National Toxicology Program
Toxicity Report Series
Number 31

NTP Technical Report
on Toxicity Studies of

Isoprene

(CAS No. 78-79-5)

Administered by Inhalation
to F344/N Rats and B6C3F$_1$ Mice

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Post Office Box 12233
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United States Department of Health and Human Services
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National Institutes of Health

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This NTP report on the toxicity studies of isoprene is based primarily on 2-week studies conducted in 1986 and on 13-week and stop-exposure studies that took place in 1988
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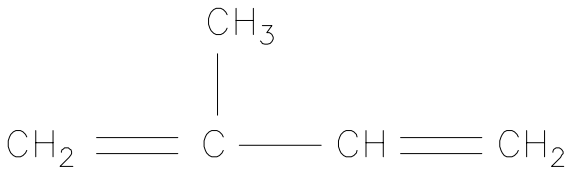
ABSTRACT

Isoprene



Molecular Formula	C_5H_8
CAS Number	78-79-5
Molecular Weight	68.1
Synonyms	isopentadiene 2-methyl-1,3-butadiene β-methylbivinyl

Isoprene, the 2-methyl analogue of 1,3-butadiene, has a high production volume and is used largely in the manufacture of synthetic rubber. Isoprene is also the major endogenous hydrocarbon exhaled in human breath. Two-week and 13-week inhalation toxicology studies were conducted in male and female F344/N rats and B6C3F$_1$ mice to characterize potential adverse effects of isoprene. Male rats and male mice were also exposed to isoprene vapors for 6 months followed by a 6-month recovery period (stop-exposure protocol) to determine if isoprene produces a carcinogenic response similar to that of 1,3-butadiene after intermediate exposure durations. In addition to histopathology, evaluations included clinical pathology, tissue glutathione analyses, forelimb and hindlimb grip strength analyses, and sperm motility and vaginal cytology. Data from inhalation teratology studies of isoprene in rats and mice are also reported. *In vitro* genetic toxicity studies included assessments of mutagenicity in *Salmonella typhimurium* and sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. In conjunction with the inhalation studies in mice, evaluations were also made of sister chromatid exchanges and chromosomal aberrations in bone marrow cells and micronuclei in peripheral blood of male mice exposed to isoprene for 12 days or 13 weeks.

Target concentrations of isoprene in the inhalation chambers were 0, 438, 875, 1,750, 3,500, and 7,000 ppm in the 2-week studies; 0, 70, 220, 700, 2,200, and 7,000 ppm in the 13-week and stop-exposure studies; and 0, 280, 1,400, and 7,000 ppm in the teratology studies. In the 2-week studies, no changes related to chemical administration were observed in survival, body weight gain,

clinical signs, hematologic or clinical chemistry parameters, or the incidence of gross or microscopic lesions in rats. In mice, there were no effects on survival; the mean body weight of males in the 7,000 ppm group was less than that of the controls. In mice, exposure to isoprene caused decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts, atrophy of the testis and thymus, cytoplasmic vacuolization of the liver, olfactory epithelial degeneration in the nasal cavity, and epithelial hyperplasia in the forestomach.

Exposure to isoprene for 13 weeks produced no discernible toxicologic effects in rats. In the stop-exposure study, interstitial cell hyperplasia of the testis was observed in all male rats in the 7,000 ppm group after 6 months of exposure. Following the 6-month recovery period, male rats exposed to 700, 2,200, or 7,000 ppm isoprene had slightly greater incidences of interstitial cell adenomas of the testes than the controls.

Exposure to isoprene for 13 weeks or 6 months produced no clear exposure-related effects on body weight gain in male or female mice; however, survival was decreased for male mice exposed to 7,000 ppm isoprene for 6 months. More notably, toxic and carcinogenic effects were induced at multiple organ sites in mice exposed to isoprene. After 6 months of exposure and 6 months of recovery, male mice exposed to 700 ppm or higher concentrations of isoprene had greater incidences of neoplasms of the liver (0 ppm, 7/30; 70 ppm, 3/30; 220 ppm, 7/29; 700 ppm, 15/30; 2,200 ppm, 18/30; 7,000 ppm, 17/28), lungs (2/30, 2/30, 1/29, 5/30, 10/30, 9/28), forestomach (0/30, 0/30, 0/30, 1/30, 4/30, 6/30), and harderian gland (2/30, 6/30, 4/30, 14/30, 13/30, 12/30) than the controls. In addition to the higher neoplasm incidences in male mice exposed to 700 ppm or greater, incidences of multiple neoplasms and/or neoplasms of greater malignancy were also higher than in the controls. Hematologic effects similar to those occurring in exposed mice in the 2-week study, plus greater mean cell volume values than in the controls, were observed after 24 days and after 13 weeks of exposure to isoprene. These hematologic effects, which were not accompanied by greater reticulocyte counts or a higher frequency of polychromatic erythrocytes than controls, were indicative of a nonresponsive, macrocytic anemia. In male mice in the stop-exposure study, partial hindlimb paralysis in the 7,000 ppm group and a dose-related decrease in grip strength were observed near the end of the 6-month exposure period. Other nonneoplastic effects in mice exposed to isoprene included spinal cord and sciatic nerve degeneration, skeletal muscle atrophy, degeneration of the olfactory epithelium, epithelial hyperplasia of the forestomach, increased estrous cycle length, testicular atrophy, and decreased epididymal weight, sperm head count, sperm concentration, and sperm motility. The inhalation teratology studies did not show

maternal or developmental toxicity in Sprague-Dawley rats at exposures of up to 7,000 ppm isoprene; in CD-1® Swiss mice, exposure to isoprene resulted in lower fetal weights and a higher percentage of fetuses per litter with supernumerary ribs.

Isoprene was not mutagenic in *Salmonella typhimurium* and did not induce sister chromatid exchanges or chromosomal aberrations in 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 thefrequency of micronucleated erythrocytes in peripheral blood.

These inhalation studies showed that isoprene caused toxic effects in the testis of rats and at multiple organ sites in mice. In F344/N rats, exposure to 7,000 ppm isoprene for 6 months caused an increase in the incidence of testicular interstitial cell hyperplasia, and after 6 months of recovery there was a marginally increased incidence of benign testicular adenomas that may have been related to isoprene administration. No-observable-adverse-effect levels (NOAELs) for isoprene-induced toxic lesions in mice were:

- 70 ppm for nonresponsive, macrocytic anemia, decreased hindlimb grip strength, olfactory epithelial degeneration, and decreases in epididymalweights, spermatid head counts, sperm concentration, and sperm motility;
- 220 ppm for forestomach epithelial hyperplasia;
- 700 ppm for increased estrous cycle length;
- and 2,200 ppm for testicular atrophy, sciatic nerve degeneration, and muscle atrophy.

A NOAEL was not achieved for spinal cord degeneration (less than 70 ppm) or developmental toxicity (less than 280 ppm, based on lower body weights of female fetuses). In addition, the 6-month inhalation exposure plus 6-month recovery (stop-exposure) study provided clear evidence of carcinogenicity of isoprene in the liver, lung, forestomach, and harderian gland of mice. Because these studies involved exposures of male rats and male mice to isoprene for only 6 months, they do not necessarily reveal the full carcinogenic potential of isoprene in these species. Most of the toxic and carcinogenic effects seen with isoprene were also caused by inhalation exposure to 1,3-butadiene.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of isoprene on November 16, 1993, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members determine if the design and conditions of these NTP studies are appropriate and ensure that this toxicity study report presents the experimental results and conclusions fully and clearly.

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SUMMARY OF PEER REVIEW COMMENTS

On November 16, 1993, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of isoprene.

Dr. Ronald L. Melnick, NIEHS, introduced the short-term toxicity studies of isoprene by reviewing the uses of isoprene and the rationale for the studies. Studies were undertaken on isoprene as part of the butadiene initiative developed in the 1980's. Isoprene and chloroprene were studied because the production volumes of these chemicals are high and because the chemicals are structurally related to butadiene. Dr. Melnick detailed the metabolism of isoprene and compared it with that for butadiene. Two-week and 13-week inhalation toxicology studies were conducted in which isoprene was administered to male and female F344/N rats and B6C3F$_1$ mice at concentrations up to 7,000 ppm. Male rats and male mice were also exposed to isoprene vapors for 6 months, followed by a 6-month recovery period, to determine if isoprene produces a carcinogenic response similar to that of 1,3-butadiene after intermediate exposure durations. In addition to histopathology, evaluations included clinical pathology, tissue glutathione concentration, forelimb and hindlimb grip strength, and sperm motility and vaginal cytology. Data from inhalation teratology studies as well as from *in vitro* and *in vivo* genetic toxicity studies were also reported.

Dr. Melnick concluded that the studies demonstrated that isoprene is toxic to the testes of rats, inducing interstitial cell hyperplasia after 6 months of exposure, and that the marginally increased incidence of testicular adenomas seen after the 6-month recovery period may have been related to isoprene administration. Exposure to isoprene for 13 weeks produced no discernible toxicologic effects in rats. In mice, isoprene caused a nonresponsive macrocytic anemia similar to that seen with butadiene and a decrease in hindlimb grip strength. Isoprene was also toxic to the forestomach, nasal cavity, testes, and spinal cord. Isoprene induced increases in the frequency of sister chromatid exchanges in bone marrow cells and in the frequency of micronucleated erythrocytes in peripheral blood. No-observable-adverse-effect levels (NOAELs) were determined for most of the toxic lesions in mice.

Isoprene was carcinogenic to the liver, lung, forestomach, and harderian gland of mice. Inhalation teratology studies did not reveal an effect in rats, but CD-1® Swiss mice exposed to isoprene had lower fetal weights and a larger percentage of fetuses per litter with supernumerary ribs at exposure concentrations that were not maternally toxic. Most of the toxic and carcinogenic effects seen with isoprene were also caused by 1,3-butadiene in mice.

Dr. Taylor, a principal reviewer, thought the report well written, the study design rigorous and highly focused, and the metabolism section quite informative. He wondered whether there had been characterization of the cytochrome P$_{450}$ isozymes associated with isoprene metabolism. Dr. Melnick said that 2E1 has been shown to be a major contributor to the oxidation of butadiene to its monoepoxide, but whether this or another isozyme contributes to isoprene metabolism is not known. Dr. Taylor suggested that more discussion of the neoplasms, especially in terms

of multiplicity, location within a site, and morphology, was needed. Dr. Melnick agreed to add more detail. Further, recognizing that these were not typical 2-year studies, Dr. Taylor said some consideration might be given to assigning a level of evidence for carcinogenicity in mice.

Dr. Ward, the second principal reviewer, also thought that consideration should be given to assigning a level of evidence in mice, in this case, **clear evidence of carcinogenic activity**. Dr. Melnick said the 6-month stop-exposure study was adequate to evaluate a carcinogenic effect, but not the full carcinogenic potential of isoprene in mice. Dr. Ward commented on the high incidences of liver, harderian gland, and lung neoplasms in control animals evaluated at 12 months and wondered whether such findings were typical for inhalation studies. Dr. Melnick responded that he couldn't answer the question, because limited NTP historical data are available for evaluations made at 12 months. Dr. Bailey asked whether there were plans to conduct a 2-year study. Dr. Melnick replied that a 2-year study was underway in rats, but no decision had been made on whether a 2-year study in mice was warranted. This concluded the discussion of the isoprene report.

INTRODUCTION

Physical Properties, Production, Use, and Exposure

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 isoprene at 25 ° C and 760 mm Hg is 1 ppm = 2.79 mg/m^3. Isoprene is obtained as a byproduct of naphtha cracking in the production of ethylene; it is also obtained through synthetic routes, including dehydrogenation of isopentane, dehydrogenation of tertiary amylenes, dimerization of propylene, and condensation of isobutylene with formaldehyde (*Kirk-Othmer*, 1981). Isoprene has been detected in tobacco smoke, and it is the monomeric unit of natural rubber and naturally occurring terpenes and steroids.

Isoprene is highly reactive, and its dimerization, halogenation, and polymerization reactions are similar to those of 1,3-butadiene. More than 95% of industrial isoprene is used in the preparation of *cis*-1,4-polyisoprene elastomers (*Kirk-Othmer*, 1981). Isoprene is also used as a comonomer with isobutylene in the production of butyl rubber. Polyisoprene elastomers are used in the manufacture of rubber tires, automotive parts, gaskets, footwear, adhesives, and flooring (*Kirk-Othmer*, 1981). About 350 million pounds of isoprene are produced annually in the United States (USITC, 1990).

Based on estimates from data compiled in a National Occupational Exposure Survey, approximately 3,700 workers are potentially exposed to isoprene annually (NIOSH, 1990). Most of these exposures involve residual monomeric isoprene in polyisoprene products. No information is available on consumer exposure or on residual concentrations of isoprene in polymeric elastomers. Human volunteers exposed to concentrations of about 60 ppm isoprene experienced irritation in the upper respiratory tract (Sandmeyer, 1981). No regulatory exposure standard has been established for isoprene.

Isoprene was identified as the major endogenous hydrocarbon in human breath (DeMaster and Nagasawa, 1978; Gelmont *et al.*, 1981); exhalation of isoprene by human subjects was estimated to be 2 to 4 mg per day (Gelmont *et al.*, 1981). Isoprene was reported to be produced endogenously by rats and mice at rates of 1.9 and 0.4 µmole/hour per kilogram body weight, respectively (Peter *et al.*, 1987). The availability and distribution of endogenous isoprene is partially controlled by the utilization of isopentenyl pyrophosphate as the activated precursor for the synthesis of biomolecules that contain isoprene units.

Metabolism and Pharmacokinetics

Isoprene has been shown to be metabolized to 3,4-epoxy-3-methyl-1-butene (Epoxide-I) and 3,4-epoxy-2-methyl-1-butene (Epoxide-II) by liver microsomal cytochrome P_{450}-dependent monooxygenases of New Zealand rabbits, Syrian golden hamsters, Wistar rats, and Swiss mice (Figure 1; Del Monte *et al.*, 1985; Longo *et al.*, 1985; Gervasi and Longo, 1990). The V_{max} value for isoprene oxidation to Epoxide-I in mice was about seven times higher than that in rats, whereas the apparent K_m values were similar in these species. Epoxide-II, which is produced at 20% to 25% the level of Epoxide-I, was oxidized to the mutagen isoprene diepoxide (2-methyl-1,2:3,4-diepoxy-butane) in hepatic microsomes from all species examined. This is similar to the biotransformation of 1,3-butadiene, which involves initial oxidation to 1,2-epoxy-3-butene followed by hydrolysis to 3-butene-1,2-diol or further oxidation to diepoxybutane (Malvoisin and Roberfroid, 1982). No isoprene diepoxide was detected after incubation of liver microsomal preparations with Epoxide-I (Del Monte *et al.*, 1985).

Peter *et al.* (1987) investigated the inhalation pharmacokinetics of isoprene in Wistar rats and $B6C3F_1$ mice. Metabolism of isoprene is linear in rats and mice at atmospheric concentrations up to about 300 ppm. Metabolic saturation occurs at about 1,500 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 µmole/hour per kg) is about three times greater than that in rats (130 µmole/hour per kg).

Metabolites of isoprene were detected in the blood, nose, lungs, liver, kidney, 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 levels of specific intermediates (*e.g.*, 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 of the disposition of inhaled isoprene, the percentage of inhaled isoprene that was metabolized in $B6C3F_1$ mice was twofold to fivefold less than the percentage that was 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 inhalation (Sun *et al.*, 1989; Bond *et al.*, 1991).

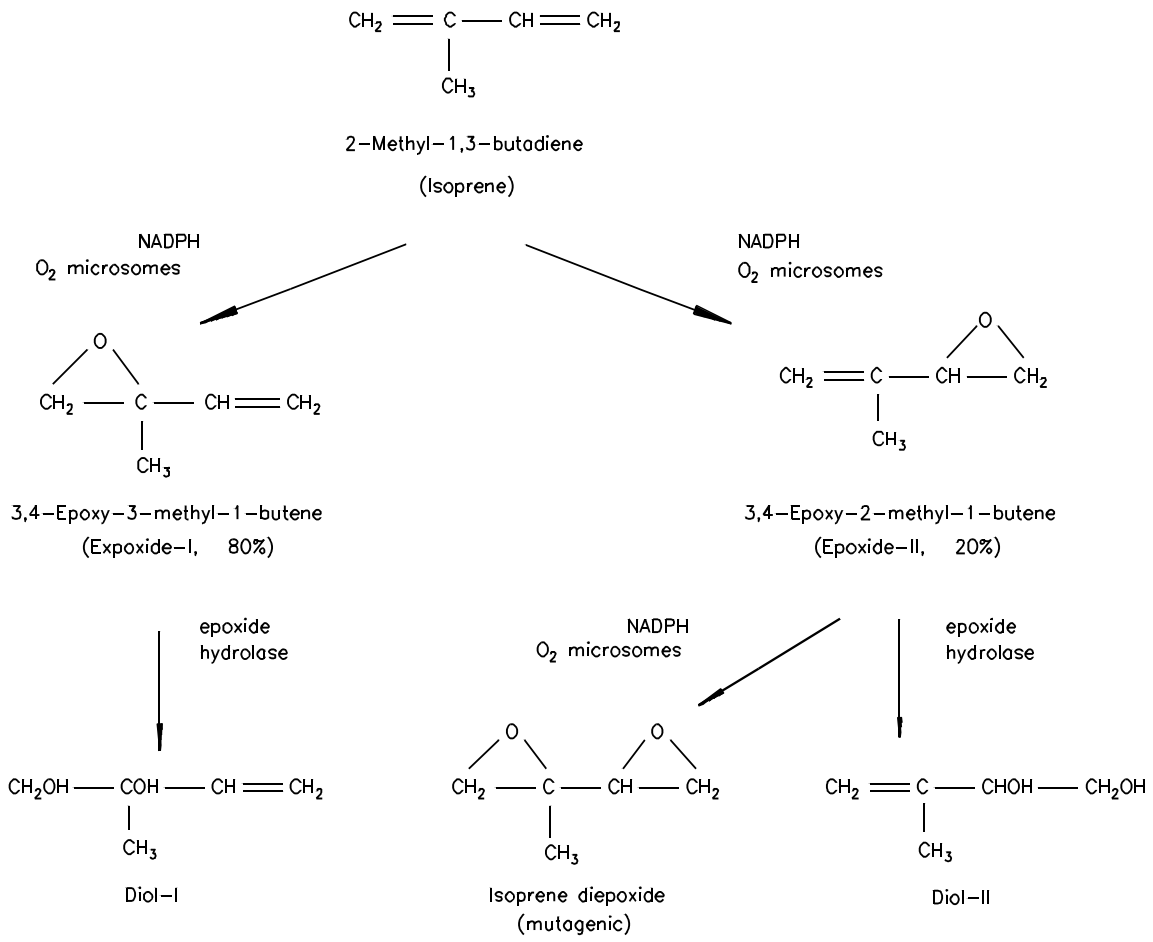

FIGURE 1 Metabolism of Isoprene in the Liver of Rabbits, Hamsters, Rats, and Mice (from Gervasi and Longo, 1990)

Toxicity

ANIMAL TOXICITY

Few toxicity studies of isoprene have been published. 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) exposed to 1,670 ppm isoprene 6 hours per day for 15 exposure days or to 6,000 ppm isoprene 6 hours per day for 6 exposure days (Gage, 1970). No body weight effects were observed in mice, rabbits, or rats exposed to concentrations of 790 to 1,750 ppm isoprene 4 hours per day for 4 to 5 months (Sandmeyer, 1981).

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 the monoepoxide intermediates of isoprene biotransformation, 3,4-epoxy-2-methyl-1-butene and 3,4-epoxy-3-methyl-1-butene, in *S. typhimurium* strains TA98 and TA100 were negative (Gervasi *et al.*, 1985). However, the diepoxide, 2-methyl-1,2:3,4-diepoxybutane, which may be generated by further epoxidation of the 2-methyl monoepoxide, is a potent *S. typhimurium* mutagen (Gervasi *et al.*, 1985). Therefore, 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 multi-step biotransformation to produce a mutagenic product.

Inhalation exposure of male B6C3F_1 mice to isoprene (6 hours per day for 12 exposure days) at concentrations ranging from 70 to 7,000 ppm produced significant increases in sister chromatid exchanges (SCEs) in bone marrow cells and 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.

Study Rationale and Design

Isoprene was selected for toxicologic evaluations because of its structural similarity to 1,3-butadiene, a potent rodent carcinogen, and its large annual production with potentially high human exposures. Long-term inhalation studies have demonstrated that 1,3-butadiene is a multiple-organ carcinogen in Sprague-Dawley rats (Owen *et al.*, 1987) and B6C3F$_1$ mice (NTP, 1984a, 1993; Huff *et al.*, 1985; Melnick *et al.*, 1990a). Particularly noteworthy in mice were the early occurrences and extensive development of lethal thymic lymphomas (as early as Week 23), the induction of uncommon hemangiosarcomas of the heart, and the development of malignant lung neoplasms at exposure concentrations as low as 6.25 ppm (Melnick *et al.*, 1990a; NTP, 1993). In experiments with reduced exposure durations, neoplastic lesions were induced at multiple organ sites in mice after only 13 weeks of exposure to 625 ppm 1,3-butadiene. Exposure of mice to 1,3-butadiene also caused a poorly regenerative macrocytic anemia, testicular and ovarian atrophy, and degenerative changes in nasal tissues.

After evaluation of the results of the 2-week inhalation toxicity studies of isoprene in F344/N rats and B6C3F$_1$ mice, a structure/activity relationship between isoprene and 1,3-butadiene became evident (Melnick *et al.*, 1990b):

- both compounds cause reductions in red blood cell counts, hemoglobin concentrations, and packed red cell volumes in mice;
- both produce olfactory epithelial degenerative changes, testicular atrophy, and forestomach epithelial hyperplasia in mice;
- both induce increases in the frequency of SCEs in bone marrow cells and in the levels of micronucleated erythrocytes in peripheral blood of mice (Tice *et al.*, 1987, 1988);
- both compounds are metabolized to monoepoxide and diepoxide intermediates by liver microsomal monooxygenases (Malvoisin *et al.*, 1979; Malvoisin and Roberfroid, 1982; Del Monte *et al.*, 1985; Longo *et al.*, 1985);
- and the diepoxide intermediates of both compounds are mutagenic in *S. typhimurium* (Wade *et al.*, 1979; Gervasi *et al.*, 1985).

Because of these similarities, 6-month exposure plus 6-month recovery (stop-exposure) studies were added to the planned 13-week studies to determine if isoprene produces a carcinogenic response similar to that of 1,3-butadiene after intermediate exposure durations. Thus, the results of the 2-week and 13-week inhalation toxicity studies and the stop-exposure inhalation studies of isoprene in rats and mice are presented in this report; data from teratology studies of isoprene in rats and mice and genetic toxicity studies of isoprene are also included.

MATERIALS AND METHODS

Procurement and Characterization of Isoprene

Six lots of isoprene were obtained from Goodyear Tire and Rubber Company (Akron, OH) for use in the 2-week and 13-week inhalation studies and the 6-month stop-exposure inhalation studies. Two additional lots of isoprene (Lots G080886 and UN1218) were used for preliminary testing and were analyzed by Midwest Research Institute (MRI; Kansas City, MO). For Lot G080886, which was obtained from Goodyear Tire and Rubber Company (Akron, OH), MRI analyzed a portion of the liquid bulk chemical; for Lot UN1218, which was obtained from Goodyears' Beaumont Chemical Plant (Beaumont, TX), MRI analyzed gas samples from the cylinder headspace. Both lots of the chemical, a clear, colorless liquid (Lot G080886) or gas (Lot UN1218), were identified as isoprene, and infrared and nuclear magnetic resonance spectra were consistent with the structure of isoprene and a literature reference (*Sadtler Standard Spectra*). Gas chromatography indicated no impurities with areas greater than 0.1% relative to the major peak area for both lots. Additional titration and colorimetric (American Oil Chemists Society Official Method Cd-8-53) analyses of Lot G080886 indicated the presence of 40 to 48 ppm *t*-butylcatechol (inhibitor) and 0.290 ± 0.003 mEq peroxide per 1,000 g of isoprene, respectively. For Lot UN1218, quantitation of limonene (the most abundant dimer) by gas chromatography with flame ionization detection (FID) indicated a concentration of less than 0.5 ng/mL (0.23 ppm) in the cylinder headspace. For Lot G080886, dimer analysis by gas chromatography/mass spectrometry indicated the presence of five dimers, all at concentrations less than 0.1%; quantitation of the dimers by gas chromatography with FID indicated a total concentration of 543 ± 21 ppm, of which 489 ± 19 ppm was determined to be limonene. Cumulative analytical data for both lots indicated purities greater than 99%.

In the 2-week, 13-week, and stop-exposure studies, all lots of isoprene used for animal exposures were sent directly to the study laboratory from Goodyear. At the study laboratory, identity and purity analyses were performed on Lots 12102-7 and 12102-11 (2-week studies) and on Lots 12299-12, 12299-84, and 12299-112 (13-week and stop-exposure studies). The identity of each lot was confirmed by infrared spectroscopy; the spectra were similar to those reported by MRI for Lots G080886 and UN1218. For each lot, gas chromatographic analyses indicated a purity greater than 99%, and limonene content, which was determined by gas chromatography, was within the acceptable limit of 1% (10,000 ppm). In addition, titration analyses of Lot 12102-127, which was

used for prestart testing in the 13-week and stop-exposure studies, detected no peroxides after 3½ months of storage.

No stability studies were performed on the bulk chemical. At the study laboratory, isoprene was stored in metal cylinders at room temperature. Bulk chemical reanalyses performed by the study laboratory with gas chromatography showed consistent purity levels throughout the studies, and limonene and peroxide contents in the bulk chemical remained within acceptable limits (less than 0.3% and 0.1 mEq/kg, respectively).

Vapor Generation System

The isoprene vapor exposures were conducted using an automated data acquisition and control system. A central computer (HP 9816; Hewlett-Packard, Palo Alto, CA) monitored and controlled the basic chamber functions (*i.e.*, isoprene concentration, airflow, vacuum, temperature, and relative humidity) in the exposure rooms. Animals were exposed and maintained in inhalation exposure chambers developed at Battelle Pacific Northwest Laboratories and commercially produced by the Harford Division of Lab Products, Inc. (Aberdeen, MD). Each chamber had an active mixing volume of 1.7 m^3.

Isoprene vapor was produced using a generator equipped with a rotary evaporator system (Büchi Rotavapor Model EL-131S, Büchi Laboratoriums Technik AG, Flavil, Switzerland). Briefly, liquid isoprene was pumped into a rotating evaporator flask immersed in a hot water bath (approximately 50° C) by introducing low pressure nitrogen (4 to 6 psi) into the vapor inlet of a cylinder of the bulk chemical. The resulting vapor moved out of the mouth of the flask into a chilled water condenser where much of the vapor was recondensed, returning to the evaporator flask. Nitrogen was metered into the bottom of the condenser and flowed out the top, becoming saturated with isoprene vapor as it passed through the condenser. The temperature of the saturated nitrogen was monitored, and the saturation vapor pressure was calculated to determine the generator output (ppm of isoprene and flow rate of saturated nitrogen). The vapor then entered a short distribution manifold where individual delivery lines carried a metered amount of the vapor to the exposure chambers. Vacuum transducer pumps connected at the chamber end of each delivery line generated the negative pressure used to move the isoprene vapor. Chamber concentration adjustments were achieved by adjusting the metering valves and/or the compressed air pressure to the pumps.

Concentration Monitoring

Isoprene vapor concentration was monitored with an automated gas chromatographic system (HP 5840; Hewlett Packard, Palo Alto, CA) equipped with a flame ionization detector and an automated 12-position stream select gas sampling valve. This system was used to measure isoprene concentrations in the exposure chambers, the control chamber, the exposure room, and the on-line standard. Calibration of the on-line chamber monitor was based on the comparison of gravimetric standards to bubbler grab samples using an independent off-line gas chromatograph. An on-line standard (2,000 ppm isoprene in nitrogen) was used to check instrument drift throughout the exposure day.

Mean chamber concentrations of isoprene during the 2-week and 13-week studies and the stop-exposure studies were calculated from daily monitoring data. The mean concentrations in all chambers for the 2-week studies were between 100% and 101% of target concentrations, with relative standard deviations ranging from 3% to 5%; at least 95% of all individual concentration measurements were within 10% of target concentrations (Table 1). The mean concentrations in all chambers for the 13-week and stop-exposure studies were between 99% and 100% of target concentrations, with relative standard deviations ranging from 5% to 7%; 99% of all individual concentration measurements were within 10% of target concentrations (Table 1).

Chamber Characterization

CONCENTRATION UNIFORMITY

During the 2-week and 13-week studies, the uniformity of vapor concentration throughout each exposure chamber was measured prior to the start of the studies and once during the studies. During the stop-exposure studies, vapor concentration uniformity was measured prior to the beginning of the studies, at the start of the studies, and after approximately 13 weeks of exposure. Vapor concentration was measured using the on-line gas chromatograph with the automatic 12-port sample valve disabled to allow continuous monitoring from a single input line. The relative standard deviations for all chamber uniformity measurements were less than 5%.

TABLE 1 Mean Chamber Concentrations of Isoprene in the 2-Week, 13-Week, and Stop-Exposure Inhalation Studies in F344/N Rats and B6C3F$_1$ Mice

Target Concentration (ppm)	Mean ± SD (ppm)	Target ± RSD[1] (%)	Maximum (ppm)	Minimum (ppm)	Samples within Range[2] (%)
2-WEEK STUDIES					
F344/N Rats					
0	< MDL[3]	—	< MDL	< MDL	100
438	436 ± 18	100 ± 4	494	378	98
875	875 ± 41	100 ± 5	1,050	768	95
1,750	1,760 ± 55	101 ± 3	1,920	1,440	99
3,500	3,530 ± 110	101 ± 3	3,880	3,030	99
7,000	6,980 ± 178	100 ± 3	7,530	6,110	99
B6C3F$_1$ Mice					
0	< MDL	—	< MDL	< MDL	100
438	436 ± 18	100 ± 4	494	382	98
875	874 ± 42	100 ± 5	1,050	768	95
1,750	1,760 ± 59	101 ± 3	1,950	1,440	99
3,500	3,530 ± 111	101 ± 3	3,880	3,030	99
7,000	6,980 ± 176	100 ± 3	7,530	6,110	99
13-WEEK STUDIES					
F344/N Rats					
0	< MDL	—	0.8	< MDL	100
70	69.2 ± 4.5	99 ± 7	76.4	0.6	99
220	219 ± 14	100 ± 7	244	4.1	99
700	695 ± 47	99 ± 7	757	19	99
2,200	2,180 ± 148	99 ± 7	2,340	149	99
7,000	6,930 ± 400	99 ± 6	7,410	641	99
B6C3F$_1$ Mice					
0	< MDL	—	0.8	< MDL	100
70	69.2 ± 4.5	99 ± 7	76.4	0.6	99
220	219 ± 14	100 ± 6	244	4.1	99
700	694 ± 47	99 ± 7	757	19	99
2,200	2,190 ± 148	99 ± 7	2,340	149	99
7,000	6,930 ± 400	99 ± 6	7,410	641	99

TABLE 1 Mean Chamber Concentrations of Isoprene in the 2-Week, 13-Week, and Stop-Exposure Inhalation Studies in F344/N Rats and B6C3F$_1$ Mice (continued)

Target Concentration (ppm)	Mean ± SD (ppm)	Target ± RSD (%)	Maximum (ppm)	Minimum (ppm)	Samples within Range (%)
STOP-EXPOSURE STUDIES					
F344/N Rats					
0	< MDL	—	0.8	< MDL	100
70	69.2 ± 3.6	99 ± 5	76.7	< MDL	99
220	220 ± 10	100 ± 5	255	4.1	99
700	698 ± 34	100 ± 5	789	19	99
2,200	2,190 ± 112	100 ± 5	2,400	149	99
7,000	6,980 ± 326	100 ± 5	7,640	641	99
B6C3F$_1$ Mice					
0	< MDL	—	0.8	< MDL	100
70	69.3 ± 3.6	99 ± 5	76.7	< MDL	99
220	220 ± 10	100 ± 5	255	4.1	99
700	698 ± 34	100 ± 5	789	19	99
2,200	2,190 ± 113	100 ± 5	2,400	149	99
7,000	6,980 ± 328	100 ± 5	7,640	641	99

[1] Target concentration ± relative standard deviation as a percent of target concentration.
[2] A sample was considered to be in range if the concentration was less than 1 ppm (for control samples) or if the sample was within 10% of the target concentration.
[3] MDL = minimum detectable limit. For the 2-week studies, MDL = 0.02 ppm. For the 13-week and stop-exposure studies, MDL = 0.03 ppm.

CONCENTRATION BUILDUP AND DECAY

Buildup and decay rates were measured prior to the start of the study without animals in the chambers and at the beginning of the exposure regimen to determine if the presence of animals in the chambers would affect the rates. The time following the start of the exposure for the isoprene concentration to reach 90% of the final stable concentration in the chamber (T_{90}) and the time following the termination of vapor generation for the isoprene concentration to decrease to 10% of the stable concentration (T_{10}) were determined.

For the 2-week studies, buildup times ranged from 9 to 12 minutes without animals and from 9 to 14 minutes with animals. A value of 12 minutes was used as the T_{90} for the 2-week studies. T_{10} values ranged from 9 to 12 minutes without animals and from 12 to 13 minutes with animals.

For the 13-week and stop-exposure studies, buildup times ranged from 8 to 9 minutes without animals in the chambers and from 10 to 13 minutes with animals. For these studies, a value of 12 minutes was also used as the T_{90}. T_{10} values ranged from 8 to 10 minutes without animals and from 10 to 12 minutes with animals.

STABILITY STUDIES

The stability of isoprene in the vapor generating system and the exposure chambers was determined by gas chromatography. For the 2-week studies, samples were taken, with and without animals present, from the 438 and 7,000 ppm chambers using gas sampling charcoal tubes (Supelco ORBO-32 charcoal tubes) and from the generator flask after a typical exposure day. Analysis of chamber samples revealed no evidence of decomposition products exceeding 1% of the isoprene concentration in either the 438 or 7,000 ppm chamber, with or without animals present. Higher than initial concentrations of peroxides and limonene were noted in the generator flask at the end of the exposure day, but this accumulation of less volatile decomposition products was expected due to the distilling effect of the vapor generation system.

For the 13-week and stop-exposure studies, samples were taken, with and without animals present, from the 70 and 7,000 ppm chambers and from the distribution line. The cumulative concentration of impurities found in the chamber and distribution samples was less than 0.2% of the total sample area. Analysis of samples collected from the 7,000 ppm chamber without animals present showed a limonene concentration of 0.009%; limonene was not detected in the 70 ppm chamber sample with or without animals or in the 7,000 ppm chamber with animals.

Toxicity Study Designs

BASE INHALATION STUDIES

F344/N rats and B6C3F$_1$ mice used in these studies were obtained from Simonsen Laboratories (Gilroy, CA) for the 2-week studies and Taconic Farms (Germantown, NY) for the 13-week and stop-exposure studies. Rats and mice used in the 2-week studies were shipped to the study laboratory at approximately 4 weeks of age; rats and mice used in the 13-week and stop-exposure studies were shipped to the study laboratory at 4 to 7 weeks of age. Animals were quarantined for 11 to 13 days and were about 5 to 6 weeks of age (2-week studies) or 6 to 8 weeks of age (13-week and stop-exposure studies) when the studies began.

During the 2-week and 13-week studies, blood samples were collected from five sentinel rats and mice of each sex 3 weeks after receipt; blood samples were also collected from five sentinel rats and mice of each sex at the end of the 13-week studies. In the stop-exposure studies, blood samples were collected from five male control rats during the exposure period, from 10 male sentinel rats and mice at the end of the exposure periods, and from 10 male control rats and mice at the end of the recovery periods. The sera were analyzed for viral and bacterial antibody titers; data showed no positive antibody titers (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). Additional details concerning study design and performance are listed in Table 2.

In the 2-week studies, groups of 10 males and 10 females per species were exposed to isoprene vapor through whole-body exposure at target concentrations of 0, 438, 875, 1,750, 3,500, or 7,000 ppm for 6 hours plus T_{90} per day, 5 days per week for 12 exposure days; additional rats and mice were used in supplemental clinicalpathology studies. The highest exposure concentration used in these studies was limited to 50% of the lower flammable level of isoprene.

Exposure concentrations for the 13-week and stop-exposure studies were based on the results of the 2-week studies. In the 13-week base studies, groups of 10 males and 10 females per species were exposed to isoprene vapor through whole-body exposure at target concentrations of 0, 70, 220, 700, 2,200, or 7,000 ppm for 6 hours plus T_{90} per day, 5 days per week for 13 weeks (excluding holidays); additional rats and mice were used in supplemental clinical pathology studies and glutathione tissue level evaluations.

