	100.0%	100.0%	99.3%	99.9%
Terminal rate	18/18 (100%)	16/16 (100%)	15/15 (100%)	15/15 (100%)
First incidence (days)	235	398	454	361
Poly-3 test	P=0.997	—	P=0.961N	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, lung, pancreas, 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 chamber control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE A4a
Historical Incidence of Mammary Gland Neoplasms in Chamber Control Male F344/N Rats[a]

Study	Incidence in Controls			
	Fibroadenoma	Adenoma	Carcinoma	Fibroadenoma, Adenoma, or Carcinoma
Historical Incidence at Battelle Pacific Northwest Laboratories				
Acetonitrile	0/48	0/48	0/48	0/48
2-Chloroacetophenone	0/50	0/50	0/50	0/50
Colbalt sulfate heptahydrate	3/50	0/50	0/50	3/50
l-Epinephrine hydrochloride	2/50	0/50	0/50	2/50
Hexachlorocyclopentadiene	0/50	0/50	0/50	0/50
Isobutyraldehyde	1/50	0/50	0/50	1/50
Molybdenum trioxide	0/50	0/50	0/50	0/50
Nitromethane	1/50	0/50	0/50	1/50
o-Chlorobenzalmalononitrile (CS-2)	1/50	0/50	0/50	1/50
Ozone	2/50	0/50	0/50	2/50
Tetrafluoroethylene	2/50	0/50	0/50	2/50
Tetrahydrofuran	0/50	0/50	0/50	0/50
Overall Historical Incidence				
Total	17/905 (1.9%)	1/905 (0.1%)	1/905 (0.1%)	19/905 (2.1%)
Standard deviation	2.0%	0.5%	0.5%	1.9%
Range	0%-6%	0%-2%	0%-2%	0%-6%

[a] Data as of 15 October 1996

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

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Pacific Northwest Laboratories			
Acetonitrile	1/48	0/48	1/48
2-Chloroacetophenone	1/49	0/49	1/49
Colbalt sulfate heptahydrate	1/50	0/50	1/50
l-Epinephrine hydrochloride	0/50	0/50	0/50
Hexachlorocyclopentadiene	0/50	0/50	0/50
Isobutyraldehyde	0/50	0/50	0/50
Molybdenum trioxide	1/50	0/50	1/50
Nitromethane	0/50	0/50	0/50
o-Chlorobenzalmalononitrile (CS-2)	1/50	0/50	1/50
Ozone	2/50	0/50	2/50
Tetrafluoroethylene	0/50	1/50	1/50
Tetrahydrofuran	1/50	0/50	1/50
Overall Historical Incidence			
Total	9/902 (1.0%)	1/902 (0.1%)	10/902 (1.1%)
Standard deviation	1.2%	0.5%	1.2%
Range	0%-4%	0%-2%	0%-4%

[a] Data as of 15 October 1996

TABLE A4c
Historical Incidence of Testicular Adenoma in Chamber Control Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Battelle Pacific Northwest Laboratories	
Acetonitrile	33/48
2-Chloroacetophenone	29/50
Colbalt sulfate heptahydrate	35/50
l-Epinephrine hydochloride	39/50
Hexachlorocyclopentadiene	38/50
Isobutyraldehyde	30/50
Molybdenum trioxide	39/50
Nitromethane	38/50
o-Chlorobenzalmalononitrile (CS-2)	31/50
Ozone	27/50
Tetrafluoroethylene	39/50
Tetrahydrofuran	23/50
Overall Historical Incidence	
Total	628/905 (69.4%)
Standard deviation	9.7%
Range	46%-83%

[a] Data as of 15 October 1996

TABLE A4d
Historical Incidence of Brain Neoplasms in Chamber Control Male F344/N Rats[a]

Study	Incidence in Controls			
	Benign Astrocytoma	Malignant Astrocytoma	Malignant Glioma	Benign Granular Cell Tumor
Historical Incidence at Battelle Pacific Northwest Laboratories				
Acetonitrile	0/48	0/48	1/48	0/48
2-Chloroacetophenone	0/49	0/49	1/49	0/49
Colbalt sulfate heptahydrate	0/50	1/50	0/50	0/50
l-Epinephrine hydrochloride	0/50	0/50	0/50	0/50
Hexachlorocyclopentadiene	0/50	0/50	0/50	0/50
Isobutyraldehyde	0/50	0/50	0/50	0/50
Molybdenum trioxide	0/50	0/50	0/50	0/50
Nitromethane	0/50	0/50	0/50	0/50
o-Chlorobenzalmalononitrile (CS-2)	0/50	0/50	0/50	0/50
Ozone	0/50	0/50	1/50	0/50
Tetrafluoroethylene	0/50	0/50	0/50	0/50
Tetrahydrofuran	0/50	0/50	0/50	0/50
Overall Historical Incidence				
Total	0/904	1/904 (0.1%)	3/904 (0.3%)	0/904
Standard deviation		0.5%	0.8%	
Range		0%-2%	0%-2%	

[a] Data as of 15 October 1996

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

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	28	29	30	30
Natural deaths	4	5	5	5
Survivors				
Died last week of study			1	
Terminal sacrifice	18	16	14	15
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, rectum	(47)	(49)	(46)	(50)
Inflammation, acute			2 (4%)	
Intestine large, cecum	(48)	(47)	(46)	(50)
Diverticulum			1 (2%)	
Inflammation, acute	1 (2%)			
Mineralization			1 (2%)	
Intestine small, duodenum	(49)	(49)	(48)	(49)
Inflammation, acute	1 (2%)		1 (2%)	1 (2%)
Necrosis	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Intestine small, ileum	(46)	(47)	(47)	(49)
Inflammation, chronic active			1 (2%)	
Necrosis			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)		5 (10%)	3 (6%)
Basophilic focus	17 (34%)	13 (26%)	13 (26%)	16 (32%)
Clear cell focus	6 (12%)	9 (18%)	7 (14%)	8 (16%)
Degeneration, cystic	9 (18%)	9 (18%)	14 (28%)	17 (34%)
Eosinophilic focus	2 (4%)	4 (8%)	3 (6%)	3 (6%)
Fatty change	15 (30%)	12 (24%)	9 (18%)	11 (22%)
Hematopoietic cell proliferation	1 (2%)			1 (2%)
Hepatodiaphragmatic nodule	1 (2%)	1 (2%)	4 (8%)	4 (8%)
Infarct				1 (2%)
Inflammation, acute			1 (2%)	
Inflammation, chronic active		1 (2%)		
Inflammation, granulomatous		1 (2%)		
Mineralization	1 (2%)			
Mixed cell focus				1 (2%)
Necrosis		1 (2%)	1 (2%)	1 (2%)
Regeneration	2 (4%)		2 (4%)	6 (12%)
Artery, inflammation, chronic active				1 (2%)
Bile duct, hyperplasia	25 (50%)	32 (64%)	36 (72%)	29 (58%)
Centrilobular, necrosis	7 (14%)	11 (22%)	10 (20%)	9 (18%)
Mesentery	(8)	(12)	(11)	(11)
Thrombosis			1 (9%)	
Artery, mineralization			1 (9%)	2 (18%)
Fat, hemorrhage	1 (13%)	1 (8%)	1 (9%)	1 (9%)
Fat, inflammation, chronic active			1 (9%)	1 (9%)
Fat, necrosis	6 (75%)	10 (83%)	8 (73%)	6 (55%)
Oral mucosa	(1)	(1)		
Pharyngeal, hyperplasia, squamous		1 (100%)		

[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 Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Atrophy	18 (36%)	23 (46%)	23 (46%)	15 (30%)
Basophilic focus	1 (2%)	4 (8%)	1 (2%)	3 (6%)
Hyperplasia	4 (8%)	6 (12%)	9 (18%)	7 (14%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)
Hyperplasia, squamous		1 (2%)	1 (2%)	1 (2%)
Inflammation, acute	1 (2%)		1 (2%)	
Mineralization			2 (4%)	
Necrosis	14 (28%)	8 (16%)	10 (20%)	12 (24%)
Stomach, glandular	(50)	(50)	(50)	(50)
Mineralization	5 (10%)	1 (2%)	4 (8%)	4 (8%)
Necrosis	6 (12%)	3 (6%)	7 (14%)	9 (18%)
Tongue			(1)	(1)
Hyperplasia			1 (100%)	
Tooth		(1)		
Developmental malformation		1 (100%)		
Cardiovascular System				
Blood vessel	(1)		(2)	(3)
Inflammation	1 (100%)			
Aorta, mineralization			2 (100%)	3 (100%)
Endothelium, hyperplasia	1 (100%)			
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	41 (82%)	44 (88%)	44 (88%)	38 (76%)
Artery, mineralization	1 (2%)		2 (4%)	4 (8%)
Atrium, thrombosis	4 (8%)	3 (6%)	3 (6%)	5 (10%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			1 (2%)
Degeneration, cystic				1 (2%)
Hyperplasia	23 (46%)	23 (46%)	19 (38%)	20 (40%)
Hypertrophy	8 (16%)	11 (22%)	4 (8%)	4 (8%)
Necrosis			1 (2%)	
Thrombosis				1 (2%)
Vacuolization cytoplasmic	2 (4%)	6 (12%)	3 (6%)	6 (12%)
Adrenal medulla	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Hyperplasia	19 (38%)	19 (38%)	25 (50%)	21 (42%)
Thrombosis				1 (2%)
Bilateral, hyperplasia			2 (4%)	
Islets, pancreatic	(50)	(50)	(50)	(49)
Hyperplasia		1 (2%)	3 (6%)	
Parathyroid gland	(48)	(46)	(45)	(47)
Cyst		1 (2%)		
Hyperplasia	5 (10%)	6 (13%)	8 (18%)	13 (28%)
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, hyperplasia	5 (10%)	6 (12%)	9 (18%)	11 (22%)
Pars intermedia, hyperplasia				1 (2%)
Thyroid gland	(49)	(50)	(49)	(50)
C-cell, hyperplasia	30 (61%)	30 (60%)	30 (61%)	34 (68%)
Follicular cell, hyperplasia	1 (2%)	1 (2%)		

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)		1 (2%)	
Preputial gland	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		1 (2%)
Inflammation, chronic active	3 (6%)	1 (2%)	1 (2%)	3 (6%)
Prostate	(50)	(50)	(50)	(50)
Hyperplasia	11 (22%)	13 (26%)	15 (30%)	17 (34%)
Inflammation, chronic active	5 (10%)	2 (4%)	1 (2%)	6 (12%)
Seminal vesicle	(47)	(48)	(47)	(50)
Inflammation, chronic active	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)	1 (2%)	
Artery, inflammation, chronic active	6 (12%)	2 (4%)	1 (2%)	
Interstitial cell, hyperplasia	15 (30%)	13 (26%)	8 (16%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Hyperplasia, histiocytic		1 (2%)		
Myelofibrosis				1 (2%)
Necrosis				2 (4%)
Lymph node	(6)	(7)	(7)	(9)
Iliac, ectasia	1 (17%)			
Renal, hemorrhage				1 (11%)
Lymph node, mandibular	(49)	(46)	(46)	(44)
Infiltration cellular, plasma cell		2 (4%)		
Lymph node, mesenteric	(50)	(49)	(49)	(50)
Infiltration cellular, plasma cell				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Depletion cellular				1 (2%)
Fibrosis	11 (22%)	14 (28%)	24 (48%)	22 (44%)
Hematopoietic cell proliferation	3 (6%)	3 (6%)	1 (2%)	5 (10%)
Hemorrhage	1 (2%)			
Necrosis	3 (6%)		2 (4%)	1 (2%)
Thymus	(47)	(44)	(49)	(47)
Atrophy				1 (2%)
Cyst	1 (2%)			
Integumentary System				
Mammary gland	(42)	(43)	(47)	(44)
Galactocele	2 (5%)	4 (9%)	2 (4%)	3 (7%)
Inflammation, chronic	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	2 (4%)			2 (4%)
Inflammation, chronic active		1 (2%)	2 (4%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy	2 (4%)	1 (2%)	6 (12%)	7 (14%)
Hyperostosis		1 (2%)	1 (2%)	2 (4%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Degeneration, focal	1 (2%)			
Gliosis		1 (2%)		
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Inflammation, chronic, focal		1 (2%)		
Inflammation, chronic active	21 (43%)	16 (32%)	22 (44%)	15 (30%)
Mineralization			2 (4%)	2 (4%)
Thrombosis		1 (2%)		1 (2%)
Alveolar epithelium, hyperplasia	7 (14%)	11 (22%)	4 (8%)	10 (20%)
Artery, mediastinum, mineralization			2 (4%)	2 (4%)
Nose	(50)	(50)	(49)	(50)
Foreign body	4 (8%)	4 (8%)	3 (6%)	2 (4%)
Inflammation, suppurative	11 (22%)	9 (18%)	10 (20%)	9 (18%)
Thrombosis	2 (4%)	4 (8%)	10 (20%)	5 (10%)
Olfactory epithelium, atrophy	1 (2%)		8 (16%)	2 (4%)
Olfactory epithelium, metaplasia	7 (14%)	2 (4%)	1 (2%)	2 (4%)
Olfactory epithelium, metaplasia, squamous	1 (2%)			
Respiratory epithelium, hyperplasia		2 (4%)	2 (4%)	2 (4%)
Respiratory epithelium, metaplasia, squamous			1 (2%)	
Trachea	(50)	(50)	(50)	(50)
Mineralization			1 (2%)	
Special Senses System				
Eye	(1)	(3)	(3)	(2)
Cataract	1 (100%)	1 (33%)	3 (100%)	2 (100%)
Hemorrhage			1 (33%)	
Cornea, inflammation, chronic active		1 (33%)	1 (33%)	
Retina, atrophy	1 (100%)	2 (67%)	3 (100%)	2 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	2 (4%)		4 (8%)	2 (4%)
Infarct	1 (2%)	1 (2%)	2 (4%)	
Mineralization			2 (4%)	3 (6%)
Nephropathy	50 (100%)	50 (100%)	50 (100%)	49 (98%)
Renal tubule, hyperplasia		2 (4%)	6 (12%)	8 (16%)
Urinary bladder	(50)	(49)	(50)	(50)
Hemorrhage				1 (2%)
Inflammation, acute				1 (2%)
Inflammation, chronic active	1 (2%)			1 (2%)
Necrosis				1 (2%)
Transitional epithelium, hyperplasia	1 (2%)			

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF ISOPRENE

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

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	16	15	18	23
Natural deaths	5	5	4	
Survivors				
Terminal sacrifice	29	30	28	27
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(48)	(49)	(50)
Intestine large, cecum	(49)	(47)	(47)	(50)
Intestine small, duodenum	(49)	(48)	(50)	(50)
Fibroma		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Hepatocellular carcinoma				1 (2%)
Hepatocellular adenoma			1 (2%)	
Histiocytic sarcoma				1 (2%)
Mesentery	(9)	(10)	(8)	(11)
Oral mucosa		(1)	(1)	
Pharyngeal, squamous cell carcinoma		1 (100%)		
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma				1 (2%)
Stomach, glandular	(50)	(49)	(50)	(50)
Tongue	(1)		(2)	
Squamous cell papilloma	1 (100%)		1 (50%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma benign			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)			
Pheochromocytoma benign	1 (2%)	5 (10%)	4 (8%)	3 (6%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma	1 (2%)	2 (4%)		
Carcinoma			2 (4%)	1 (2%)
Parathyroid gland	(48)	(43)	(43)	(45)
Pituitary gland	(50)	(50)	(50)	(50)
Sarcoma, metastatic, brain		1 (2%)		
Pars distalis, adenoma	33 (66%)	27 (54%)	36 (72%)	27 (54%)
Pars distalis, carcinoma	1 (2%)		1 (2%)	
Thyroid gland	(48)	(50)	(49)	(50)
C-cell, adenoma	6 (13%)	4 (8%)	2 (4%)	2 (4%)
C-cell, carcinoma	2 (4%)	4 (8%)	2 (4%)	
Follicular cell, adenoma			1 (2%)	
Follicular cell, carcinoma				1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
General Body System				
None				
Genital System				
Clitoral gland	(47)	(48)	(46)	(48)
Adenoma	3 (6%)	5 (10%)	5 (11%)	6 (13%)
Carcinoma	2 (4%)	4 (8%)	2 (4%)	2 (4%)
Bilateral, carcinoma		1 (2%)		
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor malignant	1 (2%)			1 (2%)
Granulosa cell tumor benign	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Polyp stromal	8 (16%)	4 (8%)	5 (10%)	7 (14%)
Polyp stromal, multiple		1 (2%)	1 (2%)	1 (2%)
Sarcoma stromal	1 (2%)	1 (2%)		
Endometrium, adenoma	1 (2%)			
Hematopoietic System				
Bone marrow	(49)	(49)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Lymph node		(6)	(4)	(2)
Lymph node, bronchial	(42)	(37)	(34)	(35)
Lymph node, mandibular	(45)	(47)	(49)	(46)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Lymph node, mediastinal	(44)	(44)	(42)	(44)
Spleen	(50)	(50)	(50)	(50)
Sarcoma	1 (2%)			
Thymus	(48)	(45)	(39)	(46)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	4 (8%)	2 (4%)	1 (2%)	3 (6%)
Fibroadenoma	12 (24%)	23 (46%)	13 (26%)	15 (30%)
Fibroadenoma, multiple	7 (14%)	12 (24%)	19 (38%)	17 (34%)
Skin	(50)	(50)	(50)	(50)
Keratoacanthoma		1 (2%)		
Squamous cell papilloma	2 (4%)	1 (2%)		
Sebaceous gland, adenoma		1 (2%)		
Subcutaneous tissue, fibroma			1 (2%)	
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, lipoma				1 (2%)
Subcutaneous tissue, sarcoma	1 (2%)	1 (2%)		1 (2%)
Musculoskeletal System				
Skeletal muscle	(1)			
Rhabdomyosarcoma	1 (100%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma benign			1 (2%)	
Carcinoma, metastatic, pituitary gland	2 (4%)		1 (2%)	
Glioma malignant				1 (2%)
Medulloblastoma malignant				1 (2%)
Meninges, granular cell tumor benign		1 (2%)		1 (2%)
Meninges, sarcoma		1 (2%)		1 (2%)
Spinal cord				(1)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)			2 (4%)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, oral mucosa		1 (2%)		
Pleura				(1)
Special Senses System				
Zymbal's gland			(1)	
Carcinoma			1 (100%)	
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Mesenchymal tumor benign		1 (2%)		
Sarcoma, metastatic, urinary bladder		1 (2%)		
Renal tubule, adenoma			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Sarcoma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Leukemia mononuclear	14 (28%)	15 (30%)	21 (42%)	21 (42%)
Mesothelioma malignant				1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	48	49	48
Total primary neoplasms	109	121	124	120
Total animals with benign neoplasms	43	43	46	41
Total benign neoplasms	79	90	94	84
Total animals with malignant neoplasms	28	27	26	31
Total malignant neoplasms	30	31	30	36
Total animals with metastatic neoplasms	2	4	1	
Total metastatic neoplasms	2	4	1	