In the stop-exposure studies, groups of 40 male rats and 40 male mice were exposed to isoprene vapor through whole-body exposure at target concentrations of 0, 70, 220, 700, 2,200, or 7,000 ppm for 6 hours plus T_{90} per day, 5 days per week for 6 months (excluding holidays). At the end of the exposure period, 10 male rats and 10 male mice per exposure group were killed and evaluated. The remaining male rats and mice were allowed to recover for an additional 6 months without isoprene exposure.

Rats and mice were housed in individual cages within the exposure chambers. During the stop-exposure recovery periods, animals where housed in cages stored on open racks. For all studies, city water (Richland, WA) was available *ad libitum*. NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form was available *ad libitum*, except during exposure periods and urine collection periods (if applicable). Animal rooms were maintained at 75 ± 3°

F and 55% ± 15% relative humidity, with approximately 15 air changes per hour and 12 hours of fluorescent light per day.

Complete necropsies were performed on all base-study animals. The brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus were weighed prior to fixation. In the stop-exposure study in mice, organ weights were not determined for five mice each in the 0 and 7,000 ppm groups killed at the end of the 6-month exposure period or for five mice each in the 0 and 7,000 ppm groups killed at the end of the 6-month recovery period; these animals were fixed by whole-body vascular perfusion with Karnovsky's fixative for electron microscopy. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

In the 2-week and 13-week studies, complete histopathologic examinations were performed on all rats and mice in the 0 and 7,000 ppm groups. In the stop-exposure studies, complete histopathologic examinations were performed on the following animals:

- rats in the 0 and 7,000 ppm groups and mice in all exposure groups killed after the 6-month exposure period,
- rats and mice in the 0 and 7,000 ppm groups and mice in the 2,200 ppm group killed after the 6-month recovery period,
- and all animals that died before the end of the studies.

Gross lesions and selected tissues were examined in the lower exposure groups to a no-observable-effect level. Additionally, lumbar spinal cord sections from five mice each in the 0 and 7,000 ppm stop-exposure groups were examined at the end of the 6-month exposure period using an electron microscope; because these examinations were noncontributory, the scheduled evaluations of spinal cord sections from five mice each in the 0 and 7,000 ppm groups were not performed at the end of the recovery period. All tissues examined microscopically are listed in Table 2.

Upon completion of the laboratory pathologist's histologic evaluation, 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 sent to an independent pathology laboratory where quality assessment was performed. The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair,

who reviewed the selected tissues for which a disagreement in diagnosis between the laboratory and quality assessment pathologists existed. 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 chair 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 exposure groups or previously rendered diagnoses. For the 6-month exposure and recovery periods of the stop-exposure studies, tissues examined in rats included the testes and lungs, and tissues examined in mice included the forestomach, nose, testes, liver, lungs, spinal cord, sciatic nerve, skeletal muscle, and harderian gland. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

SUPPLEMENTAL EVALUATIONS

Clinical Pathology Studies

In the 2-week studies of isoprene, hematology and clinical chemistry evaluations were performed on 10 supplemental-study rats and mice per exposure group (0, 438, 875, 1,750, 3,500, and 7,000 ppm); blood samples were collected from rats on Day 5 and from mice on Day 6. In addition, urine samples were collected from supplemental-study rats for evaluation on Day 4. In the 13-week studies, blood samples for hematology evaluations were collected from 10 supplemental-study rats and mice per exposure group (0, 70, 220, 700, 2,200, and 7,000 ppm) on Days 4 and 24; blood for hematology and clinical chemistry evaluations was collected from base-study rats and mice at the end of the study. Additionally, bone marrow samples were collected from supplemental-study rats and mice on Day 24 and from base-study rats and mice at the end of the 13-week studies and evaluated for bone marrow cellularity. Urine samples were collected for evaluation from base-study rats during Week 12. In the stop-exposure studies, blood for hematology evaluations was collected from 10 male rats and 10 male mice per exposure group just before the end of the 6-month exposure period.

For all hematology and clinical chemistry evaluations, animals were anesthetized with a 70% CO_2 gas mixture, and blood was collected from the retroorbital sinus. Samples for hematology analyses were collected in tubes containing potassium EDTA, and samples for clinical chemistry evaluations were collected in similar tubes devoid of anticoagulant. The latter samples were allowed to clot; samples were then centrifuged and serum was removed.

Hematology determinations were performed with an Ortho ELT-8/ds hematology analyzer (Ortho Instruments, Westwood, MA). The parameters that were evaluated are listed in Table 2. In the stop-exposure studies, manual hematocrit was determined using the microhematocrit method with a Damon/IEC MB microcentrifuge and Damon/IEC capillary reader (International Equipment Company, Needham Heights, MA). Blood smears were stained with Wright-Giemsa in a Gam Rad Model 70-9 automated slide stainer (Gam Rad West, Inc., San Juan Capistrano, CA). Differential leukocyte counts were based on classifying a minimum of 100 white blood cells. Reticulocytes were stained with New Methylene Blue and enumerated using the Miller disc method (Brecher and Schneiderman, 1950). All clinical chemistry variables were measured on an Abbott VP chemistry analyzer (Abbott Laboratories, Abbott Park, IL). The parameters evaluated are listed in Table 2.

In the 13-week studies, bone marrow samples were collected from the right femur of rats and mice. Marrow cells were flushed from the femur using Hank's balanced salt solution with added EDTA and no magnesium or calcium. A single cell suspension was assured by gently aspirating and expelling the suspension repeatedly through a 25-gauge needle and then vortexing the suspension immediately prior to the cell count. After lysis of the red blood cells, samples were analyzed for nucleated cell concentration using a Coulter ZH hematology counter (Coulter Electronics, Inc., Hialeah, FL). Cellularity, megakaryocyte concentrations, and cytologic evaluation of marrow cells were determined microscopically from marrow smears stained with Wright-Giemsa. In addition, marrow samples from base-study rats and mice were stained with Prussian blue, counterstained with safranin, and then examined microscopically to detect iron.

For the urinalysis studies, rats were placed in individual metabolism cages for overnight urine collection. Urine samples were collected in test tubes immersed in ice. During this collection period, rats had access to water but not feed. The specific gravities of samples were determined using an American Optical refractometer (American Optical, Buffalo, NY) calibrated against double-distilled water. Abbott VP methodologies were used to measure glucose, creatinine,

alkaline phosphatase, and aspartate aminotransferase concentrations. Urine protein concentrations were determined using the Coomassie Blue method (Kluwe, 1981).

TISSUE GLUTATHIONE CONCENTRATION ANALYSES

In the 13-week studies, analyses of tissue glutathione concentrations were performed on male and female rats and mice in the 0, 70, 700, and 7,000 ppm groups in the supplemental studies. Kidney, liver, lung, and thymus samples were collected immediately after exposure from five rats and five mice per group after 1 day or 12 weeks of exposure and analyzed for glutathione (nonprotein sulfhydryl) and total sulfhydryl concentrations. For these analyses, the kidney, liver, lung, and thymus were excised, debrided, and rinsed in Tris-KCl buffer to remove any exterior blood. The tissues were blotted dry, weighed, and placed in beakers containing cold Tris-KCl buffer, then coarsely chopped and homogenized with a Polytron homogenizer. All procedures were carried out in an ice bath. Methods used were similar to those described by Ellman (1959) as modified by Sedlak and Lindsay (1968).

SPERM MOTILITY AND VAGINAL CYTOLOGY EVALUATIONS

At the end of the 13-week studies, sperm motility and vaginal cytology evaluations were performed on all surviving base-study rats and mice exposed to 0, 70, 700, or 7,000 ppm isoprene. The parameters evaluated are listed in Table 2. Methods were those outlined in the National Toxicology Program's sperm morphology and vaginal cytology testing protocol (NTP, 1984b). Briefly, for the 12 days prior to sacrifice, the vaginal vaults of 10 females of each species and exposure group were lavaged, and the aspirated lavage fluid and cells were stained with toluidine blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the corpus epididymis and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five microscopic fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in phosphate buffered saline solution. Cauda were finely minced and swirled, and the tissue was incubated and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in 10% dimethyl sulfoxide in phosphate-buffered saline. Homogenization-resistant spermatid nuclei were counted using a hemacytometer.

NEUROBEHAVIORAL STUDIES

Neurobehavioral evaluations were performed on mice designated for neurobehavioral testing during the stop-exposure study. Prior to the last day of the 6-month exposure period, 120 mice (20 per exposure group: 0, 70, 220, 700, 2,200, and 7,000 ppm) were selected for forelimb and hindlimb grip strength tests. Sixty mice (10 per exposure group) were evaluated at the end of the 6-month exposure period and then killed. The remaining 10 mice per exposure group were evaluated at the following time points during the 6-month recovery period: Day 2, Months 1 and 3, and at the end of the study (Month 6).

The studies were conducted using a lexan platform equipped with a rectangular forelimb grip bar and a hindlimb T-shaped bar, both of which were attached to calibrated push-pull strain gauges. Each mouse included in the evaluations was allowed to grip the rectangular bar with its forepaws and was gently pulled back along the platform until its grip was broken. While the backward motion continued, the mouse was allowed to grasp the T-shaped bar with its hindpaws, then forced to release the bar by continued pulling. The needle deflections of the respective strain gauges were recorded and used to determine the grams of force necessary to break the animal's grip; the mean of three successive tests (both forelimb and hindlimb) was determined and used as the animal's final score.

TABLE 2 Experimental Design and Materials and Methods in the 2-Week, 13-Week, and Stop-Exposure Studies of Isoprene

2-Week Studies	13-Week Studies	Stop-Exposure Studies
EXPERIMENTAL DESIGN		
Study Laboratory		
Battelle Pacific Northwest Laboratories (Richland, WA)	Same as 2-week studies	Same as 2-week studies
Strain and Species		
F344/N rats B6C3F_1 mice	Same as 2-week studies	Same as 2-week studies
Animal Source		
Simonsen Laboratories (Gilroy, CA)	Taconic Farms (Germantown, NY)	Same as 13-week studies
Size of Study Groups		
10 males and 10 females	10 males and 10 females	40 males
Exposure Concentrations		
0, 438, 875, 1,750, 3,500, or 7,000 ppm	0, 70, 220, 700, 2,200, or 7,000 ppm	Same as 13-week studies
Exposure Durations		
6 hours plus T_{90} per day, 5 days per week for 12 days	6 hours plus T_{90} per day, 5 days per week for 13 weeks	6 hours plus T_{90} per day, 5 days per week for 6 months
Date of First Exposure		
Rats: 4 December 1986 (males), 5 December 1986 (females) Mice: 6 December 1986 (males), 7 December 1986 (females)	Rats: 7 March 1988 (males), 8 March 1988 (females) Mice: 9 March 1988	Rats: 7 March 1988 Mice: 9 March 1988
Date of Last Exposure		
Rats: 19 December 1986 (males), 20 December 1986 (females) Mice: 21 December 1986 (males), 22 December 1986 (females)	Rats: 6 June 1988 (males), 7 June 1988 (females) Mice: 8 June 1988 (males), 9 June 1988 (females)	Rats: 7 September 1988 Mice: 7 September 1988
Date of Necropsy		
Rats: 20 December 1986 (males), 21 December 1986 (females) Mice: 22 December 1986 (males), 23 December 1986 (females)	Rats: 7 June 1988 (males), 8 June 1988 (females) Mice: 9 June 1988 (males), 10 June 1988 (females)	6-Month Exposure Periods: Rats: 8 September 1988 Mice: 9 September 1988 6-Month Recovery Periods: Rats: 7, 8, or 9 March 1989 Mice: 14, 15, or 16 March 1989
Type and Frequency of Observation		
Animals were observed two times per day, 7 days per week for mortality and morbidity and up to three times per exposure day for clinical signs of toxicity. Body weights were recorded prior to the first exposure, on Day 8, and at necropsy.	Animals were observed two times per day, 7 days per week for mortality and morbidity and weekly for clinical signs of toxicity. Body weights were recorded prior to the first exposure, weekly thereafter, and at necropsy.	Animals were observed two times per day, 7 days per week for mortality and morbidity. Rats were examined for clinical signs of toxicity weekly during the first 13 weeks and monthly thereafter; mice were examined for clinical signs of toxicity once per month. Body weights were recorded prior to the first exposure, weekly for the first 13 weeks, monthly thereafter, and at necropsy.

TABLE 2 Experimental Design and Materials and Methods in the 2-Week, 13-Week, and Stop-Exposure Studies of Isoprene (continued)

2-Week Studies	13-Week Studies	Stop-Exposure Studies
Necropsy		
Complete necropsies were performed on all animals in the base studies. The brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus were weighed at necropsy.	Same as 2-week studies	Same as 2-week studies
Histopathologic Examinations		
Histopathologic examinations were performed on rats and mice in the 0 and 7,000 ppm groups. Tissues examined microscopically included: brain (three sections), glandular stomach and forestomach (mice only), gross lesions, heart, kidneys, larynx, liver, lungs, nasal cavity and turbinates (three sections), spleen, testes (with epididymis), thymus, trachea, and tracheobronchial lymph nodes. For rats, only gross lesions were examined in the lower exposure groups. For mice, tissues examined in the lower exposure groups included the thymus, nasal cavity, liver, testes, stomach, and forestomach for males and the stomach and forestomach for females.	Histopathologic examinations were performed on all rats and mice in the 0 and 7,000 ppm groups. Tissues examined microscopically included adrenal glands, brain (three sections), esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions, heart (and aorta), intestines (large: cecum, colon, rectum; small: duodenum, jejunum, ileum), kidneys, larynx, liver, lungs, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland (with adjacent skin), middle ear (all control rats and five male and female rats from the 7,000 ppm group), nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial or clitoral glands, prostate gland, salivary glands, spinal cord and sciatic nerve (if neurologic signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis and seminal vesicle), thigh muscle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies). For rats, the lung and available tracheobronchial lymph nodes were examined in the lower exposure groups. For mice, the forestomach and liver of males and females and the nasal cavity and testes of males were examined in the lower exposure groups.	Histopathologic examinations were performed on the following animals: rats in the 0 and 7,000 ppm groups and mice in all exposure groups killed after 6 months of exposure; rats and mice in the 0 and 7,000 ppm groups and mice in the 2,200 ppm group killed after 6 months of recovery; and all animals that died before the end of the studies. Tissues examined microscopically included: adrenal glands, brain (three sections), esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions, heart (and aorta), intestines (large: cecum, colon, and rectum; small: duodenum, jejunum, and ileum), kidneys, larynx, liver, lungs, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland (with adjacent skin), nasal cavity and turbinates (three sections), pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial gland, prostate gland, salivary glands, spinal cord and sciatic nerve (if neurologic signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, and urinary bladder. Additionally, lumbar spinal cord sections from five mice each in the 0 and 7,000 ppm groups were examined at the end of the exposure period using an electron microscope. For rats, the testes and lungs were examined in the lower exposure groups at the end of the exposure period and at the end of the recovery period. For mice killed after 6 months of recovery, the lungs, liver, forestomach, glandular stomach, nasal cavity, spinal cord, sciatic nerve, harderian gland, pancreatic islets, and pancreatic acini were examined in the lower exposure groups; all mice killed after 6 months of exposure received complete histopathologic examinations.

TABLE 2 Experimental Design and Materials and Methods in the 2-Week, 13-Week, and Stop-Exposure Studies of Isoprene (continued)

2-Week Studies	13-Week Studies	Stop-Exposure Studies
Clinical Pathology Studies		
Clinical pathology evaluations were performed on supplemental-study rats and mice. Hematology parameters included hematocrit (Hct), hemoglobin (Hgb) concentration, erythrocyte (RBC) count, reticulocyte count, Howell-Jolly bodies (mice), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, and leukocyte (WBC) count and differential. Clinical chemistry parameters included urea nitrogen (UN), creatinine, alanine aminotransferase (ALT), glutamate dehydrogenase, and sorbitol dehydrogenase (SDH). Urinalysis parameters (rats only) included creatinine, glucose, protein, alkaline phosphatase, aspartate aminotransferase (AST), volume, and specific gravity.	Clinical pathology evaluations were performed on supplemental- and base-study rats and mice. Hematology parameters included Hct, Hgb concentration, RBC and nucleated erythrocyte counts, reticulocyte count, Howell-Jolly bodies (mice), MCV, MCH, MCHC, platelet count, WBC count and differential, and total bone marrow cellularity. Clinical chemistry and urinalysis parameters evaluated were the same as in the 2-week studies.	Hematology evaluations were performed on rats and mice after 6 months of isoprene exposure. Hematology parameters evaluated included automated and manual Hct, Hgb concentration, RBC and nucleated erythrocyte counts, reticulocyte count, Howell-Jolly bodies (mice), MCV, MCH, MCHC, platelet count, and WBC count and differential.
Tissue Glutathione Level Analyses		
None	Tissue glutathione level analyses were performed on supplemental-study rats and mice in the 0, 70, 700, and 7,000 ppm groups. Kidney, liver, lung, and thymus samples were collected after 1 day or 12 weeks of exposure and analyzed for glutathione and total sulfhydryl concentrations.	None
Sperm Motility and Vaginal Cytology Evaluations		
None	Sperm motility and vaginal cytology evaluations were performed on base-study rats and mice in the 0, 70, 700, and 7,000 ppm groups. Males were evaluated for necropsy body and reproductive tissue weights and spermatozoal data. Females were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in various stages.	None
Neurobehavioral Evaluations		
None	None	Forelimb and hindlimb grip strength tests were performed on selected base-study mice. Ten mice per exposure group were evaluated at the end of the exposure period and killed. An additional 10 mice per group were evaluated at the following time points during the 6-month recovery period: Day 2, Months 1 and 3, and at the end of the recovery period (Month 6).

TABLE 2 Experimental Design and Materials and Methods in the 2-Week, 13-Week, and Stop-Exposure Studies of Isoprene (continued)

2-Week Studies	13-Week Studies	Stop-Exposure Studies
ANIMAL MAINTENANCE		
Time Held Before Study		
Rats: 11-12 days Mice: 12-13 days	Rats: 11-12 days Mice: 13 days	13 days
Age When Study Began		
5-6 weeks	6-8 weeks	Same as 13-week studies
Age When Killed		
7-8 weeks	19-21 weeks	6-Month Exposure Periods: 32-34 weeks 6-Month Recovery Periods: 59-61 weeks
Method of Animal Distribution		
Animals were weighed and were randomized using a computer program.	Same as 2-week studies	Same as 2-week studies
Diet		
NIH-07 Open Formula pellets (Zeigler Brothers, Inc., Gardners, PA) available *ad libitum* except during exposure periods and urine collection periods (if applicable) and softened water (City of Richland) available *ad libitum*.	Same as 2-week studies	Same as 2-week studies
Animal Room Environment		
Rats and mice were housed in individual cages in the exposure chambers. Temperature was maintained at 75° ± 3° F and relative humidity at 55% ± 15% with approximately 15 air changes per hour. Fluorescent light was provided for 12 hours per day.	Same as 2-week studies	Rats and mice were housed in individual cages in the exposure chambers. Temperature was maintained at 75° ± 3° F and relative humidity at 55% ± 15% with approximately 15 air changes per hour. Fluorescent light was provided for 12 hours per day. During the recovery periods, rats and mice were housed in chamber cage units stored on open racks.

Genetic Toxicity Studies

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. 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 2 days of incubation at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of isoprene; 10,000 μg/plate was selected as the high dose. All assays were repeated.

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. 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 to 1,600 μg/mL 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, and tests yielding equivocal or positive results were repeated.

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, and

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.

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 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. 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).

IN VIVO MOUSE CYTOGENETICS PROTOCOLS

Sister chromatid exchanges and chromosomal aberrations in the bone marrow and frequencies of micronucleated erythrocytes in the peripheral blood of male mice exposed to isoprene for 12 days or 13 weeks were evaluated. The detailed protocols, complete data tables, and statistical analyses are presented in Tice *et al.* (1988) and Shelby (1990).

Statistical Methods

CALCULATION OF INCIDENCE

The incidences of neoplasms and nonneoplastic lesions as presented in Tables A1, A2, A3, A4, B1, B2, B3, and B6 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Table B5) and of all nonneoplastic lesions are given as the ratio of the number of affected animals to the number of animals with the site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., skin, intestine, harderian gland, and mammary gland) 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.

ANALYSIS OF NEOPLASM INCIDENCES

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, other methods of statistical analysis were used, and the results of these tests are summarized in Table B5. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals. Because there were essentially no deaths among rats and mice evaluated at 6 months and rats evaluated at 12 months, no survival-adjusted methods were needed. For these data, tumor comparisons were made by the Fisher exact test and the Cochran-Armitage trend test.

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P-values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, see Haseman (1984).

ANALYSIS OF NONNEOPLASTIC LESION INCIDENCES

Because all nonneoplastic lesions in these studies were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test, a procedure based on the overall proportion of affected animals, was used.

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Clinical chemistry, hematology, sperm motility, and neurobehavioral data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into close conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across exposure levels.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

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

ANALYSIS OF CHINESE HAMSTER OVARY CELL CYTOGENETICS DATA

For the SCE data, 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, along with a trend P-value less than 0.025, 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 ($P<0.05$) in the absence of any responses reaching 20% above background led to a call of equivocal.

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 (Galloway *et al.*, 1987). For a single trial, a statistically significant ($P<0.05$) difference for one dose point and a significant trend ($P<0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend, in the absence of a statistically significant increase at any one dose point, led to a conclusion of equivocal activity. Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

Quality Assurance Methods

The animal studies of isoprene were performed in compliance with United States Food and Drug Administration Good Laboratory Practices regulations (21 CFR, Part 58). The Quality Assurance

Unit of Battelle performed audits and inspections of protocols, procedures, data, and report throughout the course of the studies.

RESULTS

2-Week Inhalation Study in F344/N Rats

All rats survived to the end of the 2-week study (Table 3). The mean body weight gain of males in the 7,000 ppm group was slightly less than that of the control group. No clinical signs considered to be related to isoprene toxicity were observed in male or female rats during the study.

TABLE 3 Survival and Body Weights of F344/N Rats in the 2-Week Inhalation Study of Isoprene

Concentration (ppm)	Survival[2]	Mean Body Weight[1] (grams) Initial	Final	Change	Final Weight Relative to Controls[3] (%)
MALE					
0	10/10	83 ± 3	160 ± 4	77 ± 2	
438	10/10	85 ± 3	158 ± 5	74 ± 3	99
875	10/10	81 ± 2	152 ± 6	71 ± 4	95
1,750	10/10	81 ± 3	152 ± 5	71 ± 3	95
3,500	10/10	79 ± 2	151 ± 4	72 ± 2	94
7,000	10/10	82 ± 3	150 ± 5	68 ± 3*	94
FEMALE					
0	10/10	78 ± 2	121 ± 3	43 ± 1	
438	10/10	79 ± 2	117 ± 3	39 ± 3	97
875	10/10	78 ± 2	122 ± 2	44 ± 1	101
1,750	10/10	77 ± 2	119 ± 2	42 ± 1	98
3,500	10/10	73 ± 2	119 ± 2	46 ± 1	98
7,000	10/10	76 ± 2	119 ± 2	43 ± 1	98

[1] Weights and weight changes are given as mean ± standard error.
[2] Number surviving at 2 weeks/number of animals per group.
[3] (Exposure group mean/control group mean) x 100.
* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

Only a few differences in hematology and urinalysis parameters between exposed and control rats were noted in the 2-week study (Table D1). These differences were minor and sporadic and were not considered to be treatment related. Clinical chemistry parameters of exposed and control rats were similar.

The absolute and relative heart weights of female rats in the 7,000 ppm group and the absolute heart weight of females in the 3,500 ppm group were slightly less than those of the controls (Table C1). In addition, the relative liver weights of male rats in the three highest exposure groups (1,750, 3,500, and 7,000 ppm) were 6% to 20% greater than the control value.

No gross or histopathologic lesions in rats in the 2-week study were attributed to isoprene exposure.

Based on the absence of mortality and the lack of life-threatening changes that could be attributed to isoprene exposure, the concentrations selected for the 13-week and stop-exposure studies in rats were 0, 70, 220, 700, 2,200, and 7,000 ppm.

13-Week Inhalation Study in F344/N Rats

All rats survived to the end of the 13-week study (Table 4). The final mean body weights and body weight gains of males and females in all exposed groups were similar to those of the control groups (Table 4 and Figure 2). No clinical signs considered to be related to isoprene toxicity were observed in male or female rats during the study.

All differences in hematology, clinical chemistry, and urinalysis parameters were minimal (Table D2). At Day 4, leukocyte numbers in female rats in the four highest exposure groups (220, 700, 2,200, and 7,000 ppm) were higher than in the controls; this transient change was accompanied by slightly higher numbers of lymphocytes and would be compatible with a physiologic (epinephrine release) response. At Week 13, erythrocyte counts, hemoglobin concentrations, and hematocrit values were slightly greater in females in the four highest exposure groups than in the controls; this difference is compatible with mild dehydration. Also at Week 13, the number of segmented neutrophils in males in the 7,000 ppm group and in females in all exposed groups were less than those in the controls. These lower numbers were not reflected in leukocyte numbers, and bone marrow cellularity counts were similar to those of the controls. This change could be compatible with a shift of neutrophils from the circulating neutrophil pool to the marginal neutrophil pool, with no difference in total blood neutrophil numbers.

Urine glucose concentrations in male rats in the 2,200 and 7,000 ppm groups were slightly greater than in the controls at Week 12; this would be compatible with a lower level of renal glucose resorption, suggesting kidney damage. However, there were no differences in other urine parameters or kidney histopathology supporting renal injury. Other hematology, clinical chemistry, and urinalysis changes were sporadic and did not suggest a treatment relationship.

Minor differences between absolute and relative organ weights of exposed and control rats were noted, but none of these differences were related to exposure. Complete organ weight data for rats in the 13-week study are presented in Appendix C, Table C2.

No exposure-related differences in glutathione concentrations in liver, kidneys, lungs, or thymus from exposed or control rats were detected after 1 day or 12 weeks of exposure (Table G1).

TABLE 4 Survival and Body Weights of F344/N Rats in the 13-Week Inhalation Study of Isoprene

Concentration (ppm)	Survival[2]	Mean Body Weight[1] (grams)			Final Weight Relative to Controls[3] (%)
		Initial	Final	Change	
MALE					
0	10/10	139 ± 5	336 ± 4	197 ± 6	
70	10/10	140 ± 5	338 ± 8	199 ± 5	101
220	10/10	137 ± 4	339 ± 5	202 ± 6	101
700	10/10	145 ± 5	337 ± 7	192 ± 5	100
2,200	10/10	141 ± 6	351 ± 6	210 ± 7	104
7,000	10/10	139 ± 4	337 ± 4	197 ± 6	100
FEMALE					
0	10/10	123 ± 1	198 ± 3	76 ± 3	
70	10/10	124 ± 1	206 ± 3	82 ± 3	104
220	10/10	124 ± 1	210 ± 4	86 ± 4	106
700	10/10	125 ± 1	203 ± 3	78 ± 4	102
2,200	10/10	122 ± 1	209 ± 4	87 ± 3	105
7,000	10/10	122 ± 2	207 ± 3	84 ± 3	104

[1] Weights and weight changes are given as mean ± standard error; differences from the control group for weights and weight changes are not significant by Williams' or Dunnett's test.
[2] Number surviving at 13 weeks/number of animals per group.
[3] (Exposure group mean/control group mean) x 100.

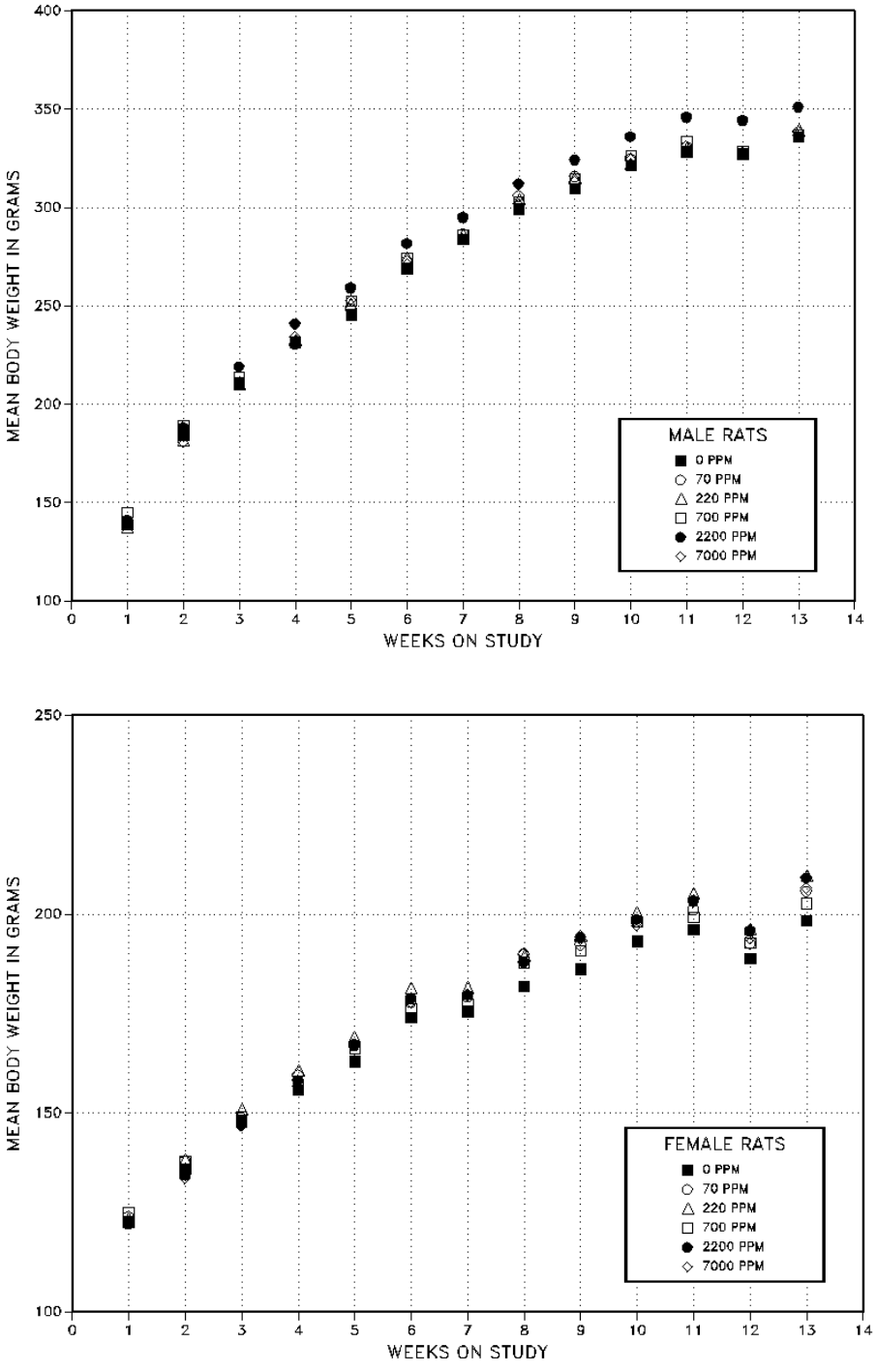

FIGURE 2 Body Weights of F344/N Rats Exposed to Isoprene by Inhalation for 13 Weeks

No treatment-related lesions were observed in rats exposed to isoprene. However, the Pathology Working Group confirmed a spectrum of inflammatory changes within the lung of control male and female rats. These changes included suppurative inflammation and alveolar macrophage infiltration in alveoli, alveolar type 2 epithelial cell hyperplasia, perivascular lymphoid hyperplasia, and peribronchial/peribronchiolar lymphoid hyperplasia. Although the etiology of these lesions is unknown, an infectious agent was suspected. Similar lesions have been observed in other NTP studies.

Sperm motility and vaginal cytology evaluations were performed on base-study rats in the 0, 70, 700, and 7,000 ppm groups at the end of the study (Tables E1 and E2). No biologically significant effects were observed.

Stop-Exposure Inhalation Study in Male F344/N Rats

One rat in the 220 ppm group scheduledfor evaluation after 6 months of recovery died before the end of the 6-month exposure period; there were no other deaths (Tables 5 and 6). At the end of the exposure period, the final mean body weightand body weight gain of rats in the 70 ppm group were greater than those of the control group (Table 5 and Figure 3); the final mean body weights and body weight gains of rats in all other exposure groups were similar to those of the control group. For rats allowed to recover for 6 months, mean body weights and body weight gains determined at the end of the exposure period and atthe end of the recovery period were similar to those of the control group(Table 6 and Figure 3). No clinical signs considered to be related to isoprene exposure were observed in any rats during the study.

TABLE 5 Body Weights of Male F344/N Rats After 6 Months of Exposure in the Stop-Exposure Inhalation Study of Isoprene

Concentration (ppm)	Number Examined[2]	Mean Body Weight[1] (grams)			Final Weight Relative to Controls[3] (%)
		Initial	Final	Change	
0	10	141 ± 4	402 ± 7	262 ± 6	
70	10	139 ± 3	434 ± 7*	295 ± 7**	108
220	10	145 ± 4	414 ± 9	269 ± 9	103
700	10	139 ± 5	415 ± 6	276 ± 7	103
2,200	10	135 ± 4	408 ± 7	273 ± 8	102
7,000	10	145 ± 3	417 ± 7	272 ± 5	104

[1] Weights and weight changes are given as mean ± standard error.
[2] Ten rats per exposure group were randomly selected and killed for evaluations after 6 months of exposure.
[3] (Exposure group mean/control group mean) x 100.
* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

TABLE 6 Survival and Body Weights of Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene After 6 Months of Exposure and 6 Months of Recovery

Concentration (ppm)	Number Examined[2]	Mean Body Weight[1] (grams)			Final Weight Relative to Controls[4] (%)
		Initial	Interim/Final[3]	Change	
6-MONTH EVALUATION					
0	30/30	135 ± 3	413 ± 5	278 ± 5	
70	30/30	137 ± 3	422 ± 4	285 ± 4	102
220	29/30[5]	139 ± 3	413 ± 5	275 ± 6	100
700	30/30	137 ± 2	410 ± 4	273 ± 4	99
2,200	30/30	138 ± 3	413 ± 4	276 ± 4	100
7,000	30/30	136 ± 3	402 ± 5	266 ± 4	97
12-MONTH EVALUATION					
0	30/30	135 ± 3	485 ± 5	350 ± 3	
70	30/30	137 ± 3	492 ± 5	355 ± 3	102
220	29/30[5]	139 ± 3	493 ± 7	354 ± 4	102
700	30/30	137 ± 2	486 ± 4	349 ± 3	100
2,200	30/30	138 ± 3	493 ± 4	355 ± 3	102
7,000	30/30	136 ± 3	491 ± 4	355 ± 3	101

[1] Weights and weight changes are given as mean ± standard error; differences from the control group for weights and weight changes are not significant by Dunnett's test.

[2] Number surviving at the end of evaluation period/number of animals per group; the number of animals per group does not include rats killed at the 6-month evaluation.

[3] Interim body weights were determined during the last week of the 6-month exposure period (Day 180); final body weights were determined at the end of the 6-month recovery period.

[4] (Exposure group mean/control group mean) x 100.

[5] One rat in this group died during the 6-month exposure period.

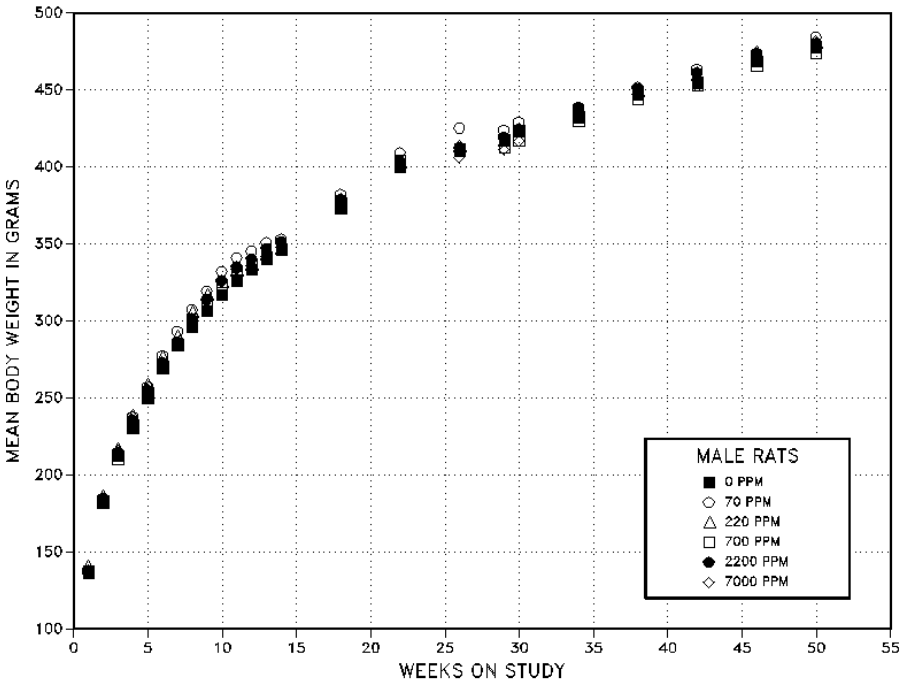

FIGURE 3 Body Weights of Male F344/N Rats During the 6-Month Exposure and Recovery Periods in the Stop-Exposure Inhalation Study of Isoprene

At the end of the 6-month exposure period, there were no significant differences between exposed groups and the control group in any of the hematology parameters evaluated (Table D3).