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: Chamber Control

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

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: Chamber Control

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	1 1 7	1 1 9	1 2 0	1 2 3	1 3 5	1 4 0	1 4 3	1 4 6	1 0 4	1 0 5	1 0 6	1 0 7	1 1 4	1 1 8	1 2 4	1 4 5	1 4 7	1 4 8	1 1 3	1 1 6	1 2 7	1 3 0	1 3 8	1 4 4	1 4 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	M	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery									+			+												+	+	9
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell papilloma																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																						X	X			2
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant			X																							1
Pheochromocytoma benign																			X							1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma	X																									1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X		X		X		X	X		X	X	X		X	X	X	X	X	X	X	X		X	X	X	33
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma				X		X			X						X						X					6
C-cell, carcinoma																	X		X							2
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	47
Adenoma							X															X				3
Carcinoma																					X					2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor malignant																										1
Granulosa cell tumor benign																							X			1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal		X			X					X					X											8
Sarcoma stromal																										1
Endometrium, adenoma																									X	1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: Chamber Control

Number of Days on Study	3 9 8	4 5 4	4 8 2	5 3 9	5 5 2	5 8 0	5 8 0	5 9 6	6 2 9	6 3 0	6 4 8	6 5 7	6 5 7	6 7 0	6 7 1	6 8 4	7 0 5	7 1 4	7 2 7	7 2 7	7 2 8	7 3 4	7 3 4	7 3 4
Carcass ID Number	1 2 2	1 2 1	1 0 9	1 3 9	1 3 5	1 2 5	1 3 6	1 0 8	1 2 6	1 0 1	1 3 7	1 3 1	1 3 4	1 5 0	1 3 2	1 0 2	1 3 3	1 4 1	1 2 8	1 4 2	1 2 9	1 0 3	1 1 0	1 1 1	1 1 2
Hematopoietic System																									
Bone marrow	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, bronchial	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	M	M	+	M	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mediastinal	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma										X															
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma								X			X	X			X		X			X		X			
Fibroadenoma, multiple										X														X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Subcutaneous tissue, fibrosarcoma																									
Subcutaneous tissue, sarcoma			X																						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
Rhabdomyosarcoma	X																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland					X																X				
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								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 Inhalation Study of Isoprene: Chamber Control

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	1 1 7	1 1 9	1 2 0	1 2 3	1 3 5	1 4 0	1 4 3	1 4 6	1 0 4	1 0 5	1 0 6	1 0 7	1 1 4	1 1 8	1 2 4	1 4 5	1 4 7	1 4 8	1 1 3	1 1 6	1 2 7	1 3 0	1 3 8	1 4 4	1 4 9	Total Tissues/ Tumors
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, bronchial	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	42
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	45
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mediastinal	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	M	+	44
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma																									1
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma	X							X				X		X											4
Fibroadenoma		X					X							X	X										12
Fibroadenoma, multiple	X							X	X												X	X			7
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma			X												X										2
Subcutaneous tissue, fibrosarcoma																							X		1
Subcutaneous tissue, sarcoma																									1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																									1
Rhabdomyosarcoma																									1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, pituitary gland																									2
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																									1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																									
Eye		+															+		+						5
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X								X							14

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: 220 ppm

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: 220 ppm

Number of Days on Study	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 4 8	3 1 1	3 1 2	3 1 5	3 1 7	3 1 8	3 2 0	3 2 8	3 3 9	3 4 3	3 4 4	3 4 4	3 0 2	3 0 3	3 0 7	3 1 3	3 1 9	3 2 2	3 2 3	3 2 3	3 3 3	3 3 4	3 4 1	3 4 2	3 4 7	3 5 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibroma																			X							1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery	+			+																+						10
Oral mucosa																										1
Pharyngeal, squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X																							1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign					X					X					X								X		X		5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma	X								X																	2
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	M	43
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, brain																										1
Pars distalis, adenoma	X		X		X	X		X			X	X			X	X		X	X						X	27
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma					X															X						4
C-cell, carcinoma						X															X		X			4
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma										X											X	X				5
Carcinoma															X	X										4
Bilateral, carcinoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal										X													X			4
Polyp stromal, multiple													X													1
Sarcoma stromal																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: 220 ppm

Number of Days on Study	3 7 0	4 8 2	5 8 7	6 0 1	6 0 2	6 2 2	6 2 6	6 3 8	6 4 3	6 6 4	6 7 0	6 7 0	6 8 5	6 8 6	6 9 3	6 9 4	6 9 5	7 1 4	7 2 7	7 2 7	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	3 3 6	3 4 9	3 0 8	3 0 1	3 0 5	3 1 4	3 2 6	3 3 7	3 4 0	3 3 0	3 0 4	3 2 9	3 0 9	3 3 5	3 2 7	3 2 4	3 1 6	3 4 6	3 2 5	3 3 8	3 0 6	3 1 0	3 1 1	3 3 5
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+				+	+									+		+	+						
Lymph node, bronchial	M	+	+	M	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	M	+	+	M
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mediastinal	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+
Integumentary System																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																		X						
Fibroadenoma		X	X					X		X	X		X	X			X					X	X		X
Fibroadenoma, multiple									X			X						X	X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma												X												
Squamous cell papilloma																								
Sebaceous gland, adenoma																								
Subcutaneous tissue, sarcoma																								
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Meninges, granular cell tumor benign																								
Meninges, sarcoma										X														
Respiratory System																								
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland							X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, oral mucosa															X									
Trachea	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Eye				+			+					+												
Urinary System																								
Kidney	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesenchymal tumor benign																								
Sarcoma, metastatic, urinary bladder																				X				
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																				X				
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 Inhalation Study of Isoprene: 220 ppm

Number of Days on Study	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 4 8	3 1 1	3 1 2	3 1 5	3 1 7	3 1 8	3 2 0	3 2 8	3 3 9	3 4 3	3 4 4	3 4 4	3 0 2	3 0 3	3 0 7	3 1 3	3 1 9	3 2 2	3 2 3	3 2 3	3 3 3	3 3 4	3 4 1	3 4 2	3 4 7	3 5 0	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node																										6
Lymph node, bronchial	M	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	M	M	+	+	M	+	+	+	M	37
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mediastinal	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	44
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	45
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																		X								2
Fibroadenoma	X		X						X	X	X	X	X	X		X	X		X		X					23
Fibroadenoma, multiple					X	X		X							X			X				X	X		X	12
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																										1
Squamous cell papilloma																							X			1
Sebaceous gland, adenoma					X																					1
Subcutaneous tissue, sarcoma							X																			1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Meninges, granular cell tumor benign																							X			1
Meninges, sarcoma																										1
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, oral mucosa																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye																+										4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Mesenchymal tumor benign																								X		1
Sarcoma, metastatic, urinary bladder																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X		X	X										X			15

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: 700 ppm

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: 700 ppm

Number of Days on Study	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	5 3 2	5 3 6	5 0 3	5 0 4	5 0 5	5 0 7	5 0 8	5 1 1	5 1 8	5 2 0	5 2 6	5 3 4	5 3 5	5 4 1	5 4 6	5 5 0	5 1 5	5 1 9	5 2 2	5 2 7	5 2 9	5 3 3	5 4 2	5 4 4	5 4 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																			X							1
Mesentery														+												8
Oral mucosa																+										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue		+																								2
Squamous cell papilloma		X																								1
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma benign																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X					X																			2
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign				X								X														4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma			X																							2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	M	+	43
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X			X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X		36
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma		X						X																		2
C-cell, carcinoma										X										X						2
Follicular cell, adenoma																									X	1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	46
Adenoma				X		X			X										X							5
Carcinoma							X																			2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal													X		X		X			X						5
Polyp stromal, multiple																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: 700 ppm

Number of Days on Study	1 1 1	4 2 7	5 0 8	5 1 0	5 3 9	5 4 7	5 6 6	5 8 0	5 8 1	6 0 4	6 1 1	6 1 7	6 3 8	6 7 2	6 9 9	7 0 8	7 1 0	7 1 0	7 1 3	7 2 1	7 2 3	7 2 7	7 3 4	7 3 4	7 3 4
Carcass ID Number	5 0 6	5 3 0	5 0 2	5 2 1	5 3 7	5 2 5	5 0 1	5 2 4	5 2 8	5 4 5	5 3 9	5 0 9	5 4 0	5 4 8	5 2 3	5 4 7	5 1 2	5 4 3	5 1 0	5 3 1	5 3 8	5 1 4	5 1 3	5 1 6	5 1 7
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+			+							+						+						
Lymph node, bronchial	M	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mediastinal	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma												X			X		X	X			X			X
Fibroadenoma, multiple					X									X					X	X		X			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma benign																									
Carcinoma, metastatic, pituitary gland																								X	
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																						+			
Zymbal's gland																									
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																								X	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	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 Inhalation Study of Isoprene: 700 ppm

Number of Days on Study	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	5 3 2	5 3 6	5 0 3	5 0 4	5 0 5	5 0 7	5 0 8	5 1 1	5 1 8	5 2 0	5 2 6	5 3 4	5 3 5	5 4 1	5 4 6	5 5 0	5 1 5	5 1 9	5 2 2	5 2 7	5 2 9	5 3 3	5 4 2	5 4 4	5 4 9	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										4
Lymph node, bronchial	+	M	+	M	+	+	M	M	+	+	M	M	M	M	M	+	M	+	+	+	+	M	+	+	+	34
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mediastinal	+	M	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	M	+	M	+	+	42
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	M	M	+	M	+	+	M	+	+	+	+	M	M	+	+	M	+	+	M	+	+	+	+	+	+	39
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma			X																							1
Fibroadenoma	X	X							X		X												X	X	X	13
Fibroadenoma, multiple			X		X	X	X	X		X				X	X	X	X	X	X		X	X				19
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																									X	1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma benign											X															1
Carcinoma, metastatic, pituitary gland																										1
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye									+																+	3
Zymbal's gland	+																									1
Carcinoma	X																									1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X		X				X	X				X				X					X	21

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: 7,000 ppm

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: 7,000 ppm

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	7 2 2	7 2 5	7 3 5	7 3 6	7 4 8	7 0 4	7 1 2	7 1 3	7 1 4	7 1 5	7 1 7	7 2 1	7 2 3	7 3 1	7 3 3	7 4 3	7 4 5	7 4 9	7 0 3	7 0 6	7 1 8	7 2 7	7 2 8	7 3 0	7 4 4	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																			X							1
Histiocytic sarcoma																										1
Mesentery				+						+							+		+							11
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma														X												1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																X										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																		X								3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X		X		X	X	X	X	X	X		X		X	X			X		X		X	X		X	27
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																							X			2
Follicular cell, carcinoma																										1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma						X				X			X				X						X			6
Carcinoma											X															2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor malignant						X																				1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal							X			X																7
Polyp stromal, multiple														X												1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Inhalation Study of Isoprene: 7,000 ppm

Number of Days on Study	1 1 4	4 1 5	4 3 2	4 6 4	4 8 2	4 9 0	4 9 7	5 9 4	5 9 4	6 2 1	6 2 5	6 3 0	6 5 2	6 5 3	6 6 4	6 6 4	6 8 1	6 8 1	7 0 9	7 1 3	7 1 3	7 1 4	7 2 7	7 3 4	7 3 4
Carcass ID Number	7 4 0 1	7 0 7	7 0 5	7 4 7	7 1 9	7 5 0	7 1 6	7 2 4	7 3 8	7 0 9	7 1 1	7 3 9	7 2 6	7 4 0	7 0 2	7 1 0	7 2 9	7 4 6	7 3 7	7 3 2	7 4 2	7 0 1	7 3 4	7 0 8	7 2 0
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																						X			
Lymph node		+											+												
Lymph node, bronchial	M	+	+	M	+	+	M	+	+	+	M	+	+	+	+	+	M	M	+	+	+	M	M	+	+
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mediastinal	+	+	M	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma			X																				X		
Fibroadenoma			X							X		X			X	X	X						X		X
Fibroadenoma, multiple											X							X	X		X	X		X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, sarcoma																		X							
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant																									
Medulloblastoma malignant					X																				
Meninges, granular cell tumor benign																									
Meninges, sarcoma																					X				
Spinal cord						+																			
Respiratory System																									
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pleura															+										
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																			+	+					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																						X			
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 Inhalation Study of Isoprene: 7,000 ppm

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	7 2 2	7 2 5	7 3 5	7 3 6	7 4 8	7 0 4	7 1 2	7 1 3	7 1 4	7 1 5	7 1 7	7 2 1	7 2 3	7 3 1	7 3 3	7 4 3	7 4 5	7 4 9	7 0 3	7 0 6	7 1 8	7 2 7	7 2 8	7 3 0	7 4 4	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node																										2
Lymph node, bronchial	+	+	+	+	M	M	+	+	+	M	+	+	+	+	+	M	M	+	+	M	+	M	+	+	+	35
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mediastinal	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	44
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma					X																					3
Fibroadenoma	X					X						X	X		X						X				X	15
Fibroadenoma, multiple					X		X	X		X	X			X		X	X	X	X	X						17
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, lipoma									X																	1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma malignant							X																			1
Medulloblastoma malignant																										1
Meninges, granular cell tumor benign									X																	1
Meninges, sarcoma																										1
Spinal cord																										1
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X																						2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pleura																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye			+			+		+										+								6
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear	X	X					X	X		X	X		X					X		X						21
Mesothelioma malignant																										1

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	1/50 (2%)	5/50 (10%)	4/50 (8%)	3/50 (6%)
Adjusted rate[b]	2.4%	11.4%	9.5%	7.3%
Terminal rate[c]	1/29 (3%)	5/30 (17%)	2/28 (7%)	1/27 (4%)
First incidence (days)	734 (T)	734 (T)	638	652
Poly-3 test[d]	P=0.617N	P=0.108	P=0.175	P=0.291
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	2/50 (4%)	5/50 (10%)	4/50 (8%)	3/50 (6%)
Adjusted rate	4.7%	11.4%	9.5%	7.3%
Terminal rate	2/29 (7%)	5/30 (17%)	2/28 (7%)	1/27 (4%)
First incidence (days)	734 (T)	734 (T)	638	652
Poly-3 test	P=0.541N	P=0.229	P=0.332	P=0.483
Clitoral Gland: Adenoma				
Overall rate	3/47 (6%)	5/48 (10%)	5/46 (11%)	6/48 (13%)
Adjusted rate	7.4%	11.8%	13.1%	15.4%
Terminal rate	2/27 (7%)	4/29 (14%)	4/26 (15%)	5/26 (19%)
First incidence (days)	596	670	708	681
Poly-3 test	P=0.284	P=0.379	P=0.322	P=0.220
Clitoral Gland: Carcinoma				
Overall rate	2/47 (4%)	5/48 (10%)	2/46 (4%)	2/48 (4%)
Adjusted rate	5.0%	11.7%	5.2%	5.1%
Terminal rate	1/27 (4%)	3/29 (10%)	1/26 (4%)	1/26 (4%)
First incidence (days)	714	482	617	594
Poly-3 test	P=0.422N	P=0.242	P=0.679	P=0.686
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	5/47 (11%)	10/48 (21%)	7/46 (15%)	8/48 (17%)
Adjusted rate	12.3%	23.3%	18.2%	20.3%
Terminal rate	3/27 (11%)	7/29 (24%)	5/26 (19%)	6/26 (23%)
First incidence (days)	596	482	617	594
Poly-3 test	P=0.449	P=0.153	P=0.341	P=0.253
Mammary Gland: Fibroadenoma				
Overall rate	19/50 (38%)	35/50 (70%)	32/50 (64%)	32/50 (64%)
Adjusted rate	42.9%	74.3%	73.7%	73.2%
Terminal rate	12/29 (41%)	23/30 (77%)	22/28 (79%)	20/27 (74%)
First incidence (days)	596	482	539	432
Poly-3 test	P=0.105	P<0.001	P=0.002	P=0.002
Mammary Gland: Carcinoma				
Overall rate	4/50 (8%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	9.4%	4.6%	2.4%	7.3%
Terminal rate	4/29 (14%)	1/30 (3%)	1/28 (4%)	2/27 (7%)
First incidence (days)	734 (T)	714	734 (T)	432
Poly-3 test	P=0.517	P=0.321N	P=0.184N	P=0.515N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	20/50 (40%)	35/50 (70%)	32/50 (64%)	32/50 (64%)
Adjusted rate	45.2%	74.3%	73.7%	73.2%
Terminal rate	13/29 (45%)	23/30 (77%)	22/28 (79%)	20/27 (74%)
First incidence (days)	596	482	539	432
Poly-3 test	P=0.127	P=0.003	P=0.004	P=0.004

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	33/50 (66%)	27/50 (54%)	36/50 (72%)	27/50 (54%)
Adjusted rate	72.2%	56.4%	78.6%	61.7%
Terminal rate	22/29 (76%)	14/30 (47%)	22/28 (79%)	18/27 (67%)
First incidence (days)	454	482	510	432
Poly-3 test	P=0.274N	P=0.078N	P=0.313	P=0.191N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	34/50 (68%)	27/50 (54%)	37/50 (74%)	27/50 (54%)
Adjusted rate	73.5%	56.4%	80.8%	61.7%
Terminal rate	22/29 (76%)	14/30 (47%)	23/28 (82%)	18/27 (67%)
First incidence (days)	454	482	510	432
Poly-3 test	P=0.230N	P=0.059N	P=0.269	P=0.155N
Thyroid Gland (C-cell): Adenoma				
Overall rate	6/48 (13%)	4/50 (8%)	2/49 (4%)	2/50 (4%)
Adjusted rate	14.4%	9.1%	4.9%	4.9%
Terminal rate	5/29 (17%)	3/30 (10%)	2/28 (7%)	1/27 (4%)
First incidence (days)	580	695	734 (T)	653
Poly-3 test	P=0.226N	P=0.334N	P=0.138N	P=0.137N
Thyroid Gland (C-cell): Carcinoma				
Overall rate	2/48 (4%)	4/50 (8%)	2/49 (4%)	0/50 (0%)
Adjusted rate	4.9%	9.0%	4.9%	0.0%
Terminal rate	2/29 (7%)	3/30 (10%)	2/28 (7%)	0/27 (0%)
First incidence (days)	734 (T)	626	734 (T)	—[e]
Poly-3 test	P=0.104N	P=0.371	P=0.691	P=0.240N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	8/48 (17%)	8/50 (16%)	4/49 (8%)	2/50 (4%)
Adjusted rate	19.2%	18.0%	9.8%	4.9%
Terminal rate	7/29 (24%)	6/30 (20%)	4/28 (14%)	1/27 (4%)
First incidence (days)	580	626	734 (T)	653
Poly-3 test	P=0.048N	P=0.554N	P=0.184N	P=0.046N
Uterus: Stromal Polyp				
Overall rate	8/50 (16%)	5/50 (10%)	6/50 (12%)	8/50 (16%)
Adjusted rate	18.4%	11.3%	14.4%	19.1%
Terminal rate	4/29 (14%)	3/30 (10%)	5/28 (18%)	3/27 (11%)
First incidence (days)	539	686	699	625
Poly-3 test	P=0.338	P=0.262N	P=0.415N	P=0.580
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	8/50 (16%)	6/50 (12%)	6/50 (12%)	8/50 (16%)
Adjusted rate	18.4%	13.4%	14.4%	19.1%
Terminal rate	4/29 (14%)	3/30 (10%)	5/28 (18%)	3/27 (11%)
First incidence (days)	539	587	699	625
Poly-3 test	P=0.383	P=0.363N	P=0.415N	P=0.580
All Organs: Mononuclear Cell Leukemia				
Overall rate	14/50 (28%)	15/50 (30%)	21/50 (42%)	21/50 (42%)
Adjusted rate	30.6%	32.1%	45.6%	46.0%
Terminal rate	3/29 (10%)	6/30 (20%)	7/28 (25%)	9/27 (33%)
First incidence (days)	539	370	427	415
Poly-3 test	P=0.120	P=0.530	P=0.102	P=0.095

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	43/50 (86%)	46/50 (92%)	41/50 (82%)
Adjusted rate	91.5%	88.6%	97.9%	90.2%
Terminal rate	28/29 (97%)	27/30 (90%)	28/28 (100%)	25/27 (93%)
First incidence (days)	454	482	510	432
Poly-3 test	P=0.458N	P=0.442N	P=0.128	P=0.564N
All Organs: Malignant Neoplasms				
Overall rate	28/50 (56%)	27/50 (54%)	26/50 (52%)	31/50 (62%)
Adjusted rate	58.0%	55.5%	56.4%	64.9%
Terminal rate	12/29 (41%)	12/30 (40%)	11/28 (39%)	13/27 (48%)
First incidence (days)	398	370	427	415
Poly-3 test	P=0.200	P=0.484N	P=0.521N	P=0.312
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	48/50 (96%)	49/50 (98%)	48/50 (96%)
Adjusted rate	98.0%	96.0%	100.0%	98.0%
Terminal rate	28/29 (97%)	28/30 (93%)	28/28 (100%)	26/27 (96%)
First incidence (days)	398	370	427	415
Poly-3 test	P=0.680	P=0.500N	P=0.505	P=0.755N

(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, pituitary gland, thyroid gland, and uterus; 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 chamber control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE B4a
Historical Incidence of Mammary Gland Neoplasms in Chamber Control Female F344/N Rats[a]

Study	Incidence in Controls			
	Fibroadenoma	Adenoma	Carcinoma	Fibroadenoma, Adenoma, or Carcinoma
Historical Incidence at Battelle Pacific Northwest Laboratories				
Acetonitrile	16/48	0/48	2/48	17/48
2-Chloroacetophenone	12/50	0/50	2/50	13/50
Colbalt sulfate heptahydrate	22/50	2/50	3/50	25/50
l-Epinephrine hydrochloride	10/50	0/50	2/50	11/50
Hexachlorocyclopentadiene	12/50	0/50	3/50	14/50
Isobutyraldehyde	27/50	0/50	1/50	27/50
Molybdenum trioxide	22/50	0/50	1/50	23/50
Nitromethane	19/50	2/50	2/50	21/50
o-Chlorobenzalmalononitrile (CS-2)	16/50	0/50	1/50	17/50
Ozone	20/50	1/50	4/50	23/50
Tetrafluoroethylene	22/50	1/50	3/50	24/50
Tetrahydrofuran	23/50	1/50	5/50	27/50
Overall Historical Incidence				
Total	315/903 (34.9%)	13/903 (1.4%)	43/903 (4.8%)	305/903 (38.8%)
Standard deviation	9.9%	1.5%	2.4%	10.5%
Range	20%-54%	0%-4%	2%-10%	22%-54%

[a] Data as of 15 October 1996

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

Study	Incidence in Controls					
	Benign Astrocytoma	Malignant Astrocytoma	Malignant Glioma	Malignant Medulloblastoma	Benign Granular Cell Tumor	Sarcoma
Historical Incidence at Battelle Pacific Northwest Laboratories						
Acetonitrile	0/48	0/48	0/48	0/48	0/48	0/48
2-Chloroacetophenone	0/49	0/49	0/49	0/49	0/49	0/49
Colbalt sulfate heptahydrate	0/50	0/50	0/50	0/50	0/50	0/50
l-Epinephrine hydrochloride	0/50	0/50	0/50	0/50	0/50	0/50
Hexachlorocyclopentadiene	0/50	0/50	0/50	0/50	0/50	0/50
Isobutyraldehyde	0/49	0/49	0/49	0/49	1/49	0/49
Molybdenum trioxide	0/50	0/50	0/50	0/50	0/50	0/50
Nitromethane	1/50	0/50	0/50	0/50	0/50	0/50
o-Chlorobenzalmalononitrile (CS-2)	0/49	0/49	0/49	0/49	1/49	0/49
Ozone	0/50	0/50	0/50	0/50	0/50	0/50
Tetrafluoroethylene	0/50	0/50	0/50	0/50	0/50	0/50
Tetrahydrofuran	0/50	0/50	0/50	0/50	0/50	0/50
Overall Historical Incidence						
Total	1/899 (0.1%)	2/899 (0.2%)	1/899 (0.1%)	0/899	2/899 (0.2%)	0/899
Standard deviation	0.5%	0.7%	0.5%		0.7%	
Range	0%-2%	0%-2%	0%-2%		0%-2%	

[a] Data as of 15 October 1996

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

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	16	15	18	23
Natural deaths	5	5	4	
Survivors				
Terminal sacrifice	29	30	28	27
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine small, ileum	(49)	(47)	(46)	(50)
Inflammation, chronic active			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)	2 (4%)	3 (6%)	4 (8%)
Basophilic focus	36 (72%)	38 (76%)	33 (66%)	33 (66%)
Clear cell focus	9 (18%)	11 (22%)	10 (20%)	11 (22%)
Degeneration, cystic		1 (2%)		1 (2%)
Degeneration, fatty		1 (2%)		
Eosinophilic focus	2 (4%)	3 (6%)	1 (2%)	4 (8%)
Fatty change	8 (16%)	6 (12%)	11 (22%)	9 (18%)
Fibrosis				1 (2%)
Hepatodiaphragmatic nodule	2 (4%)	4 (8%)	5 (10%)	6 (12%)
Inflammation, granulomatous				1 (2%)
Mixed cell focus	4 (8%)	10 (20%)	10 (20%)	5 (10%)
Necrosis				1 (2%)
Regeneration		1 (2%)	2 (4%)	3 (6%)
Bile duct, cyst	1 (2%)	1 (2%)		
Bile duct, hyperplasia	3 (6%)	9 (18%)	9 (18%)	10 (20%)
Centrilobular, necrosis	5 (10%)	5 (10%)	8 (16%)	3 (6%)
Periportal, infiltration cellular, lymphocyte			1 (2%)	
Mesentery	(9)	(10)	(8)	(11)
Fat, necrosis	9 (100%)	10 (100%)	8 (100%)	11 (100%)
Oral mucosa		(1)	(1)	
Pharyngeal, hyperplasia, squamous			1 (100%)	
Pancreas	(50)	(50)	(50)	(50)
Atrophy	18 (36%)	13 (26%)	11 (22%)	20 (40%)
Basophilic focus	1 (2%)		3 (6%)	3 (6%)
Hyperplasia		2 (4%)		
Metaplasia, hepatocyte		2 (4%)		1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum		1 (2%)		
Hyperplasia, squamous			1 (2%)	
Necrosis	5 (10%)	2 (4%)	7 (14%)	3 (6%)
Stomach, glandular	(50)	(49)	(50)	(50)
Mineralization			1 (2%)	
Necrosis	3 (6%)	3 (6%)	4 (8%)	6 (12%)
Tongue	(1)		(2)	
Hyperplasia			1 (50%)	
Tooth			(1)	(1)
Developmental malformation			1 (100%)	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 Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	35 (70%)	33 (66%)	28 (56%)	37 (74%)
Mineralization				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Degeneration, cystic	1 (2%)		1 (2%)	3 (6%)
Ectopic tissue		1 (2%)		
Hyperplasia	25 (50%)	17 (34%)	20 (40%)	13 (26%)
Hypertrophy	9 (18%)	14 (28%)	8 (16%)	14 (28%)
Inflammation, granulomatous		1 (2%)		
Necrosis	2 (4%)			1 (2%)
Thrombosis				1 (2%)
Vacuolization cytoplasmic	4 (8%)	6 (12%)	4 (8%)	6 (12%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	11 (22%)	6 (12%)	9 (18%)	9 (18%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia	2 (4%)		1 (2%)	2 (4%)
Parathyroid gland	(48)	(43)	(43)	(45)
Hyperplasia			1 (2%)	
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, hyperplasia	12 (24%)	16 (32%)	7 (14%)	12 (24%)
Thyroid gland	(48)	(50)	(49)	(50)
C-cell, hyperplasia	40 (83%)	32 (64%)	38 (78%)	40 (80%)
Follicular cell, hyperplasia	1 (2%)	1 (2%)	1 (2%)	
General Body System				
None				
Genital System				
Clitoral gland	(47)	(48)	(46)	(48)
Hyperplasia	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Inflammation, chronic active		1 (2%)		
Ovary	(50)	(50)	(50)	(50)
Cyst	5 (10%)	3 (6%)	5 (10%)	6 (12%)
Inflammation, granulomatous		1 (2%)		2 (4%)
Uterus	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)			
Hematopoietic System				
Bone marrow	(49)	(49)	(50)	(50)
Atrophy				1 (2%)
Hyperplasia, histiocytic		1 (2%)		
Hyperplasia, reticulum cell		1 (2%)		
Myelofibrosis	1 (2%)		1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Fibrosis	4 (8%)	6 (12%)	10 (20%)	4 (8%)
Hematopoietic cell proliferation	4 (8%)	5 (10%)		1 (2%)
Hemorrhage	1 (2%)	2 (4%)	4 (8%)	
Metaplasia, osseous		1 (2%)		
Necrosis	1 (2%)			1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	1 (2%)	1 (2%)	1 (2%)	
Hyperplasia	1 (2%)			2 (4%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)			
Inflammation, chronic active	1 (2%)	2 (4%)		2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy	1 (2%)			
Hyperostosis	6 (12%)	8 (16%)	11 (22%)	8 (16%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Degeneration, focal		1 (2%)		1 (2%)
Gliosis	1 (2%)			1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Inflammation, chronic active	31 (62%)	29 (58%)	25 (50%)	19 (38%)
Alveolar epithelium, hyperplasia	5 (10%)	5 (10%)	10 (20%)	8 (16%)
Nose	(50)	(50)	(50)	(50)
Foreign body	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Inflammation, suppurative	5 (10%)	8 (16%)	8 (16%)	12 (24%)
Thrombosis	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Olfactory epithelium, atrophy		1 (2%)	2 (4%)	2 (4%)
Olfactory epithelium, hyperplasia			1 (2%)	
Olfactory epithelium, metaplasia	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Respiratory epithelium, hyperplasia		1 (2%)		1 (2%)
Respiratory epithelium, metaplasia, squamous		1 (2%)	1 (2%)	1 (2%)
Special Senses System				
Eye	(5)	(4)	(3)	(6)
Cataract	5 (100%)	3 (75%)	3 (100%)	4 (67%)
Degeneration		1 (25%)		1 (17%)
Hemorrhage				1 (17%)
Retina, atrophy	5 (100%)	2 (50%)	3 (100%)	4 (67%)
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Cyst	1 (2%)			1 (2%)
Hydronephrosis		1 (2%)		
Infarct		1 (2%)	1 (2%)	
Nephropathy	46 (92%)	47 (96%)	48 (96%)	47 (94%)
Papilla, necrosis		1 (2%)		
Renal tubule, hyperplasia	4 (8%)			
Renal tubule, necrosis				1 (2%)
Transitional epithelium, hyperplasia				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, hyperplasia	1 (2%)		1 (2%)	

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Mortelmans *et al.* (1986). Isoprene 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 isoprene. The high dose was limited by toxicity to 10,000 μg/plate. 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). Isoprene 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 isoprene. The high dose in the SCE trial without S9 was limited by toxicity; in all other trials (SCE and Abs), no toxicity was apparent, and 5,000 μg/mL was selected as the high dose. A single flask per dose was used.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with isoprene in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing isoprene 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 isoprene, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no isoprene. 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 isoprene for 10 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 isoprene and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 11 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.

12-DAY MOUSE BONE MARROW, PERIPHERAL BLOOD MICRONUCLEUS, AND CELL CYCLE KINETICS PROTOCOLS

The experimental protocol is described in detail by Tice *et al.* (1988). Groups of 14 or 15 male $B6C3F_1$ mice were exposed to isoprene at concentrations of 438, 1,750, or 7,000 ppm (trial 1) and 70, 220, or 700 ppm (trial 2) 6 hours per day for a total of 12 days; 15 control animals were exposed to ambient air only. The animals were implanted subcutaneously with a BrdU tablet (McFee *et al.*, 1983) 1 hour before isoprene exposure on day 12. The use of BrdU allowed selection of the appropriate cell population (cells in the second metaphase following isoprene exposure) for scoring. Two hours before sacrifice, the animals received an intraperitoneal injection of colchicine in saline. At the time of colchicine injection, peripheral blood samples for micronucleus analysis were obtained from each animal by tail snip, and these were immediately air-dried and fixed in absolute methanol. For analysis of Abs, 10 mice per exposure group were killed 17 to 20 hours after BrdU implantation for analysis of SCEs. Five mice per exposure group were killed 24 hours after tablet implantation. One or both femurs were removed from all mice, and the marrow was flushed out with phosphate-buffered saline (pH 7.0). The cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. The slides, along with the fixed blood smears, were transported to Brookhaven National Laboratory (BNL) (Upton, NY). Slides from the processed bone marrow were prepared at BNL as described in Tice *et al.* (1988). Differential chromatid staining was used for the SCE and Abs slides, and peripheral blood smears for micronucleus analysis were stained with acridine orange.

Twenty-five second-division metaphase cells were scored for SCEs from each of four animals per treatment group. Responses were evaluated as SCEs/cell, and the data were analyzed by a trend test (Margolin *et al.*, 1986). Fifty first-division metaphase cells were scored for Abs from each of eight animals per exposure group. Responses were evaluated as the percentage of aberrant metaphase cells, excluding gaps. The data

were analyzed by a trend test (Margolin *et al.*, 1986). One thousand polychromatic erythrocytes (PCEs) and 1,000 normochromatic erythrocytes (NCEs) were scored per animal for frequency of micronucleated erythrocytes. In addition to these cytogenetic endpoints, 100 randomly selected metaphase cells per slide were scored for replication history (to provide data to calculate average cell generation time, a measure of bone marrow cell proliferation kinetics), and the percentage of cells in metaphase among 1,000 cells in each bone marrow sample was used to calculate the mitotic index. The percentage of PCEs in 1,000 erythrocytes was also determined as a measure of isoprene-induced bone marrow toxicity.

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 frequencies of SCEs, Abs, and micronucleated cells were analyzed for increasing trend over exposure groups using a one-tailed Cochran-Armitage trend test ($P < 0.05$) (Margolin *et al.*, 1986). For the mitotic index, average generation time, and percentage PCEs, a two-tailed trend test was used to determine if a treatment-related effect occurred. For all endpoints except frequency of micronucleated cells, individual animal responses were analyzed by the trend test. For data exhibiting a significant trend, pairwise comparisons between each exposure group and the concurrent chamber control were performed using either the one-tailed or two-tailed *t*-test (with Bonferroni-corrected alphas to account for multiple comparisons). For micronucleus data, the numbers of PCEs or NCEs were summed across animals within each exposure group and analyzed by a one-tailed trend test (Margolin and Risko, 1988). Pairwise comparisons between treatment groups and controls were conducted using a one-tailed Pearson chi-square test (with Bonferroni correction for multiple comparisons) to determine the minimal effective dose.

13-WEEK MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of the protocol for this assay is presented by Jauhar *et al.* (1988). At the end of a 13-week toxicity study (NTP, 1995), 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 1,000 PCEs and 10,000 NCEs in each of 10 animals per exposure group.

Log transformation of the NCE data, testing for normality by the Shapiro-Wilk test, and testing for heterogeneity of variance by Cochran's test were performed before statistical analyses. The frequency of micronucleated cells among NCEs was determined by analysis of variance with the SAS GLM procedure. The NCE data for each exposure group were compared with the concurrent solvent control by Student's *t*-test. The frequency of micronucleated cells among PCEs was analyzed by the Cochran-Armitage trend test, and individual exposure groups were compared to the concurrent solvent control by Kastenbaum-Bowman's binomial test. The percentage of PCEs among total erythrocytes was determined by an analysis of variance on ranks (classed by sex), and individual exposure groups were compared with the concurrent solvent control with a *t*-test on ranks.

RAT LUNG FIBROBLAST MICRONUCLEUS TEST PROTOCOL

The detailed protocol is described by Khan and Heddle (1991, 1992). This study was performed in conjunction with the 2-year study. Groups of 10 male and 10 female rats were exposed by inhalation to 0, 220, 700, or 7,000 ppm isoprene, 6 hours per day, 5 days per week for 4 weeks, excluding holidays. Each rat received 17 to 19 exposures with at least two consecutive exposures before sacrifice and subsequent lung cell isolation.

The cell isolation procedure was modified from Khan and Heddle (1991). Briefly, animals were anesthetized with pentobarbital, the thoracic cavity was opened aseptically, and the lungs were perfused

with Hank's balanced saline solution (HBSS). The lungs were then removed, minced, degassed under vacuum, rinsed with HBSS, and digested in 0.25% trypsin in HBSS for 80 minutes. Digestion was stopped with the addition of RPMI medium containing fetal calf serum, and the debris was allowed to settle. The supernatant was decanted and centrifuged to pellet the fibroblasts. These were plated in single-chambered slides and cultured overnight at 37.5° C. After a medium change, cultures were incubated for 72 hours with complete medium containing 3.2 μg cytochalasin B/mL. After 72 hours, hypotonic potassium chloride was added for 5 minutes, and the slides were fixed in 95% methanol. Slides were stained with acridine orange, and 1,000 binucleated cells on each of two coded slides were scored for each chamber control and exposed rat. The numbers of mononucleated cells and micronuclei in binucleated cells were recorded following the scoring criteria of Heddle *et al.* (1990).