After 6 months of exposure, liver weights of rats exposed to 7,000 ppm isoprene and kidney weights of rats in all exposed groups were greater than those of the controls (Table C3). At the end of the 6-month recovery period, the liver and kidney weights of rats in all exposed groups were similar to those of the control group (Table C4).

Testes: At the end of the 6-month exposure period, male rats in the 7,000 ppm group had a markedly greater incidence and relative severity of interstitial cell hyperplasia than the control group and the lower exposure groups (Table 7). The severity of interstitial cell hyperplasia in rats in the 7,000 ppm group varied; one male had marked, one had moderate, and three had mild interstitial cell hyperplasia. The remaining rats in the 7,000 ppm group and rats in the control and lower exposure groups had minimal hyperplasia. Because interstitial cell hyperplasia is generally uncommon in 6-month-old F344/N rats, this lesion was considered to be treatment

related. Interstitial cell proliferative lesions of the testis are uncommon in F344/N rats at 9 months of age, but the incidence increases rapidly after 1 year (Boorman *et al.*, 1990). After 6 months of recovery, the incidence of interstitial cell adenomas was slightly greater in male rats exposed to 700 ppm or greater than in rats in the 0, 70, and 220 ppm groups (Table 7). The majority of the interstitial cell adenomas occurred as single unilateral neoplasms; however, one male rat in the 7,000 ppm group had bilateral interstitial cell adenomas. At the end of the 6-month recovery period, the incidence and severity of interstitial cell hyperplasia was slightly greater in exposed rats than in the controls, although no clear concentration-related differences were evident. Because proliferative lesions involving the interstitial cells of the testis in F344/N male rats are common age-related changes, the similar incidences and severity of the hyperplasia among all groups at 12 months is not unexpected. Based on the hyperplastic lesions observed at 6 months and the adenomas observed at 12 months, the earlier development of interstitial cell proliferative lesions was considered to be related to exposure to isoprene.

TABLE 7 Testicular Lesions in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
6-MONTH EVALUATION						
Interstitial Cell Hyperplasia						
Overall rate[1]	1/10 (10%)	1/10 (10%)	3/10 (30%)	1/10 (10%)	3/10 (30%)	10/10 (100%)**
Average severity[2]	1.0	1.0	1.0	1.0	1.0	1.8
12-MONTH EVALUATION						
Interstitial Cell Hyperplasia						
Overall rate	25/30 (83%)	30/30 (100%)*	28/30 (93%)	30/30 (100%)*	29/29 (100%)*	30/30 (100%)*
Average severity	2.4	2.0	2.2	2.8	2.2	2.7
Interstitial Cell Adenoma						
Overall rate	3/30 (10%)	3/30 (10%)	4/30 (13%)	7/30 (23%)	8/29 (28%)	9/30[3] (30%)
Cochran-Armitage test[4]	P=0.021					
Fisher exact test[4]		P=0.665N	P=0.500	P=0.149	P=0.080	P=0.052

[1] Number of lesion-bearing animals/number of animals microscopically examined.
[2] Average severity is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.
[3] Includes one animal with multiple adenomas.
[4] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend is indicated by **N**.
* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test.
** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test.

Hyperplasia of interstitial cells of the testes consisted of aggregates of interstitial cells smaller than the diameter of a seminiferous tubule. Qualitative evaluations of the degree of severity for interstitial cell hyperplasia were based on the number of separate foci of hyperplasia in each testis; foci were counted, and the severity was scored as follows: minimal = one to two foci per testis; mild = three to four foci per testis; moderate = five to six foci per testis; and marked = seven or more foci per testis. When foci of hyperplasia were present in both testes, only the score for the most severely affected testis was recorded. Interstitial cell adenomas were equal in size to, or larger than, a seminiferous tubule. 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. The qualifier "multiple" was used to indicate bilateral interstitial cell adenomas.

Lungs: In control and exposed rats killed after the 6-month exposure period and in rats killed after an additional 6 months of recovery, a spectrum of inflammatory changes of the lungs similar to that noted in control rats in the 13-week study was observed (Table A4). Histopathologic evaluation of the lung revealed subtle cuffs of lymphocytes around small vessels (perivascular lymphoid hyperplasia), sometimes with larger lymphocyte clusters around the major bronchi (peribronchial lymphoid hyperplasia); in some rats, focal or multifocal accumulations of alveolar macrophages and fewer polymorphonuclear inflammatory cells in alveoli were observed in the vicinity of the small vessels with lymphoid cell cuffs. Type 2 alveolar cell hyperplasia was also observed in the alveoli with the inflammatory cell exudate.

Similar inflammatory lung lesions have been observed in control and exposed rats in inhalation studies conducted at the laboratory at which the isoprene studies were performed, as well as at other NTP study laboratories. The cause of these lung lesions has not been established, although an infectious agent is suspected. For the following reasons, the lung lesions were not considered to be related to isoprene exposure:

- neither the incidence nor the severity of the lesions was exposure related;
- the distribution of the lesions in the lung was not typical of an inhaled irritant;
- type 2 cell hyperplasia was only associated with areas of inflammation;
- control and exposed groups had similar lesions;
- and the spectrum of inflammatory lesions observed in this study has been observed in control and exposed rats in inhalation studies conducted at other study laboratories.

Teratology Study in Sprague-Dawley Rats

To assess the maternal and developmental toxicity of isoprene, teratology studies were conducted in mated female Sprague-Dawley rats exposed to 0, 280, 1,400, or 7,000 ppm isoprene vapor through whole-body exposure on gestation Days 6 through 19 (Appendix E); for comparison, 10 virgin female rats per group were exposed to isoprene vapor concurrently with the animals showing positive signs of mating.

No pregnant or virgin rats died during the study, and there were no clinical signs of toxicity. The mean body weights of exposed pregnant and virgin females were similar to those of the controls throughout the study. In addition, the gravid uterine weights, extra-gestational weight gains, absolute and relative liver weights, and absolute kidney weights of exposed dams were not affected by isoprene exposure; however, the relative kidney weight of dams in the 7,000 ppm group was slightly but significantly greater than that of the controls. No statistically significant differences in embryo/fetal parameters such as implantations per dam, resorptions per litter, fetal mortality, and fetal body weights were noted between the control and exposed groups. Gestational exposure to isoprene did not cause a significantly greater overall incidence of fetal malformations or percentage of malformed fetuses per litter. Similarly, gestational exposure did not affect the overall incidence of fetuses with variations/reduced ossifications or the overall percentage of fetuses per litter with variations/reduced ossifications, although there was an exposure-related increase in the mean percentage of fetuses per litter with reduced vertebral ossifications.

2-Week Inhalation Study in B6C3F$_1$ Mice

All mice survived to the end of the 2-week study (Table 8). The final mean body weights of male mice in all exposed groups were at least 5% less than the control value; the final mean body weight of males in the 7,000 ppm group was 15% less than that of the control group. No clinical signs considered to be related to isoprene toxicity were observed in male or female mice during the study.

TABLE 8 Survival and Body Weights of B6C3F$_1$ Mice in the 2-Week Inhalation Study of Isoprene

Concentration (ppm)	Survival[2]	Mean Body Weight[1] (grams) Initial	Final	Change	Final Weight Relative to Controls[3] (%)
MALE					
0	10/10	23.7 ± 0.5	28.7 ± 0.6	5.0 ± 0.6	
438	10/10	23.2 ± 0.4	27.1 ± 0.4**	3.9 ± 0.3	94
875	10/10	22.6 ± 0.4	26.4 ± 0.4**	3.7 ± 0.2	92
1,750	10/10	23.0 ± 0.2	27.2 ± 0.4**	4.2 ± 0.3	95
3,500	10/10	22.6 ± 0.4	26.7 ± 0.4**	4.1 ± 0.3	93
7,000	10/10	22.7 ± 0.4	24.3 ± 0.4**	1.7 ± 0.3**	85
FEMALE					
0	10/10	20.6 ± 0.3	22.6 ± 0.3	2.1 ± 0.2	
438	10/10	19.8 ± 0.4	22.6 ± 0.3	2.8 ± 0.3	100
875	10/10	19.7 ± 0.3	23.0 ± 0.2	3.4 ± 0.2**	102
1,750	10/10	19.8 ± 0.5	22.5 ± 0.4	2.7 ± 0.3	99
3,500	10/10	19.5 ± 0.4	22.9 ± 0.3	3.4 ± 0.2**	101
7,000	10/10	19.7 ± 0.4	22.3 ± 0.3	2.5 ± 0.2	98

[1] Weights and weight changes are given as mean ± standard error.
[2] Number surviving at 2 weeks/number of animals per group.
[3] (Exposure group mean/control group mean) x 100.
** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

On Day 6, a mild normocytic,normochromic, nonresponsive anemia was observed in all exposed male and female mice (Table D4). This was evidenced by lower erythrocyte counts, hemoglobin concentrations, and hematocrit values than controls, with nodifferences in mean cell volume or mean cell hemoglobin concentrationand with reticulocyte numbers no greater than in the controls (Tables 9 and D4). Considering the acute nature of these changes, and because there was no evidence of blood loss, these findings would be compatible with a mild intravascular to extravascular hemolytic process. Platelet numbers in almost all exposed groups of male and female mice were greater than in the controls; this would be compatible with a physiologic thrombocytosis related to a mobilization of platelets from the splenic pool. Differences in other hematology and clinical chemistry parameters were inconsistent and were not considered to be treatment related.

TABLE 9 Selected Hematology Data for B6C3F$_1$ Mice in the 2-Week Inhalation Study of Isoprene[1]

	Concentration (ppm)					
	0	438	875	1,750	3,500	7,000
MALE						
n	10	9	10	10	10	10
Hematocrit (%)	48.9 ± 0.6	44.9 ± 0.4**	45.2 ± 0.4**	43.2 ± 1.5**	44.6 ± 0.2**	44.7 ± 0.4**
Hemoglobin (g/dL)	16.4 ± 0.2	15.1 ± 0.1**	15.2 ± 0.1**	14.6 ± 0.5**	15.0 ± 0.1**	15.0 ± 0.1**
Erythrocytes ($10^6/\mu$L)	9.90 ± 0.11	9.11 ± 0.11**	9.19 ± 0.08**	8.76 ± 0.32**	9.02 ± 0.04**	9.19 ± 0.06**
FEMALE						
n	10	10	10	10	10	10
Hematocrit (%)	48.1 ± 0.2	45.6 ± 0.3**	45.5 ± 0.2**	45.3 ± 0.3**	45.9 ± 0.5**	45.2 ± 0.4**
Hemoglobin (g/dL)	16.4 ± 0.1	15.6 ± 0.1**	15.4 ± 0.1**	15.5 ± 0.1**	15.7 ± 0.2**	15.4 ± 0.1**
Erythrocytes ($10^6/\mu$L)	9.74 ± 0.06	9.20 ± 0.09**	9.04 ± 0.06**	9.20 ± 0.10**	9.24 ± 0.10**	9.06 ± 0.11**

[1] Data are given as mean ± standard error.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

Absolute and relative liver weights of male and female mice in all exposed groups were greater than those of the controls, and all of these differences were significant, excluding the absolute liver weight of males in the 438 ppm group (Tables 10 and C5). Absolute and relative spleen weights were significantly lower than those of the controls for all exposed males and for females in the two highest exposure groups (3,500 and 7,000 ppm). Absolute and relative testis and thymus weights in all groups of exposed males, excluding the relative testis and thymus weights of males in the 438 ppm group, were significantly less than those of the controls; absolute and relative thymus weights of female mice in all exposed groups were also less than those of the controls.

Inhalation exposure of male mice to isoprene for 2 weeks was associated with microscopic changes in the thymus, testes, liver, nasal cavity, and forestomach; microscopic lesions were also observed in the forestomach of exposed female mice (Table 11; Melnick *et al.*, 1990b). Thymic atrophy was observed in male mice exposed to 7,000 ppm isoprene and was characterized by a decrease in cellularity of the cortex. Minimal testicular atrophy was observed in mice exposed to 7,000 ppm isoprene; this lesion was multifocal and was characterized by a minimal loss of the germinal epithelium and a reduction in the number of viable cells along some of the seminiferous tubule basement membranes. Diffuse liver changes consistent with increased numbers of highly glycogenated hepatocytes were observed to similar degrees in all groups of exposed male mice.

Olfactory epithelial degeneration was observed in males in the 1,750, 3,500, and 7,000 ppm groups; the severity of this nasal lesion increased with increasing concentrations of isoprene (Table 11). Olfactory degeneration was characterized by focal loss of sensory epithelial cells and thinning of the olfactory epithelium along the dorsal meatus of the middle and posterior nasal sections. Epithelial hyperplasia of the forestomach was seen in all groups of male and female mice exposed to isoprene (Table 11). Grossly, these lesions appeared as focal, white, raised or thickened areas in the squamous mucosal surface at the anterior pole of the forestomach. Microscopically, these lesions were characterized by focal epithelial thickening with an occasional verrucous appearance.

TABLE 10 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 2-Week Inhalation Study of Isoprene[1]

	Concentration (ppm)					
	0	438	875	1,750	3,500	7,000
MALE						
n	10	10	10	10	10	10
Necropsy body wt	28.7 ± 0.6	27.1 ± 0.4**	26.4 ± 0.4**	27.2 ± 0.4**	26.7 ± 0.4**	24.3 ± 0.4**
Liver						
Absolute	1.447 ± 0.038	1.517 ± 0.029	1.566 ± 0.029*	1.625 ± 0.036**	1.665 ± 0.054**	1.634 ± 0.025**
Relative	50.32 ± 0.66	55.96 ± 0.60**	59.39 ± 0.89**	59.74 ± 0.62**	62.33 ± 1.41**	67.20 ± 0.97**
Spleen						
Absolute	0.078 ± 0.003	0.068 ± 0.001**	0.060 ± 0.002**	0.063 ± 0.002**	0.062 ± 0.001**	0.047 ± 0.002**
Relative	2.71 ± 0.10	2.51 ± 0.07*	2.27 ± 0.07**	2.31 ± 0.06**	2.33 ± 0.04**	1.93 ± 0.06**
Right testis						
Absolute	0.104 ± 0.003	0.095 ± 0.003**	0.083 ± 0.002**	0.084 ± 0.002**	0.083 ± 0.001**	0.069 ± 0.002**
Relative	3.61 ± 0.10	3.49 ± 0.08	3.14 ± 0.05**	3.08 ± 0.07**	3.11 ± 0.05**	2.84 ± 0.05**
Thymus						
Absolute	0.047 ± 0.003	0.039 ± 0.002*	0.026 ± 0.002**	0.030 ± 0.003**	0.024 ± 0.002**	0.015 ± 0.002**
Relative	1.62 ± 0.08	1.42 ± 0.06	0.99 ± 0.08**	1.10 ± 0.09**	0.91 ± 0.06**	0.60 ± 0.06**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	22.6 ± 0.3	22.6 ± 0.3	23.0 ± 0.2	22.5 ± 0.4	22.9 ± 0.3	22.3 ± 0.3
Liver						
Absolute	1.200 ± 0.023	1.290 ± 0.021*	1.365 ± 0.021**	1.334 ± 0.030**	1.424 ± 0.017**	1.438 ± 0.036**
Relative	53.05 ± 0.67	57.01 ± 0.43**	59.25 ± 0.79**	59.34 ± 1.01**	62.25 ± 0.51**	64.54 ± 1.04**
Spleen						
Absolute	0.085 ± 0.003	0.081 ± 0.005	0.074 ± 0.002	0.079 ± 0.003	0.073 ± 0.002*	0.067 ± 0.002**
Relative	3.75 ± 0.11	3.59 ± 0.22	3.21 ± 0.08	3.52 ± 0.15	3.19 ± 0.08**	3.01 ± 0.10**
Thymus						
Absolute	0.069 ± 0.003	0.054 ± 0.002**	0.046 ± 0.003**	0.048 ± 0.001**	0.049 ± 0.002**	0.035 ± 0.002**
Relative	3.03 ± 0.11	2.38 ± 0.10**	1.99 ± 0.11**	2.12 ± 0.07**	2.16 ± 0.06**	1.58 ± 0.10**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

TABLE 11 Incidence and Severity of Selected Histopathologic Lesions in B6C3F$_1$ Mice in the 2-Week Inhalation Study of Isoprene[1]

	Concentration (ppm)					
	0	438	875	1,750	3,500	7,000
MALE						
Forestomach						
Epithelial hyperplasia	0/10	3/10 (1.7)	5/10* (1.8)	10/10** (1.4)	8/10** (1.6)	9/10** (1.8)
Nasal cavity						
Olfactory epithelial degeneration	0/10	–[2]	0/10	3/10 (1.0)	6/10** (1.2)	9/10** (1.8)
Liver						
Cytoplasmic vacuolization	0/10	8/10** (2.0)	9/10** (2.0)	10/10** (2.0)	10/10** (1.8)	10/10** (2.0)
Testes						
Atrophy	0/10	–	–	–	0/10	9/10** (1.0)
Thymus						
Atrophy	0/10	–	–	–	0/10	7/9** (1.0)
FEMALE						
Forestomach						
Epithelial hyperplasia	0/10	8/10** (1.5)	7/10** (1.6)	10/10** (1.7)	9/10** (1.4)	9/10** (1.9)

[1] Adapted from Melnick *et al.* (1990). Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.
[2] Tissue was not examined at this exposure level.
* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test.
** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test.

The decreases in thymus and testis weights with increasing exposure concentration in mice in the 2-week study of isoprene are consistent with these histopathologic findings. Based on histopathologic and clinical pathology findings, the greater liver weights of exposed mice were probably not associated with glycogen accumulation in hepatocytes, but may have been associated with slight hypertrophy, which is generally not detectable by light microscopy. Female mice did not have glycogen changes in the liver, but did have liver weight changes similar to those observed in male mice.

Based on the absence of mortality and the lack of life-threatening lesions, the concentrations selected for the 13-week and stop-exposure studies in mice were 0, 70, 220, 700, 2,200, and 7,000 ppm.

13-Week Inhalation Study in B6C3F$_1$ Mice

All mice survived to the end of the 13-week study (Table 12). The final mean body weights and body weight gains of female mice in all exposed groups were lower than those of the control group. No marked differences in final mean body weights or body weight gains were noted in male mice (Table 12 and Figure 4). No significant clinical signs of toxicity related to isoprene exposure were noted during the course of the study.

TABLE 12 Survival and Body Weights of B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene

Concentration (ppm)	Survival[2]	Mean Body Weight[1] (grams) Initial	Final	Change	Final Weight Relative to Controls[3] (%)
MALE					
0	10/10	25.2 ± 0.4	36.7 ± 0.8	11.5 ± 0.6	
70	10/10	24.6 ± 0.5	36.3 ± 0.7	11.7 ± 0.6	99
220	10/10	25.1 ± 0.5	36.5 ± 0.9	11.4 ± 0.6	100
700	10/10	25.6 ± 0.3	37.6 ± 0.6	12.0 ± 0.4	103
2,200	10/10	24.5 ± 0.4	38.7 ± 1.4	14.1 ± 1.2	105
7,000	10/10	25.5 ± 0.3	35.6 ± 0.9	10.1 ± 0.9	97
FEMALE					
0	10/10	19.6 ± 0.3	33.8 ± 0.7	14.2 ± 0.5	
70	10/10	20.1 ± 0.2	29.9 ± 0.5**	9.7 ± 0.5**	88
220	10/10	20.2 ± 0.2	30.8 ± 0.5**	10.6 ± 0.6**	91
700	10/10	20.3 ± 0.3	29.9 ± 0.2**	9.7 ± 0.4**	88
2,200	10/10	20.0 ± 0.2	30.0 ± 0.5**	10.1 ± 0.5**	89
7,000	10/10	20.3 ± 0.3	29.6 ± 0.6**	9.3 ± 0.7**	87

[1] Weights and weight changes are given as mean ± standard error.
[2] Number surviving at 13 weeks/number of animals per group.
[3] (Exposure group mean/control group mean) x 100.
** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

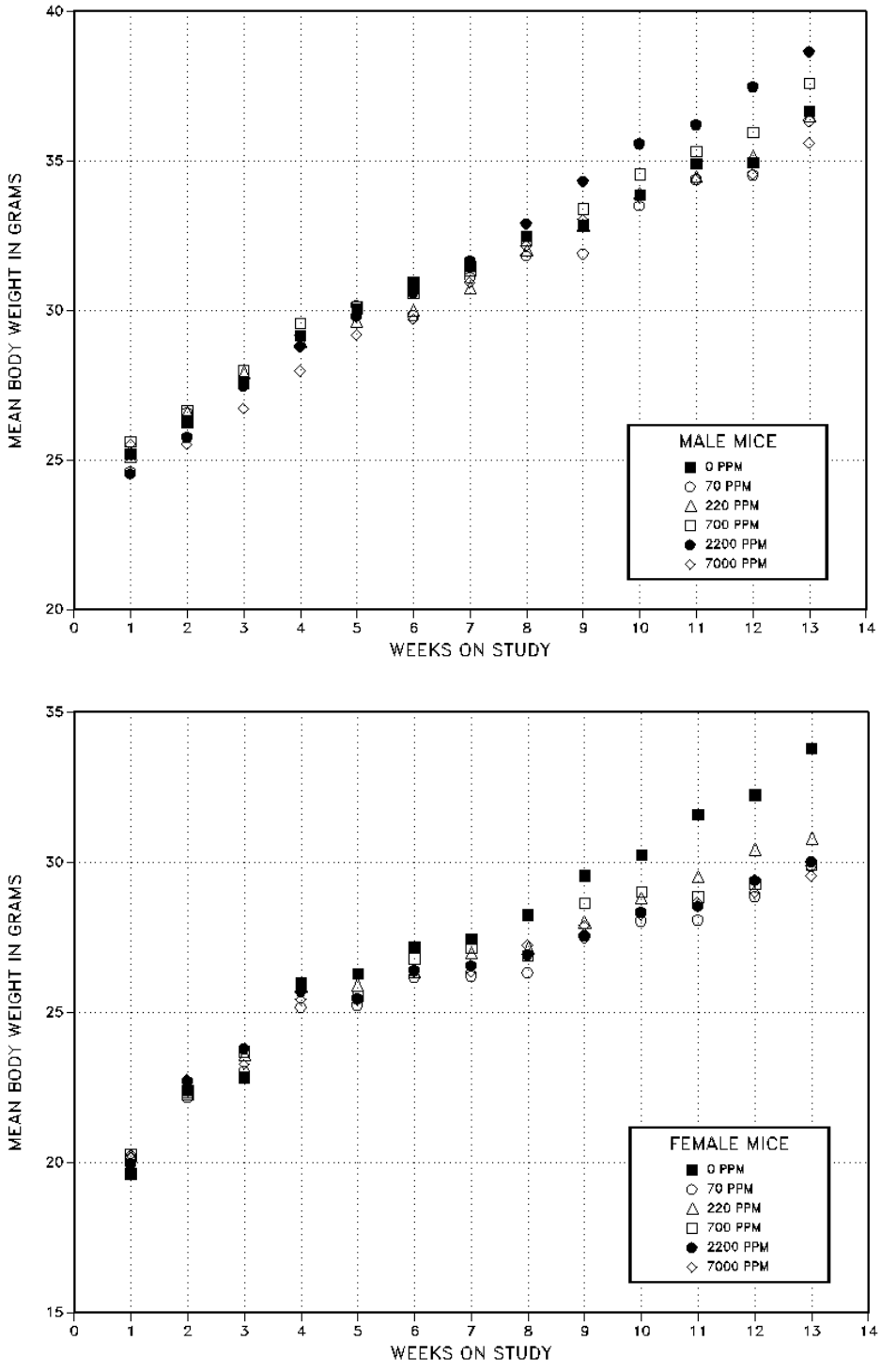

FIGURE 4 Body Weights of B6C3F$_1$ Mice Exposed to Isoprene by Inhalation for 13 Weeks

As in the 2-week study in mice, a mild normocytic, normochromic, nonresponsive anemia occurred in exposed groups of male and female mice at Day 4 (Tables 13 and D5). This was most pronounced in the 700, 2,200, and 7,000 ppm groups. However, by Day 24, the nonresponsive anemia became macrocytic, asevidenced by significantly greater mean cell volume values than in the controls, and remained so to the end of the study. In exposed male mice, increased numbers of Howell-Jolly bodies accompanied the anemia at Day 24 and Week 13, suggesting abnormal mitosis during erythroblast division (Tables 13 and D5). Leukocyte, neutrophil, lymphocyte, and bone marrow cellularity counts in male mice inthe 7,000 ppm group were sporadically lower than in the controls in during the 13-week study. Lower numbers of lymphocytes in exposed mice were the most consistent finding associated with the lower leukocyte counts and would be compatible with a stress response. Differences in otherhematology and clinical chemistry parameters were minimal and inconsistent and were not considered to be treatment related.

At the end of the 13-week study, absolute and relative testis weights of males in the 2,200 and 7,000 ppm groups were significantly less and absolute and relative liverweights of males in the 7,000 ppm group were significantly greater than those of the controls (Tables 14 and C6). The absolute liver weight of femalesin the 7,000 ppm group was also significantly greater than that of the controls. Absolute spleen weights of males and females in the three highest exposure groups (700, 2,200, and 7,000 ppm) wereless than those of the controls; in males exposed to 220 ppm isoprene or greater and females exposed to 7,000 ppm, relative spleen weights were also significantly less than those of the controls. The absolute kidney weight of female mice receiving 220 ppm isoprene or greater was significantly greater than that of the controls.

Glutathione concentrations in the lungs of female mice in the 7,000 ppm group at Day 1 and in the liver and lungs of male and female mice in the 7,000 ppm groups at Week 12 were less than the control values (Table G2); total-sulfhydryl-to-glutathione ratios in these groups were greater than in the controls at these time points.

TABLE 13 Selected Hematology Data for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene[1]

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
MALE						
n						
Day 4	10	10	10	10	10	10
Day 24	10	10	7	10	10	9
Week 13	10	10	10	10	10	10
Hematocrit (%)						
Day 4	47.5 ± 0.3	47.3 ± 0.5	45.8 ± 0.4*	43.9 ± 0.3**	44.1 ± 0.4**	43.1 ± 0.5**
Day 24	48.9 ± 0.3	49.1 ± 0.2	49.0 ± 0.4	45.9 ± 0.5**	46.2 ± 0.3**	43.8 ± 0.4**
Week 13	48.3 ± 0.4	49.1 ± 0.3	48.3 ± 0.4	44.9 ± 0.4**	45.4 ± 0.3**	42.2 ± 0.3**
Hemoglobin (g/dL)						
Day 4	16.1 ± 0.1	16.0 ± 0.1	15.4 ± 0.2**	14.9 ± 0.1**	14.9 ± 0.1**	14.7 ± 0.2**
Day 24	16.7 ± 0.1	16.8 ± 0.1	16.6 ± 0.1	15.7 ± 0.1**	15.7 ± 0.1**	15.0 ± 0.1**
Week 13	16.8 ± 0.1	17.0 ± 0.1	16.7 ± 0.1	15.7 ± 0.1**	15.7 ± 0.1**	14.6 ± 0.1**
Erythrocytes ($10^6/\mu$L)						
Day 4	10.16 ± 0.07	9.94 ± 0.11	9.63 ± 0.13**	9.25 ± 0.07**	9.21 ± 0.05**	9.09 ± 0.11**
Day 24	10.47 ± 0.08	10.46 ± 0.06	10.37 ± 0.08	9.39 ± 0.09**	9.54 ± 0.06**	8.92 ± 0.07**
Week 13	10.81 ± 0.06	10.80 ± 0.06	10.65 ± 0.06	9.76 ± 0.04**	9.72 ± 0.05**	8.80 ± 0.09**
Reticulocytes ($10^6/\mu$L)						
Day 4	0.29 ± 0.02	0.32 ± 0.03	0.38 ± 0.03	0.18 ± 0.02*	0.17 ± 0.03*	0.11 ± 0.02**
Day 24	0.20 ± 0.03	0.23 ± 0.03	0.14 ± 0.03	0.16 ± 0.02	0.13 ± 0.02	0.08 ± 0.01**[2]
Week 13	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.12 ± 0.02	0.13 ± 0.01	0.13 ± 0.02
Howell-Jolly bodies ($10^3/\mu$L)						
Day 4	18.2 ± 4.5	24.0 ± 7.3	17.3 ± 5.2	14.8 ± 3.5	23.1 ± 6.1	18.9 ± 4.3
Day 24	18.8 ± 3.0	14.6 ± 2.3	29.8 ± 7.0	36.5 ± 6.1*	46.8 ± 8.8*	66.3 ± 12.2**[2]
Week 13	5.4 ± 2.4	5.4 ± 2.4	9.5 ± 2.9	19.5 ± 5.6	29.1 ± 6.6**	23.0 ± 4.5**
Mean cell volume (fL)						
Day 4	46.8 ± 0.2	47.5 ± 0.4	47.5 ± 0.4	47.4 ± 0.4	47.8 ± 0.3	47.4 ± 0.3
Day 24	46.7 ± 0.2	47.0 ± 0.2	47.3 ± 0.2*	49.1 ± 0.2**	48.5 ± 0.2**	48.9 ± 0.3**
Week 13	44.6 ± 0.3	45.4 ± 0.3	45.2 ± 0.3	45.9 ± 0.4**	46.8 ± 0.2**	47.9 ± 0.4**
Mean cell hemoglobin (pg)						
Day 4	15.9 ± 0.1	16.0 ± 0.1	16.0 ± 0.2	16.1 ± 0.1	16.2 ± 0.1*	16.2 ± 0.1*
Day 24	16.0 ± 0.1	16.0 ± 0.1	16.0 ± 0.1	16.7 ± 0.1**	16.5 ± 0.1**	16.8 ± 0.1**
Week 13	15.6 ± 0.1	15.8 ± 0.1	15.7 ± 0.1	16.1 ± 0.1**	16.2 ± 0.1**	16.6 ± 0.1**

TABLE 13 Selected Hematology Data for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene (continued)

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
FEMALE						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 4	46.7 ± 0.3	46.1 ± 0.3	45.0 ± 0.4**	44.2 ± 0.3**	43.5 ± 0.3**	43.4 ± 0.4**
Day 24	47.2 ± 0.5	47.3 ± 0.2	46.4 ± 0.2*	44.8 ± 0.3**	44.8 ± 0.3**	43.6 ± 0.3**
Week 13	47.9 ± 0.5	47.2 ± 0.2	47.3 ± 0.4	46.5 ± 0.2**	46.3 ± 0.3**	45.1 ± 0.2**
Hemoglobin (g/dL)						
Day 4	15.8 ± 0.1	15.6 ± 0.1	15.3 ± 0.1**	15.1 ± 0.1**	14.8 ± 0.2**	14.9 ± 0.1**
Day 24	16.2 ± 0.2	16.3 ± 0.1	15.9 ± 0.1*	15.4 ± 0.1**	15.5 ± 0.1**	15.0 ± 0.1**
Week 13	16.6 ± 0.2	16.6 ± 0.1	16.4 ± 0.1	16.0 ± 0.1**	16.0 ± 0.1**	15.7 ± 0.1**
Erythrocytes ($10^6/\mu$L)						
Day 4	9.65 ± 0.09	9.68 ± 0.05	9.43 ± 0.11	9.24 ± 0.09**	9.12 ± 0.12**	9.16 ± 0.11**
Day 24	9.86 ± 0.14	9.88 ± 0.05	9.61 ± 0.06*	9.17 ± 0.07**	9.21 ± 0.07**	9.01 ± 0.08**
Week 13	10.79 ± 0.07	10.54 ± 0.03**	10.40 ± 0.12**	9.96 ± 0.05**	9.96 ± 0.07**	9.61 ± 0.04**
Reticulocytes ($10^6/\mu$L)						
Day 4	0.38 ± 0.03	0.35 ± 0.03	0.36 ± 0.03	0.20 ± 0.03**	0.17 ± 0.03**	0.15 ± 0.02**
Day 24	0.22 ± 0.02[3]	0.23 ± 0.02	0.22 ± 0.02	0.21 ± 0.01	0.19 ± 0.02	0.19 ± 0.02
Week 13	0.14 ± 0.01	0.09 ± 0.01*	0.12 ± 0.02	0.14 ± 0.02	0.13 ± 0.01	0.13 ± 0.01
Howell-Jolly bodies ($10^3/\mu$L)						
Day 4	21.1 ± 3.3	15.5 ± 4.2	18.0 ± 5.6	21.1 ± 5.0	13.9 ± 3.8	11.0 ± 2.3*
Day 24	13.3 ± 3.7[3]	16.7 ± 6.7	26.1 ± 4.4	41.1 ± 6.4*	32.5 ± 7.5	27.0 ± 4.5
Week 13	12.9 ± 3.1	9.4 ± 3.3	12.5 ± 2.1	20.0 ± 5.8	17.9 ± 4.4	35.5 ± 4.3
Mean cell volume (fL)						
Day 4	48.4 ± 0.2	47.6 ± 0.3	47.6 ± 0.3*	47.9 ± 0.3	47.7 ± 0.4	47.5 ± 0.3*
Day 24	47.9 ± 0.3	47.9 ± 0.3	48.4 ± 0.2	48.8 ± 0.3*	48.6 ± 0.2*	48.5 ± 0.2
Week 13	44.4 ± 0.4	44.8 ± 0.3	45.5 ± 0.2*	46.6 ± 0.2**	46.2 ± 0.1**	47.1 ± 0.2**
Mean cell hemoglobin (pg)						
Day 4	16.4 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.3 ± 0.1	16.3 ± 0.1	16.3 ± 0.1
Day 24	16.5 ± 0.1	16.5 ± 0.1	16.5 ± 0.0	16.8 ± 0.1**	16.8 ± 0.0**	16.7 ± 0.1**
Week 13	15.4 ± 0.1	15.7 ± 0.1*	15.7 ± 0.1*	16.0 ± 0.1**	16.1 ± 0.1**	16.3 ± 0.1**

[1] Data are given as mean ± standard error.
[2] n=8.
[3] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE 14 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene[1]

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
MALE						
n	10	10	10	10	10	10
Necropsy body wt	36.4 ± 0.8	36.1 ± 0.7	37.2 ± 0.8	37.4 ± 0.6	39.2 ± 1.4	36.7 ± 0.9
Right kidney						
Absolute	0.316 ± 0.010	0.330 ± 0.009	0.329 ± 0.010	0.337 ± 0.010	0.331 ± 0.007	0.317 ± 0.009
Relative	8.71 ± 0.29	9.16 ± 0.22	8.86 ± 0.19	9.02 ± 0.30	8.53 ± 0.29	8.65 ± 0.24
Liver						
Absolute	1.597 ± 0.047	1.525 ± 0.034	1.620 ± 0.061	1.643 ± 0.054	1.708 ± 0.049	2.010 ± 0.043**
Relative	43.95 ± 1.03	42.37 ± 0.97	43.60 ± 1.30	43.98 ± 1.60	43.83 ± 1.06	54.91 ± 1.27**
Spleen						
Absolute	0.074 ± 0.003	0.074 ± 0.004	0.068 ± 0.003	0.065 ± 0.002*	0.061 ± 0.003**	0.049 ± 0.002**
Relative	2.04 ± 0.08	2.05 ± 0.09	1.83 ± 0.06*	1.74 ± 0.07**	1.57 ± 0.07**	1.34 ± 0.06**
Right testis						
Absolute	0.120 ± 0.004	0.124 ± 0.002	0.120 ± 0.003	0.117 ± 0.001	0.106 ± 0.002**	0.077 ± 0.003**
Relative	3.31 ± 0.10	3.44 ± 0.07	3.22 ± 0.06	3.14 ± 0.06	2.73 ± 0.09**	2.10 ± 0.09**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	34.0 ± 0.7	30.8 ± 0.6**	32.3 ± 0.8**	30.1 ± 0.4**	30.4 ± 0.5**	29.9 ± 0.6**
Right kidney						
Absolute	0.223 ± 0.004	0.236 ± 0.006	0.240 ± 0.005*	0.239 ± 0.004*	0.239 ± 0.005*	0.239 ± 0.006*
Relative	6.59 ± 0.19	7.67 ± 0.14**	7.46 ± 0.19**	7.95 ± 0.17**	7.88 ± 0.15**	8.01 ± 0.24**
Liver						
Absolute	1.511 ± 0.047	1.514 ± 0.048	1.652 ± 0.066	1.559 ± 0.058	1.587 ± 0.060	1.724 ± 0.072*
Relative	44.50 ± 1.15	49.19 ± 1.38*	51.21 ± 1.76**	51.76 ± 1.65**	52.22 ± 1.58**	57.51 ± 1.61**
Spleen						
Absolute	0.111 ± 0.011	0.095 ± 0.002	0.110 ± 0.006	0.090 ± 0.001*	0.089 ± 0.003**	0.081 ± 0.002**
Relative	3.29 ± 0.36	3.09 ± 0.06	3.41 ± 0.15	2.99 ± 0.05	2.93 ± 0.05	2.71 ± 0.07*

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

Inhalation exposure of mice to isoprene for 13 weeks was associated with microscopic changes in the forestomach, nasal cavity, liver, and testis (Table 15). Epithelial hyperplasia of the forestomach was observed in male and female mice exposed to 700, 2,200, or 7,000 ppm isoprene. The epithelial hyperplasia was similar to that seen in mice in the 2-week study and was characterized by a focally thickened and folded epithelial cell layer. Occasionally, intraepithelial microabscesses and submucosal infiltrates of mixed inflammatory cells were observed in areas of hyperplasia

Olfactory epithelial degeneration was observed in all male mice exposed to 7,000 ppm isoprene. Olfactory epithelial degeneration was similar to that observed in mice in the 2-week study; this lesion was characterized by a loss of sensory epithelial cells and thinning of the olfactory epithelium along the dorsal meatus of the middle nasal section and, sometimes, the posterior nasal section. Cytoplasmic vacuolization of hepatocytes in the liver was present in a few males exposed to 2,200 ppm and in all males exposed to 7,000 ppm isoprene. The cytoplasmic vacuolization was similar to that observed in male mice in the 2-week study and was characterized by enlarged hepatocytes with prominent clear spaces in the cytoplasm. Greater liver weights in exposed mice were probably due to slight hepatocellular hypertrophy, which was undetectable by microscopic examination. As glycogen accumulation was only observed in males, the similarly greater liver weights of exposed females would suggest that these changes were due to a slight hepatocellular hypertrophy.