Raw data obtained from the lung fibroblast micronucleus assay were tabulated and the mean, standard deviation, and standard error of the mean for the number of mononucleated cells/1,000 binucleated cells and micronuclei/1,000 binucleated cells were calculated. A two-way analysis of variance was used to analyze the measurements. Intergroup differences were delineated by Tukey's studentized range test.

RESULTS

In vitro mutagenicity tests with isoprene yielded negative results. No increase in mutations was noted in *S. typhimurium* strain TA98, TA100, TA1535, or TA1537 treated with isoprene (100 to 10,000 μg/plate), with or without induced rat or hamster liver S9 metabolic activation enzymes (Table C1, Mortelmans *et al.*, 1986). Also, no increases in SCEs or Abs were noted in cultured CHO cells treated with isoprene, with or without S9 (Tables C2 and C3). Concentrations of isoprene may have been reduced by evaporation during incubation in these *in vitro* tests.

In contrast to the negative results seen in these *in vitro* experiments, *in vivo* tests for chromosomal effects in mice yielded mostly positive results. Bone marrow cells from male mice exposed to isoprene for 12 days via inhalation showed significantly increased levels of SCEs; however, the incidence of bone marrow cells with Abs in male mice treated for 12 days with isoprene was not increased (Table C4; Tice *et al.*, 1988; Shelby, 1990). The frequencies of micronucleated NCEs and PCEs were also significantly elevated in peripheral blood samples obtained from the male mice exposed for 12 days to isoprene (Table C5; Tice *et al.*, 1988). The negative bone marrow Abs test results and the positive peripheral blood micronucleus results were from the same animals. These apparently conflicting results may imply that numerical chromosomal damage, not detected by traditional Abs tests, was induced by isoprene, or, perhaps, that the Abs test was not sufficiently sensitive to detect a rather low level of induced structural damage. Further evidence of the ability of isoprene to increase the frequency of micronucleated erythrocytes in mice comes from the results of a 13-week study in which males and females showed significantly elevated frequencies of micronucleated NCEs and PCEs after inhalation of isoprene (Table C6; NTP, 1995).

Analysis of average generation time and mitotic index data from mice exposed for 12 days to isoprene indicates no change in the percentage of bone marrow cells engaged in division but a significant lengthening, at the high dose of 7,000 ppm isoprene, of the cell cycle duration of proliferating cells. The percentage of circulating PCEs decreased in an exposure-related fashion in the 12-day study (Table C5; Tice *et al.*, 1988). However, in the mice exposed for 13 weeks, the percentage of PCEs among erythrocytes in peripheral blood was increased (Table C6). This may represent an adaptation to cytotoxicity with chronic exposure and result from increased erythropoiesis to compensate for depletion of the erythrocyte population.

In male and female rats exposed to isoprene by inhalation for 4 weeks, no significant increase in the frequency of micronucleated lung fibroblasts was observed (Table C7).

TABLE C1
Mutagenicity of Isoprene in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/plate[b]					
		S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100	0	90 ± 6.0	95 ± 10.4	111 ± 9.8	101 ± 5.5	105 ± 11.6	97 ± 3.5
	100	103 ± 6.7	83 ± 3.7	109 ± 5.4	87 ± 8.5	90 ± 4.7	93 ± 7.8
	333	111 ± 9.9	85 ± 8.7	108 ± 4.4	117 ± 5.1	101 ± 6.4	80 ± 3.5
	1,000	92 ± 4.1	81 ± 3.8	110 ± 2.7	102 ± 1.9	109 ± 10.5	94 ± 3.9
	3,333	98 ± 13.6	73 ± 7.5	102 ± 8.1	79 ± 2.4	97 ± 6.4	90 ± 6.7
	10,000	83 ± 3.7[c]	56 ± 9.2[c]	84 ± 12.2[c]	85 ± 5.4	90 ± 6.4[c]	94 ± 6.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		377 ± 7.5	288 ± 2.0	1,379 ± 19.9	1,071 ± 51.0	399 ± 24.4	491 ± 19.1
TA1535	0	28 ± 5.4	20 ± 3.7	9 ± 3.3	7 ± 0.9	7 ± 0.3	5 ± 0.3
	100	23 ± 2.5	16 ± 2.5	9 ± 1.3	5 ± 0.0	10 ± 1.2	5 ± 0.0
	333	24 ± 4.3	15 ± 2.0	11 ± 2.5	8 ± 2.8	12 ± 1.5	6 ± 0.3
	1,000	20 ± 4.6	12 ± 3.7	8 ± 0.9	7 ± 1.0	6 ± 0.6	6 ± 0.7
	3,333	14 ± 0.7	10 ± 0.0	6 ± 0.3	6 ± 0.3	11 ± 1.2	6 ± 1.5
	10,000	9 ± 2.2[c]	0 ± 0.0[c]	5 ± 1.2[c]	6 ± 1.5	5 ± 1.8[c]	2 ± 0.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		440 ± 6.9	282 ± 15.0	482 ± 29.5	331 ± 20.1	162 ± 6.1	136 ± 10.9
TA1537	0	6 ± 1.2	7 ± 1.8	8 ± 0.9	5 ± 2.0	8 ± 1.2	6 ± 1.5
	100	5 ± 1.2	3 ± 0.6	6 ± 0.9	6 ± 1.2	8 ± 0.9	6 ± 0.7
	333	5 ± 1.5	4 ± 0.7	7 ± 0.7	5 ± 1.2	9 ± 1.0	4 ± 0.3
	1,000	7 ± 1.8	4 ± 0.9	5 ± 0.9	7 ± 1.2	7 ± 0.7	4 ± 0.6
	3,333	5 ± 0.9	2 ± 0.0	5 ± 1.0	7 ± 1.2	3 ± 0.6	2 ± 0.3
	10,000	4 ± 1.0[c]	4 ± 1.0[c]	4 ± 1.2[c]	3 ± 0.9	5 ± 0.9[c]	6 ± 0.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		317 ± 31.7	178 ± 49.6	457 ± 9.5	224 ± 47.5	163 ± 25.6	141 ± 12.3
TA98	0	16 ± 1.2	15 ± 1.2	27 ± 4.6	22 ± 1.7	24 ± 1.0	22 ± 4.4
	100	16 ± 1.8	13 ± 2.5	22 ± 5.1	21 ± 2.1	26 ± 1.5	16 ± 4.0
	333	16 ± 1.5	16 ± 4.4	22 ± 4.4	16 ± 1.3	22 ± 3.7	20 ± 0.9
	1,000	17 ± 2.1	13 ± 2.8	21 ± 3.0	22 ± 2.7	19 ± 2.0	15 ± 4.9
	3,333	13 ± 0.3	13 ± 1.8	20 ± 2.3	22 ± 1.5	22 ± 1.0	17 ± 2.2
	10,000	10 ± 3.4[c]	2 ± 2.3[c]	17 ± 3.7[c]	17 ± 1.9	18 ± 1.5[c]	13 ± 2.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		388 ± 21.7	300 ± 21.9	1,128 ± 60.2	926 ± 57.1	285 ± 17.7	465 ± 14.7

[a] Study was performed at SRI International. The detailed protocol and these data are presented by Mortelmans *et al.* (1986). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Isoprene[a]

Compound	Concentration (µg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Summary: Negative								
Dimethylsulfoxide[c]		50	1,033	475	0.45	9.5	26.0	
Mitomycin-C[d]	0.0007	50	1,039	667	0.64	13.3	26.0	39.61
	0.0050	10	207	247	1.19	24.7	26.0	159.50
Isoprene	50	50	1,027	373	0.36	7.5	26.0	-21.02
	160	50	1,034	373	0.36	7.5	26.0	-21.55
	500	50	1,041	428	0.41	8.6	26.0	-10.59
	1,600	50	1,036	428	0.41	8.6	26.0	-10.16
					P=0.769[e]			
+S9								
Summary: Negative								
Dimethylsulfoxide		50	1,047	398	0.38	8.0	26.0	
Cyclophosphamide[d]	0.1	50	1,049	485	0.46	9.7	26.0	21.63
	0.6	10	210	137	0.65	13.7	26.0	71.62
Isoprene	160	50	1,048	391	0.37	7.8	26.0	-1.85
	500	50	1,046	347	0.33	6.9	26.0	-12.73
	1,600	50	1,047	390	0.37	7.8	26.0	-2.01
	5,000	50	1,046	391	0.37	7.8	26.0	-1.67
					P=0.587			

[a] Study was performed at Environmental Health Research & Testing, Inc. 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 C3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Isoprene[a]

Compound	Concentration (µg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
-S9					
Harvest time: 12 hours					
Summary: Negative					
Dimethylsulfoxide[b]		200	1	0.01	0.5
Mitomycin-C[c]	0.125	200	54	0.27	21.5
	0.250	50	33	0.66	34.0
Isoprene	1,600	200	3	0.02	1.5
	3,000	200	1	0.01	0.5
	5,000	200	3	0.02	1.5
					P=0.276[d]
+S9					
Harvest time: 13 hours					
Summary: Negative					
Dimethylsulfoxide		200	4	0.02	1.5
Cyclophosphamide[c]	5.0	200	29	0.15	13.5
	7.5	50	23	0.46	42.0
Isoprene	1,600	200	4	0.02	2.0
	3,000	200	6	0.03	3.0
	5,000	200	3	0.02	1.5
					P=0.394

[a] Study was performed at Environmental Health Research and Testing, Inc. 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 C4
Induction of Sister Chromatid Exchanges and Chromosomal Aberrations in Male Mouse Bone Marrow Cells Following Treatment with Isoprene by Inhalation for 12 Days[a]

	Concentration (ppm)	Number of Mice	SCEs/Cell	Number of Mice	Abs/Cell	Cells with Abs (%)
Trial 1[b]						
	Chamber Control	4	4.40 ± 0.215	8	0.02 ± 0.008	2.25 ± 0.701
	438	4	14.84 ± 1.909*	8	0.04 ± 0.011	3.75 ± 1.031
	1,750	4	11.61 ± 0.261*	8	0.05 ± 0.015	4.50 ± 1.296
	7,000	4	13.98 ± 0.577*	8	0.04 ± 0.007	3.50 ± 0.732
			P=0.046[c]		P=0.455	P=0.368
Trial 2[d]						
	Chamber Control	4	4.67 ± 0.345	8		1.25 ± 0.366
	70	4	4.89 ± 0.244	8		0.50 ± 0.327
	220	4	9.18 ± 0.772*	8		0.75 ± 0.366
	700	4	13.50 ± 1.226*	8		1.00 ± 0.655
			P<0.001			P>0.05

* Significantly different (P≤0.017, α=0.05; Bonferroni-corrected for three pairwise comparisons) from the chamber control group by the one-tailed *t*-test
[a] Study was performed at Battelle Pacific Northwest Laboratories. Data are given as mean ± standard error. SCE=sister chromatid exchange. Abs=chromosomal aberrations.
[b] The detailed protocol under which all these data were generated is presented by Tice *et al*. (1988); trial 1 data are included in this publication.
[c] Significance tested by the one-tailed trend test; significant at P≤0.05 (Margolin *et al*., 1986)
[d] These data are presented by Shelby (1990).

TABLE C5
Frequency of Micronuclei in Male Mouse Peripheral Blood Cells and Bone Marrow Cell Cycle Kinetics Data Following Treatment with Isoprene by Inhalation for 12 Days[a]

Concentration (ppm)	No. of Mice	Micronucleated Cells/1,000 Cells		PCEs (%)	Average Generation Time (hours)	Mitotic Index (%)
		PCEs	NCEs			
Chamber Control	15	2.00 ± 0.338	1.47 ± 0.236	3.91 ± 0.191	11.68 ± 0.342	1.15 ± 0.156
438	15	12.00 ± 0.662*	5.20 ± 0.619*	3.00 ± 0.116*	12.98 ± 0.498	1.09 ± 0.141
1,750	15	15.60 ± 1.068*	6.40 ± 0.689*	2.87 ± 0.143*	12.73 ± 0.483	1.05 ± 0.110
7,000	14	16.93 ± 1.003*	6.93 ± 0.934*	1.64 ± 0.095*	13.72 ± 0.361*	1.30 ± 0.140
		P<0.001[b]	P<0.001[b]	P<0.001[c]	P=0.010[c]	P=0.271[c]

* Significantly different (P≤0.05) from the chamber control group by *t*-test (a one-tailed *t*-test was used for the micronucleus data, to determine if a significant increase occurred; a two-tailed *t*-test was used to analyze the percent PCE, average generation time, and mitotic index data, to determine if any treatment-related effect occurred.)
[a] Micronucleus analysis was performed at Battelle Pacific Northwest Laboratories; kinetics data analysis was performed at Brookhaven National Laboratory. The detailed protocol and cell cycle kinetics data are presented by Tice *et al*. (1988). Data are given as mean ± standard error. PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte
[b] Significance of micronucleated cells/1,000 cells tested by the one-tailed trend test, significant at P≤0.05 (Margolin and Risko, 1988)
[c] Significance of percent PCEs, average generation time, and mitotic index tested by the two-tailed trend test, using individual animal data

TABLE C6
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Isoprene by Inhalation for 13 Weeks[a]

	Concentration (ppm)	Number of Mice	Micronucleated Cells/1,000 Cells[b]		PCEs[b] (%)
			PCEs	NCEs	
Male					
	Chamber Control	10	2.46 ± 0.53	1.74 ± 0.18	2.12 ± 0.08
	70	10	1.50 ± 0.21	1.82 ± 0.17	2.50 ± 0.08
	220	10	2.05 ± 0.41	1.97 ± 0.10	2.45 ± 0.11
	700	10	8.78 ± 1.16**	6.23 ± 0.28	2.63 ± 0.13**
	2,200	10	7.18 ± 0.76**	7.19 ± 0.17	2.83 ± 0.10**
	7,000	10	11.78 ± 0.91**	10.09 ± 0.42	3.23 ± 0.19**
			P<0.001[c]	P<0.001[d]	P<0.001[d]
Female					
	Chamber Control	10	1.54 ± 0.22	1.28 ± 0.06	2.27 ± 0.09
	70	10	1.77 ± 0.47	0.34 ± 0.07	2.15 ± 0.11
	220	10	2.80 ± 0.30*	2.39 ± 0.23**	2.43 ± 0.09**
	700	10	6.35 ± 0.91**	4.71 ± 0.21**	2.64 ± 0.11**
	2,200	10	7.05 ± 0.71**	5.34 ± 0.64**	2.62 ± 0.11**
	7,000	10	9.29 ± 1.05**	6.43 ± 0.24**	2.59 ± 0.12**
			P<0.001	P<0.001	P=0.008

* Significantly different (P≤0.05) from the chamber control group by Kastenbaum-Bowman's binomial test (PCEs) or Student's *t*-test (NCEs and percent PCEs)
** P≤0.01
[a] Study was performed at USDA Western Regional Center. The detailed protocol is presented by Jauhar *et al*. (1988). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte
[b] Data are given as mean ± standard error.
[c] Significance of PCEs/1,000 cells tested by the Cochran-Armitage trend test, using individual animal data
[d] Significance was tested by linear contrasts from analysis of variance (NCEs/1,000 cells and percent PCEs)

TABLE C7
Frequency of Micronuclei in Lung Fibroblasts of Rats Following Treatment with Isoprene by Inhalation for 4 Weeks[a]

	Concentration (ppm)	Number of Rats	Micronuclei/1,000 Binucleated Cells[b]
Male			
	Chamber Control	10	19 ± 3
	220	10	19 ± 2
	700	10	15 ± 1
	7,000	10	20 ± 2
Female			
	Chamber Control	10	20 ± 2
	220	10	25 ± 2
	700	10	22 ± 2
	7,000	10	27 ± 2

[a] Study conducted at Battelle Pacific Northwest Laboratories. The protocol is presented by Khan and Heddle (1991, 1992).
[b] Mean ± standard error. Data analyzed by two-way analysis of variance and pairwise comparisons were performed using Tukey's studentized range test; results were not significant.