TABLE 15 Incidence and Severity of Selected Histopathologic Lesions in B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene[1]

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
MALE						
Forestomach						
Epithelial hyperplasia	0/10	0/10	0/10	9/10** (3.0)	8/10** (2.6)	9/10** (1.7)
Nasal cavity						
Olfactory epithelial degeneration	0/10	0/10	0/10	0/10	0/10	10/10** (1.7)
Liver						
Cytoplasmic vacuolization	0/10	0/10	0/10	0/10	3/10 (2.0)	10/10** (1.8)
Testes						
Atrophy	0/10	0/10	0/10	0/10	0/10	2/10 (1.0)
FEMALE						
Forestomach						
Epithelial hyperplasia	0/10	0/10	0/10	10/10** (3.4)	9/10** (3.1)	10/10** (3.2)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.
** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test.

Unlike the 2-week study, treatment-related thymic atrophy was not present and thymus weights were not decreased after 13 weeks. This was probably due to compensation on the part of mice for initial stress related to inhalation exposure.

The testicular atrophy observed in the 2-week study was minimal and was associated with lower testicular weights in exposed mice. At the end of the 13-week study, the testicular weight of high-dose male mice was also less than that of the controls, and minimal morphologic changes were detected in 2 of 10 high-dose mice (Table 15).

Seminiferous tubule atrophy was observed in two males exposed to 7,000 ppm isoprene; this lesion was confined to a few scattered tubules and was characterized by vacuolation and minimal loss and pyknosis of germinal epithelial cells. The left testis weight and the number of spermatid heads per gram of testis were significantly lower in male mice exposed to 7,000 ppm isoprene than in the controls (Table E3). For males in the 700 and 7,000 ppm groups, left epididymal and cauda epididymal weights, sperm motility, sperm concentration, spermatid count, and the number of spermatid heads per testis were also lower than in the controls. In addition to these changes in males, the average estrous cycle length of females exposed to 7,000 ppm isoprene was significantly longer than that of the control group (Table E4).

Stop-Exposure Inhalation Study in Male B6C3F$_1$ Mice

Ten male mice per group were killed and evaluated after 6 months of exposure in the stop-exposure study of isoprene (Table 16). During the exposure period, one mouse each in the 0, 70, and 700 ppm groups, two mice in the 2,200 ppm group, and six mice in the 7,000 ppm group died or were killed moribund (Table 17). During the recovery period, two mice each in the 0, 220, 700, and 2,200 ppm groups, one mouse in the 70 ppm group, and three mice in the 7,000 ppm group died or were killed moribund (Table 17). Of the early deaths that occurred during the stop-exposure study, one death each in the 0, 700, 2,200, and 7,000 ppm groups was attributed to lymphoma, and one death in the 7,000 ppm group was attributed to hepatocellular carcinoma. Additionally, one death in the 2,200 ppm group was attributed to forestomach squamous cell carcinoma, and one death in the 700 ppm group was attributed to histiocytic lymphoma. Three mice in the 7,000 ppm group were also killed due to hindlimb paralysis. Estimates of the probabilities of survival are shown in the Kaplan-Meier survival curve in Figure 5.

At the end of the 6-month exposure period, the final mean body weight and body weight gain of mice in the 7,000 ppm group were less than those of the control group (Table 16 and Figure 6); the final mean body weights and body weight gains of mice in all other exposed groups were similar to those of the control group. For mice allowed to recover for 6 months, mean body weights and body weight gains determined at the end of the exposure period and at the end of the recovery period were similar to or greater than those of the control group (Table 17 and Figure 6).

Several significant clinical signs of toxicity were noted in mice during the stop-exposure study. Near the end of the exposure period, abnormal posture and impaired hindlimb function were observed primarily in mice in the 7,000 ppm group; however, over the course of the recovery period, these clinical signs subsided, and the affected animals gradually returned to a clinically normal state. During the recovery period, emaciation and tachypnea were noted in a few mice in the higher exposure groups.

A macrocytic, nonresponsive anemia similar to that observed in mice in the 13-week study was present in male mice at the end of the exposure period. This was evidenced by lower erythrocyte counts, hemoglobin concentrations, and hematocrit values and greater mean cell volume values in mice in the 700, 2,200, and 7,000 ppm groups than in the controls (Table D6).

TABLE 16 Body Weights of Male B6C3F$_1$ Mice Killed After 6 Months of Exposure in the Stop-Exposure Inhalation Study of Isoprene

Concentration (ppm)	Number Examined[2]	Mean Body Weight[1] (grams)			Final Weight Relative to Controls[3] (%)
		Initial	Final	Change	
0	10	25.2 ± 0.4	45.0 ± 1.2	19.8 ± 1.0	
70	10	25.4 ± 0.4	45.0 ± 1.1	19.7 ± 1.0	100
220	10	25.0 ± 0.4	43.1 ± 1.3	18.1 ± 1.3	96
700	10	24.5 ± 0.4	47.8 ± 0.8	23.3 ± 0.7	106
2,200	10	24.6 ± 0.4	46.9 ± 1.0	22.3 ± 0.9	104
7,000	10	24.6 ± 0.5	37.4 ± 2.8**	12.8 ± 2.5**	83

[1] Weights and weight changes are given as mean ± standard error.
[2] Ten mice per exposure group were randomly selected for evaluations after 6 months of exposure and killed.
[3] (Exposure group mean/control group mean) x 100.
** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

TABLE 17 Survival and Body Weights of Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene After 6 Months of Exposure and 6 Months of Recovery

Concentration (ppm)	Survival[2]	Mean Body Weight[1] (grams)			Final Weight Relative to Controls[4] (%)
		Initial	Interim/Final[3]	Change	
6-MONTH EVALUATION					
0	29/30	25.6 ± 0.2	44.4 ± 0.6	18.9 ± 0.5	
70	29/30	24.8 ± 0.2*	44.4 ± 0.6	19.6 ± 0.5	100
220	30/30	25.7 ± 0.2	45.5 ± 0.6	19.8 ± 0.5	102
700	29/30	24.9 ± 0.2*	47.4 ± 0.7*	22.6 ± 0.6**	107
2,200	28/30	24.8 ± 0.2*	47.6 ± 0.6*	22.7 ± 0.6**	107
7,000	24/30	24.8 ± 0.2*	46.6 ± 1.5*	21.8 ± 1.4**	105
12-MONTH EVALUATION					
0	27/30[5]	25.6 ± 0.2	51.4 ± 1.0	25.8 ± 0.8	
70	28/30	24.8 ± 0.2*	53.5 ± 0.9	28.7 ± 0.7*	104
220	28/30	25.7 ± 0.2	53.0 ± 1.0	27.3 ± 0.6	103
700	27/30	24.9 ± 0.2*	55.2 ± 0.8*	30.3 ± 0.5**	108
2,200	26/30	24.8 ± 0.2*	54.9 ± 0.6*	30.1 ± 0.6**	107
7,000	21/30*[5]	24.8 ± 0.2*	53.4 ± 1.7	28.6 ± 1.4*	104

[1] Weights and weight changes are given as mean ± standard error.
[2] Number surviving at the end of evaluation period/number of animals per group; the number of animals per group does not include mice killed at the 6-month evaluation.
[3] Interim body weights were determined during the last week of the 6-month exposure period (Day 178); final body weights were determined at the end of the 6-month recovery period.
[4] (Exposure group mean/control group mean) x 100.
[5] Mean final body weight and body weight change of this group do not include data for five mice removed for electron microscopy at the end of the recovery period.
* Significantly different ($P \leq 0.05$) from the control group by the life table test (survival only) or Dunnett's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

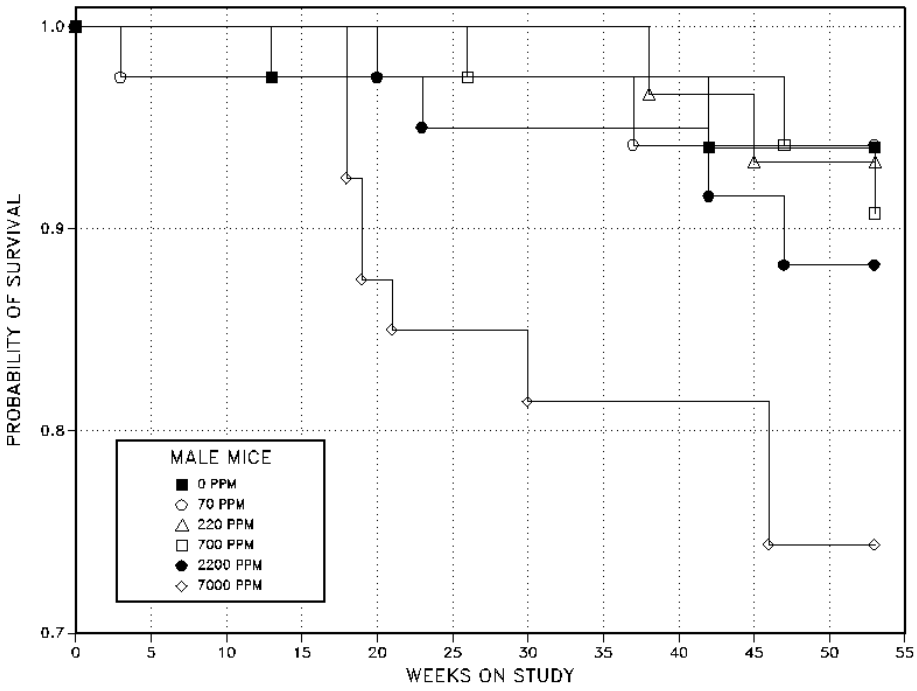

FIGURE 5 Kaplan-Meier Survival Curves for Male B6C3F$_1$ Mice During the 6-Month Exposure and Recovery Periods in the Stop-Exposure Inhalation Study of Isoprene

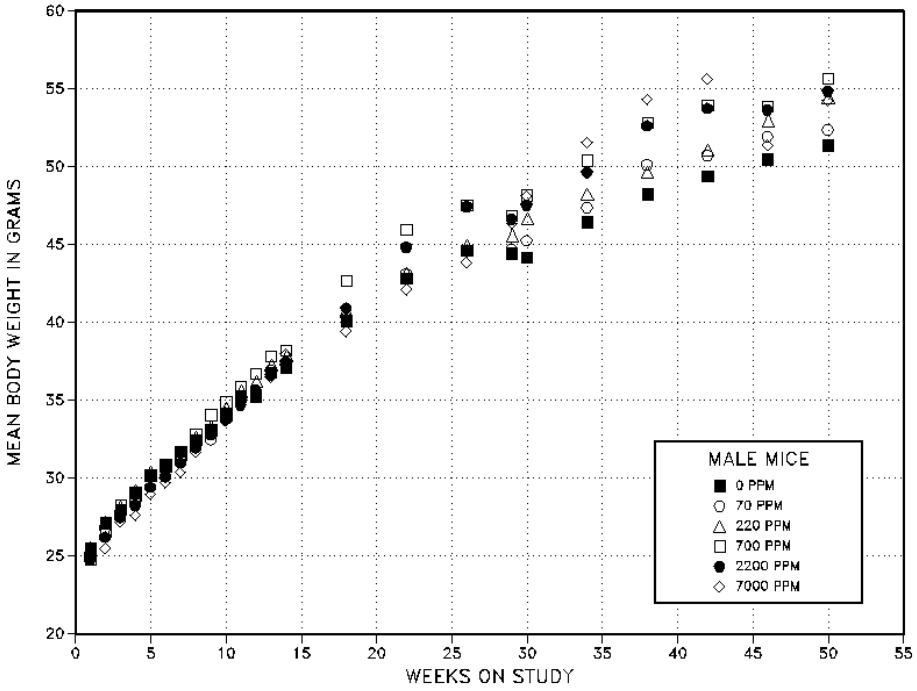

FIGURE 6 Body Weights of Male B6C3F$_1$ Mice During the 6-Month Exposure and Recovery Periods in the Stop-Exposure Inhalation Study of Isoprene

At the end of the 6-month exposure period and at the end of the 6-month recovery period, absolute liver weights of mice in the three highest exposure groups were significantly greater than the control values (Tables 18, C7, and C8); relative liver weights of mice in the 7,000 ppm group at the end of the exposure period and in the three highest exposure groups at the end of the recovery period were also greater than the relative liver weight of the controls. For mice in the 7,000 ppm group, absolute and relative testis weights were significantly less than those of the controls at the end of the exposure period (Table 18); however, by the end of the recovery period, absolute and relative testis weights for mice in this group were similar to those of the control group.

At the end of the exposure period, the absolute and relative spleen weights of mice in the 2,200 and 7,000 ppm groups and the relative spleen weight of mice in the 700 ppm group were significantly less than those of the controls. However, by the end of the recovery period, the absolute spleen weight of mice in the 2,200 ppm group and the absolute and relative spleen weights of mice in the 7,000 ppm group were greater than those of the controls. At both time points in the study, absolute brain weights of mice in the 7,000 ppm group were less than the control values; after 6 months of recovery, relative brain weights of mice in the three highest exposure groups were also significantly less than the relative brain weight of the controls.

To assess the neurobehavioral effects of isoprene exposure, the forelimb and hindlimb grip strengths of mice were tested at the end of the 6-month exposure period and at several time points during the 6-month recovery period. At the end of the exposure period, forelimb and hindlimb grip strengths of mice in the 220, 700, 2,200, and 7,000 ppm groups were significantly less than those of the control group (Table 19). Hindlimb grip strengths of mice in the three highest exposure groups remained significantly lower than in the controls at Day 2 of the recovery period, and after 1 month of recovery, the hindlimb grip strength of mice in the 2,200 ppm group was still significantly less than that of the control group (Table 19). However, at Months 3 and 6 of the recovery period, no significant differences in hindlimb grip strengths were observed between the control group and exposed groups. In addition, no significant differences in forelimb grip strengths were noted between the control group and exposed groups at any time point during the recovery period (Table 19).

TABLE 18 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene[1]

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
6-MONTH EVALUATION						
n	5	10	10	10	10	5
Necropsy body wt	44.0 ± 2.0	45.9 ± 1.2	44.5 ± 1.3	48.5 ± 1.0	47.2 ± 1.0	43.6 ± 4.0
Liver						
Absolute	1.613 ± 0.079	1.760 ± 0.092	1.704 ± 0.062	1.943 ± 0.045*	1.851 ± 0.042*	1.952 ± 0.153*
Relative	36.64 ± 0.38	38.15 ± 1.12	38.48 ± 1.44	40.14 ± 1.01	39.27 ± 0.85	45.07 ± 1.32**
Spleen						
Absolute	0.074 ± 0.002	0.073 ± 0.003	0.071 ± 0.002	0.069 ± 0.002	0.061 ± 0.002**[2]	0.059 ± 0.002**
Relative	1.70 ± 0.12	1.58 ± 0.05	1.60 ± 0.05	1.44 ± 0.05**	1.30 ± 0.04**[2]	1.38 ± 0.10**
Right testis						
Absolute	0.119 ± 0.004	0.128 ± 0.004	0.115 ± 0.007	0.122 ± 0.003	0.118 ± 0.001	0.086 ± 0.014**
Relative	2.71 ± 0.11	2.81 ± 0.10	2.58 ± 0.15	2.52 ± 0.05	2.50 ± 0.05	1.92 ± 0.19**
12-MONTH EVALUATION						
n	22	28	28	27	26	16
Necropsy body wt	51.4 ± 1.0	53.5 ± 0.9	53.0 ± 1.0	55.2 ± 0.8*	54.9 ± 0.6*	53.4 ± 1.7
Liver						
Absolute	2.317 ± 0.125	2.455 ± 0.086	2.432 ± 0.140	2.931 ± 0.133**	2.951 ± 0.161**	3.338 ± 0.243**
Relative	44.86 ± 2.06	45.89 ± 1.42	45.66 ± 2.60	53.19 ± 2.55*	54.10 ± 3.28*	62.94 ± 4.69**
Spleen						
Absolute	0.089 ± 0.005	0.098 ± 0.007	0.099 ± 0.005	0.104 ± 0.004	0.123 ± 0.015**	0.136 ± 0.014**
Relative	1.75 ± 0.12	1.86 ± 0.16	1.85 ± 0.08	1.90 ± 0.09	2.23 ± 0.25	2.58 ± 0.27**
Right testis						
Absolute	0.123 ± 0.003	0.124 ± 0.003	0.126 ± 0.001	0.126 ± 0.002	0.127 ± 0.002	0.121 ± 0.003
Relative	2.41 ± 0.06	2.34 ± 0.05	2.38 ± 0.04	2.28 ± 0.03	2.31 ± 0.03	2.28 ± 0.05

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE 19 Forelimb and Hindlimb Grip Strength Data for Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene[1]

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
n						
End of exposure period	9	10	10	9	10	10
Recovery periods	10	10	10	10	10	10
Forelimb Grip Strength						
End of exposure period	124.1 ± 6.9	109.9 ± 6.4	100.9 ± 5.0*	102.2 ± 2.8*	98.9 ± 2.9**	96.2 ± 9.1**
Recovery periods						
Day 2	87.0 ± 4.1	84.2 ± 4.3	76.5 ± 4.6	83.6 ± 4.5	90.7 ± 5.1	86.5 ± 2.8
Month 1	88.5 ± 5.8	93.4 ± 5.5	92.5 ± 4.1	86.7 ± 4.5	90.1 ± 5.0	101.2 ± 5.2
Month 3	96.8 ± 4.2	87.7 ± 6.9	95.7 ± 4.8	98.3 ± 4.0	97.5 ± 8.5	101.2 ± 5.3
Month 6	75.9 ± 2.0	73.7 ± 3.6	74.4 ± 5.2[2]	68.1 ± 4.9[2]	82.6 ± 4.6[2]	77.1 ± 5.1[2]
Hindlimb Grip Strength						
End of exposure period	85.2 ± 8.7	73.9 ± 6.4	60.3 ± 3.3*	58.2 ± 4.3*	52.5 ± 3.7**	33.2 ± 4.0**
Recovery periods						
Day 2	73.0 ± 5.7	67.4 ± 3.0	61.9 ± 3.8	53.7 ± 3.3**	48.0 ± 5.2**	38.5 ± 3.9**
Month 1	106.0 ± 6.0	96.5 ± 4.1	92.2 ± 5.3	90.8 ± 5.1	79.2 ± 3.0**	94.8 ± 3.7
Month 3	114.5 ± 6.2	103.4 ± 6.5	101.9 ± 4.6	98.4 ± 2.4	99.1 ± 4.9	103.3 ± 3.6
Month 6	103.5 ± 6.2	103.5 ± 3.9	92.6 ± 3.2[2]	98.8 ± 3.6[2]	90.8 ± 4.6[2]	104.2 ± 4.8[2]

[1] Data are given as g grip strength (mean ± standard error of three trials).
[2] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

Stomach: At the end of the 6-month exposure period, focal hyperplasia of the forestomach epithelium was observed in most mice in the 700, 2,200, and 7,000 ppm groups (Table 20); a squamous cell papilloma was observed in one mouse in the 700 ppm group. At the end of the recovery period, the incidence of hyperplasia in mice in the 700, 2,200, and 7,000 ppm groups was greater than the control incidence. In addition, the incidence of squamous cell papillomas and the combined incidence of squamous cell papillomas or carcinomas were significantly greater in mice in the 7,000 ppm group than in mice in the control group. Squamous cell papillomas occurred in one mouse in the 700 ppm group and in two mice in the 2,200 ppm group, while two other mice in the 2,200 ppm group had squamous cell carcinomas (Table 20).

Forestomach epithelial hyperplasia was typically a focal lesion consisting of thickened epithelium forming blunt rugose folds of varying lengths. Shallow ulcers were sometimes present in the center of the hyperplastic lesions with submucosal infiltrates of inflammatory cells. The papillomas were generally more complex, with a short stalk and branching papillae consisting of well-differentiated stratified squamous epithelium overlying a fibrovascular stroma. The squamous cell carcinomas

exhibited invasion of the forestomach mucosa by cords and sheet of anaplastic epithelial cells with broad areas of intervening fibrous connective tissue.

TABLE 20 Forestomach Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
6-MONTH EVALUATION						
Epithelial Hyperplasia						
Overall rate[1]	0/10 (0%)	0/10 (0%)	0/10 (0%)	8/10 (80%)**	10/10 (100%)**	9/10 (90%)**
Squamous Cell Papilloma						
Overall rate	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	0/10 (0%)	0/10 (0%)
12-MONTH EVALUATION						
Epithelial Hyperplasia						
Overall rate	1/30 (3%)	2/30 (7%)	0/29 (0%)	8/30 (27%)	9/30 (30%)	6/28 (21%)
Logistic regression test[2]	P=0.050	P=0.513	P=0.493N	P=0.011	P=0.008	P=0.061
Squamous Cell Papilloma						
Overall rate	0/30 (0%)	0/30 (0%)	0/30 (0%)	1/30 (3%)	2/30 (7%)	5/30 (17%)
Adjusted rate[3]	0.0%	0.0%	0.0%	3.7%	7.7%	20.6%
Terminal rate[4]	0/27 (0%)	0/28 (0%)	0/28 (0%)	1/27 (4%)	2/26 (8%)	3/21 (14%)
First incidence (days)	)[5]	)	)	371 (T)	371 (T)	128
Logistic regression test	P=0.001	)	)	P=0.500	P=0.229	P=0.053
Squamous Cell Carcinoma						
Overall rate	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	2/30 (7%)	1/30 (3%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	7.4%	4.8%
Terminal rate	0/27 (0%)	0/28 (0%)	0/28 (0%)	0/27 (0%)	1/26 (4%)	1/21 (5%)
First incidence (days)	)	)	)	)	326	371 (T)
Logistic regression test	P=0.159	)	)	)	P=0.236	P=0.450
Squamous Cell Papilloma or Carcinoma						
Overall rate	0/30 (0%)	0/30 (0%)	0/30 (0%)	1/30 (3%)	4/30 (13%)	6/30 (20%)
Adjusted rate	0.0%	0.0%	0.0%	3.7%	14.8%	25.0%
Terminal rate	0/27 (0%)	0/28 (0%)	0/28 (0%)	1/27 (4%)	3/26 (12%)	4/21 (19%)
First incidence (days)	)	)	)	371 (T)	326	128
Logistic regression test	P<0.001	)	)	P=0.500	P=0.060	P=0.025

(T)Terminal sacrifice.

[1] Number of lesion-bearing animals/number of animals necropsied.

[2] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions occurring in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by **N**.

[3] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.

[4] Observed incidence at terminal kill.

[5] Not applicable; no lesions in animal group.

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

Liver: Two mice in the lowest exposure group (70 ppm) had hepatocellular adenomas at the 6-month evaluation (Table 21). After an additional 6 months of recovery, the incidence of hepatocellular adenomas in mice in the 700, 2,200, and 7,000 ppm groups and the incidence of hepatocellular carcinomas in mice in the 7,000 ppm group were significantly greater than the incidences in the controls (Table 21). In mice in the 700, 2,200, and 7,000 ppm groups, the combined incidence of hepatocellular adenomas or carcinomas was also significantly greater than the control incidence. The incidence of multiple liver neoplasms also increased with increasing exposure concentration (Table B3). In addition, the incidence of altered hepatocellular foci in the 700, 2,200, and 7,000 ppm groups was slightly greater than in the controls at the end of the 6-month recovery period.

In general, altered hepatocellular foci (basophilic, eosinophilic, or mixed cell types) consisted of hepatocytes with altered cytoplasmic staining properties usually associated with changes in the amounts of rough or smooth endoplasmic reticulum, ribosomes, glycogen, or lipids. Although the cells and their nuclei were often slightly enlarged, the hepatic plates were generally minimally altered within foci, and the lobular architecture was maintained. Hepatocellular adenomas were discrete, expansile masses that were larger than hepatic lobules and compressed the adjacent parenchyma. Hepatic plates within the adenomas were not organized in a normal lobular pattern and often intersected at near-right angles with plates in the adjacent normal liver. Hepatocellular carcinomas were larger than the adenomas and consisted of markedly disorganized hepatocytes that formed solid clusters, glandular structures, or broad trabeculae several layers thick. Neoplastic hepatocytes generally showed moderate to marked pleomorphism and atypia.

TABLE 21 Liver Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
6-MONTH EVALUATION						
Basophilic Focus						
Overall rate[1]	0/10 (0%)	0/10 (0%)	1/10 (10%)	2/10 (20%)	0/10 (0%)	1/10 (10%)
Hepatocellular Adenoma						
Overall rate	0/10 (0%)	2/10 (20%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
12-MONTH EVALUATION						
Basophilic Focus						
Overall rate	3/30 (10%)	1/30 (3%)	1/29 (3%)	2/30 (7%)	5/30 (17%)	3/28 (11%)
Logistic regression test[2]	P=0.139	P=0.290N	P=0.290N	P=0.500N	P=0.331	P=0.543
Eosinophilic Focus						
Overall rate	1/30 (3%)	0/30 (0%)	0/29 (0%)	6/30 (20%)	5/30 (17%)	3/28 (11%)
Logistic regression test	P=0.084	P=0.493N	P=0.493N	P=0.054	P=0.091	P=0.217
Mixed Cell Focus						
Overall rate	0/30 (0%)	0/30 (0%)	1/29 (3%)	1/30 (3%)	2/30 (7%)	3/28 (11%)
Logistic regression test	P=0.011	)[3]	P=0.507	P=0.500	P=0.229	P=0.079
Hepatocellular Adenoma						
Overall rate	4/30 (13%)	2/30 (7%)	6/29 (21%)	15/30 (50%)	18/30 (60%)	16/28 (57%)
Adjusted rate[4]	14.8%	7.1%	21.4%	55.6%	69.2%	72.7%
Terminal rate[5]	4/27 (15%)	2/28 (7%)	6/28 (21%)	15/27 (56%)	18/26 (69%)	15/21 (71%)
First incidence (days)	371 (T)	371 (T)	371 (T)	371 (T)	371 (T)	317
Logistic regression test	P<0.001	P=0.317N	P=0.388	P=0.002	P<0.001	P<0.001
Hepatocellular Carcinoma						
Overall rate	4/30 (13%)	1/30 (3%)	3/29 (10%)	5/30 (17%)	4/30 (13%)	9/28 (32%)
Adjusted rate	14.8%	3.6%	10.7%	18.5%	15.4%	42.9%
Terminal rate	4/27 (15%)	1/28 (4%)	3/28 (11%)	5/27 (19%)	4/26 (15%)	9/21 (43%)
First incidence (days)	371 (T)	371 (T)	371 (T)	371 (T)	371 (T)	371 (T)
Logistic regression test	P<0.001	P=0.166N	P=0.480N	P=0.500	P=0.627	P=0.034
Hepatocellular Adenoma or Carcinoma						
Overall rate	7/30 (23%)	3/30 (10%)	7/29 (24%)	15/30 (50%)	18/30 (60%)	17/28 (61%)
Adjusted rate	25.9%	10.7%	25.0%	55.6%	69.2%	77.2%
Terminal rate	7/27 (26%)	3/28 (11%)	7/28 (25%)	15/27 (56%)	18/26 (69%)	16/21 (76%)
First incidence (days)	371 (T)	371 (T)	371 (T)	371 (T)	371 (T)	317
Logistic regression test	P<0.001	P=0.135N	P=0.590N	P=0.027	P=0.002	P<0.001

(T)Terminal sacrifice.

[1] Number of lesion-bearing animals/number of animals microscopically examined.

[2] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions occurring in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by **N**.

[3] Not applicable; no lesions in animal group.

[4] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.

[5] Observed incidence at terminal kill.

Harderian Gland: After 6 months of isoprene exposure, minimal hyperplasia of the harderian gland was observed in one mouse each in the 220 and 2,200 ppm groups (Table 22). After 6 months of recovery, the incidences of harderian gland adenomas in mice in the 700, 2,200, and 7,000 ppm groups were significantly greater than the incidence in the controls (Table 22). In addition, at the end of the 6-month recovery period, a harderian gland carcinoma was present in one mouse in the 2,200 ppm group that also had an adenoma. The incidence of multiple harderian gland adenomas also increased with increasing exposure concentration (Table B3).

Hyperplasia of the harderian gland consisted of a focal change, with increases in the size and number of cells in the glandular acinus. Minimal or no compression of adjacent tissue was observed. Adenomas were usually larger than hyperplastic lesions and caused compression of the surrounding tissue. Compared to adenomas, cells in the carcinoma were pleomorphic, with some cells containing vacuoles. Multiple foci of necrosis were also present in the carcinoma.

Lungs: One mouse in the 7,000 ppm group had an alveolar/bronchiolar adenoma after 6 months of exposure to isoprene (Table 23); alveolar epithelial hyperplasia was present in one mouse each in the control and 2,200 ppm groups. After 6 months of recovery, the incidences of alveolar/bronchiolar adenomas and the combined incidences of alveolar/bronchiolar adenomas or carcinomas in mice in the 2,200 and 7,000 ppm groups were significantly greater than the control incidence (Table 23). The incidence of alveolar epithelial hyperplasia in mice in the 7,000 ppm group was significantly greater than that in the controls at the end of the 6-month recovery period. These proliferative lesions may represent preneoplastic changes in the lung. The incidences of multiple lung neoplasms were greater in the 2,200 and 7,000 ppm groups than in the controls (Table B3).

Alveolar epithelial hyperplasia consisted of a focal increase in the cellularity of the alveolar epithelium with retention of the alveolar architecture. In contrast, the alveolar/bronchiolar adenomas exhibited distortion of alveolar structure due to the formation of complex, irregular papillary patterns lined by relatively uniform cuboidal or columnar cells. The alveolar/bronchiolar carcinomas were similar to the adenomas but consisted of heterogeneous cell populations with varying degrees of cellular pleomorphism and atypia. Carcinomas were larger, highly anaplastic neoplasms, often containing areas of hemorrhage or necrosis.

TABLE 22 Harderian Gland Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
6-MONTH EVALUATION						
Hyperplasia						
Overall rate[1]	0/10 (0%)	0/10 (0%)	1/10 (10%)	0/10 (0%)	1/10 (10%)	0/10 (0%)
12-MONTH EVALUATION						
Hyperplasia						
Overall rate	1/30 (3%)	0/30 (0%)	2/29 (7%)	2/30 (7%)	2/30 (7%)	2/28 (7%)
Logistic regression test[2]	P=0.356	P=0.493N	P=0.457	P=0.494	P=0.500	P=0.454
Adenoma						
Overall rate	2/30 (7%)	6/30 (20%)	4/30 (13%)	14/30 (47%)	13/30[3] (43%)	12/30 (40%)
Adjusted rate[4]	7.4%	21.4%	14.3%	50.0%	48.1%	54.5%
Terminal rate[5]	2/27 (7%)	6/28 (21%)	4/28 (14%)	13/27 (48%)	12/26 (46%)	11/21 (52%)
First incidence (days)	371 (T)	371 (T)	371 (T)	367	289	317
Logistic regression test	P<0.001	P=0.140	P=0.351	P<0.001	P=0.001	P<0.001

(T)Terminal sacrifice.

[1] Number of lesion-bearing animals/number of animals necropsied.

[2] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions occurring in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by **N**.

[3] One mouse with an adenoma also had a carcinoma.

[4] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.

[5] Observed incidence at terminal kill.

TABLE 23 Lung Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
6-MONTH EVALUATION						
Alveolar Epithelial Hyperplasia						
Overall rate[1]	1/10 (10%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	0/10 (0%)
Alveolar/bronchiolar Adenoma						
Overall rate	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)
12-MONTH EVALUATION						
Alveolar Epithelial Hyperplasia						
Overall rate	0/30 (0%)	1/30 (3%)	0/29 (0%)	3/30 (10%)	4/30 (13%)	7/28 (25%)
Logistic regression test[2]	P<0.001	P=0.507	)[3]	P=0.120	P=0.057	P=0.003
Alveolar/bronchiolar Adenoma						
Overall rate	2/30 (7%)	2/30 (7%)	1/29 (3%)	4/30 (13%)	10/30 (33%)	8/28 (29%)
Adjusted rate[4]	7.4%	7.1%	3.6%	14.8%	37.0%	38.1%
Terminal rate[5]	2/27 (7%)	2/28 (7%)	1/28 (4%)	4/27 (15%)	9/26 (35%)	8/21 (38%)
First incidence (days)	371 (T)	371 (T)	371 (T)	371 (T)	326	371 (T)
Logistic regression test	P<0.001	P=0.683N	P=0.487N	P=0.334	P=0.011	P=0.013
Alveolar/bronchiolar Carcinoma						
Overall rate	0/30 (0%)	0/30 (0%)	0/29 (0%)	1/30 (3%)	1/30 (3%)	3/28 (11%)
Adjusted rate	0.0%	0.0%	0.0%	3.7%	3.8%	14.3%
Terminal rate	0/27 (0%)	0/28 (0%)	0/28 (0%)	1/27 (4%)	1/26 (4%)	3/21 (14%)
First incidence (days)	)	)	)	371 (T)	371 (T)	371 (T)
Logistic regression test	P=0.003	)	)	P=0.500	P=0.492	P=0.079
Alveolar/bronchiolar Adenoma or Carcinoma						
Overall rate	2/30 (7%)	2/30 (7%)	1/29 (3%)	5/30 (17%)	10/30 (33%)	9/28 (32%)
Adjusted rate	7.4%	7.1%	3.6%	18.5%	37.0%	42.9%
Terminal rate	2/27 (7%)	2/28 (7%)	1/28 (4%)	5/27 (19%)	9/26 (35%)	9/21 (43%)
First incidence (days)	371 (T)	371 (T)	371 (T)	371 (T)	326	371 (T)
Logistic regression test	P<0.001	P=0.683N	P=0.487N	P=0.211	P=0.011	P=0.006

(T)Terminal sacrifice.

[1] Number of lesion-bearing animals/number of animals microscopically examined.

[2] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by **N**.

[3] Not applicable; no lesions in animal group.

[4] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.

[5] Observed incidence at terminal kill.

Nose: After 6 months of exposure to isoprene, mild to minimal olfactory epithelial degeneration was observed in all mice in the 7,000 ppm group and in one mouse each in the 700 and 2,200 ppm groups (Table 24). No evidence of resolution of the olfactory epithelial degeneration was observed during the recovery phase of the study; there was some evidence of progression of the lesion. The incidence of olfactory lesions in mice exposed to 700 or 2,200 ppm was similar to that in the controls at the 6-month evaluation; however, minimal olfactory epithelial lesions were observed in three male mice exposed to 1,750 ppm in the 2-week study. At the end of the 6-month recovery period, the incidence of mild to moderate olfactory epithelial degeneration in mice in the 220, 700, 2,200, and 7,000 ppm groups was significantly greater than in the controls (Table 24). Degeneration was characterized by focal loss of the olfactory epithelium, with single layers of columnar, cuboidal, or respiratory epithelial cells covering the defect. Bowman's glands were prominent and dilated and were filled with neutrophils and eosinophilic debris. Dilated Bowman's glands were lined by ciliated epithelial cells. Chronic inflammation characterized by fibrosis of the lamina propria was observed, along with mixed cell inflammatory infiltrate. Degeneration was minimal to moderate in severity and usually affected the olfactory epithelium at the dorsal meatus of the middle and posterior nasal section.