APPENDIX D
URINARY VINYL LACTIC ACID – BIOMARKER OF EXPOSURE

TABLE D1
Urinary Biomarker Data for Rats in the 2-Year Inhalation Study of Isoprene[a]

	Chamber Control	220 ppm	700 ppm	7,000 ppm
n	10	10	10	10
Male				
Urine excretion (g)				
3 Months	11.79 ± 1.41	11.81 ± 1.27	12.70 ± 1.74	9.69 ± 0.79
6 Months	5.57 ± 0.41	5.80 ± 0.60	5.72 ± 0.85	6.00 ± 0.52
12 Months	9.35 ± 0.66	8.45 ± 0.53	9.15 ± 0.80	8.28 ± 0.64
18 Months	11.50 ± 0.99	10.69 ± 1.32	13.74 ± 1.72	12.48 ± 1.80
Urine creatinine (mg/dL)				
3 Months	74.7 ± 8.6	74.1 ± 7.1	74.2 ± 8.7	92.0 ± 8.6
6 Months	104.9 ± 11.5	108.5 ± 6.8	109.4 ± 5.8	115.9 ± 7.1
12 Months	95.8 ± 5.3	100.1 ± 4.7	99.3 ± 6.4	103.0 ± 4.0
18 Months	89.6 ± 6.9	90.9 ± 8.9	88.8 ± 7.7	82.7 ± 6.0
Urine VLA/urine (mg/total sample)				
3 Months	—[b]	3.89 ± 0.08	9.01 ± 0.29**	23.95 ± 0.89**
6 Months	—	3.62 ± 0.28	9.24 ± 0.79**	30.67 ± 1.91**
12 Months	—	5.03 ± 0.24	13.14 ± 0.58**	35.71 ± 2.45**
18 Months	—	5.33 ± 0.46	14.46 ± 0.55**	36.91 ± 1.97**
Urine VLA/creatinine (μg/μg)				
3 Months	—	0.488 ± 0.009	1.124 ± 0.042**	2.874 ± 0.112**
6 Months	—	0.598 ± 0.024	1.584 ± 0.085**	4.617 ± 0.227**
12 Months	—	0.606 ± 0.012	1.533 ± 0.084**	4.247 ± 0.143**
18 Months	—	0.590 ± 0.033	1.321 ± 0.069**	3.928 ± 0.171**
Urine VLA/creatinine/isoprene (ng/μg/ppm)				
3 Months	—	2.218 ± 0.043	1.606 ± 0.059**	0.411 ± 0.016**
6 Months	—	2.718 ± 0.108	2.263 ± 0.121*	0.660 ± 0.032**
12 Months	—	2.755 ± 0.056	2.190 ± 0.120**	0.607 ± 0.020**
18 Months	—	2.682 ± 0.148	1.887 ± 0.099**	0.561 ± 0.024**

TABLE D1
Urinary Biomarker Data for Rats in the 2-Year Inhalation Study of Isoprene

	Chamber Control	220 ppm	700 ppm	7,000 ppm
n	10	10	10	10
Female				
Urine excretion (g)				
3 Months	10.07 ± 1.69	10.20 ± 1.18	12.25 ± 1.32	11.09 ± 2.00
6 Months	3.51 ± 0.41	4.00 ± 0.32	8.13 ± 3.66	3.86 ± 0.51
12 Months	5.70 ± 0.68	5.58 ± 0.76	8.02 ± 1.09	7.67 ± 1.03
18 Months	6.78 ± 0.62	6.77 ± 0.61	7.52 ± 0.84	6.50 ± 0.76
Urine creatinine (mg/dL)				
3 Months	45.8 ± 7.9	44.7 ± 6.2	41.2 ± 5.9	47.2 ± 7.2
6 Months	94.2 ± 8.7	92.7 ± 6.6	85.0 ± 10.1	106.9 ± 10.2
12 Months	74.4 ± 5.5	79.4 ± 5.0	75.0 ± 8.1	78.6 ± 5.7
18 Months	88.7 ± 6.1	76.2 ± 5.2	77.8 ± 6.0	92.2 ± 8.3
Urine VLA/urine (mg/total sample)				
3 Months	—	2.87 ± 0.12	6.21 ± 0.31**	14.54 ± 0.78**
6 Months	—	2.62 ± 0.12	5.78 ± 0.34**	16.51 ± 0.54**
12 Months	—	3.10 ± 0.32	8.60 ± 0.47**	24.71 ± 1.87**
18 Months	—	3.51 ± 0.19	9.73 ± 0.57**	30.05 ± 1.33**
Urine VLA/creatinine (μg/μg)				
3 Months	—	0.722 ± 0.021	1.433 ± 0.094**	3.526 ± 0.155**
6 Months	—	0.740 ± 0.023	1.490 ± 0.062**	4.573 ± 0.304**
12 Months	—	0.745 ± 0.028	1.637 ± 0.066**	4.524 ± 0.323**
18 Months	—	0.711 ± 0.017	1.782 ± 0.041**	5.446 ± 0.135**
Urine VLA/creatinine/isoprene (ng/μg/ppm)				
3 Months	—	3.282 ± 0.094	2.047 ± 0.135**	0.504 ± 0.022**
6 Months	—	3.364 ± 0.105	2.129 ± 0.088**	0.653 ± 0.043**
12 Months	—	3.386 ± 0.127	2.339 ± 0.095**	0.646 ± 0.046**
18 Months	—	3.232 ± 0.079	2.546 ± 0.059**	0.778 ± 0.019**

* Significantly different ($P \leq 0.05$) from the 220 ppm group by Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data. VLA = vinyl lactic acid
[b] Less than the limit of detection (5 μg vinyl lactic acid/mL urine)

APPENDIX E
CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION OF ISOPRENE

Isoprene was obtained from the Goodyear Tire and Rubber Company (Akron, OH) in three lots (BC21028-F1-161-1, BC31019-F3010H-1, and BC40827-F603-1), which were used during the 2-year study. Identity and purity analyses were conducted by the study laboratory. Reports on analyses performed in support of the isoprene studies are on file at the National Institute of Environmental Health Sciences.

All lots of the chemical, a clear, colorless liquid, were identified as isoprene by infrared and nuclear magnetic resonance spectroscopy (proton and C^{13}) and by gas chromatography/mass spectrometry analysis of headspace samples; a helium carrier gas was used with two systems:

A) J&W DB 5 column with electron multiplier detection at 1,776 volts and an oven temperature of 30° C for 10 minutes, and

B) J&W DB 624 deactivated fused silica column with electron multiplier detection at 1,847 volts and an oven temperature program of –20° to 0° C at 1° C per minute, then 0° to 200° C at 20° C per minute with no temperature hold.

All spectra were consistent with the literature spectra (*Aldrich*, 1983; McLafferty and Stauffer, 1989) of isoprene; the nuclear magnetic resonance C^{13} chemical shift data were consistent with shift values expected for isoprene. The infrared and nuclear magnetic spectra are presented in Figures E1 and E2.

The purity of lot BC21028-F1-161-1 was determined by gas chromatography, gas chromatography/mass spectrometry analysis for *t*-butylcatechol and dimers, potentiometric titration for peroxides, and determination of polymer content. For potentiometric titration, a mixture of acetic acid and chloroform (2:1) was stirred under a steady spray of nitrogen; bulk isoprene and then sodium iodide were added. The mixture was then titrated with sodium thiosulfate while being stirred under a stream of nitrogen. Polymer content was determined by timed evaporation under a nitrogen stream until the net weight change was less than 1 mg per 60 seconds. Gas chromatography was performed with flame ionization detection and a helium carrier gas; heptane was added to the isoprene solutions as a reference. Two systems were used:

A) J&W DB 624 column with an oven temperature program of –20° to 0° C at 1° C per minute, 0° to 200° C at 10° C per minute, and then 200° to 250° C at 25° C per minute with a 3-minute hold at 250° C, and

B) Restek Rtx-1701 column with an oven temperature of 30° C for 5 minutes, then 30° to 250° C at 15° C per minute with a 3-minute hold at 250° C.

Analyses for *t*-butylcatechol and total dimers were performed with gas chromatography/mass spectrometry with electron multiplier detection at 1,847 volts and gas chromatography with flame ionization detection. Each system used a J&W DB 5 column and an oven temperature program of 90° C for 1 minute, 90° to 120° C at 5° C per minute, and then 120° to 250° C at 25° C per minute with a 6.8-minute hold at 250° C. A helium carrier gas was used.

The total concentration of isoprene dimers ranged from 0.11% to 0.13%, well below the rejection limit of 0.3%. No other impurities with areas greater than 0.1% were detected. Concentrations of the inhibitor *t*-butylcatechol ranged from 185 to 246 ppm. Potentiometric titration was performed on the barrel of

lot BC21028-F1-161-1, which gas chromatography/mass spectrometry indicated contained the lowest concentration of inhibitor; titration indicated a peroxide concentration of approximately 0.02 meq/kg. Headspace samples taken from the storage barrels were analyzed with gas chromatography by system A; results indicated that isoprene accounted for greater than 99% of the peak area, and the concentrations of volatile impurities were not elevated. The polymer content of 0.025% to 0.070% was well below the rejection limit of 0.5%. The overall purity of greater than 99% for lot BC21028-F1-161-1 was in agreement with data provided by the manufacturer.

Additional analyses of lot BC21028-F1-161-1 were performed with gas chromatography/mass spectrometry by system B described for the identity analyses to further characterize impurities indicated by gas chromatography. No impurities were conclusively identified; however, one minor peak eluting just before the major peak had a retention time similar to that of 1,4-pentadiene or a pentadiene isomer.

The manufacturer indicated that lots BC31019-F3010H-1 and BC40827-F603-1 contained 99.8% and 99.7% isoprene, respectively. Lot BC31019-F3010H-1 contained 0.124% *cis*- and 0.032% *trans*-2-pentene, less than 0.1 ppm cyclopentadiene, 1 ppm alpha acetylenes, 217 ppm isoprene dimers, 213 ppm *t*-butylcatechol, and 16 ppm total sulfur. Lot BC40827-F603-1 contained 0.175% *cis*- and 0.054% *trans*-2-pentene, 2.44 ppm cyclopentadiene, 5 ppm alpha acetylenes, 189 ppm isoprene dimers, 130 ppm *t*-butylcatechol, and less than 1 ppm total sulfur.

Throughout the 2-year study, total isoprene dimer concentrations and *t*-butylcatechol concentrations were monitored with gas chromatography; peroxides and polymers were also monitored. The bulk chemical was initially stored in the original shipping containers at approximately 22° C. Because the rate of dimer formation was increased by approximately 1% per 361 days during the first four analyses, the bulk chemical was subsequently stored at 16° C to limit dimer formation to a maximum total concentration of 2%.

VAPOR GENERATION AND EXPOSURE SYSTEM

A diagram of the isoprene generation and delivery system is shown in Figure E3. Isoprene vapor was generated with a rotary evaporation system (Büchi Rotavapor, Model EL-131S, Büchi Labratoriums Technik AG, Flavil, Switzerland). Isoprene was pumped from the bulk reservoir by a liquid micrometering pump into a rotating flask partially emersed in a hot water bath. Isoprene vapor passed from the flask into a chilled water condenser in which much of the vapor condensed and returned to the evaporator flask. Uncondensed vapor was carried to the top of the condenser column by a metered stream of nitrogen that entered the bottom of the condenser column. Vapor temperature was monitored at the top of the condenser column by a temperature sensor. The total output of the generator was calculated from the metered nitrogen flow and an estimate of the isoprene vapor pressure at the exit temperature.

After exiting the condenser column, the isoprene vapor was further diluted with nitrogen. The vapor then entered a short distribution manifold from which individual delivery lines carried metered amounts of vapor to each exposure chamber. Flow to each chamber was regulated by vacuum pumps located at the chamber end of each delivery line. A three-way valve, mounted between the distribution manifold and each chamber, directed vapor to the exposure chamber exhaust until a stable concentration of isoprene vapor was built up in the distribution line. At each chamber, the vapor was further diluted with charcoal- and HEPA-filtered air to the appropriate isoprene concentration.

The study laboratory designed the inhalation exposure chamber (Hazleton 2000, Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A small particle detector (Type CN, Gardners Associates, Schenectady, NY) was used with and without

animals in the exposure chambers to ensure that isoprene vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

VAPOR CONCENTRATION MONITORING

The isoprene concentrations in the exposure chambers were monitored by an on-line gas chromatograph (Model 5890, Hewlett-Packard, Palo Alto, CA). Samples were drawn from each exposure chamber, an on-line standard, and a charcoal-filtered air blank approximately every 15 minutes using a 12-port stream select valve. The gas chromatograph was checked against an on-line standard of isoprene in nitrogen (Matheson Gas Products, Portland, OR) throughout the day for instrument drift and was calibrated monthly against gravimetrically prepared standards. Additionally, the gas chromatograph was calibrated by a comparison of chamber concentration data to data from grab samples, which were collected with charcoal sampling tubes (ORBO™-32, Supelco, Bellefonte, PA), extracted with toluene containing heptane as an internal standard, and analyzed by an off-line gas chromatograph. The volumes of gas were sampled at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standards of isoprene in toluene. A summary of the chamber concentrations for the 2-year study is in Table E1.

CHAMBER ATMOSPHERE CHARACTERIZATION

The times for the exposure concentrations to build up to 90% of the final exposure concentration (T_{90}) and the decay to 10% of the exposure concentration (T_{10}) were measured with and without animals present. At a chamber airflow rate of 10 air changes per hour, the theoretical value for both T_{90} and T_{10} is approximately 12.5 minutes; the T_{90} value chosen for the study was 12 minutes. Actual T_{90} values were 8 or 9 minutes without animals and 10 or 11 minutes with animals in the chambers. T_{10} values were 9 or 10 minutes without animals and ranged from 9 to 12 minutes with animals in the chambers.

The uniformity of isoprene vapor concentration in the exposure chambers without animals was measured before the study began; concentration uniformity with animals present in the chambers was measured periodically during the study. The vapor concentration was determined with the on-line gas chromatograph used for concentration monitoring; the automatic 12-port sampling valve was disabled to allow continuous monitoring from a single line. Samples were collected from several positions in each chamber. Chamber concentration uniformity was maintained throughout the study.

The persistence of isoprene in the 7,000 ppm exposure chamber following exposure was monitored during the study with and without animals present. The concentration of isoprene in the exposure chamber fell to less than 1% of the beginning concentration within approximately 22 minutes without animals present; with animals present, the time to decay to less than 1% of the initial concentration was 26 minutes.

To ensure that the purity of the isoprene vapor entering the exposure chambers was greater than 99% and that the concentration of isoprene dimers in the distribution line remained less than 0.05%, a second on-line gas chromatograph (Hewlett-Packard Model 5890) was used to analyze samples drawn from the distribution lines approximately every 9 minutes with a 12-port sample valve. The purity monitor was checked against an on-line standard of limonene in nitrogen before and after the exposure period on each exposure day for instrument drift. The purity monitor was calibrated every 6 months by a comparison of distribution line concentration data to data from grab samples, as described for the vapor concentration monitoring system. A diffusion tube standard generator (Model 360, Thermo Electron, Hopkinton, MA) provided the on-line standard of limonene, which was also used to calculate dimer concentration. Effluent was drawn from the generator through bubblers containing dimethylformamide; samples were analyzed on an off-line gas chromatograph calibrated with gravimetrically prepared standards of limonene in dimethylformamide.

During prestudy testing, a purity of 99.95% or greater was maintained in the distribution line during generation.

Isoprene from the vapor generator reservoir, evaporation flask, distribution manifold, and exposure chambers was tested for stability before and during the study with gas chromatography. Because isoprene can undergo a variety of addition, isomerization, and polymerization reactions, samples were analyzed for isoprene, *cis*- and *trans*-2-pentene, 1,4-pentadiene, limonene, cyclopentadiene, alpha acetylenes, total peroxides, total sulfur, and isoprene dimers. During the prestudy testing, vapor was spiked with approximately 2.5% total isoprene dimer and approximately 600 ppm *t*-butylcatechol. Grab samples were collected from the distribution line and from 70 and 7,000 ppm chambers with charcoal sampling tubes and analyzed for isoprene dimers, volatile organics, and semivolatile organics with gas chromatography; samples were also collected from the distribution line during a similated failure of the vapor generator. The two online gas chromatographs were used to analyze chamber samples for trace impurities eluting ahead of isoprene and distribution line samples for isoprene dimers. Grab samples from the evaporation flask and generator reservoir were analyzed by gas chromatography for *t*-butylcatechol and dimer concentrations, peroxides, and nonvolatile residues. During the 2-year study, samples from the generator reservoir, evaporation flask, distribution manifold, and the 0, 220, and 7,000 ppm exposure chambers (with animals present) were collected during an exposure period. Samples from the distribution manifold were analyzed for isoprene dimers, volatile organics, and semivolatile organics; samples from the evaporation flask and reservoir were analyzed for impurities, *t*-butylcatechol and dimer concentrations, peroxide content, and nonvolatile residue.

No impurities with areas greater than 0.1% relative to the major peak area were detected in the distribution manifold or 7,000 ppm chamber; no impurities with areas greater than 1.0% were detected in the 70 and 220 ppm exposure chambers. Concentrations of isoprene dimers and *t*-butylcatechol in the exposure chambers were less than 0.006% and 0.02% of the isoprene concentration by volume, respectively. The total concentration of impurities was approximately 0.1%. During prestudy testing, total isoprene dimers were present at 2.6% (by weight) in the generator reservoir and 5.0% in the evaporation flask at the beginning of an exposure period; at the end of exposure, the reservoir contained 2.0% and the evaporation flask contained 42.8% isoprene dimers. The reservoir contained 777 ppm *t*-butylcatechol at the beginning of exposure and 571 ppm at the end; the evaporation flask contained 1,551 and 12,336 ppm *t*-butylcatechol, respectively. The distribution manifold contained less than 0.05% total isoprene dimers and no *t*-butylcatechol. Because these measurements indicated that the generator efficiently suppressed the distribution of isoprene dimers and *t*-butylcatechol into the distribution manifold, the rejection limit for total isoprene dimers in the bulk chemical to be used in the 2-year study was increased to 2%. During the study, the generator reservoir contained 0.7% total isoprene dimers at the beginning and end of the exposure period and the evaporation flask contained 1.1% and 13.1%, respectively. The generator reservoir contained 205 ppm *t*-butylcatechol at the beginning and 209 ppm at the end of exposure; the evaporation flask contained 351 and 3,967 ppm *t*-butylcatechol, respectively. These results indicated that no degradation of isoprene occurred as a result of vapor generation during the study.