Testes: Atrophy of the seminiferous tubules was present in five mice in the 7,000 ppm group and one mouse in the 220 ppm group after 6 months of exposure to isoprene (Table 24). After 6 months of recovery, the incidences of atrophy of the seminiferous tubules in exposed and control mice were similar. Testicular atrophy was focal and was characterized by a loss or minimal decrease in the apparent number of germinal cells.

Spinal Cord: At the end of the 6-month exposure period, degeneration of the spinal cord white matter was present in all mice exposed to 7,000 ppm and in one mouse exposed to 2,200 ppm (Table 24). After 6 months of recovery, the incidence of spinal cord degeneration in mice in all exposed groups was significantly greater than in the controls. Degeneration was of minimal severity at each time point. The spinal cord degeneration was a subtle lesion characterized by dilated clear spaces in the white matter; some of the clear spaces contained eosinophilic globules or "ovoids" measuring approximately 2 to 3 microns in diameter. Spinal cord degeneration most likely accounted for the hindlimb dysfunction discussed above (Table 19).

TABLE 24 Selected Histopathologic Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene[1]

	Concentration (ppm)					
	0	70	220	700	2,200	7,000
6-MONTH EVALUATION						
Nasal Turbinate: Olfactory Epithelial Degeneration						
Overall rate[1]	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	1/10 (10%)	10/10 (100%)**
Testes: Atrophy						
Overall rate	0/10 (0%)	0/10 (0%)	1/10 (10%)	0/10 (0%)	0/10 (0%)	5/10 (50%)*
Spinal Cord: Degeneration						
Overall rate	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	10/10 (100%)**
Sciatic Nerve: Degeneration						
Overall rate	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	2/10 (20%)
Skeletal Muscle: Atrophy						
Overall rate	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	4/10 (40%)*
12-MONTH EVALUATION						
Nasal Turbinate: Olfactory Epithelial Degeneration						
Overall rate	1/30 (3%)	2/30 (7%)	5/29 (17%)	11/30 (37%)	25/30 (83%)	28/28 (100%)
Logistic regression test[2]	P<0.001	P=0.510	P=0.030	P=0.001	P<0.001	P<0.001
Testes: Atrophy						
Overall rate	0/30 (0%)	0/30 (0%)	0/29 (0%)	0/30 (0%)	0/30 (0%)	3/29 (10%)
Logistic regression test	P=0.016	)[3]	)	)	)	P=0.253
Spinal Cord: Degeneration						
Overall rate	4/30 (13%)	20/30 (67%)	19/29 (66%)	28/30 (93%)	17/29 (59%)	13/28 (46%)
Logistic regression test	P=0.522N	P<0.001	P<0.001	P<0.001	P<0.001	P=0.005
Sciatic Nerve: Degeneration						
Overall rate	0/30 (0%)	0/30 (0%)	0/29 (0%)	1/29 (3%)	1/28 (4%)	3/28 (11%)
Logistic regression test	P=0.038	)	)	P=0.492	P=0.492	P=0.173

[1] Number of lesion-bearing animals/number of animals microscopically examined.

[2] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions occurring in animals dying prior to terminal kill as nonfatal. A negative trend is indicated by **N**.

[3] Not applicable; no lesions in animal group.

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test.

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

Sciatic Nerve: Minimal sciatic nerve degeneration was noted in two mice after 6 months of exposure to 7,000 ppm isoprene (Table 24). In addition, one mouse each in the 700 and 2,200 ppm groups and three males in the 7,000 ppm group had sciatic nerve degeneration after 6 months of recovery from isoprene exposure. Sciatic nerve degeneration was characterized by scattered dilated clear spaces or vacuoles containing granules of eosinophilic debris within the nerve.

Skeletal Muscle: Skeletal muscle atrophy was present in four mice after 6 months of exposure to 7,000 ppm isoprene but was not observed in mice at the end of the recovery period (Table 24). Skeletal muscle atrophy was minimal and may have been secondary to spinal cord degeneration. The atrophy was characterized by scattered fibers which were small and angular.

Teratology Study in CD-1® Swiss Mice

To assess the maternal and developmental toxicity of isoprene, teratology studies were conducted in mated female CD-1® Swiss mice exposed to 0, 280, 1,400, or 7,000 ppm isoprene vapor through whole-body exposure on gestation Days 6 through 17 (Appendix E); for comparison, 10 virgin female mice per group were exposed to isoprene vapor concurrently with the positive-control mated animals.

No pregnant or virgin mice died during the study, and there were no clinical signs of toxicity. The mean body weights of exposed virgin mice were similar to control values throughout the study. However, exposure-related decreases were noted for the mean body weights of exposed dams on gestation Days 12, 15, and 18, and the mean body weight of dams in the 7,000 ppm group was significantly less than that of the control group on gestation Days 15 and 18. The gravid uterine weight of dams exposed to 7,000 ppm isoprene was significantly less than that of the control group; in addition, the relative liver weight of dams in the 1,400 ppm group and the relative liver and relative kidney weights of dams in the 7,000 ppm group were significantly greater than those of the control group (Appendix E).

In the teratology study in mice, gestational exposure to isoprene did not affect the number of litters with resorptions or resorptions per litter. In addition, no statistically significant differences in fetal mortality or the number of live fetuses per litter were noted between the control and exposed groups. However, fetal body weights decreased with increasing exposure concentration, and the body weights of male fetuses in the 1,400 and 7,000 ppm groups and female fetuses in all exposed groups were significantly less than those of the controls (Appendix E). Gestational exposure to isoprene did not significantly increase the total number of fetal malformations or the percentage of malformed fetuses per litter. There were no statistically significant differences between the control and exposed groups in the overall incidence of fetal variations/reduced ossifications. However, the mean percentage of fetuses per litter with variations/reduced ossifications (mostly supernumerary ribs) increased with increasing exposure concentration and was significantly greater at the highest exposure level than in the controls.

Genetic Toxicity Studies

Results of mutagenicity tests of isoprene (100 to 10,000 µg/plate) in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without Aroclor 1254-induced rat or hamster liver S9 were negative (Table F1; Mortelmans *et al.*, 1986). No induction of sister chromatid exchanges (SCEs) or chromosomal aberrations (Abs) was observed in Chinese hamster ovary cells treated with isoprene with or without S9 (Tables F2 and F3).

Results of the *in vivo* cytogenetics studies are presented in detail by Tice *et al.* (1988) and Shelby (1990). Briefly, significantly greater numbers of SCEs in bone marrow cells and micronucleated polychromatic and normochromatic erythrocytes in peripheral blood were observed in mice exposed to isoprene for 12 days or 13 weeks than in unexposed mice. However, no difference in the number of Abs was noted in mouse bone marrow cells.

DISCUSSION

Toxicity studies of isoprene, the 2-methyl analogue of 1,3-butadiene, were conducted in rats and mice to characterize potential adverse effects induced by this high production chemical in two mammalian species and to determine if isoprene exposure produces effects similar to those of 1,3-butadiene. A comparison of dose-response relationships for the toxicologic effects resulting from inhalation exposure to these chemicals is important because long-term inhalation studies have shown that 1,3-butadiene is carcinogenic at multiple organ sites in laboratory animals (NTP, 1984a, 1993; Huff *et al.*, 1985; Owen *et al.*, 1987; Melnick *et al.*, 1990a). Relevant to the present studies on isoprene are the findings that the sites of 1,3-butadiene-induced carcinogenicity and the magnitude of response were different in mice from those in rats. For example, in mice there were early occurrences and extensive development of thymic lymphomas, induction of uncommon hemangiosarcomas of the heart, and development of malignant lung neoplasms at exposure concentrations as low as 6.25 ppm (Melnick *et al.*, 1990a; NTP, 1993); however, in rats exposed to 8,000 ppm 1,3-butadiene for 2 years, the incidences of neoplasms of the hematopoietic system, heart, or lung were not greater than in the controls (Owen *et al.*, 1987). Based on the carcinogenic effects of 1,3-butadiene in laboratory animals and the findings of excess lymphatic and hematopoietic cancers in workers exposed to 1,3-butadiene (Meinhardt *et al.*, 1982; Divine, 1990; Matanoski *et al.*, 1990), the United States Occupational Safety and Health Administration (OSHA) has proposed lowering the occupational standard for this chemical from 1,000 ppm to 2 ppm (OSHA, 1990); meanwhile, there is no OSHA standard or American Conference of Governmental Industrial Hygienists threshold limit value for isoprene.

Both 1,3-butadiene and isoprene are metabolized to mono- and diepoxide intermediates by liver microsomal cytochrome P_{450}-dependent monooxygenase (Malvoisin *et al.*, 1979; Malvoisin and Roberfroid, 1982; Del Monte *et al.*, 1985; Longo *et al.*, 1985), and the metabolic elimination rates of 1,3-butadiene and isoprene are two to three times greater in mice than in rats (Kreiling *et al.*, 1986; Peter *et al.*, 1987). In contrast to 1,3-butadiene, isoprene may be metabolized to two monoepoxide intermediates, and only the minor intermediate (20%), 3,4-epoxy-2-methyl-1-butene, was found to be oxidized to isoprene diepoxide (see Figure 1 in the Introduction). The primary metabolite of 1,3-butadiene metabolism, 1,2-epoxy-3-butene, can be further oxidized to diepoxybutane. The epoxide intermediates of isoprene and 1,3-butadiene metabolism may be detoxified by hydrolysis (catalyzed by epoxide hydrolase) or by conjugation with glutathione (catalyzed by glutathione-S-transferase). Isoprene diepoxide and the mono- and diepoxide

intermediates of 1,3-butadiene metabolism have been shown to be mutagenic in *Salmonella typhimurium*; the monoepoxide intermediates of isoprene metabolism were not mutagenic (Gervasi *et al.*, 1985). Thus, based on their metabolic profiles and mutagenicity patterns, it is expected that exposure to 1,3-butadiene would result in a greater body burden of mutagenic epoxides than would exposure to an equivalent concentration of isoprene. Although the mechanism of 1,3-butadiene-induced carcinogenicity is not fully understood, the epoxide intermediates discussed above are thought to be important because they have been shown to induce local neoplasms in rats and mice when administered by skin application or subcutaneous injection (IARC, 1992).

2-WEEK INHALATION STUDIES IN RATS AND MICE

In the 2-week studies reported here, groups of male and female rats and mice were exposed to isoprene vapors at concentrations of up to 7,000 ppm for 6 hours per day, 5 days per week for 12 exposures. The upper exposure level was limited by the lower flammable limit value of 1.5% for isoprene in air. In rats, no chemical-related changes were observed in survival, body weight gains, clinical signs, clinical pathology parameters, or the incidence of gross or microscopic lesions. Organ weight differences between exposed and control rats were not associated with any histopathologic changes.

In mice exposed to isoprene for 2 weeks, there were no differences in survival between exposed and control groups, but a lower body weight gain than in the controls was observed in the 7,000 ppm exposure group of males. Toxicity in mice included hematologic effects (lower hematocrit values, hemoglobin concentrations, and erythrocyte counts than in the controls), testicular atrophy, thymic atrophy, olfactory epithelial degeneration, and forestomach epithelial hyperplasia. Similar hematologic changes and lesions in the testis, nose, and forestomach were observed in mice exposed to 1,3-butadiene (NTP, 1984a; Melnick *et al.*, 1990c). Further, in conjunction with these studies, additional groups of mice were exposed to 438, 1,750, or 7,000 ppm isoprene and evaluated for cytogenetic effects. Exposure to isoprene induced increases in the frequency of sister chromatid exchanges (SCEs) in bone marrow cells and in the levels of micronucleated erythrocytes in peripheral blood at all exposures studied (Tice *et al.*, 1988). Similar effects, but with a greater magnitude of response, were observed in mice exposed to 1,3-butadiene (Tice *et al.*, 1987). However, unlike 1,3-butadiene, isoprene did not induce chromosomal aberrations in bone marrow cells.

As a consequence of the numerous similarities between isoprene and 1,3-butadiene (structure, species sensitivity, organ toxicity, genetic toxicity, and metabolism), 6-month exposure studies with 6-month recovery periods were added to the planned 13-week studies of isoprene in rats and mice to evaluate the potential reversibility or progression of exposure-related lesions in these two species. It was expected that if isoprene has effects similar to those of 1,3-butadiene, such an expanded exposure protocol would detect a carcinogenic response. The 6-month exposure duration was selected because 3-month and 6-month stop-exposure studies using 625 ppm 1,3-butadiene produced multiple organ carcinogenicity in male mice at the same sites that were identified in the 2-year study of this gas (hematopoietic system, heart, lung, forestomach, harderian gland), and high incidences of lymphomas were detected within approximately 25 weeks after the start of exposure to 1,3-butadiene (Melnick *et al.*, 1990a).

13-WEEK AND 6-MONTH INHALATION STUDIES IN RATS

Exposure to isoprene for 13 weeks produced no discernible toxicologic effects in rats. These results were not totally unexpected, since no treatment-related gross or microscopic changes or effects on growth, survival, hematologic or blood biochemical parameters, urinary measurements, or neuromuscular functions were reported in male or female Sprague-Dawley rats exposed to 1,3-butadiene (1,000 to 8,000 ppm) 6 hours per day, 5 days per week for 13 weeks (Crouch *et al.*, 1979). Interstitial cell hyperplasia of the testis was observed in all male rats exposed to 7,000 ppm isoprene for 6 months. Following the 6-month recovery period, the incidence of benign testicular adenomas was marginally greater in this group than in the controls. To determine whether this greater incidence was related to isoprene administration, a long-term study of isoprene in a different strain of rat would be necessary. The incidence of interstitial cell proliferative lesions increases rapidly in untreated F344/N rats 1 year of age and older, and by 18 months, the incidence of testicular adenomas is greater than 80% (Boorman *et al.*, 1990). In untreated Sprague-Dawley rats of similar age, testicular neoplasms are uncommon. Furthermore, because the incidence of interstitial cell neoplasms of the testis was greater in Sprague-Dawley rats exposed to 8,000 ppm 1,3-butadiene for 2 years than in the controls (Owen *et al.*, 1987), it is particularly important that the carcinogenic potential of isoprene in the testis be fully evaluated. Two-year exposure durations to 1,3-butadiene were necessary to produce neoplastic effects in rats (Owen *et al.*, 1987).

13-WEEK AND 6-MONTH INHALATION STUDIES IN MICE

In mice exposed to isoprene, toxic and carcinogenic effects were induced at multiple organ sites. Exposure to isoprene for 13 weeks or 6 months produced no clear exposure-related effects on body weight gains in male or female mice. Although the body weight gain of male mice killed after 6 months of exposure to 7,000 ppm isoprene was less than that of the controls, the body weight gain of the larger group of males exposed to 7,000 ppm for 6 months and then allowed to recover for 6 months was similar to that of the controls at the end of the exposure portion of the study. Body weight differences in female mice exposed to isoprene for 13 weeks were not exposure-related. There was a larger number of mortalities in mice exposed to 7,000 ppm isoprene for 6 months than in the controls or in the other exposure groups. Some of the early deaths, as well as clinical signs of toxicity (tachypnea and emaciation) in the highest exposure group, may have been related to the development of lung and liver neoplasms.

Partial hindlimb paralysis was observed near the end of the 6-month exposure period, primarily in mice in the 7,000 ppm group. During the recovery period, mice that were affected with partial hindlimb paralysis gradually returned to a clinically normal state. An assessment of hindlimb function showed an exposure-related decrease in grip strength that was largely resolved by 1 month after exposure ended. At 3 and 6 months post-exposure, hindlimb grip strength measurements of exposed groups were similar to those of control mice. Histopathology revealed skeletal muscle atrophy, sciatic nerve degeneration, and spinal cord degeneration after 6 months of exposure to 7,000 ppm isoprene. At the end of the 6-month recovery period, there was no identifiable muscle atrophy; however, incidences of minimal spinal cord degeneration in all of the exposed groups were greater than the incidence in the controls. Thus, these studies did not achieve a no-observable-adverse-effect level (NOAEL) for spinal cord degeneration. No hindlimb dysfunction or spinal cord degeneration was observed in mice exposed to 1,3-butadiene.

Hematologic effects seen in male and female mice in the 2-week study (lower erythrocyte counts, hemoglobin concentrations, and hematocrit values than in the controls) were reproduced in the 13-week study. The changes were not accompanied by higher reticulocyte counts or frequencies of polychromatic erythrocytes in peripheral blood. In contrast to the 2-week studies, values for mean cell volume were greater in exposed mice than in the controls after 24 days or 13 weeks of exposure to 220 ppm isoprene or greater. Mean erythrocyte volume was also greater in mice exposed to 625 ppm 1,3-butadiene than in the controls (Melnick *et al.*, 1990c). In addition, bone

marrow cytotoxicity due to exposure to isoprene was evidenced by a lower number and rate of dividing cells in the bone marrow (Tice *et al.*, 1988). Thus, these findings indicate that like 1,3-butadiene, isoprene suppresses hematopoiesis in the bone marrow of mice and induces a nonresponsive, macrocytic anemia.

Neither clinical chemistry and urinalysis data nor microscopic examination of stained tissue sections revealed evidence of liver necrosis or kidney damage in mice exposed to isoprene for 13 weeks or 6 months. Tissue glutathione concentrations in the liver and lung of male and female mice exposed to 7,000 ppm isoprene for 12 weeks were approximately 40% to 60% lower than those in the controls. The lower spleen weights in mice exposed to isoprene for 13 weeks or 6 months were not associated with any histologic changes. The greater liver weights were probably due to slight hepatocellular hypertrophy. After 6 months of exposure plus 6 months of recovery, there was an exposure-related increase in the incidence of hepatocellular neoplasms. The incidences in the 700, 2,200, and 7,000 ppm groups were significantly greater than the control incidence. Furthermore, the incidence of hepatocellular carcinomas in males exposed to 7,000 ppm was greater than in the controls. The increases in neoplasm multiplicity and the greater tendency to malignant neoplasia in the liver further demonstrate the carcinogenic potential of isoprene in this organ. The incidences of hepatocellular neoplasms in male and female mice exposed to 1,3-butadiene were marginally greater than the incidences in the controls (Melnick *et al.*, 1990a). The conclusion that these greater incidences were related to the administration of 1,3-butadiene was strengthened by the detection of activated K-*ras* oncogenes with specific codon 13 mutations in liver neoplasms obtained from mice exposed to this gas (Goodrow *et al.*, 1990); activated K-*ras* oncogenes have rarely been detected in liver tumors from untreated B6C3F$_1$ mice. Oncogene analyses have not been completed on tumor tissues obtained from mice exposed to isoprene.

Exposure-related decreases in testis weights were observed in mice after 2 weeks, 13 weeks, and 6 months of exposure to isoprene; however, at the end of the 6-month recovery period in the stop-exposure study, the mean testis weights of previously exposed mice were similar to those of the controls. Testicular atrophy was also observed in mice exposed to 7,000 ppm isoprene, and this effect was resolved during the 6-month recovery period. Testicular atrophy was induced in mice exposed to 625 ppm 1,3-butadiene or greater (Melnick and Huff, 1992). In male mice exposed to 700 or 7,000 ppm isoprene for 13 weeks, lower epididymal weights, spermatid head counts, sperm concentration, and sperm motility than in the controls were also observed. A concentration-

related increase in sperm head abnormalities was observed in mice exposed to 200 to 5,000 ppm 1,3-butadiene; however, no effect on male fertility was detected at these exposure concentrations (Morrissey *et al.*, 1990).

Olfactory epithelial degeneration and chronic inflammation of the olfactory epithelium were observed in male mice exposed to 2,200 or 7,000 ppm isoprene for 13 weeks or to 7,000 ppm isoprene for 6 months. These lesions did not regress during the 6-month recovery period. Nasal lesions, including atrophy and chronic inflammation of the olfactory epithelium, were also observed in male mice exposed to 1,250 ppm 1,3-butadiene (NTP, 1984a). Nasal lesions induced by exposure to 1,3-butadiene or isoprene showed no evidence of progression to neoplasia.

In addition to the liver neoplasms discussed above, exposure of male mice to isoprene produced increased incidences of lung, forestomach, and harderian gland neoplasms. No histopathologic changes were detected in the lungs of isoprene-exposed mice killed after 6 months of exposure; however, after the 6-month recovery period, the incidences of hyperplasia of the alveolar epithelium in the 700, 2,200, and 7,000 ppm groups were greater than in the controls, and the incidences of alveolar/bronchiolar adenomas plus carcinomas were significantly greater in the 2,200 and 7,000 ppm groups than in the controls. Alveolar epithelial hyperplasia may represent an early preneoplastic change in the development of lung neoplasms. Lung carcinomas were diagnosed in one mouse in each of the 700 and 2,200 ppm groups and in three mice in the 7,000 ppm group. Alveolar epithelial hyperplasia was also observed in mice exposed to 1,3-butadiene, and the incidences of lung neoplasms in male mice exposed to 62.5 ppm 1,3-butadiene or greater for up to 2 years, to 200 ppm 1,3-butadiene for 40 weeks, or to 625 ppm 1,3-butadiene for 13 or 26 weeks were greater than the control incidences (Melnick *et al.*, 1990a; NTP, 1993). In female mice exposed to 1,3-butadiene, greater incidences of lung neoplasms were even observed at the 6.25 ppm exposure level. Thus, both of these epoxide-forming chemicals are carcinogenic to the mouse lung, with 1,3-butadiene appearing to be more active at lower concentrations.

Isoprene caused epithelial hyperplasia of the forestomach in mice after 2 weeks of exposure to 438 ppm or greater and after 13 weeks or 6 months of exposure to 700 ppm or greater. After the 6-month recovery period, exposure-related increases in the incidences of epithelial hyperplasia and forestomach neoplasms (squamous cell papillomas and squamous cell carcinomas) were observed. The incidence of forestomach neoplasms in the 7,000 ppm group was significantly greater than the control incidence. In mice exposed to 200 ppm 1,3-butadiene or greater, the incidence of

forestomach neoplasms was greater than in the controls (Melnick *et al.*, 1990a; NTP, 1993). Squamous cell neoplasms of the forestomach were detected as late as Week 88 and Week 105 in mice that were exposed to 625 ppm 1,3-butadiene for 13 weeks and then held in control chambers to allow time for progression or regression of lesions induced by 1,3-butadiene. This observation indicates that a 13-week or shorter exposure duration may induce forestomach lesions that persist and progress to malignant neoplasms in the absence of further exposure to 1,3-butadiene. A similar relationship between the duration of exposure and development of forestomach neoplasms may exist in mice exposed to isoprene.

Incidences of harderian gland adenomas in mice exposed to 700, 2,200, or 7,000 ppm isoprene for 6 months were similarly greater than the control values. In mice exposed to 62.5 ppm 1,3-butadiene or greater, incidences of harderian gland neoplasms were higher than in the controls. In the stop-exposure studies of 1,3-butadiene, incidences of harderian gland neoplasms after 13 or 26 weeks of exposure to 625 ppm 1,3-butadiene and after 40 weeks of exposure to 200 ppm 1,3-butadiene were greater than the control incidences. Thus, as with the induction of lung neoplasms, isoprene appears to be less active than 1,3-butadiene in inducing harderian gland neoplasms.

COMPARISONS OF TOXICITY AND CARCINOGENICITY BETWEEN ISOPRENE AND 1,3-BUTADIENE

Isoprene appears to be less active than 1,3-butadiene in inducing lung, forestomach, and harderian gland neoplasms. This difference may be due in part to differences in experimental design. Evaluations of the carcinogenicity of 1,3-butadiene were made after 2 years of continuous exposure and at the end of a 2-year period that included 13 to 52 weeks of exposure followed by an extended recovery period; evaluations of the carcinogenicity of isoprene were made at the end of 1 year, after 6 months of exposure followed by 6 months of recovery. The exposures to isoprene may not have been of sufficient duration to reveal the full carcinogenic potential of this chemical. Exposure to isoprene did not result in increased incidences of lymphomas or hemangiosarcomas of the heart, as were observed in mice exposed to 1,3-butadiene (NTP, 1984a, 1993; Huff *et al.*, 1985; Melnick *et al.*, 1990a). Lymphomas were observed as early as 23 weeks after exposure to 1,3-butadiene began. Thus, the exposure regimen for isoprene should have been sufficient to detect a carcinogenic response in the hematopoietic system of mice if isoprene is as active as 1,3-butadiene. Metabolic and mutagenic differences may distinguish the carcinogenicity of these two chemicals. Isoprene metabolism in mice deviates from linearity above 300 ppm, and

saturation at about 2,000 ppm limits the production of epoxide intermediates at higher concentrations (Peter *et al.*, 1987). In addition to differences in experimental design, differences in potency or sites of carcinogenicity between isoprene and 1,3-butadiene under conditions of linear metabolism may also reflect differences in the mutagenic activity of the monoepoxide intermediates (the monoepoxide intermediates of isoprene metabolism were not mutagenic in *S. typhimurium*) or in the levels of production of the corresponding diepoxide intermediates. The concentrations of mutagenic epoxides in the tissues of mice exposed to approximately 600 ppm 1,3-butadiene may not be reached in the tissues of mice exposed to concentrations of isoprene near metabolic saturation.

Metabolic saturation may account for the nearly flat dose-response curves for cytogenetic effects (frequency of SCEs and levels of micronucleated erythrocytes) and the induction of lung, liver, and harderian gland neoplasms in mice exposed to concentrations of isoprene greater than 2,000 ppm. For most of these endpoints, effects at 700 ppm were not very different from those at about 2,000 ppm. Toxic effects which were more severe or which occurred at a higher incidence in the 7,000 ppm group (*e.g.*, hindlimb paralysis, muscle atrophy, sciatic nerve degeneration, spinal cord degeneration, olfactory epithelial degeneration, and effects on the testis and estrous cycle) probably reflect the involvement of the parent compound.

As with 1,3-butadiene, species differences were observed between the toxicologic and carcinogenic effects of isoprene in rats and mice. The basis for species differences resulting from exposure to 1,3-butadiene are not fully understood. A physiologically based pharmacokinetic model of the uptake, tissue distribution, and metabolism of inhaled 1,3-butadiene did not reveal species differences of sufficient magnitude to account for the different carcinogenic response observed in rats and mice (Kohn and Melnick, 1993). Evidently, other factors are crucial for 1,3-butadiene-induced carcinogenesis. Similar models of isoprene metabolism in rats and mice have not been reported. Because 2-year exposures were necessary to demonstrate the carcinogenicity of 1,3-butadiene in rats, the 6-month exposure plus 6-month recovery protocol must be considered inadequate to evaluate the carcinogenic potential of isoprene in this species.

INHALATION TERATOLOGY STUDIES IN RATS AND MICE

Teratology studies of isoprene showed that inhalation exposures of up to 7,000 ppm did not result in apparent maternal or developmental toxicity in Sprague-Dawley rats. In CD-1® Swiss mice, the lower mean body weight of dams exposed to 7,000 ppm isoprene was indicative of maternal toxicity at this exposure concentration. Developmental toxicity was caused by gestational exposure to 280, 1,400, or 7,000 ppm isoprene, as evidenced by exposure-related reductions in fetal body weights and greater incidences of supernumerary ribs. The body weights of male fetuses in the 1,400 and 7,000 ppm groups and of female fetuses at all exposure levels were significantly less than those of the controls; in the 7,000 ppm group only, the percentage of fetuses per litter with supernumerary ribs was significantly greater than that in the controls.

1,3-Butadiene also exhibited a species difference in developmental toxicity. Developmental effects were not exhibited in Sprague-Dawley rats administered gestational exposures of 40, 200, or 1,000 ppm 1,3-butadiene; however, in CD-1® Swiss mice, fetal body weights at all exposure levels were less than those of the controls, and greater incidences of fetal variations (supernumerary ribs and reduced ossification of sternebrae) than in the controls occurred in the 200 and 1,000 ppm groups (Morrissey *et al.*, 1990). The latter effects, however, were accompanied by reductions in maternal weight gain.

CONCLUSIONS

In conclusion, isoprene caused toxic effects in the testis of rats and at multiple organ sites in mice. In F344/N rats, exposure to 7,000 ppm isoprene for 6 months caused an increase in the incidence of testicular interstitial cell hyperplasia, and after 6 months of recovery there was a marginal increase in the incidence of benign testicular adenomas that may have been related to isoprene administration. NOAELs for isoprene-induced toxic lesions in mice were:

- 70 ppm for nonresponsive, macrocytic anemia, decreased hindlimb grip strength, olfactory epithelial degeneration, and decreases in epididymal weights, spermatid head counts, sperm concentration, and sperm motility;
- 220 ppm for forestomach epithelial hyperplasia;
- 700 ppm for increased estrous cycle length;
- and 2,200 ppm for testicular atrophy, sciatic nerve degeneration, and muscle atrophy.

A NOAEL was not achieved for spinal cord degeneration (less than 70 ppm) or developmental toxicity (less than 280 ppm, based on lower body weights of female fetuses). In addition, the 6-

month inhalation exposure plus 6-month recovery (stop-exposure) study provided clear evidence of carcinogenicity of isoprene in the liver, lung, forestomach, and harderian gland of mice. Because these studies involved exposures of male rats and male mice to isoprene for only 6 months, they do not necessarily reveal the full carcinogenic potential of isoprene in these species. Most of the toxic and carcinogenic effects seen with isoprene were also caused by inhalation exposure to 1,3-butadiene.

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APPENDIX A

Summary of Lesions in Rats

Table A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Inhalation Study of Isoprene A-2

Table A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Inhalation Study of Isoprene A-4

Table A3 Summary of the Incidence of Neoplasms in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene A-6

Table A4 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene A-9

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	1 (10%)		2 (20%)	1 (10%)		1 (10%)
Mesentery	(1)	(1)				
Necrosis	1 (100%)					
Pancreas	(10)			(1)	(3)	(10)
Accessory spleen				1 (100%)	2 (67%)	
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Cardiomyopathy, focal	5 (50%)					5 (50%)
Endocrine System						
Pituitary gland	(10)					(10)
Pars distalis, cyst	1 (10%)					
General Body System						
None						
Genital System						
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Degeneration				1 (10%)		
Hematopoietic System						
Lymph node		(2)				
Iliac, pigmentation		1 (50%)				
Renal, congestion		1 (50%)				
Lymph node, bronchial	(10)	(9)	(7)	(7)	(8)	(9)
Congestion	9 (90%)	9 (100%)	7 (100%)	7 (100%)	8 (100%)	8 (89%)
Hyperplasia	8 (80%)					
Lymph node, mandibular	(8)					(8)
Congestion						1 (13%)
Hyperplasia	1 (13%)					
Lymph node, mesenteric	(10)	(1)			(1)	(10)
Congestion	1 (10%)	1 (100%)			1 (100%)	
Hyperplasia	1 (10%)					
Pigmentation, hemosiderin	1 (10%)					
Lymph node, mediastinal	(10)					(9)
Congestion	8 (80%)					9 (100%)
Hyperplasia	9 (90%)					1 (11%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Developmental malformation					1 (10%)	

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
Integumentary System						
Skin	(10)		(1)			(10)
Inflammation, chronic			1 (100%)			
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage, focal	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Alveolar epithelium, hyperplasia, focal	9 (90%)					
Alveolus, infiltration cellular, focal, histiocyte	9 (90%)					
Alveolus, inflammation, focal, suppurative	6 (60%)					
Peribronchial, hyperplasia, focal, lymphoid	6 (60%)					
Perivascular, hyperplasia, focal, lymphoid	9 (90%)					
Trachea	(10)	(1)				(10)
Submucosa, infiltration cellular, focal, mononuclear cell	1 (10%)					
Special Senses System						
None						
Urinary System						
None						

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

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	1 (10%)	1 (10%)	1 (10%)			3 (30%)
Pharynx	(10)					(10)
Palate, inflammation, suppurative	1 (10%)					
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	
(10)						
Cardiomyopathy, focal	2 (20%)					
Endocrine System						
None						
General Body System						
None						
Genital System						
Ovary	(10)		(3)			(10)
Follicle, cyst			1 (33%)			
Periovarian tissue, cyst	2 (20%)		2 (67%)			
Uterus	(10)					(10)
Dilatation						1 (10%)
Hematopoietic System						
Lymph node, bronchial	(10)	(4)	(10)	(7)	(7)	(10)
Congestion	7 (70%)	4 (100%)	10 (100%)	7 (100%)	7 (100%)	7 (70%)
Hyperplasia	4 (40%)					
Lymph node, mandibular	(9)					(10)
Congestion	1 (11%)					
Hyperplasia	1 (11%)					1 (10%)
Lymph node, mediastinal	(9)					(10)
Congestion	8 (89%)					8 (80%)
Hyperplasia	7 (78%)					4 (40%)
Integumentary System						
Skin	(10)					(10)
Subcutaneous tissue, hemorrhage	1 (10%)					

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
Musculoskeletal System						
Skeletal muscle	(1)					
Diaphragm, hernia	1 (100%)					
Nervous System						
None						
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage, focal	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Alveolar epithelium, hyperplasia, focal	8 (80%)					
Alveolus, infiltration cellular, focal, histiocyte	8 (80%)					1 (10%)
Alveolus, inflammation, focal, suppurative	5 (50%)					
Peribronchial, hyperplasia, focal, lymphoid	4 (40%)					
Perivascular, hyperplasia, focal, lymphoid	6 (60%)					
Perivascular, hyperplasia, lymphoid	1 (10%)					
Special Senses System						
Harderian gland	(10)					(10)
Hyperplasia, focal, lymphoid	1 (10%)					
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Bilateral, mineralization, focal	9 (90%)				1 (10%)	8 (80%)
Bilateral, pelvis, dilatation						1 (10%)

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

TABLE A3 Summary of the Incidence of Neoplasms in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	40	40	40	40	40	40
6-Month evaluation	10	10	10	10	10	10
Early deaths						
Natural deaths			1			
Survivors						
Terminal sacrifice	30	30	29	30	30	30
Animals examined microscopically	40	40	40	40	40	40
6-MONTH EVALUATION						
Alimentary System						
None						
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						

TABLE A3 Summary of the Incidence of Neoplasms in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
6-MONTH EVALUATION (continued)						
Special Senses System						
None						
Urinary System						
None						
12-MONTH EVALUATION						
Alimentary System						
Pharynx	(30)		(1)			(30)
Palate, papilloma						1 (3%)
Cardiovascular System						
None						
Endocrine System						
Adrenal medulla	(28)		(2)			(30)
Ganglioneuroma			1 (50%)			
Pituitary gland	(30)	(8)	(4)	(4)	(4)	(30)
Adenoma	6 (20%)	7 (88%)	3 (75%)	4 (100%)	3 (75%)	8 (27%)
General Body System						
None						
Genital System						
Epididymis	(30)		(2)		(1)	(30)
Testes	(30)	(30)	(30)	(30)	(29)	(30)
Interstitial cell, adenoma	3 (10%)	3 (10%)	4 (13%)	7 (23%)	8 (28%)	8 (27%)
Interstitial cell, adenoma, multiple						1 (3%)
Hematopoietic System						
Spleen	(30)	(3)	(2)	(3)	(3)	(30)
Integumentary System						
Mammary gland	(2)			(1)		(1)
Fibroadenoma				1 (100%)		
Skin	(30)		(1)	(2)		(30)
Sarcoma			1 (100%)			
Musculoskeletal System						
None						

TABLE A3 Summary of the Incidence of Neoplasms in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
12-MONTH EVALUATION (continued)						
Nervous System						
None						
Respiratory System						
Lung	(30)	(30)	(30)	(30)	(30)	(30)
Alveolar/bronchiolar adenoma	1 (3%)	1 (3%)				1 (3%)
Special Senses System						
None						
Urinary System						
None						
Systemic Lesions						
Multiple organs[2]	(30)	(30)	(30)	(30)	(30)	(30)
Leukemia mononuclear		2 (7%)				
Mesothelioma benign			1 (3%)			1 (3%)
Mesothelioma NOS					1 (3%)	
Neoplasm Summary						
Total animals with primary neoplasms[3]	8	10	7	7	5	12
Total primary neoplasms	9	10	7	8	6	17
Total animals with benign neoplasms	8	8	6	7	5	12
Total benign neoplasms	9	8	6	8	5	17
Total animals with malignant neoplasms		2	1			
Total malignant neoplasms		2	1			
Total animals with neoplasms uncertain-benign or malignant					1	
Total uncertain neoplasms					1	

[1] Number of animals examined microscopically at site and number of animals with neoplasm.
[2] Number of animals with any tissue examined microscopically.
[3] Primary neoplasms: all neoplasms except metastatic neoplasms.