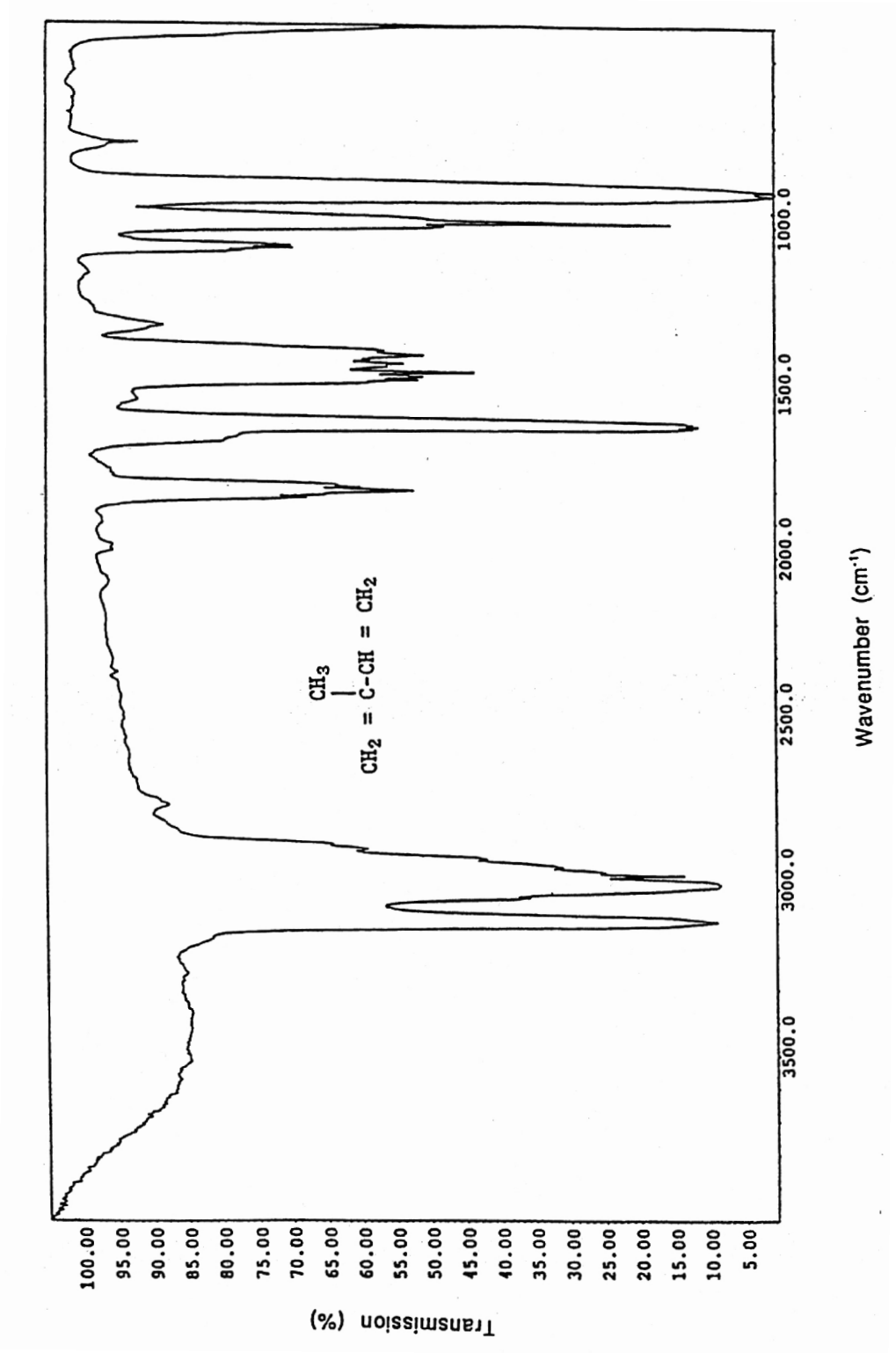

FIGURE E1
Infrared Absorption Spectrum of Isoprene

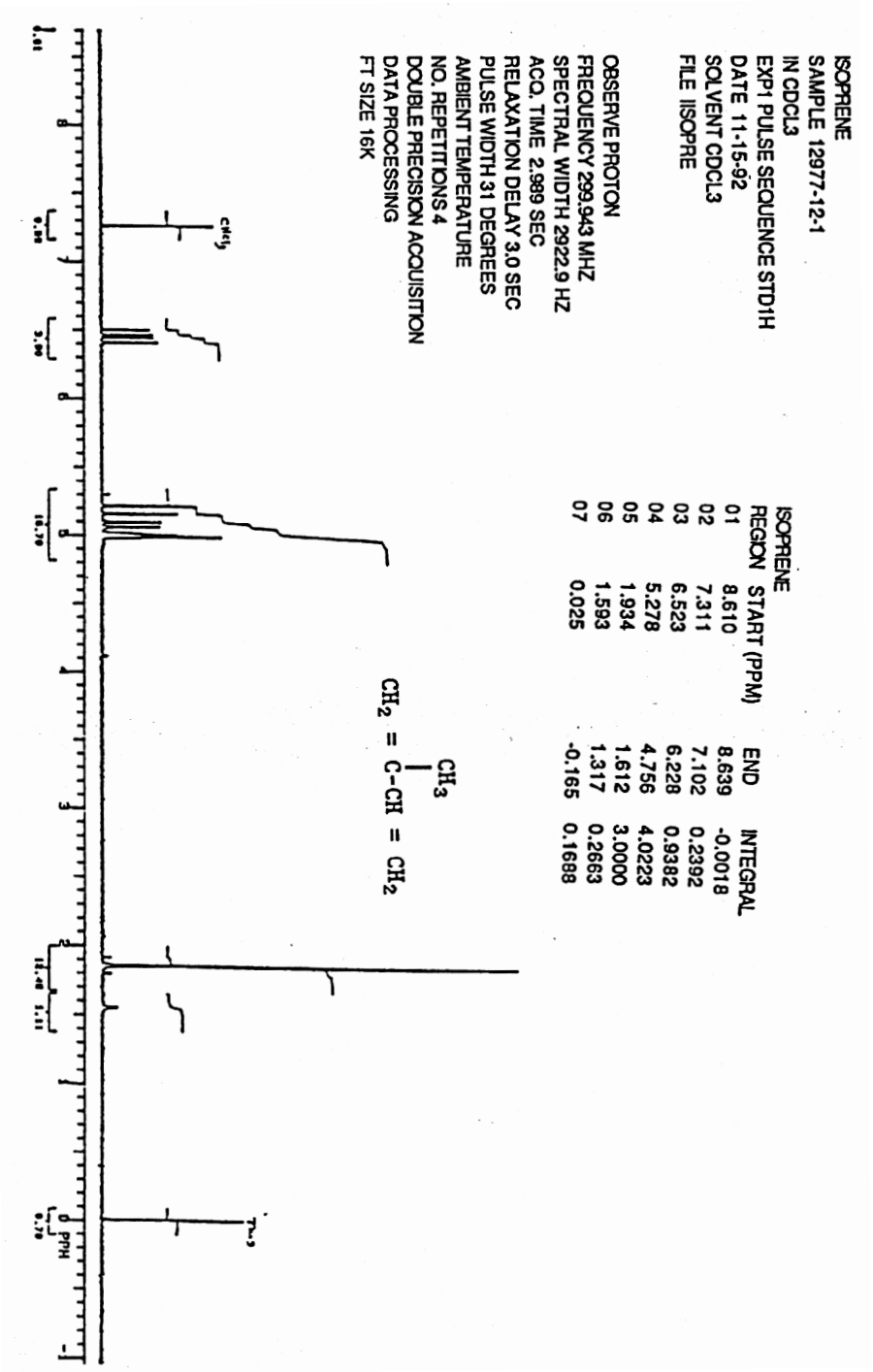

FIGURE E2
Nuclear Magnetic Resonance Spectrum of Isoprene

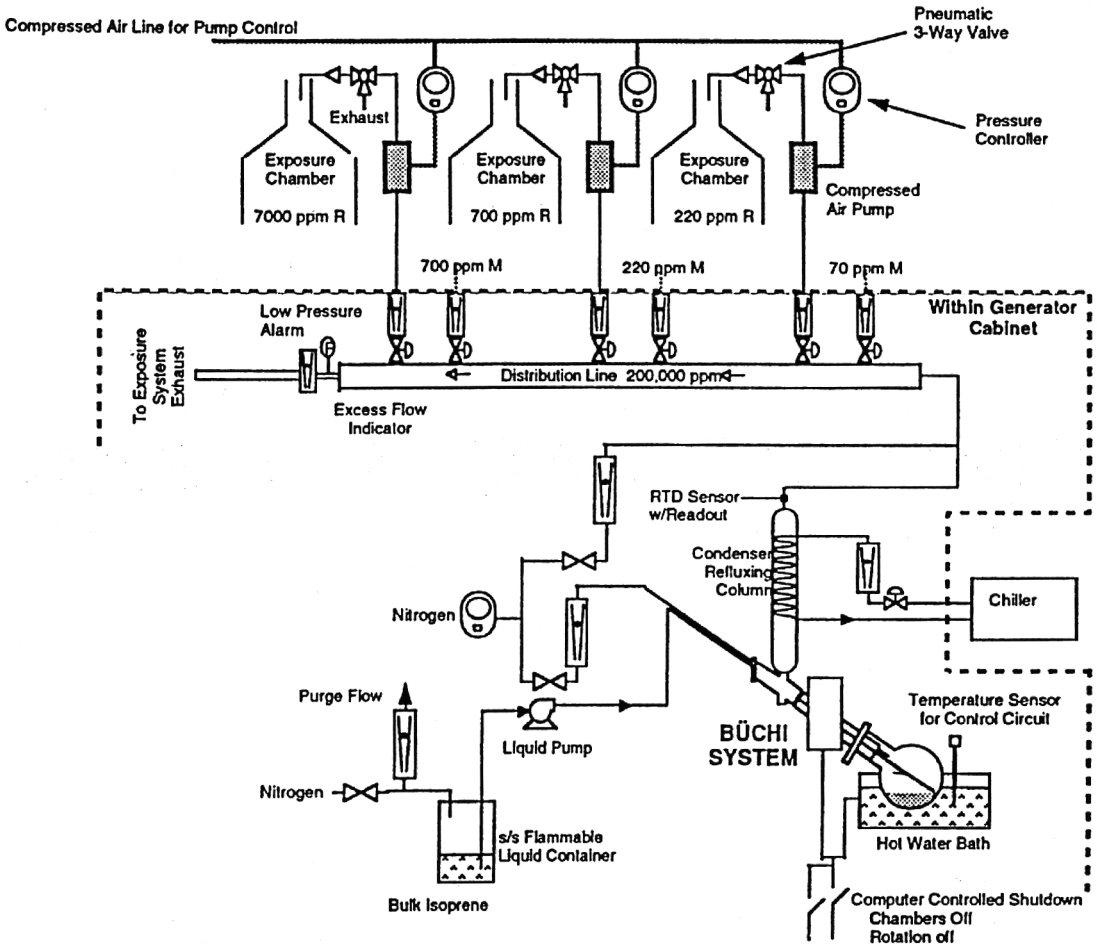

FIGURE E3
Schematic of the Generation and Delivery System

TABLE E1
Summary of Chamber Concentrations in the 2-Year Inhalation Study of Isoprene

Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
220	10,168	220 ± 9
700	10,264	699 ± 27
7,000	10,361	6,980 ± 285

[a] Mean ± standard deviation

APPENDIX F
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE F1
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 F2
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
d-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 F3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.91 ± 0.51	22.1 - 23.6	24
Crude fat (% by weight)	5.38 ± 0.19	5.00 - 5.80	24
Crude fiber (% by weight)	3.22 ± 0.37	2.60 - 4.30	24
Ash (% by weight)	6.25 ± 0.15	5.72 - 6.54	24
Amino Acids (% of total diet)			
Arginine	1.273 ± 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	0.273 ± 0.034	0.210 - 0.320	11
Vitamins			
Vitamin A (IU/kg)	6,673 ± 382	5,500 - 7,260	24
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)	17.39 ± 3.61	14.0 - 26.0	23
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 (%)	1.15 ± 0.06	1.03 - 1.27	24
Phosphorus (%)	0.89 ± 0.03	0.840 - 0.970	24
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 - 0.370	12
Magnesium (%)	0.165 ± 0.010	0.148 - 0.180	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 ± 9.73	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 F4
Contaminant Levels in NIH-07 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.53 ± 0.17	0.10 - 0.80	24
Cadmium (ppm)	0.05 ± 0.02	0.04 - 0.13	24
Lead (ppm)	0.23 ± 0.06	0.20 - 0.40	24
Mercury (ppm)	<0.02		24
Selenium (ppm)	0.34 ± 0.10	0.10 - 0.50	24
Aflatoxins (ppb)	<5.0		24
Nitrate nitrogen (ppm)[c]	7.18 ± 2.30	2.90 - 11.0	24
Nitrite nitrogen (ppm)[c]	1.42 ± 0.90	0.30 - 3.50	24
BHA (ppm)[d]	0.90 ± 0.41	0.01 - 2.00	24
BHT (ppm)[d]	1.70 ± 1.16	0.18 - 5.0	24
Aerobic plate count (CFU/g)	125,750 ± 124,542	13,000 - 460,000	24
Coliform (MPN/g)	149 ± 570	3 - 2,800	24
Escherichia coli (MPN/g)	7 ± 3.6	3 - 10	24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[e]	12.24 ± 3.82	4.0 - 23.0	24
N-Nitrosodimethylamine (ppb)[e]	10.45 ± 3.57	3.0 - 21.0	24
N-Nitrosopyrrolidine (ppb)[e]	1.80 ± 0.78	1.0 - 4.0	24
Pesticides (ppm)			
α-BHC	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.10		24
Estimated PCBs	<0.20		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.10		24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion	0.13 ± 0.17	0.02 - 0.83	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

[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 G
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 during the 2-year study. 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
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	6, 12, and 18 months, study termination
RCV/SDA (rat coronavirus/ sialodacryoadenitis virus)	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
M. arthritidis	Study termination
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	6, 12, and 18 months, study termination
KRV (Kilham rat virus)	6, 12, and 18 months, study termination

RESULTS

Five rats had positive titers to *M. arthritidis* at the end of the 2-year study. Further evaluation of samples positive for *M. arthritidis* by immunoblot and Western blot procedures indicated that the positive titers may have been due to cross reaction with antibodies of nonpathogenic *Mycoplasma* or other agents. Only sporadic samples were positive and there were no clinical findings or histopathologic changes of *M. arthritidis* infection in animals with potitive titers. Accordingly, *M. arthritidis*-positive titers were considered false positives.

APPENDIX H
DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR ISOPRENE

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DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR ISOPRENE

INTRODUCTION

Isoprene (2-methyl-1,3-butadiene), a monomeric chemical used in the production of synthetic rubber, is metabolized to monoepoxide and diepoxide intermediates. These metabolites may be responsible for the toxic and carcinogenic effects of isoprene in rats and mice that were identified in this report or reported previously (Melnick *et al.*, 1994; Placke *et al.*, 1996). To provide a better understanding of the relationship between exposure to isoprene and tissue concentrations of the parent compound and its metabolites, a first-generation, physiologically based pharmacokinetic (PBPK) model was developed for isoprene. Information obtained from this model may be useful in relating target organ dosimetry to adverse effects resulting from exposure to the parent compound. A validated model that incorporates species-specific biochemical and physiological parameter values can be useful for both low-dose and interspecies extrapolations and can accommodate different routes of administration or different exposure regimens.

The model presented in this report includes descriptions of isoprene absorption after intraperitoneal injection or inhalation exposure, followed by its tissue distribution, metabolism, and elimination (including metabolites) in rats. The metabolism of isoprene is similar to that of 1,3-butadiene and involves initial cytochrome P_{450}-mediated oxidation to 3,4-epoxy-3-methyl-1-butene (Epoxide I) or 3,4-epoxy-2-methyl-1-butene (Epoxide II), as shown in Figure H1. The monoepoxide intermediates may be hydrolyzed by epoxide hydrolase (EH) to the corresponding diols (Diol I and Diol II), conjugated with glutathione by glutathione-S-transferase (GST), or further oxidized to isoprene diepoxide (2-methyl-1,2:3,4-diepoxybutane). Oxidation of isoprene to Epoxide I occurs at a rate nearly four times that of isoprene oxidation to Epoxide II. Consistent with this metabolic scheme is the finding that vinyl lactic acid (VLA; 2-hydroxy-2-methyl-3-butenoic acid), an oxidation product of Diol I, was identified as the major urinary metabolite of isoprene metabolism in F344/N rats (Buckley *et al.*, 1999).

The PBPK model developed here incorporates the metabolic processes described above in characterizing the disposition of isoprene in rats. Physiological parameters (cardiac output, alveolar ventilation, tissue blood flows, and tissue volumes) were available from the literature and were not optimized to fit the data. Kinetic parameter values for isoprene metabolism that were available in the literature were used directly; other biochemical parameters were obtained by optimization or assumed to be equivalent to those for 1,3-butadiene metabolism.

MATERIALS AND METHODS

Physiological parameters: The PBPK model developed here includes compartments for blood, liver, lung, kidney, fat, gastrointestinal tract, slowly perfused tissues, and rapidly perfused tissues (Figure H2). The distribution of isoprene and isoprene epoxides between blood and tissues is limited by blood flow, compartment partition coefficients, and tissue permeability. Physiological parameters, including rates for cardiac output and alveolar ventilation, tissue blood flows, and tissue volumes that were obtained from the literature and used in the model are listed in Table H1.

Partition coefficients: The tissue:blood partition coefficients for isoprene were taken from Filser *et al.* (1996). Partition coefficients for the two isoprene monoepoxides (Table H2) were extrapolated from estimated octanol/water partition coefficients (K_{ow}). The K_{ow} for these epoxides were assumed to be the same and were estimated from atomic fragments and group factors of isoprene (Lyman *et al.*, 1990). The

linear regression equations of Fiserova-Bergerova and Diaz (1986) were used to calculate partition coefficients in different tissues from the K_{ow}.

The blood:air partition coefficient could not be calculated using this technique and was therefore obtained by optimization. The intraperitoneal injection study of Buckley *et al*. (1999) indicated that 94% of the radioactivity recovered in breath from animals administered [^{14}C]-isoprene was the parent compound. Hence, the proportion of epoxides in the exhaled breath of the rats was assumed to be 6% of the recovered radioactivity. These data, along with the simulated blood concentrations of isoprene and isoprene epoxides, were used to optimize the blood:air partition coefficients.

Biochemical parameters: The kinetic parameters for the metabolism of isoprene (Figure H1) were either obtained from the literature, optimized to reproduce the uptake of isoprene from closed chambers, or assumed to be equivalent to those for 1,3-butadiene metabolism; these parameter values are listed in Table H3. K_m and V_{max} values for isoprene oxidation in rats were obtained by optimization to fit the inhalation toxicokinetic data of Peter *et al*. (1990). In those studies, male Wistar rats were placed in closed desiccator jars with varying initial concentrations of isoprene (5 to 5,000 ppm), and the time-dependent declines of isoprene in the gas phase were measured for approximately 10 hours. Oxidation of isoprene to the monoepoxides was assumed to occur in the liver, lung, and kidney, whereas the oxidation of the monoepoxides to isoprene diepoxide was assumed to occur only in the liver. The K_m value for isoprene oxidation (2 μM) was assumed to be the same in all metabolizing tissues. The whole-body V_{max} was optimized to be distributed as 88% in the liver, 7% in the kidney, and 5% in the lung. Based on the *in vitro* kinetic data of Gervasi and Longo (1990), 75% of isoprene oxidation was assumed to result in the formation of Epoxide I and 25% in the formation of Epoxide II.

The V_{max} value reported by Gervasi and Longo (1990) for the oxidation of Epoxide II to isoprene dioxide in rat liver microsomes (0.3 nmol/mg microsomal protein/minute) was used to estimate this activity in the intact liver. The V_{max} for oxidation of Epoxide I was assumed to be 83% of the V_{max} for oxidation of Epoxide II, based on measured rates of formation of isoprene diepoxide from these monoepoxides by rat liver microsomes (Bogaards *et al*., 1996). The K_m for oxidation of the monoepoxides to isoprene diepoxide was assumed to be 600 μM, based on kinetic data for this activity in liver microsomes prepared from phenobarbital-treated rats (Gervasi and Longo, 1990).

Enzymatic rates for hydrolysis of isoprene monoepoxides and for conjugation with glutathione were included in the model and were assumed to take place in the liver and lung. The rates of these activities are not available in the literature and were assumed to be the same as those reported for hydrolysis and glutathione conjugation of epoxybutene in rat tissues (Csanády *et al*., 1992). Because approximately 95% of the isoprene metabolites identified in rat urine were derived from the hydrolysis of the monoepoxide intermediates and shown not to contain mercapturic acid derivatives (Buckley *et al*., 1999), the rate constant corresponding to GST activity was optimized to result in approximately 5% of the monoepoxide intermediates converted to glutathione conjugates.

The rate equations for intraperitoneal injection and inhalation absorption of isoprene and the differential equations used to estimate the time-dependent concentrations of isoprene and isoprene monoepoxides (Epoxide I plus Epoxide II) in the blood, kidney, liver, and lung are shown in Table H4. The SCoP program package (Kootsey *et al*., 1986; Kohn *et al*., 1994; available from Simulation Resources, Berrien Springs, MI) was used for both optimizations and simulations.