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	40	40	40	40	40	40
6-Month evaluation	10	10	10	10	10	10
Early deaths						
Natural deaths			1			
Survivors						
Terminal sacrifice	30	30	29	30	30	30
Animals examined microscopically	40	40	40	40	40	40
6-MONTH EVALUATION						
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	3 (30%)	4 (40%)	1 (10%)	3 (30%)	3 (30%)	
Inflammation, focal						1 (10%)
Sinusoid, congestion, focal						1 (10%)
Mesentery			(1)	(1)		(1)
Accessory spleen						1 (100%)
Necrosis				1 (100%)		
Pancreas	(10)	(10)	(10)	(10)	(10)	(10)
Accessory spleen						1 (10%)
Atrophy						1 (10%)
Atrophy, focal	1 (10%)					
Infiltration cellular, mixed cell						2 (20%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, mixed cell						1 (10%)
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Cardiomyopathy						3 (30%)
Cardiomyopathy, multifocal	7 (70%)					1 (10%)
Endocrine System						
Pituitary gland	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia	1 (10%)					
Pars distalis, cyst	1 (10%)					1 (10%)
Thyroid gland	(10)	(10)	(10)	(10)	(10)	(10)
Cyst	2 (20%)					1 (10%)
General Body System						
None						
Genital System						
Preputial gland	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, suppurative	1 (10%)					
Prostate	(10)	(10)	(10)	(10)	(10)	(10)
Corpora amylacea	1 (10%)					2 (20%)
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Interstitial cell, hyperplasia	1 (10%)	1 (10%)	3 (30%)	1 (10%)	3 (30%)	10 (100%)

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
6-MONTH EVALUATION (continued)						
Hematopoietic System						
Lymph node		(2)				
Pancreatic, congestion		1 (50%)				
Lymph node, bronchial	(9)	(10)	(10)	(10)	(10)	(10)
Congestion	9 (100%)					8 (80%)
Hyperplasia	1 (11%)					
Lymph node, mandibular	(9)	(10)	(10)	(10)	(10)	(10)
Congestion				1 (10%)		2 (20%)
Hyperplasia	1 (11%)					
Lymph node, mediastinal	(8)	(10)	(10)	(10)	(10)	(6)
Congestion	8 (100%)	1 (10%)	1 (10%)		1 (10%)	6 (100%)
Hyperplasia	4 (50%)					
Pigmentation, hemosiderin	2 (25%)					
Integumentary System						
Mammary gland	(1)	(10)	(10)	(10)	(10)	(2)
Hyperplasia	1 (100%)					1 (50%)
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Subcutaneous tissue, hemorrhage			1 (10%)			
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Congestion	1 (10%)					
Hemorrhage		10 (100%)	10 (100%)	10 (100%)	10 (100%)	1 (10%)
Hemorrhage, multifocal	10 (100%)					9 (90%)
Alveolar epithelium, hyperplasia						1 (10%)
Alveolus, hemorrhage						1 (10%)
Alveolus, infiltration cellular, focal, histiocyte	1 (10%)					
Alveolus, infiltration cellular, multifocal, histiocyte	1 (10%)					
Alveolus, infiltration cellular, histiocyte			1 (10%)			1 (10%)
Peribronchial, hyperplasia, focal, lymphoid						3 (30%)
Peribronchial, hyperplasia, lymphoid						1 (10%)
Peribronchial, hyperplasia, lymphoid, multifocal	5 (50%)					
Perivascular, hyperplasia, focal, lymphoid	1 (10%)	1 (10%)				1 (10%)
Perivascular, hyperplasia, lymphoid, multifocal	5 (50%)	1 (10%)	2 (20%)	1 (10%)	3 (30%)	

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
6-MONTH EVALUATION (continued)						
Respiratory System (continued)						
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Turbinate, infiltration cellular, mixed cell						1 (10%)
Trachea	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, mononuclear cell, multifocal						1 (10%)
Special Senses System						
Harderian gland	(2)					(2)
Hyperplasia, lymphoid	2 (100%)					
Hyperplasia, lymphoid, multifocal						2 (100%)
Urinary System						
None						
12-MONTH EVALUATION						
Alimentary System						
Intestine small, jejunum	(30)					(30)
Hemorrhage, multifocal						1 (3%)
Inflammation, multifocal, suppurative						1 (3%)
Intestine small, ileum	(30)					(30)
Hyperplasia, lymphoid						1 (3%)
Liver	(30)	(5)	(12)	(5)	(7)	(30)
Clear cell focus			1 (8%)			
Fatty change	1 (3%)		3 (25%)	4 (80%)		
Fatty change, focal					1 (14%)	
Fatty change, multifocal			2 (17%)	1 (20%)		
Fibrosis	1 (3%)				1 (14%)	1 (3%)
Fibrosis, focal					1 (14%)	
Fibrosis, multifocal	1 (3%)		1 (8%)			1 (3%)
Hepatodiaphragmatic nodule	2 (7%)	5 (100%)	7 (58%)	1 (20%)	5 (71%)	6 (20%)
Inflammation	1 (3%)					
Inflammation, multifocal	2 (7%)					
Necrosis, focal			1 (8%)			1 (3%)
Necrosis, multifocal	1 (3%)		2 (17%)			3 (10%)
Mesentery	(1)	(2)	(1)	(1)	(2)	
Congestion			1 (100%)			
Necrosis	1 (100%)	2 (100%)		1 (100%)	2 (100%)	
Pancreas	(30)		(1)			(30)
Atrophy	8 (27%)					8 (27%)
Atrophy, multifocal	1 (3%)					1 (3%)
Infiltration cellular, focal, mixed cell	1 (3%)					
Infiltration cellular, mixed cell	2 (7%)					

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
12-MONTH EVALUATION (continued)						
Cardiovascular System						
Heart	(30)		(1)			(30)
Cardiomyopathy	1 (3%)					9 (30%)
Cardiomyopathy, focal	2 (7%)					
Cardiomyopathy, multifocal	7 (23%)					1 (3%)
Endocrine System						
Adrenal cortex	(30)		(1)			(30)
Vacuolization cytoplasmic, focal	1 (3%)					
Islets, pancreatic	(30)		(1)			(30)
Hyperplasia	4 (13%)					6 (20%)
Pituitary gland	(30)	(8)	(4)	(4)	(4)	(30)
Hemorrhage, focal				1 (25%)		
Hyperplasia	10 (33%)	1 (13%)			1 (25%)	11 (37%)
Pars distalis, cyst			1 (25%)			1 (3%)
Thyroid gland	(30)					(30)
Cyst						2 (7%)
General Body System						
None						
Genital System						
Epididymis	(30)		(2)		(1)	(30)
Hemorrhage	1 (3%)					
Hypospermia	1 (3%)					
Inflammation, chronic	1 (3%)					1 (3%)
Preputial gland	(29)			(2)		(30)
Hyperplasia	1 (3%)					
Infiltration cellular, mixed cell	2 (7%)			2 (100%)		1 (3%)
Inflammation, suppurative	2 (7%)			2 (100%)		
Prostate	(30)		(1)			(30)
Inflammation, suppurative						2 (7%)
Testes	(30)	(30)	(30)	(30)	(29)	(30)
Degeneration	1 (3%)		1 (3%)			
Granuloma			1 (3%)			
Inflammation			1 (3%)			
Interstitial cell, hyperplasia	25 (83%)	30 (100%)	28 (93%)	30 (100%)	29 (100%)	30 (100%)
Hematopoietic System						
Lymph node	(1)					
Pancreatic, congestion	1 (100%)					
Lymph node, bronchial	(23)		(1)		(1)	(27)
Congestion	8 (35%)					11 (41%)
Hyperplasia					1 (100%)	6 (22%)
Lymph node, mandibular	(26)		(2)	(1)		(30)
Congestion	1 (4%)					1 (3%)
Hyperplasia	7 (27%)		1 (50%)			5 (17%)

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
12-MONTH EVALUATION (continued)						
Hematopoietic System (continued)						
Lymph node, mediastinal	(27)	(5)	(2)	(1)		(24)
Congestion	12 (44%)	2 (40%)				11 (46%)
Hyperplasia	1 (4%)	4 (80%)	1 (50%)	1 (100%)		8 (33%)
Pigmentation, hemosiderin	4 (15%)					2 (8%)
Spleen	(30)	(3)	(2)	(3)	(3)	(30)
Accessory spleen	2 (7%)	1 (33%)	1 (50%)	2 (67%)	3 (100%)	2 (7%)
Capsule, hyperplasia, lymphoid				1 (33%)		
Integumentary System						
Mammary gland	(2)			(1)		(1)
Hyperplasia						1 (100%)
Skin	(30)		(1)	(2)		(30)
Inflammation, chronic				1 (50%)		
Musculoskeletal System						
None						
Nervous System						
Brain	(30)	(1)	(1)			(30)
Hemorrhage		1 (100%)				
Respiratory System						
Larynx	(30)					(30)
Infiltration cellular, mixed cell	1 (3%)					3 (10%)
Lung	(30)	(30)	(30)	(30)	(30)	(30)
Congestion			1 (3%)			
Fibrosis, multifocal			1 (3%)			
Hemorrhage	5 (17%)		1 (3%)			4 (13%)
Hemorrhage, multifocal	25 (83%)	30 (100%)	26 (87%)	24 (80%)	22 (73%)	26 (87%)
Alveolar epithelium, hyperplasia	3 (10%)			1 (3%)		
Alveolar epithelium, hyperplasia, focal					1 (3%)	2 (7%)
Alveolar epithelium, hyperplasia, multifocal	2 (7%)	15 (50%)	13 (43%)	11 (37%)	11 (37%)	3 (10%)
Alveolus, hemorrhage	2 (7%)				1 (3%)	2 (7%)
Alveolus, infiltration cellular, focal, histiocyte	1 (3%)		1 (3%)	1 (3%)	1 (3%)	1 (3%)
Alveolus, infiltration cellular, multifocal, histiocyte	1 (3%)	17 (57%)	18 (60%)	16 (53%)	15 (50%)	18 (60%)
Alveolus, infiltration cellular, histiocyte						1 (3%)
Alveolus, inflammation, granulomatous						1 (3%)
Peribronchial, hyperplasia, focal, lymphoid		2 (7%)	3 (10%)			1 (3%)
Peribronchial, hyperplasia, lymphoid, multifocal		8 (27%)	7 (23%)	7 (23%)	4 (13%)	1 (3%)
Perivascular, hyperplasia, focal, lymphoid				2 (7%)	2 (7%)	1 (3%)
Perivascular, hyperplasia, lymphoid, multifocal	10 (33%)	22 (73%)	23 (77%)	19 (63%)	14 (47%)	23 (77%)

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
12-MONTH EVALUATION (continued)						
Respiratory System (continued)						
Nose	(30)		(1)			(30)
Turbinate, hemorrhage			1 (100%)			
Turbinate, infiltration cellular, multifocal, mixed cell						1 (3%)
Turbinate, infiltration cellular, mixed cell	1 (3%)					
Turbinate, inflammation, suppurative	1 (3%)					1 (3%)
Special Senses System						
Eye	(30)		(1)	(1)	(1)	(30)
Cataract				1 (100%)	1 (100%)	
Retina, degeneration				1 (100%)	1 (100%)	
Harderian gland	(3)					(4)
Hemorrhage	1 (33%)					
Hyperplasia, lymphoid	2 (67%)					3 (75%)
Hyperplasia, lymphoid, multifocal						1 (25%)
Urinary System						
Kidney	(30)		(2)	(2)	(6)	(30)
Nephropathy, chronic				1 (50%)		
Bilateral, cyst				1 (50%)		
Bilateral, nephropathy, chronic	23 (77%)		1 (50%)	1 (50%)	6 (100%)	29 (97%)
Urinary bladder	(30)	(1)	(3)		(7)	(30)
Calculus microscopic observation only	2 (7%)		3 (100%)		5 (71%)	12 (40%)

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

APPENDIX B

Summary of Lesions in Mice

Table B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene B-2

Table B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene B-4

Table B3 Summary of the Incidence of Neoplasms in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene B-6

Table B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery B-10

Table B5 Statistical Analysis of Primary Neoplasms in Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery B-36

Table B6 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene B-40

TABLE B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)		(2)	(10)	(9)	(10)
Basophilic focus			1 (50%)			
Necrosis	1 (10%)					
Vacuolization cytoplasmic					3 (33%)	10 (100%)
Stomach, forestomach	(10)		(1)	(9)	(8)	(10)
Epithelium, hyperplasia				9 (100%)	8 (100%)	9 (90%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Testes	(10)		(1)	(10)	(9)	(10)
Atrophy						2 (20%)
Hematopoietic System						
Spleen	(10)		(1)	(10)	(9)	(10)
Pigmentation						1 (10%)
Pigmentation, melanin					1 (11%)	
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

TABLE B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
Respiratory System						
Lung	(10)		(1)	(10)	(9)	(10)
Hemorrhage	1 (10%)					
Nose	(10)					(10)
Turbinate, olfactory epithelium, degeneration						10 (100%)
Special Senses System						
None						
Urinary System						
Kidney	(10)		(1)	(10)	(9)	(10)
Bilateral, hydronephrosis	1 (10%)					

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

TABLE B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(1)	(1)	(10)	(10)	(10)
Vacuolization cytoplasmic						1 (10%)
Stomach, forestomach	(10)			(10)	(10)	(10)
Epithelium, hyperplasia				10 (100%)	9 (90%)	10 (100%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Ovary	(10)		(1)			(10)
Abscess	1 (10%)					
Hematopoietic System						
Lymph node	(1)					
Renal, hyperplasia	1 (100%)					
Spleen	(10)	(1)	(1)	(10)	(10)	(10)
Pigmentation, melanin				1 (10%)	1 (10%)	
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

TABLE B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
Respiratory System						
Nose	(10)					(10)
Turbinate, inflammation, suppurative						1 (10%)
Special Senses System						
None						
Urinary System						
None						

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

TABLE B3 Summary of the Incidence of Neoplasms in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	40	40	40	40	40	40
6-Month evaluation	10	10	10	10	10	10
Early deaths						
Moribund sacrifice	1	1	1	1	1	5
Natural death	1	1	1	2	3	4
Accidentally killed	1					
Survivors						
Terminal sacrifice	27	28	28	27	26	21
Animals examined microscopically	40	40	40	40	40	40
6-MONTH EVALUATION						
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatocellular adenoma		1 (10%)				
Hepatocellular adenoma, multiple		1 (10%)				
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Squamous cell papilloma				1 (10%)		
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

TABLE B3 Summary of the Incidence of Neoplasms in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
6-MONTH EVALUATION (continued)						
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma						1 (10%)
Special Senses System						
None						
Urinary System						
None						
12-MONTH EVALUATION						
Alimentary System						
Liver	(30)	(30)	(29)	(30)	(30)	(28)
Hemangioma					2 (7%)	
Hepatocellular carcinoma	3 (10%)	1 (3%)	3 (10%)	4 (13%)	1 (3%)	9 (32%)
Hepatocellular carcinoma, multiple	1 (3%)			1 (3%)	3 (10%)	
Hepatocellular adenoma	4 (13%)	2 (7%)	3 (10%)	12 (40%)	13 (43%)	5 (18%)
Hepatocellular adenoma, multiple			3 (10%)	3 (10%)	5 (17%)	11 (39%)
Squamous cell carcinoma, metastatic, stomach, forestomach					1 (3%)	
Mesentery		(2)		(2)	(4)	(2)
Squamous cell carcinoma, metastatic, stomach, forestomach					1 (25%)	
Stomach, forestomach	(30)	(30)	(29)	(30)	(30)	(28)
Squamous cell carcinoma					2 (7%)	1 (4%)
Squamous cell papilloma				1 (3%)	2 (7%)	4 (14%)
Squamous cell papilloma, multiple						1 (4%)
Tooth	(1)		(2)			
Odontoma	1 (100%)		1 (50%)			
Cardiovascular System						
Heart	(30)	(2)	(1)	(3)	(30)	(28)
Endocrine System						
None						
General Body System						
None						
Genital System						
None						

TABLE B3 Summary of the Incidence of Neoplasms in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
12-MONTH EVALUATION (continued)						
Hematopoietic System						
Bone marrow	(30)	(2)	(1)	(3)	(30)	(28)
Lymph node, bronchial	(23)	(1)	(1)	(2)	(27)	(23)
Squamous cell carcinoma, metastatic, stomach, forestomach					1 (4%)	
Lymph node, mandibular	(18)	(1)		(2)	(25)	(15)
Lymph node, mesenteric	(30)	(3)	(1)	(3)	(29)	(27)
Squamous cell carcinoma, metastatic, stomach, forestomach					1 (3%)	
Lymph node, mediastinal	(13)	(2)	(1)	(3)	(14)	(17)
Squamous cell carcinoma, metastatic, stomach, forestomach					1 (7%)	
Spleen	(30)	(5)	(1)	(3)	(30)	(28)
Thymus	(30)	(2)	(1)	(3)	(30)	(26)
Integumentary System						
Skin	(30)	(4)	(1)	(4)	(30)	(29)
Squamous cell papilloma				1 (25%)		
Sebaceous gland, adenoma				1 (25%)		
Subcutaneous tissue, sarcoma						1 (3%)
Musculoskeletal System						
Skeletal muscle	(30)	(30)	(29)	(30)	(30)	(28)
Nervous System						
Brain	(30)	(2)	(1)	(3)	(30)	(28)
Meningioma malignant, metastatic, spinal cord						1 (4%)
Spinal cord	(30)	(30)	(29)	(30)	(29)	(28)
Meningioma malignant						1 (4%)
Respiratory System						
Lung	(30)	(30)	(29)	(30)	(30)	(28)
Alveolar/bronchiolar adenoma	2 (7%)	2 (7%)	1 (3%)	4 (13%)	8 (27%)	4 (14%)
Alveolar/bronchiolar adenoma, multiple					2 (7%)	4 (14%)
Alveolar/bronchiolar carcinoma				1 (3%)		3 (11%)
Alveolar/bronchiolar carcinoma, multiple					1 (3%)	
Squamous cell carcinoma, metastatic, stomach, forestomach					1 (3%)	
Nose	(30)	(30)	(29)	(30)	(30)	(28)

TABLE B3 Summary of the Incidence of Neoplasms in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
12-MONTH EVALUATION (continued)						
Special Senses System						
Harderian gland	(30)	(30)	(29)	(30)	(30)	(28)
Adenoma	2 (7%)	6 (20%)	4 (14%)	13 (43%)	11 (37%)	7 (25%)
Adenoma, multiple				1 (3%)	2 (7%)	5 (18%)
Carcinoma					1 (3%)	
Urinary System						
Kidney	(30)	(3)	(1)	(4)	(30)	(28)
Systemic Lesions						
Multiple organs[2]	(30)	(30)	(30)	(30)	(30)	(30)
Lymphoma malignant					1 (3%)	
Lymphoma malignant histiocytic				1 (3%)		1 (3%)
Lymphoma malignant lymphocytic	1 (3%)			1 (3%)	1 (3%)	1 (3%)
Neoplasm Summary						
Total animals with primary neoplasms[3]						
6-Month evaluation		2		1		1
12-Month evaluation	12	10	11	24	26	24
Total primary neoplasms						
6-Month evaluation		2		1		1
12-Month evaluation	14	11	15	44	55	58
Total animals with benign neoplasms						
6-Month evaluation		2		1		1
12-Month evaluation	9	9	10	22	25	23
Total benign neoplasms						
6-Month evaluation		2		1		1
12-Month evaluation	9	10	12	36	45	41
Total animals with malignant neoplasms						
12-Month evaluation	5	1	3	8	9	14
Total malignant neoplasms						
12-Month evaluation	5	1	3	8	10	17
Total animals with metastatic neoplasms						
12-Month evaluation					1	1
Total metastatic neoplasms						
12-Month evaluation					6	1

[1] Number of animals examined microscopically at site and number of animals with neoplasm.
[2] Number of animals with any tissue examined microscopically
[3] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 0 ppm

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

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

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 0 ppm (continued)

Number of Days on Study	3 7 3	3 7 3	3 7 3	3 7 3	3 7 3	
Carcass ID Number	0 3 0	0 3 1	0 3 4	0 3 5	0 3 6	**Total Tissues/ Tumors**
Alimentary System						
Esophagus	+	+	+	+	+	30
Gallbladder	+	+	+	+	+	29
Intestine large, colon	+	+	+	+	+	30
Intestine large, rectum	+	+	+	+	+	29
Intestine large, cecum	+	+	+	+	+	30
Intestine small, duodenum	+	+	+	+	+	29
Intestine small, jejunum	+	+	+	+	+	30
Intestine small, ileum	+	+	+	+	+	30
Liver	+	+	+	+	+	30
Hepatocellular carcinoma		X				3
Hepatocellular carcinoma, multiple						1
Hepatocellular adenoma	X					4
Pancreas	+	+	+	+	+	30
Pharynx	+	+	+	+	+	30
Salivary glands	+	+	+	+	+	30
Stomach, forestomach	+	+	+	+	+	30
Stomach, glandular	+	+	+	+	+	30
Tongue	+	+	+	+	+	30
Tooth						1
Odontoma						1
Cardiovascular System						
Blood vessel	+	+	+	+	+	30
Heart	+	+	+	+	+	30
Endocrine System						
Adrenal cortex	+	+	+	+	+	30
Adrenal medulla	+	+	+	+	+	29
Islets, pancreatic	+	+	+	+	+	30
Parathyroid gland	M	+	+	+	+	25
Pituitary gland	+	+	+	M	M	28
Thyroid gland	+	+	+	+	+	30
General Body System						
None						
Genital System						
Epididymis	+	+	+	+	+	30
Penis						1
Preputial gland	+	+	+	+	+	30
Prostate	+	+	M	+	+	28
Seminal vesicle	+	+	+	+	+	30
Testes	+	+	+	+	+	30

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 0 ppm (continued)

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

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 0 ppm (continued)

Number of Days on Study	3 7 3	3 7 3	3 7 3	3 7 3	3 7 3	
Carcass ID Number	0 3 0	0 3 1	0 3 4	0 3 5	0 3 6	**Total Tissues/ Tumors**
Hematopoietic System						
Bone marrow	+	+	+	+	+	30
Lymph node						1
Lymph node, bronchial	+	+	+	+	+	23
Lymph node, mandibular	+	+	M	+	M	18
Lymph node, mesenteric	+	+	+	+	+	30
Lymph node, mediastinal	+	M	+	M	+	13
Spleen	+	+	+	+	+	30
Thymus	+	+	+	+	+	30
Integumentary System						
Mammary gland	M	M	M	M	M	
Skin	+	+	+	+	+	30
Musculoskeletal System						
Bone	+	+	+	+	+	30
Skeletal muscle	+	+	+	+	+	30
Nervous System						
Brain	+	+	+	+	+	30
Peripheral nerve	+	+	+	+	+	30
Spinal cord	+	+	+	+	+	30
Respiratory System						
Larynx	+	+	+	+	+	30
Lung	+	+	+	+	+	30
Alveolar/bronchiolar adenoma						2
Nose	+	+	+	+	+	30
Trachea	+	+	+	+	+	30
Special Senses System						
Eye	+	+	+	+	+	30
Harderian gland	+	+	+	+	+	30
Adenoma						2
Zymbal's gland	+	+	+	+	+	30
Urinary System						
Kidney	+	+	+	+	+	30
Urinary bladder	+	+	+	+	+	30
Systemic Lesions						
Multiple organs	+	+	+	+	+	30
Lymphoma malignant lymphocytic						1

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 70 ppm

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

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 70 ppm (continued)

Number of Days on Study	3 7 3	3 7 3	3 7 3	3 7 3	3 7 3	
Carcass ID Number	0 6 6	0 6 7	0 6 8	0 6 9	0 7 0	**Total Tissues/ Tumors**
Alimentary System						
Esophagus						2
Gallbladder						1
Intestine large, colon						2
Intestine large, rectum						2
Intestine large, cecum						2
Intestine small, duodenum						2
Intestine small, jejunum						1
Intestine small, ileum						1
Liver	+	+	+	+	+	30
Hepatocellular carcinoma						1
Hepatocellular adenoma		X				2
Mesentery						2
Pancreas	+	+	+	+	+	30
Pharynx						2
Salivary glands						2
Stomach, forestomach	+	+	+	+	+	30
Stomach, glandular	+	+	+	+	+	30
Tongue						2
Cardiovascular System						
Blood vessel						2
Heart						2
Endocrine System						
Adrenal cortex						2
Adrenal medulla						2
Islets, pancreatic	+	+	+	+	+	30
Parathyroid gland						2
Pituitary gland						2
Thyroid gland						2
General Body System						
None						
Genital System						
Epididymis						2
Preputial gland		+				3
Prostate						2
Seminal vesicle						2
Testes	+	+	+	+	+	30

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 70 ppm (continued)

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

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 70 ppm (continued)

Number of Days on Study	3 7 3	3 7 3	3 7 3	3 7 3	3 7 3	
Carcass ID Number	0 6 6	0 6 7	0 6 8	0 6 9	0 6 7	**Total Tissues/ Tumors**
Hematopoietic System						
Bone marrow						2
Lymph node						1
Lymph node, bronchial						1
Lymph node, mandibular						1
Lymph node, mesenteric						3
Lymph node, mediastinal						2
Spleen						5
Thymus						2
Integumentary System						
Mammary gland						
Skin						4
Musculoskeletal System						
Bone						2
Skeletal muscle	+	+	+	+	+	30
Nervous System						
Brain						2
Peripheral nerve	+	+	+	+	+	30
Spinal cord	+	+	+	+	+	30
Respiratory System						
Larynx						2
Lung	+	+	+	+	+	30
Alveolar/bronchiolar adenoma		X				2
Nose	+	+	+	+	+	30
Trachea						2
Special Senses System						
Eye						2
Harderian gland	+	+	+	+	+	30
Adenoma			X	X		6
Zymbal's gland						2
Urinary System						
Kidney						3
Urinary bladder					+	5
Systemic Lesions						
Multiple organs	+	+	+	+	+	30

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 220 ppm

Number of Days on Study	260	309	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372
Carcass ID Number	08 5	10 6	08 1	08 2	08 3	08 4	08 6	08 7	08 8	08 9	09 0	09 1	09 2	09 3	09 4	09 5	09 6	09 7	09 8	09 9	10 0	10 1	10 2	10 3	10 4	
Alimentary System																										
Esophagus	A	+																								
Gallbladder	A	+																								
Intestine large, colon	A	+																								
Intestine large, rectum	A	+																								
Intestine large, cecum	A	+																								
Intestine small, duodenum	A	+																								
Intestine small, jejunum	A	+																								
Intestine small, ileum	A	+																								
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hepatocellular carcinoma								X							X											
Hepatocellular adenoma							X	X										X								
Hepatocellular adenoma, multiple			X									X														
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pharynx	+	+																								
Salivary glands	A	+																								
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Tongue	+	+																								
Tooth		+																		+						
Odontoma																				X						
Cardiovascular System																										
Blood vessel	A	M																								
Heart	A	+																								
Endocrine System																										
Adrenal cortex	A	+																								
Adrenal medulla	A	+																								
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Parathyroid gland	A	M																								
Pituitary gland	A	+																								
Thyroid gland	A	+																								
General Body System																										
None																										
Genital System																										
Epididymis	A	+																								
Preputial gland	A	+																								
Prostate	A	+																								
Seminal vesicle	A	+																								
Testes	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 220 ppm (continued)

Number of Days on Study	372	372	372	372	372	
Carcass ID Number	10 5	10 7	10 8	10 9	11 0	**Total Tissues/ Tumors**
Alimentary System						
Esophagus						1
Gallbladder						1
Intestine large, colon						1
Intestine large, rectum						1
Intestine large, cecum						1
Intestine small, duodenum						1
Intestine small, jejunum						1
Intestine small, ileum						1
Liver	+	+	+	+	+	29
Hepatocellular carcinoma				X		3
Hepatocellular adenoma						3
Hepatocellular adenoma, multiple				X		3
Pancreas	+	+	+	+	+	29
Pharynx						2
Salivary glands						1
Stomach, forestomach	+	+	+	+	+	29
Stomach, glandular	+	+	+	+	+	29
Tongue						2
Tooth						2
Odontoma						1
Cardiovascular System						
Blood vessel						
Heart						1
Endocrine System						
Adrenal cortex						1
Adrenal medulla						1
Islets, pancreatic	+	+	+	+	+	29
Parathyroid gland						
Pituitary gland						1
Thyroid gland						1
General Body System						
None						
Genital System						
Epididymis						1
Preputial gland						1
Prostate						1
Seminal vesicle						1
Testes	+	+	+	+	+	29

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 220 ppm (continued)

Number of Days on Study	260	309	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372	372
Carcass ID Number	08 5	10 6	08 1	08 2	08 3	08 4	08 6	08 7	08 8	08 9	09 0	09 1	09 2	09 3	09 4	09 5	09 6	09 7	09 8	09 9	10 0	10 1	10 2	10 3	10 4	
Hematopoietic System																										
Bone marrow	A	+																								
Lymph node, bronchial	A	+																								
Lymph node, mandibular	A	M																								
Lymph node, mesenteric	A	+																								
Lymph node, mediastinal	A	+																								
Spleen	A	+																								
Thymus	A	+																								
Integumentary System																										
Mammary gland	A	M																								
Skin	A	+																								
Musculoskeletal System																										
Bone	A	+														+										
Skeletal muscle	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Nervous System																										
Brain	A	+																								
Peripheral nerve	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spinal cord	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Respiratory System																										
Larynx	A	+																								
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma														X												
Nose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	A	+																								
Special Senses System																										
Eye	+	+																								
Harderian gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma								X			X						X									
Zymbal's gland	+	+																								
Urinary System																										
Kidney	A	+																								
Urinary bladder	A	+																								
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 220 ppm (continued)

Number of Days on Study	3 3 3 3 3 7 7 7 7 7 2 2 2 2 2	
Carcass ID Number	1 1 1 1 1 0 0 0 0 1 5 7 8 9 0	**Total Tissues/ Tumors**
Hematopoietic System		
Bone marrow		1
Lymph node, bronchial		1
Lymph node, mandibular		
Lymph node, mesenteric		1
Lymph node, mediastinal		1
Spleen		1
Thymus		1
Integumentary System		
Mammary gland		
Skin		1
Musculoskeletal System		
Bone		2
Skeletal muscle	+ + + + +	29
Nervous System		
Brain		1
Peripheral nerve	+ + + + +	29
Spinal cord	+ + + + +	29
Respiratory System		
Larynx		1
Lung	+ + + + +	29
Alveolar/bronchiolar adenoma		1
Nose	+ + + + +	29
Trachea		1
Special Senses System		
Eye		2
Harderian gland	+ + + + +	29
Adenoma	X	4
Zymbal's gland		2
Urinary System		
Kidney		1
Urinary bladder		1
Systemic Lesions		
Multiple organs	+ + + + +	30

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 700 ppm

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

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 700 ppm (continued)

Number of Days on Study	3 7 2	3 7 2	3 7 2	3 7 2	3 7 2	
Carcass ID Number	1 4 5	1 4 6	1 4 7	1 4 8	1 4 9	**Total Tissues/ Tumors**
Alimentary System						
Esophagus						3
Gallbladder						2
Intestine large, colon						3
Intestine large, rectum						3
Intestine large, cecum						4
Intestine small, duodenum						2
Intestine small, jejunum						3
Intestine small, ileum						3
Liver	+	+	+	+	+	30
Hepatocellular carcinoma						4
Hepatocellular carcinoma, multiple						1
Hepatocellular adenoma		X	X	X	X	12
Hepatocellular adenoma, multiple						3
Mesentery						2
Pancreas	+	+	+	+	+	30
Pharynx						3
Salivary glands						3
Stomach, forestomach	+	+	+	+	+	30
Squamous cell papilloma						1
Stomach, glandular	+	+	+	+	+	30
Tongue						3
Cardiovascular System						
Blood vessel						3
Heart						3
Endocrine System						
Adrenal cortex						3
Adrenal medulla						3
Islets, pancreatic	+	+	+	+	+	30
Parathyroid gland						3
Pituitary gland						3
Thyroid gland						3
General Body System						
None						
Genital System						
Epididymis						3
Preputial gland						6
Prostate						3
Seminal vesicle						3
Testes	+	+	+	+	+	30

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 700 ppm (continued)

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

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 700 ppm (continued)

Number of Days on Study	3 7 2	3 7 2	3 7 2	3 7 2	3 7 2	
Carcass ID Number	1 4 5	1 4 6	1 4 7	1 4 8	1 4 9	**Total Tissues/ Tumors**
Hematopoietic System						
Bone marrow						3
Lymph node, bronchial						2
Lymph node, mandibular						2
Lymph node, mesenteric						3
Lymph node, mediastinal						3
Spleen						3
Thymus						3
Integumentary System						
Mammary gland						
Skin		+				4
Squamous cell papilloma		X				1
Sebaceous gland, adenoma		X				1
Musculoskeletal System						
Bone						3
Skeletal muscle	+	+	+	+	+	30
Nervous System						
Brain						3
Peripheral nerve	+	+	+	+	+	29
Spinal cord	+	+	+	+	+	30
Respiratory System						
Larynx						3
Lung	+	+	+	+	+	30
Alveolar/bronchiolar adenoma						4
Alveolar/bronchiolar carcinoma						1
Nose	+	+	+	+	+	30
Trachea						3
Special Senses System						
Eye						3
Harderian gland	+	+	+	+	+	30
Adenoma		X		X	X	13
Adenoma, multiple						1
Zymbal's gland						3
Urinary System						
Kidney						4
Urinary bladder						3
Systemic Lesions						
Multiple organs	+	+	+	+	+	30
Lymphoma malignant histiocytic						1
Lymphoma malignant lymphocytic						1

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 2,200 ppm

Number of Days on Study	136	160	289	326	371	771	771	771	771	771	771	771	771	771	771	771	771	771	771	771	771	771	771	771	771
Carcass ID Number	165	163	182	179	161	262	267	268	269	270	272	273	274	275	276	277	280	285	287	288	289	290	291	292	293
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Gallbladder	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma														X											
Hepatocellular carcinoma																X									
Hepatocellular carcinoma, multiple											X	X							X						
Hepatocellular adenoma					X	X		X				X	X	X					X	X		X		X	X
Hepatocellular adenoma, multiple									X		X				X	X									
Squamous cell carcinoma, metastatic, Stomach, forestomach				X																					
Mesentery			+	+				+																+	
Squamous cell carcinoma, metastatic, Stomach, forestomach				X																					
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma				X																					
Squamous cell papilloma																	X					X			
Stomach, glandular	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
General Body System																									
None																									

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 2,200 ppm (continued)

Number of Days on Study	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	
Carcass ID Number	1 9 4	1 9 6	1 9 7	1 9 8	1 9 9	**Total Tissues/ Tumors**
Alimentary System						
Esophagus	+	+	+	+	+	29
Gallbladder	+	+	+	+	+	27
Intestine large, colon	+	+	+	+	+	29
Intestine large, rectum	+	+	+	+	+	28
Intestine large, cecum	+	+	+	+	+	27
Intestine small, duodenum	+	+	+	+	+	27
Intestine small, jejunum	+	+	+	+	+	28
Intestine small, ileum	+	+	+	+	+	27
Liver	+	+	+	+	+	30
Hemangioma			X			2
Hepatocellular carcinoma						1
Hepatocellular carcinoma, multiple						3
Hepatocellular adenoma				X	X	13
Hepatocellular adenoma, multiple			X			5
Squamous cell carcinoma, metastatic, Stomach, forestomach						1
Mesentery						4
Squamous cell carcinoma, metastatic, Stomach, forestomach						1
Pancreas	+	+	+	+	+	30
Pharynx	+	+	+	+	+	30
Salivary glands	+	+	+	+	+	30
Stomach, forestomach	+	+	+	+	+	30
Squamous cell carcinoma					X	2
Squamous cell papilloma						2
Stomach, glandular	+	+	+	+	+	29
Tongue	+	+	+	+	+	30
Cardiovascular System						
Blood vessel	+	+	+	+	+	29
Heart	+	+	+	+	+	30
Endocrine System						
Adrenal cortex	+	+	+	+	+	30
Adrenal medulla	+	+	+	+	+	30
Islets, pancreatic	+	+	+	+	+	30
Parathyroid gland	+	M	+	+	+	24
Pituitary gland	+	+	+	+	+	29
Thyroid gland	+	+	+	+	+	29
General Body System						
None						

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 2,200 ppm (continued)

Number of Days on Study	1 3 6	1 6 0	2 8 9	3 2 6	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1
Carcass ID Number	1 6 5	1 6 3	1 8 2	1 7 9	1 6 1	1 6 2	1 6 7	1 6 8	1 6 9	1 7 0	1 7 2	1 7 3	1 7 4	1 7 5	1 7 6	1 7 7	1 8 0	1 8 5	1 8 7	1 8 8	1 8 9	1 9 0	1 9 1	1 9 2	1 9 3
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, bronchial	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	M
Squamous cell carcinoma, metastatic, Stomach, forestomach				X																					
Lymph node, mandibular	+	+	M	M	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, Stomach, forestomach				X																					
Lymph node, mediastinal	M	+	+	+	M	M	+	M	M	M	M	+	+	+	M	+	M	M	M	M	M	M	M	M	+
Squamous cell carcinoma, metastatic, Stomach, forestomach				X																					
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Larynx	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X			X		X		X												X	X	
Alveolar/bronchiolar adenoma, Multiple																						X			
Alveolar/bronchiolar carcinoma, Multiple																						X			
Squamous cell carcinoma, metastatic, Stomach, forestomach				X																					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 2,200 ppm (continued)

Number of Days on Study	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	
Carcass ID Number	1 9 4	1 9 6	1 9 7	1 9 8	1 9 9	**Total Tissues/ Tumors**
Genital System						
Epididymis	+	+	+	+	+	30
Preputial gland	+	+	+	+	+	29
Prostate	+	+	+	+	+	29
Seminal vesicle	+	+	+	+	+	30
Testes	+	+	+	+	+	30
Hematopoietic System						
Bone marrow	+	+	+	+	+	30
Lymph node, bronchial	+	+	+	+	+	27
Squamous cell carcinoma, metastatic, Stomach, forestomach						1
Lymph node, mandibular	+	M	+	+	+	25
Lymph node, mesenteric	+	+	+	+	+	29
Squamous cell carcinoma, metastatic, Stomach, forestomach						1
Lymph node, mediastinal	+	+	+	+	+	14
Squamous cell carcinoma, metastatic, Stomach, forestomach						1
Spleen	+	+	+	+	+	30
Thymus	+	+	+	+	+	30
Integumentary System						
Mammary gland	M	M	M	M	M	
Skin	+	+	+	+	+	30
Musculoskeletal System						
Bone	+	+	+	+	+	30
Skeletal muscle	+	+	+	+	+	30
Nervous System						
Brain	+	+	+	+	+	30
Peripheral nerve	+	+	+	+	+	28
Spinal cord	+	+	+	+	+	29
Respiratory System						
Larynx	+	+	+	+	+	28
Lung	+	+	+	+	+	30
Alveolar/bronchiolar adenoma	X				X	8
Alveolar/bronchiolar adenoma, multiple				X		2
Alveolar/bronchiolar carcinoma, multiple						1
Squamous cell carcinoma, metastatic, stomach, forestomach						1
Nose	+	+	+	+	+	30
Trachea	+	+	+	+	+	30

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 2,200 ppm (continued)

Number of Days on Study	136	160	289	326	371	371	371	371	371	371	371	371	371	371	371	371	371	371	371	371	371	371	371	371	371
Carcass ID Number	16505	16603	16822	17079	16101	16612	16627	16688	16699	17070	17072	17073	17074	17075	17076	17077	18020	18085	18087	18088	18089	19090	19091	19092	19093
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X					X	X						X	X	X	X		X		X			
Adenoma, multiple														X											
Carcinoma																									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant		X																							
Lymphoma malignant lymphocytic		X																							

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 2,200 ppm (continued)

Number of Days on Study	337 171	337 171	337 171	337 171	337 171	
Carcass ID Number	19 94	19 96	19 97	19 98	19 99	**Total Tissues/ Tumors**
Special Senses System						
Eye	+	+	+	+	+	30
Harderian gland	+	+	+	+	+	30
Adenoma	X			X		11
Adenoma, multiple			X			2
Carcinoma				X		1
Zymbal's gland	+	+	+	+	+	30
Urinary System						
Kidney	+	+	+	+	+	30
Urinary bladder	+	+	+	+	+	30
Systemic Lesions						
Multiple organs	+	+	+	+	+	30
Lymphoma malignant						1
Lymphoma malignant lymphocytic						1

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 7,000 ppm

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

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 7,000 ppm (continued)

Number of Days on Study	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	
Carcass ID Number	2 3 4	2 3 6	2 3 7	2 3 8	2 3 9	**Total Tissues/ Tumors**
Alimentary System						
Esophagus	+	+	+	+	+	28
Gallbladder	+	+	+	+	+	24
Intestine large, colon	+	+	+	+	+	28
Intestine large, rectum	+	+	+	+	+	28
Intestine large, cecum	+	+	+	+	+	27
Intestine small, duodenum	+	+	+	+	+	26
Intestine small, jejunum	+	+	+	+	+	26
Intestine small, ileum	+	+	+	+	+	26
Liver	+	+	+	+	+	28
Hepatocellular carcinoma	X					9
Hepatocellular adenoma				X	X	5
Hepatocellular adenoma, multiple						11
Mesentery			+			2
Pancreas	+	+	+	+	+	28
Pharynx	+	+	+	+	+	30
Salivary glands	+	+	+	+	+	28
Stomach, forestomach	+	+	+	+	+	28
Squamous cell carcinoma						1
Squamous cell papilloma		X				4
Squamous cell papilloma, multiple	X					1
Stomach, glandular	+	+	+	+	+	27
Tongue	+	+	+	+	+	30
Cardiovascular System						
Blood vessel	+	+	+	+	+	28
Heart	+	+	+	+	+	28
Endocrine System						
Adrenal cortex	+	+	+	+	+	28
Adrenal medulla	+	+	+	+	+	28
Islets, pancreatic	+	+	+	+	+	28
Parathyroid gland	+	+	+	+	+	25
Pituitary gland	+	+	+	M	+	25
Thyroid gland	+	+	+	+	+	28
General Body System						
None						
Genital System						
Epididymis	+	+	+	+	+	29
Penis						2
Preputial gland	+	+	+	+	+	27
Prostate	+	+	+	+	+	27
Seminal vesicle	+	+	+	+	+	28
Testes	+	+	+	+	+	29

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 7,000 ppm (continued)

Number of Days on Study	1 2 3	1 2 3	1 2 3	1 2 8	1 2 8	1 4 6	2 0 9	3 1 7	3 1 7	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1	3 7 1
Carcass ID Number	2 0 2	2 0 7	2 3 5	2 0 6	2 3 3	2 1 0	2 1 7	2 0 9	2 0 4	2 0 1	2 0 1	2 1 3	2 1 3	2 1 4	2 1 6	2 1 8	2 1 9	2 2 0	2 2 1	2 2 2	2 2 5	2 2 7	2 2 8	2 2 9	2 3 0	2 3 1
Hematopoietic System																									
Bone marrow	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, bronchial	M	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	M	+	+	+
Lymph node, mandibular	M	+	A	M	+	M	+	+	M	+	+	+	M	+	M	M	+	+	M	M	M	M	+	M	+
Lymph node, mesenteric	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mediastinal	M	+	A	M	+	M	+	+	+	M	M	M	+	+	+	+	+	+	M	+	+	+	M	+	M
Spleen	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	+	A	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, sarcoma																			X						
Musculoskeletal System																									
Bone	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Meningioma malignant, metastatic, spinal cord										X															
Peripheral nerve	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Meningioma malignant										X															
Respiratory System																									
Larynx	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma										X		X	X								X				
Alveolar/bronchiolar adenoma, multiple																X				X				X	
Alveolar/bronchiolar carcinoma																							X	X	
Nose	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X		X		X						X	X	X	
Adenoma, multiple									X						X		X								
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic															X										
Lymphoma malignant lymphocytic							X																		

TABLE B4 Individual Animal Tumor Pathology of Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery: 7,000 ppm (continued)

Number of Days on Study	3 3 3 3 3 7 7 7 7 7 1 1 1 1 1	
Carcass ID Number	2 2 2 2 2 3 3 3 3 3 4 6 7 8 9	**Total Tissues/ Tumors**
Hematopoietic System		
Bone marrow	+ + + + +	28
Lymph node, bronchial	+ M + + +	23
Lymph node, mandibular	+ M + M +	15
Lymph node, mesenteric	+ + + + M	27
Lymph node, mediastinal	M + M M +	17
Spleen	+ + + + +	28
Thymus	+ + + + +	26
Integumentary System		
Mammary gland	M M M M M	
Skin	+ + + + +	29
Subcutaneous tissue, sarcoma		1
Musculoskeletal System		
Bone	+ + + + +	29
Skeletal muscle	+ + + + +	28
Nervous System		
Brain	+ + + + +	28
Meningioma malignant, metastatic, spinal cord		1
Peripheral nerve	+ + + + +	28
Spinal cord	+ + + + +	28
Meningioma malignant		1
Respiratory System		
Larynx	+ + + + +	28
Lung	+ + + + +	28
Alveolar/bronchiolar adenoma		4
Alveolar/bronchiolar adenoma, multiple	X	4
Alveolar/bronchiolar carcinoma	X	3
Nose	+ + + + +	28
Trachea	+ + + + +	28
Special Senses System		
Eye	+ + + + +	30
Harderian gland	+ + + + +	28
Adenoma	X	7
Adenoma, multiple	X X	5
Zymbal's gland	+ + + + +	30
Urinary System		
Kidney	+ + + + +	28
Urinary bladder	+ + + + +	28
Systemic Lesions		
Multiple organs	+ + + + +	30
Lymphoma malignant histiocytic		1
Lymphoma malignant lymphocytic		1

TABLE B5 Statistical Analysis of Primary Neoplasms in Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
Harderian Gland: Adenoma or Carcinoma						
Overall rate[1]	2/30 (7%)	6/30 (20%)	4/30 (13%)	14/30 (47%)	13/30 (43%)	12/30 (40%)
Adjusted rate[2]	7.4%	21.4%	14.3%	50.0%	48.1%	54.5%
Terminal rate[3]	2/27 (7%)	6/28 (21%)	4/28 (14%)	13/27 (48%)	12/26 (46%)	11/21 (52%)
First incidence (days)	371 (T)	371 (T)	371 (T)	367	289	317
Life table test[4]	P<0.001	P=0.140	P=0.351	P<0.001	P=0.001	P<0.001
Logistic regression test[4]	P<0.001	P=0.140	P=0.351	P<0.001	P=0.001	P<0.001
Cochran-Armitage test[4]	P=0.011					
Fisher exact test[4]		P=0.127	P=0.335	P<0.001	P=0.001	P=0.002
Liver: Hemangioma						
Overall rate	0/30 (0%)	0/30 (0%)	0/29 (0%)	0/30 (0%)	2/30 (7%)	0/28 (0%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	7.7%	0.0%
Terminal rate	0/27 (0%)	0/28 (0%)	0/28 (0%)	0/27 (0%)	2/26 (8%)	0/21 (0%)
First incidence (days)	)[5]	)	)	)	371 (T)	)
Life table test	P=0.615	)	)	)	P=0.229	)
Logistic regression test	P=0.615	)	)	)	P=0.229	)
Cochran-Armitage test	P=0.647					
Fisher exact test		)	)	)	P=0.246	)
Liver: Hepatocellular Adenoma						
Overall rate	4/30 (13%)	2/30 (7%)	6/29 (21%)	15/30 (50%)	18/30 (60%)	16/28 (57%)
Adjusted rate	14.8%	7.1%	21.4%	55.6%	69.2%	72.7%
Terminal rate	4/27 (15%)	2/28 (7%)	6/28 (21%)	15/27 (56%)	18/26 (69%)	15/21 (71%)
First incidence (days)	371 (T)	371 (T)	371 (T)	371 (T)	371 (T)	317
Life table test	P<0.001	P=0.317N	P=0.388	P=0.002	P<0.001	P < 0.0001
Logistic regression test	P<0.001	P=0.317N	P=0.388	P=0.002	P<0.001	P < 0.0001
Cochran-Armitage test	P<0.001					
Fisher exact test		P=0.335N	P=0.343	P=0.002	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma						
Overall rate	4/30 (13%)	1/30 (3%)	3/29 (10%)	5/30 (17%)	4/30 (13%)	9/28 (32%)
Adjusted rate	14.8%	3.6%	10.7%	18.5%	15.4%	42.9%
Terminal rate	4/27 (15%)	1/28 (4%)	3/28 (11%)	5/27 (19%)	4/26 (15%)	9/21 (43%)
First incidence (days)	371 (T)	371 (T)	371 (T)	371 (T)	371 (T)	371 (T)
Life table test	P<0.001	P=0.166N	P=0.480N	P=0.500	P=0.627	P=0.034
Logistic regression test	P<0.001	P=0.166N	P=0.480N	P=0.500	P=0.627	P=0.034
Cochran-Armitage test	P=0.004					
Fisher exact test		P=0.177N	P=0.520N	P=0.500	P=0.647N	P=0.080
Liver: Hepatocellular Adenoma or Carcinoma						
Overall rate	7/30 (23%)	3/30 (10%)	7/29 (24%)	15/30 (50%)	18/30 (60%)	17/28 (61%)
Adjusted rate	25.9%	10.7%	25.0%	55.6%	69.2%	77.2%
Terminal rate	7/27 (26%)	3/28 (11%)	7/28 (25%)	15/27 (56%)	18/26 (69%)	16/21 (76%)
First incidence (days)	371 (T)	371 (T)	371 (T)	371 (T)	371 (T)	317
Life table test	P<0.001	P=0.135N	P=0.590N	P=0.027	P=0.002	P < 0.0001
Logistic regression test	P<0.001	P=0.135N	P=0.590N	P=0.027	P=0.002	P < 0.0001
Cochran-Armitage test	P<0.001					
Fisher exact test		P=0.149N	P=0.592	P=0.030	P=0.004	P = 0.0004

TABLE B5 Statistical Analysis of Primary Neoplasms in Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
Lung: Alveolar/bronchiolar Adenoma						
Overall rate	2/30 (7%)	2/30 (7%)	1/29 (3%)	4/30 (13%)	10/30 (33%)	8/28 (29%)
Adjusted rate	7.4%	7.1%	3.6%	14.8%	37.0%	38.1%
Terminal rate	2/27 (7%)	2/28 (7%)	1/28 (4%)	4/27 (15%)	9/26 (35%)	8/21 (38%)
First incidence (days)	371 (T)	371 (T)	371 (T)	371 (T)	326	371 (T)
Life table test	P<0.001	P=0.683N	P=0.487N	P=0.334	P=0.011	P=0.013
Logistic regression test	P<0.001	P=0.683N	P=0.487N	P=0.334	P=0.011	P=0.013
Cochran-Armitage test	P=0.002					
Fisher exact test		P=0.694N	P=0.513N	P=0.335	P=0.011	P=0.030
Lung: Alveolar/bronchiolar Carcinoma						
Overall rate	0/30 (0%)	0/30 (0%)	0/29 (0%)	1/30 (3%)	1/30 (3%)	3/28 (11%)
Adjusted rate	0.0%	0.0%	0.0%	3.7%	3.8%	14.3%
Terminal rate	0/27 (0%)	0/28 (0%)	0/28 (0%)	1/27 (4%)	1/26 (4%)	3/21 (14%)
First incidence (days)	)	)	)	371 (T)	371 (T)	371 (T)
Life table test	P=0.003	)	)	P=0.500	P=0.492	P=0.079
Logistic regression test	P=0.003	)	)	P=0.500	P=0.492	P=0.079
Cochran-Armitage test	P=0.007					
Fisher exact test		)	)	P=0.500	P=0.500	P=0.106
Lung: Alveolar/bronchiolar Adenoma or Carcinoma						
Overall rate	2/30 (7%)	2/30 (7%)	1/29 (3%)	5/30 (17%)	10/30 (33%)	9/28 (32%)
Adjusted rate	7.4%	7.1%	3.6%	18.5%	37.0%	42.9%
Terminal rate	2/27 (7%)	2/28 (7%)	1/28 (4%)	5/27 (19%)	9/26 (35%)	9/21 (43%)
First incidence (days)	371 (T)	371 (T)	371 (T)	371 (T)	326	371 (T)
Life table test	P<0.001	P=0.683N	P=0.487N	P=0.211	P=0.011	P = 0.0006
Logistic regression test	P<0.001	P=0.683N	P=0.487N	P=0.211	P=0.011	P = 0.0006
Cochran-Armitage test	P<0.001					
Fisher exact test		P=0.694N	P=0.513N	P=0.212	P=0.011	P=0.015
Stomach (Forestomach): Squamous Cell Papilloma						
Overall rate	0/30 (0%)	0/30 (0%)	0/30 (0%)	1/30 (3%)	2/30 (7%)	5/30 (17%)
Adjusted rate	0.0%	0.0%	0.0%	3.7%	7.7%	20.6%
Terminal rate	0/27 (0%)	0/28 (0%)	0/28 (0%)	1/27 (4%)	2/26 (8%)	3/21 (14%)
First incidence (days)	)	)	)	371 (T)	371 (T)	128
Life table test	P<0.001	)	)	P=0.500	P=0.229	P=0.021
Logistic regression test	P=0.001	)	)	P=0.500	P=0.229	P=0.053
Cochran-Armitage test	P<0.001					
Fisher exact test		)	)	P=0.500	P=0.246	P=0.026
Stomach (Forestomach): Squamous Cell Carcinoma						
Overall rate	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	2/30 (7%)	1/30 (3%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	7.4%	4.8%
Terminal rate	0/27 (0%)	0/28 (0%)	0/28 (0%)	0/27 (0%)	1/26 (4%)	1/21 (5%)
First incidence (days)	)	)	)	)	326	371 (T)
Life table test	P=0.124	)	)	)	P=0.236	P=0.450
Logistic regression test	P=0.159	)	)	)	P=0.236	P=0.450
Cochran-Armitage test	P=0.180					
Fisher exact test		)	)	)	P=0.246	P=0.500

TABLE B5 Statistical Analysis of Primary Neoplasms in Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
Stomach (Forestomach): Squamous Cell Papilloma or Carcinoma						
Overall rate	0/30 (0%)	0/30 (0%)	0/30 (0%)	1/30 (3%)	4/30 (13%)	6/30 (20%)
Adjusted rate	0.0%	0.0%	0.0%	3.7%	14.8%	25.0%
Terminal rate	0/27 (0%)	0/28 (0%)	0/28 (0%)	1/27 (4%)	3/26 (12%)	4/21 (19%)
First incidence (days)	)	)	)	371 (T)	326	128
Life table test	P<0.001	)	)	P=0.500	P=0.060	P = 0.010
Logistic regression test	P<0.001	)	)	P=0.500	P=0.060	P=0.025
Cochran-Armitage test	P<0.001					
Fisher exact test		)	)	P=0.500	P=0.056	P=0.012
All Organs: Hemangioma						
Overall rate	0/30 (0%)	0/30 (0%)	0/30 (0%)	0/30 (0%)	2/30 (7%)	0/30 (0%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	7.7%	0.0%
Terminal rate	0/27 (0%)	0/28 (0%)	0/28 (0%)	0/27 (0%)	2/26 (8%)	0/21 (0%)
First incidence (days)	)	)	)	)	371 (T)	)
Life table test	P=0.615	)	)	)	P=0.229	)
Logistic regression test	P=0.615	)	)	)	P=0.229	)
Cochran-Armitage test	P=0.655					
Fisher exact test		)	)	)	P=0.246	)
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, or NOS)						
Overall rate	1/30 (3%)	0/30 (0%)	0/30 (0%)	2/30 (7%)	1/30 (3%)	2/30 (7%)
Adjusted rate	3.3%	0.0%	0.0%	6.8%	3.4%	8.7%
Terminal rate	0/27 (0%)	0/28 (0%)	0/28 (0%)	0/27 (0%)	0/26 (0%)	1/21 (5%)
First incidence (days)	90	)	)	176	160	209
Life table test	P=0.151	P=0.507N	P=0.500N	P=0.508	P=0.760	P=0.453
Logistic regression test	P=0.571	P=0.367N	P=0.581N	P=0.229	P=0.852	P=0.719
Cochran-Armitage test	P=0.201					
Fisher exact test		P=0.500N	P=0.500N	P=0.500	P=0.754N	P=0.500
All Organs: Benign Neoplasms						
Overall rate	9/30 (30%)	9/30 (30%)	10/30 (33%)	22/30 (73%)	25/30 (83%)	23/30 (77%)
Adjusted rate	33.3%	32.1%	35.7%	78.6%	89.3%	95.8%
Terminal rate	9/27 (33%)	9/28 (32%)	10/28 (36%)	21/27 (78%)	23/26 (88%)	20/21 (95%)
First incidence (days)	371 (T)	371 (T)	371 (T)	367	289	128
Life table test	P<0.001	P=0.576N	P=0.539	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.576N	P=0.539	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001					
Fisher exact test		P=0.611N	P=0.500	P<0.001	P<0.001	P<0.001
All Organs: Malignant Neoplasms						
Overall rate	5/30 (17%)	1/30 (3%)	3/30 (10%)	8/30 (27%)	9/30 (30%)	14/30 (47%)
Adjusted rate	17.7%	3.6%	10.7%	27.5%	32.1%	63.5%
Terminal rate	4/27 (15%)	1/28 (4%)	3/28 (11%)	6/27 (22%)	7/26 (27%)	13/21 (62%)
First incidence (days)	90	371 (T)	371 (T)	176	160	209
Life table test	P<0.001	P=0.098N	P=0.337N	P=0.279	P=0.176	P = 0.002
Logistic regression test	P<0.001	P=0.096N	P=0.436N	P=0.197	P=0.177	P = 0.006
Cochran-Armitage test	P<0.001					
Fisher exact test		P=0.097N	P=0.353N	P=0.266	P=0.180	P=0.013

TABLE B5 Statistical Analysis of Primary Neoplasms in Male B6C3F$_1$ Mice After 6 Months of Inhalation Exposure to Isoprene and 6 Months of Recovery (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
All Organs: Benign or Malignant Neoplasms						
Overall rate	12/30 (40%)	10/30 (33%)	11/30 (37%)	24/30 (80%)	26/30 (87%)	24/30 (80%)
Adjusted rate	42.7%	35.7%	39.3%	82.7%	89.7%	96.0%
Terminal rate	11/27 (41%)	10/28 (36%)	11/28 (39%)	22/27 (81%)	23/26 (88%)	20/21 (95%)
First incidence (days)	90	371 (T)	371 (T)	176	160	128
Life table test	P<0.001	P=0.359N	P=0.458N	P=0.003	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.390N	P=0.501N	P=0.002	P<0.001	P<0.001
Cochran-Armitage test	P<0.001					
Fisher exact test		P=0.395N	P=0.500N	P=0.002	P<0.001	P=0.002

(T)Terminal sacrifice

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

[2] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.

[3] Observed incidence at terminal kill.

[4] Beneath the control incidence are the P-values associated with the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between the controls and that exposed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a lower incidence in an exposure group is indicated by **N**.

[5] Not applicable; no neoplasms in animal group.

TABLE B6 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	40	40	40	40	40	40
6-Month evaluation	10	10	10	10	10	10
Early deaths						
Moribund sacrifice	1	1	1	1	1	5
Natural death	1	1	1	2	3	4
Accidentally killed	1					
Survivors						
Terminal sacrifice	27	28	28	27	26	21
Animals examined microscopically	40	40	40	40	40	40
6-MONTH EVALUATION						
Alimentary System						
Gallbladder	(10)	(9)	(9)	(10)	(10)	(9)
Inflammation, suppurative				1 (10%)		
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Basophilic focus			1 (10%)	2 (20%)		1 (10%)
Vacuolization cytoplasmic	2 (20%)	2 (20%)	3 (30%)	4 (40%)	3 (30%)	3 (30%)
Mesentery	(1)					
Necrosis	1 (100%)					
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, hyperplasia				8 (80%)	10 (100%)	9 (90%)
Stomach, glandular	(10)	(10)	(10)	(10)	(9)	(10)
Inflammation, suppurative		1 (10%)				
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Preputial gland	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, suppurative		1 (10%)				
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy			1 (10%)			5 (50%)

TABLE B6 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
6-MONTH EVALUATION (continued)						
Hematopoietic System						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Pigmentation, melanin		1 (10%)		1 (10%)	1 (10%)	
Integumentary System						
None						
Musculoskeletal System						
Skeletal muscle	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy						4 (40%)
Inflammation			1 (10%)		1 (10%)	
Nervous System						
Brain	(10)	(10)	(10)	(10)	(10)	(10)
Cyst epithelial inclusion			1 (10%)			
Peripheral nerve	(10)	(10)	(10)	(10)	(10)	(10)
Sciatic, degeneration						2 (20%)
Spinal cord	(10)	(10)	(10)	(10)	(10)	(10)
Degeneration					1 (10%)	10 (100%)
Meninges, cyst epithelial inclusion						1 (10%)
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage	1 (10%)					
Metaplasia, osseous	1 (10%)					
Alveolar epithelium, hyperplasia	1 (10%)				1 (10%)	
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Turbinate, olfactory epithelium, degeneration				1 (10%)	1 (10%)	10 (100%)
Turbinate, olfactory epithelium, inflammation, chronic						5 (50%)
Special Senses System						
Harderian gland	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia			1 (10%)		1 (10%)	
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Bilateral, fibrosis	1 (10%)					
Bilateral, hydronephrosis	1 (10%)				1 (10%)	

TABLE B6 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
12-MONTH EVALUATION						
Alimentary System						
Gallbladder	(29)	(1)	(1)	(2)	(27)	(24)
Inflammation, suppurative					1 (4%)	
Intestine large, cecum	(30)	(2)	(1)	(4)	(27)	(27)
Parasite				1 (25%)		
Intestine small, duodenum	(29)	(2)	(1)	(2)	(27)	(26)
Serosa, inflammation, chronic					1 (4%)	
Intestine small, jejunum	(30)	(1)	(1)	(3)	(28)	(26)
Parasite						1 (4%)
Intestine small, ileum	(30)	(1)	(1)	(3)	(27)	(26)
Parasite						1 (4%)
Liver	(30)	(30)	(29)	(30)	(30)	(28)
Angiectasis				2 (7%)	1 (3%)	2 (7%)
Basophilic focus	3 (10%)	1 (3%)	1 (3%)	2 (7%)	5 (17%)	3 (11%)
Clear cell focus				2 (7%)		1 (4%)
Deformity	1 (3%)					
Eosinophilic focus	1 (3%)			6 (20%)	5 (17%)	3 (11%)
Hematopoietic cell proliferation					2 (7%)	1 (4%)
Hyperplasia, focal					1 (3%)	
Infarct		1 (3%)				
Inflammation, chronic					1 (3%)	
Mixed cell focus			1 (3%)	1 (3%)	2 (7%)	3 (11%)
Necrosis			1 (3%)	1 (3%)		2 (7%)
Vacuolization cytoplasmic	20 (67%)	23 (77%)	24 (83%)	16 (53%)	21 (70%)	11 (39%)
Bile duct, hyperplasia						1 (4%)
Mesentery		(2)		(2)	(4)	(2)
Angiectasis				1 (50%)		
Necrosis		2 (100%)		2 (100%)	2 (50%)	2 (100%)
Pancreas	(30)	(30)	(29)	(30)	(30)	(28)
Angiectasis						1 (4%)
Focal cellular change	2 (7%)	1 (3%)		5 (17%)	3 (10%)	6 (21%)
Inflammation, chronic		2 (7%)			1 (3%)	1 (4%)
Duct, cyst		1 (3%)				1 (4%)
Salivary glands	(30)	(2)	(1)	(3)	(30)	(28)
Inflammation, suppurative	2 (7%)					
Stomach, forestomach	(30)	(30)	(29)	(30)	(30)	(28)
Angiectasis					2 (7%)	
Infiltration cellular, mast cell			1 (3%)			
Epithelium, hyperplasia	1 (3%)	2 (7%)		8 (27%)	9 (30%)	6 (21%)
Stomach, glandular	(30)	(30)	(29)	(30)	(29)	(27)
Hyperplasia		1 (3%)		3 (10%)	2 (7%)	4 (15%)
Inflammation, suppurative				1 (3%)		2 (7%)
Necrosis						2 (7%)
Pigmentation, hemosiderin		1 (3%)		1 (3%)		
Ulcer	1 (3%)			1 (3%)		
Tooth	(1)		(2)			
Deformity			1 (50%)			

TABLE B6 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
12-MONTH EVALUATION (continued)						
Cardiovascular System						
Heart	(30)	(2)	(1)	(3)	(30)	(28)
Pericardium, hemorrhage	1 (3%)					
Endocrine System						
Adrenal cortex	(30)	(2)	(1)	(3)	(30)	(28)
Hematopoietic cell proliferation					1 (3%)	
Hemorrhage	1 (3%)					
Hyperplasia	6 (20%)				8 (27%)	6 (21%)
Hyperplasia, focal						1 (4%)
Hypertrophy, focal	5 (17%)				6 (20%)	4 (14%)
Islets, pancreatic	(30)	(30)	(29)	(30)	(30)	(28)
Angiectasis						1 (4%)
Hyperplasia	9 (30%)	9 (30%)	12 (41%)	12 (40%)	16 (53%)	13 (46%)
Pituitary gland	(28)	(2)	(1)	(3)	(29)	(25)
Pars distalis, cyst	2 (7%)		1 (100%)			
Pars intermedia, hyperplasia	1 (4%)					
Thyroid gland	(30)	(2)	(1)	(3)	(29)	(28)
Cyst	1 (3%)				1 (3%)	1 (4%)
Follicular cell, hyperplasia					1 (3%)	
General Body System						
None						
Genital System						
Epididymis	(30)	(2)	(1)	(3)	(30)	(29)
Hyperplasia						1 (3%)
Inflammation		1 (50%)				1 (3%)
Preputial gland	(30)	(3)	(1)	(6)	(29)	(27)
Angiectasis				1 (17%)		1 (4%)
Atrophy					1 (3%)	1 (4%)
Hemorrhage		1 (33%)				
Inflammation, chronic				1 (17%)		1 (4%)
Inflammation, suppurative	1 (3%)	2 (67%)			1 (3%)	
Necrosis		1 (33%)				
Prostate	(28)	(2)	(1)	(3)	(29)	(27)
Hyperplasia					2 (7%)	
Inflammation, suppurative				2 (67%)		
Seminal vesicle	(30)	(2)	(1)	(3)	(30)	(28)
Inflammation, suppurative				1 (33%)		
Testes	(30)	(30)	(29)	(30)	(30)	(29)
Atrophy						3 (10%)
Mineralization						1 (3%)

TABLE B6 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
12-MONTH EVALUATION (continued)						
Hematopoietic System						
Bone marrow	(30)	(2)	(1)	(3)	(30)	(28)
Angiectasis					1 (3%)	
Hyperplasia	1 (3%)			1 (33%)	2 (7%)	4 (14%)
Lymph node	(1)	(1)				
Iliac, hyperplasia	1 (100%)	1 (100%)				
Inguinal, hyperplasia	1 (100%)					
Lymph node, mesenteric	(30)	(3)	(1)	(3)	(29)	(27)
Hyperplasia		1 (33%)		1 (33%)	1 (3%)	
Necrosis		1 (33%)				
Lymph node, mediastinal	(13)	(2)	(1)	(3)	(14)	(17)
Hyperplasia					1 (7%)	
Spleen	(30)	(5)	(1)	(3)	(30)	(28)
Accessory spleen		1 (20%)				
Hematopoietic cell proliferation	2 (7%)	1 (20%)			2 (7%)	4 (14%)
Infiltration cellular, histiocyte				1 (33%)		
Necrosis		1 (20%)				
Pigmentation, melanin	1 (3%)	2 (40%)				
Thymus	(30)	(2)	(1)	(3)	(30)	(26)
Atrophy				1 (33%)	1 (3%)	
Necrosis		2 (100%)				1 (4%)
Integumentary System						
Skin	(30)	(4)	(1)	(4)	(30)	(29)
Cyst epithelial inclusion					1 (3%)	
Hemorrhage		1 (25%)			1 (3%)	
Inflammation, suppurative		2 (50%)				1 (3%)
Prepuce, inflammation, chronic	2 (7%)	1 (25%)		1 (25%)	2 (7%)	2 (7%)
Sebaceous gland, hyperplasia						1 (3%)
Musculoskeletal System						
Bone	(30)	(2)	(2)	(3)	(30)	(29)
Osteopetrosis			1 (50%)			
Nervous System						
Brain	(30)	(2)	(1)	(3)	(30)	(28)
Hemorrhage				2 (67%)		
Mineralization	1 (3%)				2 (7%)	
Necrosis				1 (33%)		
Peripheral nerve	(30)	(30)	(29)	(29)	(28)	(28)
Sciatic, degeneration				1 (3%)	1 (4%)	3 (11%)
Spinal cord	(30)	(30)	(29)	(30)	(29)	(28)
Degeneration	4 (13%)	20 (67%)	19 (66%)	28 (93%)	17 (59%)	13 (46%)
Meninges, cyst epithelial inclusion			1 (3%)			
Meninges, hemorrhage	1 (3%)					

TABLE B6 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the Stop-Exposure Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
12-MONTH EVALUATION (continued)						
Respiratory System						
Larynx	(30)	(2)	(1)	(3)	(28)	(28)
Inflammation						1 (4%)
Lung	(30)	(30)	(29)	(30)	(30)	(28)
Congestion	3 (10%)					2 (7%)
Hemorrhage	2 (7%)	2 (7%)	2 (7%)	1 (3%)	2 (7%)	2 (7%)
Thrombosis				1 (3%)		1 (4%)
Alveolar epithelium, hyperplasia		1 (3%)		3 (10%)	4 (13%)	7 (25%)
Nose	(30)	(30)	(29)	(30)	(30)	(28)
Inflammation						2 (7%)
Turbinate, inflammation			1 (3%)	1 (3%)		
Turbinate, inflammation, suppurative						1 (4%)
Turbinate, olfactory epithelium, degeneration	1 (3%)	2 (7%)	5 (17%)	11 (37%)	25 (83%)	28 (100%)
Turbinate, olfactory epithelium, inflammation, chronic					1 (3%)	25 (89%)
Special Senses System						
Eye	(30)	(2)	(2)	(3)	(30)	(30)
Inflammation, chronic						1 (3%)
Harderian gland	(30)	(30)	(29)	(30)	(30)	(28)
Hyperplasia	1 (3%)		2 (7%)	2 (7%)	2 (7%)	2 (7%)
Urinary System						
Kidney	(30)	(3)	(1)	(4)	(30)	(28)
Bilateral, fibrosis	1 (3%)					1 (4%)
Bilateral, fibrosis, focal						1 (4%)
Bilateral, hydronephrosis	1 (3%)	1 (33%)		1 (25%)		1 (4%)
Bilateral, metaplasia, osseous	1 (3%)				1 (3%)	
Bilateral, nephropathy						1 (4%)
Bilateral, cortex, pelvis, inflammation, suppurative				1 (25%)		
Bilateral, pelvis, inflammation, suppurative	1 (3%)	1 (33%)		1 (25%)		
Bilateral, renal tubule, hyperplasia	1 (3%)				2 (7%)	
Urinary bladder	(30)	(5)	(1)	(3)	(30)	(28)
Calculus microscopic observation only	1 (3%)	1 (20%)				
Dilatation	1 (3%)	2 (40%)		1 (33%)		
Inflammation, suppurative	2 (7%)					1 (4%)
Transitional epithelium, hyperplasia	1 (3%)			1 (33%)		

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

APPENDIX C

Organ Weights and Organ-Weight-to-Body-Weight Ratios

Table C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 2-Week Inhalation Study of Isoprene C-2

Table C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of Isoprene C-4

Table C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats After 6 Months of Exposure in the Stop-Exposure Inhalation Study of Isoprene C-6

Table C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats After 6 Months of Recovery in the Stop-Exposure Inhalation Study of Isoprene C-7

Table C5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F_1 Mice in the 2-Week Inhalation Study of Isoprene C-8

Table C6 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F_1 Mice in the 13-Week Inhalation Study of Isoprene C-10

Table C7 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F_1 Mice After 6 Months of Exposure in the Stop-Exposure Inhalation Study of Isoprene C-12

Table C8 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F_1 Mice After 6 Months of Recovery in the Stop-Exposure Inhalation Study of Isoprene C-13

TABLE C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 2-Week Inhalation Study of Isoprene[1]