RESULTS AND DISCUSSION

Model simulations of the time courses of changes in isoprene concentrations in the gas phase of closed desiccator jars (6.4 L) occupied by two Wistar rats are shown in Figure H3. The model provided a

reasonably good fit to the experimental data with each of the different initial concentrations of isoprene, and it reproduced the exhalation of endogenously produced isoprene by untreated rats. The rate of change of isoprene concentration is determined by the ventilation rate, blood flow, compartment volumes, tissue partition coefficients of isoprene, and the rates of isoprene metabolism to its monoepoxide intermediates. The optimized fits to the data had a correlation coefficient of greater than 0.93 for all exposure concentrations. Model predictions of isoprene monoepoxide concentrations in the blood of rats during a 1-week exposure (6 hr/day) to isoprene are shown in Figure H4. The bottom line in this figure reflects monoepoxide formation from endogenously produced isoprene.

In a disposition study (Buckley *et al.*, 1999), male F344 rats were administered 64 mg ^{14}C-labeled isoprene/kg body weight by intraperitoneal injection, and urine, feces, and exhaled breath were collected at 3, 6, and 24 hours post-exposure. Radiolabel was excreted primarily in exhaled breath (>50%) and in urine (>30%). Greater than 95% of the recovered radioactivity in the breath traps was the parent compound. Nearly 90% of the total radioactivity recovered in the urine was identified as metabolites originating from the hydrolysis of Epoxide I. The major urinary metabolite (>60%) was identified as VLA. Optimizations of the lag time for the appearance of isoprene metabolites in urine and of the absorption rate for intraperitoneally injected [^{14}C]-isoprene led to a good fit of the model's predictions to the recovery-adjusted percentage of administered dose that was exhaled or excreted in the urine (Table H5).

Model predictions of the concentrations of isoprene metabolites in blood following 6-hour exposure to isoprene vapors were compared to results from an inhalation study reported by Dahl *et al.* (1987). In that study, blood samples were taken from male F344 rats exposed to 18, 280, 1,480, or 8,200 ppm [^{14}C]-isoprene for 6 hours, fractionated by vacuum line cryogenic distillation, and analyzed for radioactivity. The predicted and measured concentrations of isoprene metabolites in blood are presented in Table H6. This comparison shows that at exposure concentrations ranging from 280 to 8,200 ppm, the model underpredicted the concentrations of total isoprene metabolites in rat blood after 6-hour exposures. The model predictions ranged from 35% to 73% of the measured values. This discrepancy may be due to incorrect assumptions in the model that would affect blood concentrations of volatile metabolites (e.g., the kinetics of isoprene monoepoxide metabolism may not be the same as those of epoxybutene) or to possible incomplete trapping of isoprene by the distillation procedure.

To further assess the validity of the model, predictions of VLA excreted in the urine of rats exposed to isoprene were compared to data obtained in the 2-year inhalation toxicology study (Table D1). Sixteen-hour post-exposure urine samples were collected from exposed and control F344/N rats at 3, 6, 12, and 18 months of the 2-year isoprene study and analyzed for urine weight and concentrations of creatinine and VLA. The assumption was made that 70% of Diol-I would be metabolized into VLA, based on the metabolite analyses of rat urine after intraperitoneal injection of [^{14}C]-isoprene (Buckley *et al.*, 1999). The model underpredicted the amounts of VLA that were measured in the 16-hour urine samples taken at 3 months of the study (Table H7). At the 220 and 700 ppm exposures the model predictions were 91% and 62%, respectively, of the measured values. Discrepancies between observed and predicted values may be a consequence of incorrect assumptions used to estimate parameter values that were not available in the literature or to strain differences (Wistar rat versus F344/N rat) in rates of isoprene metabolism. An attempt to optimize the epoxide hydrolase activity (increasing its V_{max} by a factor of 4) resulted in only a slight improvement in the predicted values (Table H7).

The model was also used to predict cumulative concentrations of isoprene and isoprene monoepoxides in the blood, liver, lung, and kidney of rats exposed to isoprene by inhalation for 5 days followed by 2 nonexposure days (Table H8). These results were used in the dose-response modeling section of the Discussion. The relative proportion of isoprene epoxides in each of these tissues was about two times greater for 700 ppm rats compared to 220 ppm rats and two times greater for 7,000 ppm rats compared to 700 ppm rats. The lack of proportionality between increasing exposure concentration and increasing tissue

concentrations of the epoxide intermediates reflects the nonlinearity in metabolism caused by saturation of cytochrome P_{450} over this exposure range.

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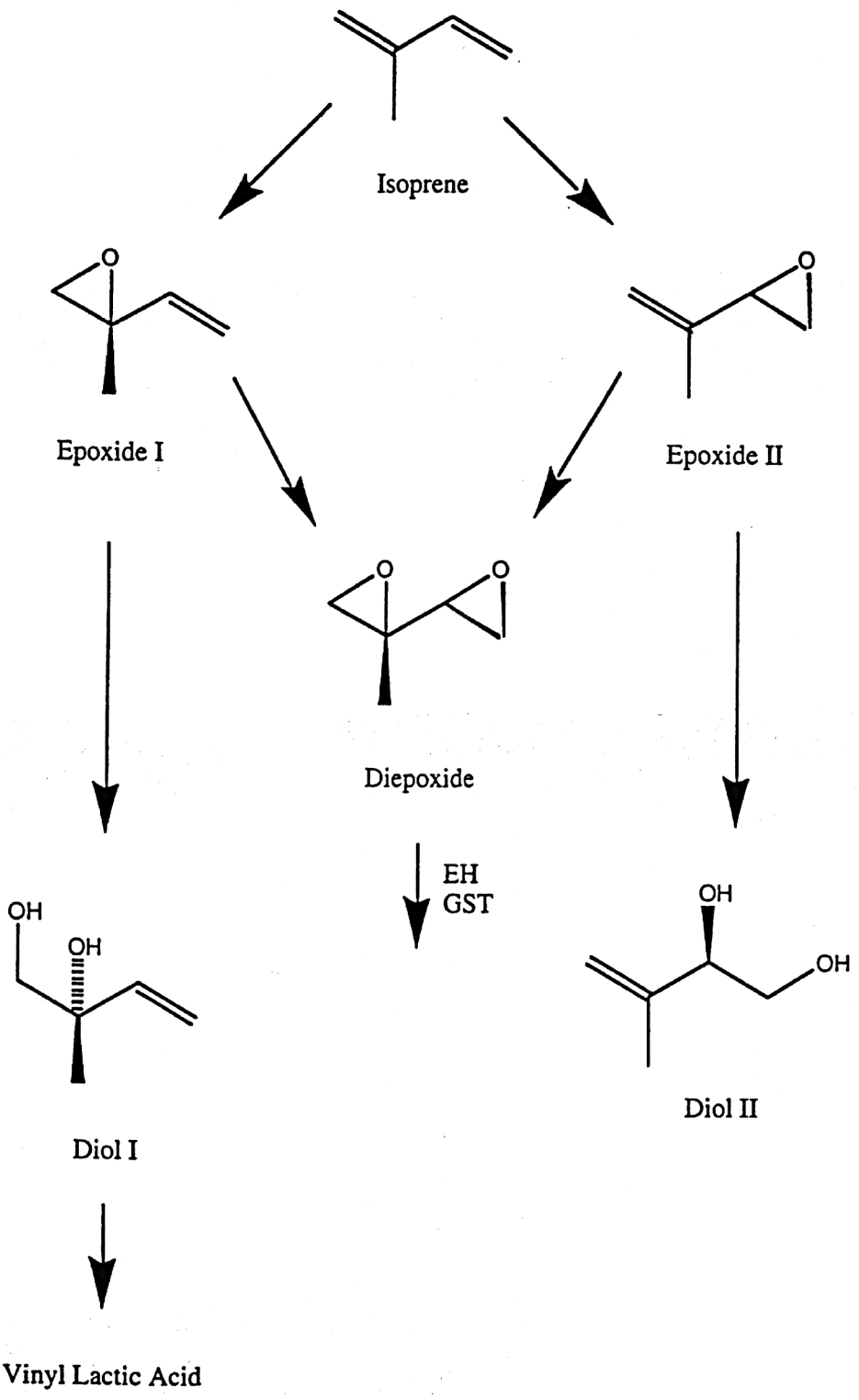

FIGURE H1
Scheme for Metabolism of Isoprene

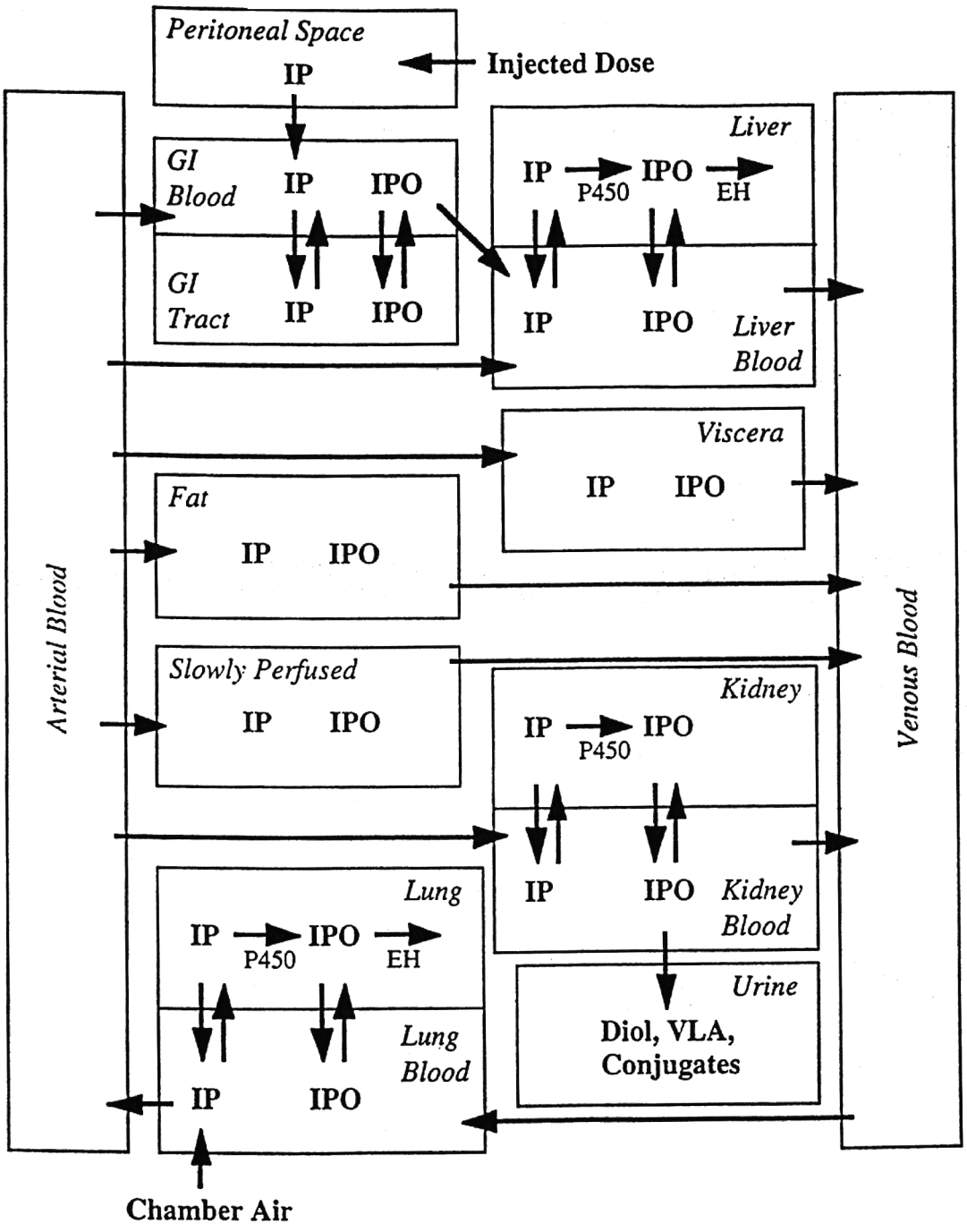

FIGURE H2
Diagram of the Isoprene Physiologically Based Pharmacokinetic Model

TABLE H1
Rat Physiological Parameters used in the Isoprene Pharmacokinetic Model: Compartment Volumes, Blood Flow, and Ventilation for Rats

Parameter	Value	Source
Body Weight	0.250 kg	Peter *et al.*, 1987
Cardiac Output	5.57 L/hr	14.7 L/hr/kg 0.7
Ventilation Rate	7.58 L/hr	20 L/hr/kg 0.7
Tissue Volumes as Fraction of Body Weight		
Blood	0.054	Davies and Morris, 1993
Fat	0.07	Delp *et al.,* 1991
Liver	0.037	Brown *et al.*, 1997
Viscera	0.1677	Residual calculation
Slow	0.542	Delp *et al.*, 1991; skin+muscle
Kidney	0.0148	Davies and Morris, 1993
Alveolar	0.0515	Schmidt-Nielsen, 1979
GI	0.0371	Average of literature values
Tubule	0.02	Pitts, 1974 (by analogy to humans)
Lung	0.0052	Average of literature values
Tissue Blood Flows as Fraction of Cardiac Output		
Fat	0.065	Delp *et al.*, 1991
Liver	0.039	Average of literature values
Viscera	0.286	Residual calculation
Slow	0.334	Delp *et al.*, 1991; skin+muscle
Kidney	0.133	Delp *et al.*, 1991
GI	0.143	Average of literature values

TABLE H2
Tissue:Air and Tissue:Blood Partition Coefficients for Isoprene and Isoprene Monoepoxide used in the Isoprene Pharmacokinetic Model

Parameter	Isoprene	Isoprene Monoepoxide
Air Partition	2.33	200
Fat Partition	26.4	7.60
GI Partition	0.64	0.82
Kidney Partition	0.75	0.80
Liver Partition	0.83	0.90
Lung Partition	0.83	1.10
Viscera Partition	0.79	0.80
Slow Partition	0.64	0.82

TABLE H3
Biochemical Parameters used in the Isoprene Pharmacokinetic Model

Parameter[a]	Value	Source
Endogenous IP	1.9 μmol/kg/hr	Peter *et al.*, 1987
Liver V cyt IP1	1,177.8 μmol/hr/L	Optimized to fit desiccator data
Liver K_m cyt IP1	2 μM	Optimized to fit desiccator data
Liver V cyt IP2	392.599 μmol/hr/L	25% of Liver V cyt IP1 (Gervasi and Longo, 1990)
Liver K_m cyt IP2	2 μM	Assumed same as Liver K_m cyt IP1
Liver V cyt IPO1	242 μmol/hr/L	Gervasi and Longo, 1990
Liver K_m cyt IPO1	600 μM	Gervasi and Longo, 1990
Liver V cyt IPO2	302.4 μmol/hr/L	83% of Liver V cyt IPO1 (Bogaards *et al.*, 1996)
Liver K_m cyt IPO2	600 μM	Assumed same as Liver K_m cyt IP01
Liver V EH1	2,500 μmol/hr/L	1,3-Butadiene-based value (Csanády *et al.*,1992)
Liver K_m EH1	260 μM	1,3-Butadiene-based value (Csanády *et al.*,1992)
Liver V EH2	2,500 μmol/hr/L	Assumed same as Liver V EH1
Liver K_m EH2	260 μM	Assumed same as Liver K_m EH2
Liver k GST1	50 L^{-1}/hr	Optimized GST to represent 5% of total metabolites
Liver k GST2	50 L^{-1}/hr	Assumed same as Liver k GST1
Lung V cyt IP1	630 μmol/hr/L	Optimized to fit desiccator data
Lung K_m cyt IP1	2 μM	Assumed same as Liver K_m
Lung V cyt IP2	210 μmol/hr/L	25% of Lung V cyt IP1 (Gervasi and Longo, 1990)
Lung K_m cyt IP2	2 μM	Assumed same as Liver K_m
Kidney V cyt IP1	440 μmol/hr/L	Optimized to fit desiccator data
Kidney K_m cyt IP1	2 μM	Assumed same as Liver K_m
Kidney V cyt IP2	146 μmol/hr/L	25% of kidney V cyt IP1 (Gervasi and Longo, 1990)
Kidney K_m cyt IP2	2 μM	Assumed same as Liver K_m
Lung k hydr 1	237 L^{-1}/hr	1,3-Butadiene-based value (Csanády *et al.*,1992)
Lung k hydr 2	237 L^{-1}/hr	1,3-Butadiene-based value (Csanády *et al.*,1992)

[a] Parameters are expressed as: 1) the organ where activity occurs (liver, lung, or kidney); 2) the biological function ($V = V_{max}$; K_m = Michaelis-Menten constant; k = apparent first-order rate constant); 3) enzyme (cyt = cytochrome P_{450}-mediated oxidation; EH = epoxide hydrolase; GST = glutathione-S-transferase); and 4) substrate (IP1 = isoprene with metabolism at the methyl-substituted vinyl group; IP2 = isoprene with metabolism at the nonmethyl-substituted vinyl group; IPO1 = epoxide I; IPO2 = epoxide II).

TABLE H4
Differential Equations used in the Isoprene Pharmacokinetic Model

Ventilation = Ventilation Rate × Body Weight$^{0.7}$ × animals
Blood Flow = Cardiac Output × Body Weight$^{0.7}$ × animals

Absorption from the GI tract

GI Blood IP´ = K_{abs} × GI Lumen IP

Uptake from chamber

Alveolar IP´ = Ventilation Air IP / Air Volume – Ventilation Alveolar IP / Alveolar Volume + Blood Flow Lung Blood IP / (Lung Blood Volume × Air Partition IP) – Blood Flow × Alveolar IP / (Alveolar Volume)

Distribution of isoprene through the blood

Lung Blood IP´ = Blood Flow Alveolar IP / Alveolar Volume – Blood Flow Lung Blood IP / (Lung Blood Volume Air Partition IP) + Blood Flow × Venous IP / Venous Volume – Blood Flow × Lung Blood IP / Lung Blood Volume + Blood Flow × Lung IP / (Lung Partition IP × Lung Volume) – Blood Flow × Lung Blood IP / Lung Blood Volume

Arterial IP´ = Endogenous IP + Blood Flow Lung Blood IP / Lung Blood Volume – Liver Flow Arterial IP / Arterial Volume – Kidney Flow × Arterial IP / Arterial Volume – Fat Flow × Arterial IP / Arterial Volume – Slow Flow × Arterial IP / Arterial Volume – GI Tract Flow × Arterial IP / Arterial Volume – Viscera Flow × Arterial IP / Arterial Volume

Venous IP´ = Blood Flow Venous IP / Venous Volume + (Liver Flow + GI Tract Flow) Liver Blood IP / Liver Blood Volume + Kidney Flow × Kidney Blood IP / Kidney Blood Volume + Fat Flow × Fat Blood IP / Fat Blood Volume + Slow Flow × Slow Blood IP / Slow Blood Volume Viscera + Flow × Viscera Blood IP / Viscera Blood Volume

Liver Blood IP´ = Liver Flow × Arterial IP / Arterial Volume + GI Tract Flow × GI Tract Blood IP / GI Tract Blood Volume – (Liver Flow + GI Tract Flow) × Liver Blood IP / Liver Blood Volume + (Liver Flow + GI Tract Flow) × Liver IP / (Liver Partition IP × Liver Volume) – (Liver Flow + GI Tract Flow) × Liver Blood IP / Liver Blood Volume

Kidney Blood IP´ = Kidney Flow × Arterial IP / Arterial Volume – Kidney Flow × Kidney Blood IP / Kidney Blood Volume + Kidney Flow × Kidney IP / (Kidney Partition IP × Kidney Volume) – Kidney Flow × Kidney Blood / Kidney Blood Volume

Tissue concentrations of isoprene

Lung IP´ = Blood Flow × Lung Blood IP / Lung Blood Volume – Blood Flow × Lung IP / (Lung Partition IP × Lung Volume) – Lung V cyt IP1 × Lung Volume × Lung IP / (Lung K_m cyt IP1 × Lung Volume + Lung IP) – Lung V cyt IP2 × Lung Volume × Lung IP / (Lung K_m cyt IP2 × Lung Volume + Lung IP)

Liver IP´ = (Liver Flow + GI Tract Flow) × Liver Blood IP / Liver Blood Volume – (Liver Flow + GI Tract Flow) × Liver IP / (Liver Partition IP × Liver Volume) – Liver V cyt IP1 × Liver Volume × Liver IP / (Liver K_m cyt IP1 × Liver Volume + Liver IP) – Liver V cyt IP2 × Liver Volume × Liver IP / (Liver K_m cyt IP2 × Liver Volume + Liver IP)

Kidney IP´ = Kidney Flow × Kidney Blood IP / Kidney Blood Volume – Kidney Flow × Kidney IP / (Kidney Partition IP × Kidney Volume) – Kidney V cyt IP1 × Kidney Volume × Kidney IP / (Kidney K_m cyt IP1 × Kidney Volume + Kidney IP) – Kidney V cyt IP2 × Kidney Volume × Kidney IP / (Kidney K_m cyt IP2 × Kidney Volume + Kidney IP)

Fat IP´ = Fat Flow × Arterial IP / Arterial Volume – Fat Flow × Fat IP / (Fat Volume × Fat Partition IP)

Viscera IP´ = Viscera Flow × Arterial IP / Arterial Volume – Viscera Flow × Viscera IP / (Viscera Volume × Viscera Partition IP)

Slow IP´ = Slow Flow × Arterial IP / Arterial Volume – Slow Flow × Slow IP / (Slow Volume × Slow Partition IP)

TABLE H4
Differential Equations used in the Isoprene Pharmacokinetic Model

Distribution of isoprene epoxides through the blood

Lung Blood IPO1´ = Blood Flow × Alveolar IPO1 / Alveolar Volume – Blood Flow × Lung Blood IPO1 / (Lung Blood Volume × Air Partition IPO) + Blood Flow × Venous IPO1 / Venous Volume – Blood Flow × Lung Blood IPO1 / Lung Blood Volume + Blood Flow × Lung IPO1 / (Lung Partition IPO × Lung Volume) – Blood Flow × Lung Blood IPO1 / Lung Blood Volume

Arterial IPO1´ = Endogenous IPO1 + Blood Flow × Lung Blood IPO1 / Lung Blood Volume – Liver Flow × Arterial IPO1 / Arterial Volume – Kidney Flow × Arterial IPO1 / Arterial Volume – Fat Flow × Arterial IPO1 / Arterial Volume – Slow Flow × Arterial IPO1 / Arterial Volume – GI Tract Flow × Arterial IPO1 / Arterial Volume – Viscera Flow × Arterial IPO1 / Arterial Volume

Venous IPO1´ = Blood Flow × Venous IPO1 / Venous Volume + (Liver Flow + GI Tract Flow) × Liver Blood IPO1 / Liver Blood Volume + Kidney Flow × Kidney Blood IPO1 / Kidney Blood Volume + Fat Flow × Fat Blood IPO1 / Fat Blood Volume + Slow Flow × Slow Blood IPO1 / Slow Blood Volume Viscera + Flow × Viscera Blood IPO1 / Viscera Blood Volume

Liver Blood IPO1´ = Liver Flow × Arterial IPO1 / Arterial Volume + GI Tract Flow × GI Tract Blood IPO1 / GI Tract Blood Volume – (Liver Flow + GI Tract Flow) × Liver Blood IPO1 / Liver Blood Volume + (Liver Flow + GI Tract Flow) × Liver IPO1 / (Liver Partition IPO × Liver Volume)

Kidney Blood IPO1´ = Kidney Flow × Arterial IPO1 / Arterial Volume – Kidney Flow × Kidney Blood IPO1 / Kidney Blood Volume + Kidney Flow × Kidney IPO1 / (Kidney Partition IPO × Kidney Volume) – Kidney Flow × Kidney Blood / Kidney Blood Volume

Lung Blood IPO2´ = Blood Flow × Alveolar IPO2 / Alveolar Volume – Blood Flow × Lung Blood IPO2 / (Lung Blood Volume × Air Partition IPO) + Blood Flow × Venous IPO2 / Venous Volume – Blood Flow × Lung Blood IPO2 / Lung Blood Volume + Blood Flow × Lung IPO2 / (Lung Partition IPO × Lung Volume) – Blood Flow × Lung Blood IPO2 / Lung Blood Volume

Arterial IPO2´ = Endogenous IPO2 + Blood Flow × Lung Blood IPO2 / Lung Blood Volume – Liver Flow × Arterial IPO2 / Arterial Volume – Kidney Flow × Arterial IPO2 / Arterial Volume – Fat Flow × Arterial IPO2 / Arterial Volume – Slow Flow × Arterial IPO2 / Arterial Volume – GI Tract Flow × Arterial IPO2 / Arterial Volume – Viscera Flow × Arterial IPO2 / Arterial Volume

Venous IPO2´ = Blood Flow × Venous IPO2 / Venous Volume + (Liver Flow + GI Tract Flow) × Liver Blood IPO2 / Liver Blood Volume + Kidney Flow × Kidney Blood IPO2 / Kidney Blood Volume + Fat Flow × Fat Blood IPO2 / Fat Blood Volume + Slow Flow × Slow Blood IPO2 / Slow Blood Volume Viscera + Flow × Viscera Blood IPO2 / Viscera Blood Volume

Liver Blood IPO2´ = Liver Flow × Arterial IPO2 / Arterial Volume + GI Tract Flow × GI Tract Blood IPO2 / GI Tract Blood Volume – (Liver Flow + GI Tract Flow) × Liver Blood IPO2 / Liver Blood Volume + (Liver Flow + GI Tract Flow) × Liver IPO2 / (Liver Partition IPO × Liver Volume) – (Liver Flow + GI Tract Flow) × Liver Blood IPO2 / Liver Blood Volume + (Liver Flow + GI Tract Flow) × Liver IPO2 / (Liver Volume × Liver Partition)

Kidney Blood IPO2´ = Kidney Flow × Arterial IPO2 / Arterial Volume – Kidney Flow × Kidney Blood IPO2 / Kidney Blood Volume + Kidney Flow × Kidney IPO2 / (Kidney Partition IPO × Kidney Volume) – Kidney Flow × Kidney Blood / Kidney Blood Volume

Tissue concentrations of isoprene epoxides

Lung IPO1´ = Blood Flow × Lung Blood IPO1 / Lung Blood Volume – Blood Flow × Lung IPO1 / (Lung Partition IPO × Lung Volume) + Lung V cyt IP1 × Lung Volume × Lung IPO / (Lung K_m cyt IP1 × Lung Volume + Lung IP) – Lung V EH1 × Lung Volume × Lung IPO1 / (Lung K_m EH1 × Lung Volume + Lung IPO1) – k GST1 × Lung Volume × Lung IPO1

Liver IPO1´ = (Liver Flow + GI Tract Flow) × Liver Blood IPO1 / Liver Blood Volume – (Liver Flow + GI Tract Flow) × Liver IP / (Liver Partition IPO × Liver Volume) + Liver V cyt IP1 × Liver Volume × Liver IP / (Liver K_m cyt IP1 × Liver Volume + Liver IPO1) – Liver V EH2 × Liver Volume × Liver IPO1 / (Liver K_m EH1 × Liver Volume + Liver IPO1) – k GST1 × Liver Volume × Liver IPO1

Kidney IPO1´ = Kidney Flow × Kidney Blood IPO1 / Kidney Blood Volume – Kidney Flow × Kidney IPO1 / (Kidney Partition IPO × Kidney Volume) + Kidney V cyt IP1 × Kidney Volume × Kidney IPO1 / (Kidney K_m cyt IP1 × Kidney Volume + Kidney IPO1)

Fat IPO1´ = Fat Flow × Arterial IPO1 / Arterial Volume – Fat Flow × Fat IPO1 / (Fat Volume × Fat Partition IPO)

Viscera IPO1´ = Viscera Flow × Arterial IPO1 / Arterial Volume – Viscera Flow × Viscera IPO1 / (Viscera Volume × Viscera Partition IPO)

TABLE H4
Differential Equations used in the Isoprene Pharmacokinetic Model

Tissue concentrations of isoprene epoxides (continued)

Slow IPO1´ = Slow Flow × Arterial IPO1 / Arterial Volume – Slow Flow × Slow IPO1 / (Slow Volume × Slow Partition IPO)

Lung IPO2´ = Blood Flow × Lung Blood IPO2 / Lung Blood Volume – Blood Flow × Lung IPO2 / (Lung Partition IPO × Lung Volume) + Lung V cyt IP1 × Lung Volume × Lung IPO / (Lung K_m cyt IP1 × Lung Volume + Lung IP) – Lung V EH2 × Lung Volume × Lung IPO2 / (Lung Km EH2 × Lung Volume + Lung IPO2) – k GST2 × Lung Volume × Lung IPO2

Liver IPO2´ = (Liver Flow + GI Tract Flow) × Liver Blood IPO2 / Liver Blood Volume – (Liver Flow + GI Tract Flow) × Liver IP / (Liver Partition IPO × Liver Volume) + Liver V cyt IP1 × Liver Volume × Liver IP / (Liver K_m cyt IP1 × Liver Volume + Liver IPO2) – Liver V EH2 × Liver Volume × Liver IPO2 / (Liver K_m EH2 × Liver Volume + Liver IPO2) – k GST2 × Liver Volume × Liver IPO2

Kidney IPO2´ = Kidney Flow × Kidney Blood IPO2 / Kidney Blood Volume – Kidney Flow × Kidney IPO2 / (Kidney Partition IPO × Kidney Volume) + Kidney V cyt IP1 × Kidney Volume × Kidney IPO2 / (Kidney K_m cyt IP1 × Kidney Volume + Kidney IPO2)

Fat IPO2´ = Fat Flow × Arterial IPO2 / Arterial Volume – Fat Flow × Fat IPO2 / (Fat Volume × Fat Partition IPO)

Viscera IPO2´ = Viscera Flow × Arterial IPO2 / Arterial Volume – Viscera Flow × Viscera IPO2 / (Viscera Volume × Viscera Partition IPO)

Slow IPO2´ = Slow Flow × Arterial IPO2 / Arterial Volume – Slow Flow × Slow IPO2 / (Slow Volume × Slow Partition IPO)

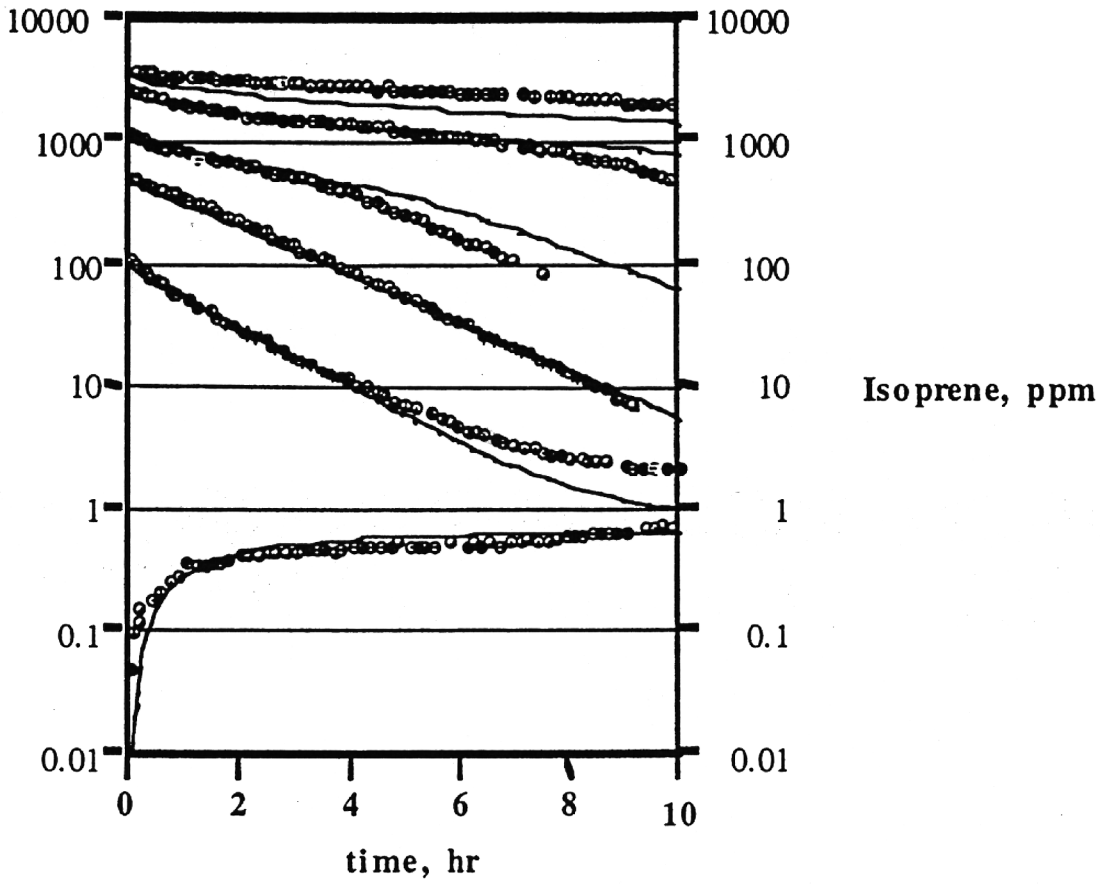

FIGURE H3
Simulations of Isoprene Uptake in Rats
(Circles represent the observed time course concentrations of isoprene in the gas phase of closed 6.4 L desiccator chambers occupied by two Wistar rats. The lines are the optimized fits of the pharmacokinetic model to the observed data.)

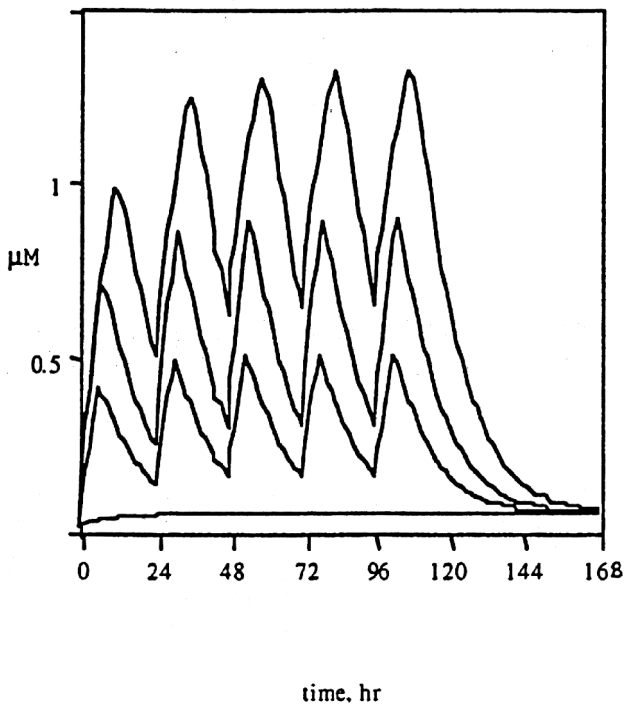

FIGURE H4
Model Predictions of Isoprene Monoepoxide Concentrations in the Blood of Rats Exposed to 0, 220, 700, or 7,000 ppm Isoprene for 5 Days (6 hr/day)

TABLE H5
Radioactivity (Isoprene and Isoprene Metabolites) Recovered in Urine or Breath from F344/N Rats Administered 64 mg/kg [^{14}C]-Isoprene by Intraperitoneal Injection: Model Predictions versus Experimental Data

Time (hr)	Urine		Breath	
	Model	Measured	Model	Measured
6	2.6	5.2 ± 1.3	58.2	57 ± 4.8
12	18.9	19.0 ± 0.4	61.0	59 ± 0.5
24	34.9	35.0 ± 3.6	67.6	62 ± 3.2

TABLE H6
Concentration of Isoprene Metabolites (μM) in Blood of F344/N Rats Immediately Following Single 6-Hour Inhalation Exposures: Model Predictions versus Experimental Data

Exposure (ppm)	Predicted	Observed
18	17.3	9
280	173	235
1,480	210	603
8,200	213	614

TABLE H7
Excretion of Vinyl Lactic Acid (μmol) in 16-Hour Urine Samples of F344/N Rats after 3 Months of Inhalation Exposure to Isoprene: Model Predictions versus Experimental Data

Exposure (ppm)	Measured	Model[a]	Model[b]
0	0	1.5	1.8
220	36.9	33.5	40.2
700	90.7	56.1	69.2
7,000	220.7	81.9	109

[a] Epoxide hydrolase activity assumed to be the same as that measured with epoxybutene (Csanády *et al.*, 1992)
[b] Optimized epoxide hydrolase activity

TABLE H8
Predicted 1-Week Cumulative Levels of Isoprene and Isoprene Monoepoxides (μmol/L • 7 days) in Blood, Kidney, Liver, and Lung of Rats Exposed to Isoprene by Inhalation for 5 Days Followed by 2 Nonexposure Days

Exposure	Isoprene				Isoprene Monoepoxides			
(ppm)	Blood	Kidney	Liver	Lung	Blood	Kidney	Liver	Lung
0	38.7	9.6	2.7	13.9	426	366	350	331
220	584	290	65.8	366	4,920	4,130	4,190	3,820
700	2,160	1,420	927	1,390	9,620	7,950	8,340	7,460
7,000	26,200	19,300	20,300	17,300	17,400	14,400	15,100	13,500