	0 ppm	438 ppm	875 ppm	1,750 ppm	3,500 ppm	7,000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	160 ± 4	158 ± 5	152 ± 6	152 ± 5	151 ± 4	150 ± 5
Brain						
Absolute	1.677 ± 0.014	1.675 ± 0.015	1.625 ± 0.031	1.653 ± 0.015	1.642 ± 0.016	1.652 ± 0.019
Relative	10.54 ± 0.19	10.66 ± 0.28	10.80 ± 0.25	10.95 ± 0.29	10.93 ± 0.22	11.10 ± 0.29
Heart						
Absolute	0.568 ± 0.006	0.562 ± 0.014	0.541 ± 0.013	0.567 ± 0.024	0.537 ± 0.008	0.557 ± 0.020
Relative	3.57 ± 0.06	3.56 ± 0.05	3.59 ± 0.06	3.75 ± 0.18	3.57 ± 0.05	3.71 ± 0.04
Right kidney						
Absolute	0.731 ± 0.013	0.763 ± 0.026	0.709 ± 0.026	0.745 ± 0.023	0.743 ± 0.017	0.740 ± 0.026
Relative	4.59 ± 0.08	4.82 ± 0.05	4.68 ± 0.06	4.90 ± 0.04**	4.93 ± 0.07**	4.94 ± 0.06**
Liver						
Absolute	7.887 ± 0.189	8.034 ± 0.250	7.516 ± 0.320	7.947 ± 0.294	8.109 ± 0.228	8.389 ± 0.403
Relative	49.41 ± 0.65	50.77 ± 0.55	49.53 ± 0.55	52.22 ± 0.82*	53.77 ± 0.86**	55.86 ± 1.51**
Lungs						
Absolute	1.182 ± 0.064	1.269 ± 0.056	1.137 ± 0.065	1.210 ± 0.047	1.183 ± 0.039	1.345 ± 0.099
Relative	7.42 ± 0.43	8.15 ± 0.55	7.48 ± 0.27	8.04 ± 0.43	7.88 ± 0.31	9.02 ± 0.66*
Spleen						
Absolute	0.462 ± 0.008	0.449 ± 0.012	0.434 ± 0.018	0.442 ± 0.012	0.452 ± 0.012	0.432 ± 0.013
Relative	2.90 ± 0.04	2.84 ± 0.05	2.86 ± 0.05	2.91 ± 0.05	3.00 ± 0.05	2.89 ± 0.05
Right testis						
Absolute	0.904 ± 0.025	0.858 ± 0.032	0.842 ± 0.035	0.833 ± 0.045	0.834 ± 0.024	0.815 ± 0.044
Relative	5.66 ± 0.10	5.41 ± 0.09	5.56 ± 0.10	5.45 ± 0.17	5.53 ± 0.11	5.41 ± 0.14
Thymus						
Absolute	0.412 ± 0.012	0.422 ± 0.014	0.386 ± 0.016	0.418 ± 0.010	0.420 ± 0.016	0.404 ± 0.013
Relative	2.59 ± 0.09	2.68 ± 0.10	2.55 ± 0.09	2.77 ± 0.09	2.80 ± 0.12	2.71 ± 0.06

TABLE C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 2-Week Inhalation Study of Isoprene (continued)

	0 ppm	438 ppm	875 ppm	1,750 ppm	3,500 ppm	7,000 ppm
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	121 ± 3	117 ± 3	122 ± 2	119 ± 2	119 ± 2	119 ± 2
Brain						
Absolute	1.607 ± 0.018	1.595 ± 0.015	1.607 ± 0.011	1.598 ± 0.014	1.596 ± 0.009	1.580 ± 0.012
Relative	13.29 ± 0.21	13.70 ± 0.38	13.20 ± 0.17	13.44 ± 0.21	13.46 ± 0.18	13.31 ± 0.16
Heart						
Absolute	0.479 ± 0.007	0.470 ± 0.011	0.491 ± 0.008	0.465 ± 0.010	0.454 ± 0.007*	0.441 ± 0.006**
Relative	3.96 ± 0.06	4.02 ± 0.04	4.03 ± 0.06	3.90 ± 0.06	3.83 ± 0.06	3.71 ± 0.02**
Right kidney						
Absolute	0.576 ± 0.013	0.595 ± 0.012	0.619 ± 0.014	0.602 ± 0.011	0.599 ± 0.015	0.602 ± 0.010
Relative	4.76 ± 0.08	5.09 ± 0.08*	5.07 ± 0.08*	5.06 ± 0.06*	5.04 ± 0.11*	5.07 ± 0.06*
Liver						
Absolute	5.715 ± 0.119	5.436 ± 0.227	5.751 ± 0.115	5.655 ± 0.105	5.821 ± 0.079	5.794 ± 0.125
Relative	47.22 ± 0.79	46.21 ± 0.84	47.15 ± 0.55	47.50 ± 0.78	49.04 ± 0.66	48.70 ± 0.44
Lungs						
Absolute	0.916 ± 0.035	0.869 ± 0.034	0.986 ± 0.046	0.859 ± 0.024	0.988 ± 0.059	0.995 ± 0.056
Relative	7.62 ± 0.42	7.42 ± 0.23	8.09 ± 0.36	7.21 ± 0.17	8.34 ± 0.54	8.36 ± 0.44
Spleen						
Absolute	0.375 ± 0.005	0.335 ± 0.013	0.376 ± 0.009	0.358 ± 0.006	0.363 ± 0.004	0.346 ± 0.006*
Relative	3.10 ± 0.05	2.85 ± 0.06**	3.08 ± 0.06	3.01 ± 0.05	3.06 ± 0.04	2.91 ± 0.03*
Thymus						
Absolute	0.343 ± 0.010	0.327 ± 0.016	0.359 ± 0.006	0.348 ± 0.012	0.366 ± 0.010	0.331 ± 0.006
Relative	2.85 ± 0.11	2.78 ± 0.09	2.95 ± 0.05	2.92 ± 0.09	3.09 ± 0.10	2.78 ± 0.05

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	342 ± 4	344 ± 9	345 ± 5	342 ± 7	358 ± 7	340 ± 4
Brain						
Absolute	1.902 ± 0.011	1.902 ± 0.023	1.922 ± 0.013	1.889 ± 0.019	1.915 ± 0.025	1.899 ± 0.022
Relative	5.56 ± 0.07	5.55 ± 0.08	5.58 ± 0.05	5.53 ± 0.09	5.36 ± 0.05	5.58 ± 0.06
Heart						
Absolute	0.938 ± 0.016	0.926 ± 0.034	0.928 ± 0.012	0.913 ± 0.015	0.938 ± 0.023	0.916 ± 0.016
Relative	2.74 ± 0.04	2.69 ± 0.03	2.69 ± 0.03	2.67 ± 0.04	2.62 ± 0.03	2.69 ± 0.04
Right kidney						
Absolute	1.094 ± 0.017	1.076 ± 0.040	1.098 ± 0.016	1.094 ± 0.026	1.150 ± 0.031	1.189 ± 0.032*
Relative	3.20 ± 0.04	3.12 ± 0.05	3.19 ± 0.03	3.19 ± 0.04	3.21 ± 0.05	3.49 ± 0.07**
Liver						
Absolute	11.185 ± 0.172	10.798 ± 0.583	10.813 ± 0.196	10.795 ± 0.311	11.745 ± 0.337	11.034 ± 0.390
Relative	32.71 ± 0.60	31.23 ± 1.00	31.40 ± 0.50	31.50 ± 0.54	32.78 ± 0.60	32.41 ± 1.06
Lungs						
Absolute	1.863 ± 0.094	1.791 ± 0.115	1.775 ± 0.102	1.740 ± 0.054	1.847 ± 0.077	1.733 ± 0.052
Relative	5.46 ± 0.32	5.19 ± 0.25	5.14 ± 0.27	5.08 ± 0.11	5.16 ± 0.20	5.09 ± 0.14
Spleen						
Absolute	0.703 ± 0.014	0.669 ± 0.018	0.686 ± 0.013	0.689 ± 0.011	0.702 ± 0.016	0.680 ± 0.013
Relative	2.05 ± 0.04	1.95 ± 0.02*	1.99 ± 0.03	2.02 ± 0.03	1.96 ± 0.02	2.00 ± 0.03
Right testis						
Absolute	1.387 ± 0.022	1.368 ± 0.027	1.378 ± 0.027	1.325 ± 0.049	1.383 ± 0.025	1.404 ± 0.022
Relative	4.05 ± 0.05	3.99 ± 0.06	4.00 ± 0.05	3.87 ± 0.11	3.86 ± 0.03	4.13 ± 0.07
Thymus						
Absolute	0.388 ± 0.018	0.356 ± 0.012	0.363 ± 0.013	0.363 ± 0.013	0.390 ± 0.017	0.362 ± 0.013
Relative	1.14 ± 0.05	1.04 ± 0.03	1.05 ± 0.03	1.06 ± 0.03	1.09 ± 0.04	1.06 ± 0.04

TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	201 ± 3	208 ± 3	214 ± 4*	204 ± 3	212 ± 4	208 ± 3
Brain						
Absolute	1.774 ± 0.010	1.719 ± 0.014*	1.770 ± 0.010	1.747 ± 0.011	1.740 ± 0.024	1.754 ± 0.011
Relative	8.86 ± 0.16	8.28 ± 0.09*	8.30 ± 0.15*	8.57 ± 0.13	8.23 ± 0.14**	8.45 ± 0.12
Heart						
Absolute	0.631 ± 0.007	0.621 ± 0.009	0.627 ± 0.008	0.622 ± 0.006	0.617 ± 0.013	0.607 ± 0.011
Relative	3.15 ± 0.05	2.99 ± 0.04**	2.94 ± 0.05*	3.05 ± 0.03*	2.91 ± 0.03**	2.92 ± 0.05**
Right kidney						
Absolute	0.706 ± 0.015	0.679 ± 0.016	0.736 ± 0.014	0.709 ± 0.014	0.737 ± 0.020	0.771 ± 0.018**
Relative	3.52 ± 0.08	3.27 ± 0.05	3.45 ± 0.09	3.48 ± 0.08	3.48 ± 0.06	3.71 ± 0.07
Liver						
Absolute	6.221 ± 0.189	6.236 ± 0.212	6.263 ± 0.118	5.807 ± 0.136	6.040 ± 0.165	5.771 ± 0.077
Relative	31.02 ± 0.86	29.99 ± 0.84	29.33 ± 0.49	28.44 ± 0.61**	28.51 ± 0.55**	27.79 ± 0.38**
Lungs						
Absolute	1.277 ± 0.036	1.113 ± 0.047**	1.190 ± 0.019**	1.135 ± 0.021**	1.162 ± 0.023**	1.137 ± 0.016**
Relative	6.37 ± 0.15	5.35 ± 0.18**	5.57 ± 0.09**	5.57 ± 0.14**	5.49 ± 0.09**	5.48 ± 0.10**
Spleen						
Absolute	0.432 ± 0.008	0.418 ± 0.011	0.425 ± 0.009	0.404 ± 0.009*	0.400 ± 0.007*	0.397 ± 0.007**
Relative	2.16 ± 0.05	2.01 ± 0.06*	1.99 ± 0.04*	1.98 ± 0.05**	1.89 ± 0.03**	1.91 ± 0.03**
Thymus						
Absolute	0.278 ± 0.011	0.246 ± 0.010	0.280 ± 0.012	0.266 ± 0.016	0.264 ± 0.011	0.273 ± 0.010
Relative	1.39 ± 0.06	1.18 ± 0.04*	1.31 ± 0.05	1.30 ± 0.07	1.24 ± 0.05	1.31 ± 0.05

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats After 6 Months of Exposure in the Stop-Exposure Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
n	10	10	10	10	10	10
Necropsy body wt	403 ± 7	437 ± 7**	417 ± 9	419 ± 7	410 ± 7	419 ± 7
Brain						
Absolute	1.948 ± 0.014	1.917 ± 0.017	1.935 ± 0.015	1.949 ± 0.016	1.938 ± 0.019	1.955 ± 0.015
Relative	4.84 ± 0.05	4.39 ± 0.07**	4.66 ± 0.09	4.66 ± 0.04	4.73 ± 0.05	4.67 ± 0.06
Heart						
Absolute	1.003 ± 0.020	1.060 ± 0.021	1.014 ± 0.025	1.061 ± 0.018	1.011 ± 0.024	1.039 ± 0.015
Relative	2.49 ± 0.02	2.42 ± 0.02	2.43 ± 0.02	2.54 ± 0.05	2.46 ± 0.03	2.48 ± 0.03
Right kidney						
Absolute	1.147 ± 0.021	1.256 ± 0.026*	1.213 ± 0.028*	1.287 ± 0.025**	1.258 ± 0.032**	1.352 ± 0.019**
Relative	2.85 ± 0.03	2.87 ± 0.05	2.91 ± 0.04	3.08 ± 0.06**	3.07 ± 0.04**	3.23 ± 0.04**
Liver						
Absolute	11.096 ± 0.207	12.650 ± 0.301**	11.983 ± 0.252	11.994 ± 0.490	11.323 ± 0.234	12.707 ± 0.368**
Relative	27.55 ± 0.42	28.90 ± 0.35	28.78 ± 0.44	28.59 ± 0.83	27.62 ± 0.35	30.29 ± 0.49**
Lungs						
Absolute	1.965 ± 0.106	1.920 ± 0.046	1.841 ± 0.033	1.925 ± 0.043	1.813 ± 0.036	1.914 ± 0.043
Relative	4.89 ± 0.29	4.39 ± 0.10	4.43 ± 0.09	4.61 ± 0.12	4.42 ± 0.06	4.57 ± 0.09
Spleen						
Absolute	0.686 ± 0.008	0.745 ± 0.013*	0.705 ± 0.013	0.703 ± 0.015	0.696 ± 0.014	0.702 ± 0.015
Relative	1.71 ± 0.03	1.70 ± 0.02	1.69 ± 0.02	1.68 ± 0.02	1.70 ± 0.02	1.68 ± 0.03
Right testis						
Absolute	1.468 ± 0.018	1.502 ± 0.015	1.479 ± 0.026	1.459 ± 0.015	1.467 ± 0.017	1.502 ± 0.031
Relative	3.65 ± 0.06	3.44 ± 0.05*	3.55 ± 0.02	3.49 ± 0.04	3.59 ± 0.07	3.59 ± 0.07
Thymus						
Absolute	0.361 ± 0.021	0.364 ± 0.016	0.375 ± 0.019	0.360 ± 0.021	0.349 ± 0.009	0.348 ± 0.018
Relative	0.90 ± 0.05	0.83 ± 0.03	0.90 ± 0.04	0.86 ± 0.04	0.85 ± 0.03	0.83 ± 0.05

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/N Rats After 6 Months of Recovery in the Stop-Exposure Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
n	30	30	29	30	30	30
Necropsy body wt	485 ± 5	492 ± 5	493 ± 7	486 ± 4	493 ± 4	491 ± 4
Brain						
Absolute	1.971 ± 0.013	1.984 ± 0.008	1.980 ± 0.010	1.955 ± 0.009	1.971 ± 0.010	1.995 ± 0.012
Relative	4.08 ± 0.04	4.04 ± 0.04	4.04 ± 0.04	4.03 ± 0.03	4.01 ± 0.03	4.07 ± 0.03
Heart						
Absolute	1.234 ± 0.016	1.214 ± 0.013	1.248 ± 0.014	1.230 ± 0.018	1.238 ± 0.011	1.239 ± 0.010
Relative	2.55 ± 0.01	2.47 ± 0.02*	2.54 ± 0.02	2.53 ± 0.03	2.51 ± 0.01	2.53 ± 0.02
Right kidney						
Absolute	1.505 ± 0.021	1.518 ± 0.018	1.538 ± 0.024	1.511 ± 0.023	1.542 ± 0.018	1.540 ± 0.022
Relative	3.10 ± 0.03	3.08 ± 0.02	3.12 ± 0.03	3.11 ± 0.04	3.13 ± 0.03	3.14 ± 0.03
Liver						
Absolute	15.520 ± 0.363	15.993 ± 0.291	16.146 ± 0.341	16.366 ± 0.376	15.885 ± 0.196	15.872 ± 0.304
Relative	31.92 ± 0.49	32.42 ± 0.37	32.71 ± 0.35	33.63 ± 0.68*	32.23 ± 0.23	32.29 ± 0.47
Lungs						
Absolute	2.330 ± 0.075	2.410 ± 0.053	2.395 ± 0.067	2.300 ± 0.073	2.279 ± 0.043	2.339 ± 0.058
Relative	4.79 ± 0.12	4.90 ± 0.10	4.87 ± 0.13	4.74 ± 0.15	4.63 ± 0.08	4.77 ± 0.11
Spleen						
Absolute	1.017 ± 0.025	1.065 ± 0.053	1.050 ± 0.021	0.977 ± 0.025	0.995 ± 0.018	0.994 ± 0.017
Relative	2.10 ± 0.05	2.16 ± 0.11	2.13 ± 0.04	2.01 ± 0.05	2.02 ± 0.03	2.02 ± 0.03
Right testis						
Absolute	1.572 ± 0.025	1.524 ± 0.025	1.619 ± 0.019[2]	1.579 ± 0.014	1.576 ± 0.018	1.604 ± 0.018
Relative	3.25 ± 0.05	3.10 ± 0.05	3.30 ± 0.05[2]	3.25 ± 0.04	3.20 ± 0.04	3.27 ± 0.03
Thymus						
Absolute	0.299 ± 0.011	0.352 ± 0.012**	0.371 ± 0.014**	0.352 ± 0.012**	0.348 ± 0.009*	0.291 ± 0.010
Relative	0.62 ± 0.02	0.71 ± 0.02**	0.75 ± 0.03**	0.72 ± 0.02**	0.71 ± 0.02*	0.59 ± 0.02

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=28.

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

TABLE C5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 2-Week Inhalation Study of Isoprene[1]

	0 ppm	438 ppm	875 ppm	1,750 ppm	3,500 ppm	7,000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	28.7 ± 0.6	27.1 ± 0.4**	26.4 ± 0.4**	27.2 ± 0.4**	26.7 ± 0.4**	24.3 ± 0.4**
Brain						
Absolute	0.461 ± 0.004	0.454 ± 0.007	0.443 ± 0.004	0.456 ± 0.003	0.451 ± 0.003	0.430 ± 0.005**
Relative	16.07 ± 0.20	16.76 ± 0.15*	16.82 ± 0.24*	16.80 ± 0.18*	16.92 ± 0.18**	17.70 ± 0.32**
Heart						
Absolute	0.132 ± 0.003	0.127 ± 0.004	0.121 ± 0.003*	0.121 ± 0.002*	0.118 ± 0.002**	0.112 ± 0.004**
Relative	4.59 ± 0.05	4.69 ± 0.15	4.59 ± 0.10	4.45 ± 0.06	4.42 ± 0.05	4.60 ± 0.13
Right kidney						
Absolute	0.278 ± 0.006	0.271 ± 0.007	0.271 ± 0.006	0.273 ± 0.004	0.276 ± 0.005	0.259 ± 0.004*
Relative	9.67 ± 0.11	10.00 ± 0.20	10.27 ± 0.17*	10.05 ± 0.12*	10.34 ± 0.11**	10.65 ± 0.13**
Liver						
Absolute	1.447 ± 0.038	1.517 ± 0.029	1.566 ± 0.029*	1.625 ± 0.036**	1.665 ± 0.054**	1.634 ± 0.025**
Relative	50.32 ± 0.66	55.96 ± 0.60**	59.39 ± 0.89**	59.74 ± 0.62**	62.33 ± 1.41**	67.20 ± 0.97**
Lungs						
Absolute	0.179 ± 0.005	0.177 ± 0.009	0.166 ± 0.005	0.174 ± 0.003	0.169 ± 0.003	0.159 ± 0.004**
Relative	6.23 ± 0.14	6.53 ± 0.30	6.29 ± 0.19	6.41 ± 0.09	6.33 ± 0.08	6.54 ± 0.14
Spleen						
Absolute	0.078 ± 0.003	0.068 ± 0.001**	0.060 ± 0.002**	0.063 ± 0.002**	0.062 ± 0.001**	0.047 ± 0.002**
Relative	2.71 ± 0.10	2.51 ± 0.07*	2.27 ± 0.07**	2.31 ± 0.06**	2.33 ± 0.04**	1.93 ± 0.06**
Right testis						
Absolute	0.104 ± 0.003	0.095 ± 0.003**	0.083 ± 0.002**	0.084 ± 0.002**	0.083 ± 0.001**	0.069 ± 0.002**
Relative	3.61 ± 0.10	3.49 ± 0.08	3.14 ± 0.05**	3.08 ± 0.07**	3.11 ± 0.05**	2.84 ± 0.05**
Thymus						
Absolute	0.047 ± 0.003	0.039 ± 0.002*	0.026 ± 0.002**	0.030 ± 0.003**	0.024 ± 0.002**	0.015 ± 0.002**
Relative	1.62 ± 0.08	1.42 ± 0.06	0.99 ± 0.08**	1.10 ± 0.09**	0.91 ± 0.06**	0.60 ± 0.06**

TABLE C5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 2-Week Inhalation Study of Isoprene (continued)

	0 ppm	438 ppm	875 ppm	1,750 ppm	3,500 ppm	7,000 ppm
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	22.6 ± 0.3	22.6 ± 0.3	23.0 ± 0.2	22.5 ± 0.4	22.9 ± 0.3	22.3 ± 0.3
Brain						
Absolute	0.469 ± 0.003	0.458 ± 0.004	0.453 ± 0.004	0.462 ± 0.006	0.457 ± 0.006	0.450 ± 0.004**
Relative	20.75 ± 0.17	20.27 ± 0.24	19.67 ± 0.18**	20.57 ± 0.24	19.98 ± 0.21	20.25 ± 0.28
Heart						
Absolute	0.112 ± 0.002	0.111 ± 0.002	0.114 ± 0.002	0.112 ± 0.002	0.108 ± 0.002	0.104 ± 0.002*
Relative	4.95 ± 0.06	4.91 ± 0.06	4.95 ± 0.10	4.98 ± 0.08	4.72 ± 0.08*	4.68 ± 0.09*
Right kidney						
Absolute	0.190 ± 0.005	0.183 ± 0.004	0.197 ± 0.003	0.197 ± 0.004	0.196 ± 0.005	0.201 ± 0.004
Relative	8.39 ± 0.13	8.09 ± 0.12	8.55 ± 0.15	8.76 ± 0.11	8.56 ± 0.14	9.03 ± 0.13**
Liver						
Absolute	1.200 ± 0.023	1.290 ± 0.021*	1.365 ± 0.021**	1.334 ± 0.030**	1.424 ± 0.017**	1.438 ± 0.036**
Relative	53.05 ± 0.67	57.01 ± 0.43**	59.25 ± 0.79**	59.34 ± 1.01**	62.25 ± 0.51**	64.54 ± 1.04**
Lungs						
Absolute	0.169 ± 0.003	0.162 ± 0.005	0.168 ± 0.004	0.166 ± 0.003	0.158 ± 0.002*	0.158 ± 0.004*
Relative	7.48 ± 0.18	7.16 ± 0.20	7.29 ± 0.14	7.39 ± 0.09	6.91 ± 0.08*	7.10 ± 0.15*
Spleen						
Absolute	0.085 ± 0.003	0.081 ± 0.005	0.074 ± 0.002	0.079 ± 0.003	0.073 ± 0.002*	0.067 ± 0.002**
Relative	3.75 ± 0.11	3.59 ± 0.22	3.21 ± 0.08	3.52 ± 0.15	3.19 ± 0.08**	3.01 ± 0.10**
Thymus						
Absolute	0.069 ± 0.003	0.054 ± 0.002**	0.046 ± 0.003**	0.048 ± 0.001**	0.049 ± 0.002**	0.035 ± 0.002**
Relative	3.03 ± 0.11	2.38 ± 0.10**	1.99 ± 0.11**	2.12 ± 0.07**	2.16 ± 0.06**	1.58 ± 0.10**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

TABLE C6 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	36.4 ± 0.8	36.1 ± 0.7	37.2 ± 0.8	37.4 ± 0.6	39.2 ± 1.4	36.7 ± 0.9
Brain						
Absolute	0.463 ± 0.006	0.464 ± 0.005	0.465 ± 0.005	0.460 ± 0.008	0.442 ± 0.006*	0.427 ± 0.004**
Relative	12.78 ± 0.28	12.91 ± 0.28	12.56 ± 0.22	12.31 ± 0.26	11.40 ± 0.36**	11.69 ± 0.29**
Heart						
Absolute	0.154 ± 0.005	0.155 ± 0.002	0.150 ± 0.006	0.146 ± 0.004	0.144 ± 0.005	0.139 ± 0.003*
Relative	4.24 ± 0.11	4.31 ± 0.10	4.04 ± 0.14	3.91 ± 0.10*	3.70 ± 0.12**	3.79 ± 0.08**
Right kidney						
Absolute	0.316 ± 0.010	0.330 ± 0.009	0.329 ± 0.010	0.337 ± 0.010	0.331 ± 0.007	0.317 ± 0.009
Relative	8.71 ± 0.29	9.16 ± 0.22	8.86 ± 0.19	9.02 ± 0.30	8.53 ± 0.29	8.65 ± 0.24
Liver						
Absolute	1.597 ± 0.047	1.525 ± 0.034	1.620 ± 0.061	1.643 ± 0.054	1.708 ± 0.049	2.010 ± 0.043**
Relative	43.95 ± 1.03	42.37 ± 0.97	43.60 ± 1.30	43.98 ± 1.60	43.83 ± 1.06	54.91 ± 1.27**
Lungs						
Absolute	0.235 ± 0.006	0.239 ± 0.007	0.243 ± 0.008	0.243 ± 0.005	0.227 ± 0.005	0.233 ± 0.011
Relative	6.47 ± 0.14	6.64 ± 0.18	6.54 ± 0.15	6.50 ± 0.15	5.86 ± 0.23	6.32 ± 0.17
Spleen						
Absolute	0.074 ± 0.003	0.074 ± 0.004	0.068 ± 0.003	0.065 ± 0.002*	0.061 ± 0.003**	0.049 ± 0.002**
Relative	2.04 ± 0.08	2.05 ± 0.09	1.83 ± 0.06*	1.74 ± 0.07**	1.57 ± 0.07**	1.34 ± 0.06**
Right testis						
Absolute	0.120 ± 0.004	0.124 ± 0.002	0.120 ± 0.003	0.117 ± 0.001	0.106 ± 0.002**	0.077 ± 0.003**
Relative	3.31 ± 0.10	3.44 ± 0.07	3.22 ± 0.06	3.14 ± 0.06	2.73 ± 0.09**	2.10 ± 0.09**
Thymus						
Absolute	0.041 ± 0.004	0.039 ± 0.002	0.041 ± 0.002	0.039 ± 0.003[2]	0.045 ± 0.005	0.035 ± 0.004[2]
Relative	1.11 ± 0.09	1.07 ± 0.05	1.11 ± 0.06	1.04 ± 0.07[2]	1.15 ± 0.11	0.97 ± 0.10[2]

TABLE C6 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	34.0 ± 0.7	30.8 ± 0.6**	32.3 ± 0.8**	30.1 ± 0.4**	30.4 ± 0.5**	29.9 ± 0.6**
Brain						
Absolute	0.473 ± 0.002	0.480 ± 0.003	0.485 ± 0.003	0.471 ± 0.005	0.475 ± 0.004	0.466 ± 0.004
Relative	13.98 ± 0.32	15.63 ± 0.26**	15.11 ± 0.42**	15.68 ± 0.28**	15.66 ± 0.14**	15.65 ± 0.38**
Heart						
Absolute	0.136 ± 0.003	0.144 ± 0.002	0.146 ± 0.003	0.135 ± 0.003	0.135 ± 0.003	0.134 ± 0.003
Relative	4.01 ± 0.08	4.68 ± 0.07**	4.54 ± 0.09**	4.49 ± 0.08**	4.45 ± 0.07**	4.49 ± 0.09**
Right kidney						
Absolute	0.223 ± 0.004	0.236 ± 0.006	0.240 ± 0.005*	0.239 ± 0.004*	0.239 ± 0.005*	0.239 ± 0.006*
Relative	6.59 ± 0.19	7.67 ± 0.14**	7.46 ± 0.19**	7.95 ± 0.17**	7.88 ± 0.15**	8.01 ± 0.24**
Liver						
Absolute	1.511 ± 0.047	1.514 ± 0.048	1.652 ± 0.066	1.559 ± 0.058	1.587 ± 0.060	1.724 ± 0.072*
Relative	44.50 ± 1.15	49.19 ± 1.38*	51.21 ± 1.76**	51.76 ± 1.65**	52.22 ± 1.58**	57.51 ± 1.61**
Lungs						
Absolute	0.246 ± 0.007	0.226 ± 0.006	0.242 ± 0.004	0.229 ± 0.004	0.223 ± 0.007*	0.237 ± 0.007
Relative	7.24 ± 0.15	7.34 ± 0.14	7.54 ± 0.22	7.62 ± 0.16	7.34 ± 0.14	7.95 ± 0.26**
Spleen						
Absolute	0.111 ± 0.011	0.095 ± 0.002	0.110 ± 0.006	0.090 ± 0.001*	0.089 ± 0.003**	0.081 ± 0.002**
Relative	3.29 ± 0.36	3.09 ± 0.06	3.41 ± 0.15	2.99 ± 0.05	2.93 ± 0.05	2.71 ± 0.07*
Thymus						
Absolute	0.053 ± 0.002	0.048 ± 0.002	0.049 ± 0.002	0.049 ± 0.002	0.051 ± 0.002	0.044 ± 0.002**
Relative	1.56 ± 0.07	1.55 ± 0.05	1.52 ± 0.08	1.64 ± 0.06	1.69 ± 0.06	1.46 ± 0.07

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE C7 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F$_1$ Mice After 6 Months of Exposure in the Stop-Exposure Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
n	5	10	10	10	10	5
Necropsy body wt	44.0 ± 2.0	45.9 ± 1.2	44.5 ± 1.3	48.5 ± 1.0	47.2 ± 1.0	43.6 ± 4.0
Brain						
Absolute	0.460 ± 0.008	0.468 ± 0.006	0.455 ± 0.004	0.458 ± 0.005	0.449 ± 0.005	0.425 ± 0.010**
Relative	10.54 ± 0.48	10.24 ± 0.26	10.31 ± 0.28	9.47 ± 0.13	9.54 ± 0.25	10.06 ± 0.85
Heart						
Absolute	0.174 ± 0.007	0.179 ± 0.008	0.184 ± 0.006	0.178 ± 0.006	0.170 ± 0.004	0.154 ± 0.009
Relative	3.99 ± 0.19	3.91 ± 0.12	4.16 ± 0.16	3.68 ± 0.10	3.61 ± 0.07	3.59 ± 0.18
Right kidney						
Absolute	0.346 ± 0.014	0.375 ± 0.013	0.350 ± 0.011	0.359 ± 0.007	0.352 ± 0.010	0.315 ± 0.020
Relative	7.88 ± 0.12	8.09 ± 0.18	7.89 ± 0.17	7.42 ± 0.12	7.48 ± 0.27	7.32 ± 0.30
Liver						
Absolute	1.613 ± 0.079	1.760 ± 0.092	1.704 ± 0.062	1.943 ± 0.045*	1.851 ± 0.042*	1.952 ± 0.153*
Relative	36.64 ± 0.38	38.15 ± 1.12	38.48 ± 1.44	40.14 ± 1.01	39.27 ± 0.85	45.07 ± 1.32**
Lungs						
Absolute	0.267 ± 0.017	0.262 ± 0.008	0.265 ± 0.006	0.278 ± 0.007	0.276 ± 0.007	0.241 ± 0.021
Relative	6.08 ± 0.27	5.71 ± 0.15	5.99 ± 0.18	5.73 ± 0.09	5.85 ± 0.12	5.58 ± 0.28
Spleen						
Absolute	0.074 ± 0.002	0.073 ± 0.003	0.071 ± 0.002	0.069 ± 0.002	0.061 ± 0.002**[2]	0.059 ± 0.002**
Relative	1.70 ± 0.12	1.58 ± 0.05	1.60 ± 0.05	1.44 ± 0.05**	1.30 ± 0.04**[2]	1.38 ± 0.10**
Right testis						
Absolute	0.119 ± 0.004	0.128 ± 0.004	0.115 ± 0.007	0.122 ± 0.003	0.118 ± 0.001	0.086 ± 0.014**
Relative	2.71 ± 0.11	2.81 ± 0.10	2.58 ± 0.15	2.52 ± 0.05	2.50 ± 0.05	1.92 ± 0.19**
Thymus						
Absolute	0.045 ± 0.006	0.052 ± 0.006	0.044 ± 0.003	0.053 ± 0.005	0.046 ± 0.004	0.049 ± 0.009
Relative	1.02 ± 0.11	1.11 ± 0.11	0.98 ± 0.05	1.09 ± 0.09	0.96 ± 0.07	1.10 ± 0.14

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

TABLE C8 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F$_1$ Mice After 6 Months of Recovery in the Stop-Exposure Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
n	22	28	28	27	26	16
Necropsy body wt	51.4 ± 1.0	53.5 ± 0.9	53.0 ± 1.0	55.2 ± 0.8*	54.9 ± 0.6*	53.4 ± 1.7
Brain						
Absolute	0.458 ± 0.003	0.466 ± 0.004	0.466 ± 0.003	0.460 ± 0.002	0.458 ± 0.002	0.437 ± 0.004**
Relative	8.97 ± 0.17	8.77 ± 0.16	8.87 ± 0.18	8.37 ± 0.10**	8.36 ± 0.09**	8.30 ± 0.24**
Heart						
Absolute	0.205 ± 0.004	0.208 ± 0.004	0.209 ± 0.004	0.216 ± 0.005	0.216 ± 0.003	0.214 ± 0.006
Relative	4.01 ± 0.09	3.90 ± 0.06	3.95 ± 0.06	3.90 ± 0.06	3.94 ± 0.06	4.03 ± 0.06
Right kidney						
Absolute	0.418 ± 0.011	0.427 ± 0.009	0.415 ± 0.010	0.424 ± 0.009	0.433 ± 0.008	0.415 ± 0.012
Relative	8.15 ± 0.19	8.01 ± 0.17	7.81 ± 0.11	7.66 ± 0.09	7.90 ± 0.12	7.81 ± 0.18
Liver						
Absolute	2.317 ± 0.125	2.455 ± 0.086	2.432 ± 0.140	2.931 ± 0.133**	2.951 ± 0.161**	3.338 ± 0.243**
Relative	44.86 ± 2.06	45.89 ± 1.42	45.66 ± 2.60	53.19 ± 2.55*	54.10 ± 3.28*	62.94 ± 4.69**
Lungs						
Absolute	0.308 ± 0.007	0.324 ± 0.007	0.311 ± 0.008	0.317 ± 0.008	0.310 ± 0.006	0.348 ± 0.018*
Relative	6.01 ± 0.12	6.06 ± 0.08	5.87 ± 0.11	5.75 ± 0.11	5.65 ± 0.10	6.56 ± 0.33
Spleen						
Absolute	0.089 ± 0.005	0.098 ± 0.007	0.099 ± 0.005	0.104 ± 0.004	0.123 ± 0.015**	0.136 ± 0.014**
Relative	1.75 ± 0.12	1.86 ± 0.16	1.85 ± 0.08	1.90 ± 0.09	2.23 ± 0.25	2.58 ± 0.27**
Right testis						
Absolute	0.123 ± 0.003	0.124 ± 0.003	0.126 ± 0.001	0.126 ± 0.002	0.127 ± 0.002	0.121 ± 0.003
Relative	2.41 ± 0.06	2.34 ± 0.05	2.38 ± 0.04	2.28 ± 0.03	2.31 ± 0.03	2.28 ± 0.05
Thymus						
Absolute	0.043 ± 0.003	0.050 ± 0.003	0.049 ± 0.003	0.060 ± 0.003**	0.050 ± 0.003	0.051 ± 0.006
Relative	0.83 ± 0.05	0.94 ± 0.05	0.93 ± 0.05	1.09 ± 0.06**	0.90 ± 0.05	0.93 ± 0.10

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

APPENDIX D

Hematology, Clinical Chemistry, and Urinalysis Results

Table D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 2-Week Inhalation Study of Isoprene D-2

Table D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Inhalation Study of Isoprene D-4

Table D3 Hematology Data for Male F344/N Rats After 6 Months of Exposure in the Stop-Exposure Inhalation Study of Isoprene D-8

Table D4 Hematology and Clinical Chemistry Data for B6C3F_1 Mice in the 2-Week Inhalation Study of Isoprene D-9

Table D5 Hematology and Clinical Chemistry Data for B6C3F_1 Mice in the 13-Week Inhalation Study of Isoprene D-11

Table D6 Hematology Data for Male B6C3F_1 Mice After 6 Months of Exposure in the Stop-Exposure Inhalation Study of Isoprene D-15

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 2-Week Inhalation Study of Isoprene[1]

	0 ppm	438 ppm	875 ppm	1,750 ppm	3,500 ppm	7,000 ppm
MALE						
Hematology						
n	10	10	10	10	10	10
Hematocrit (%)	42.3 ± 0.5	43.8 ± 0.7	42.3 ± 0.9	43.5 ± 0.3	43.8 ± 0.5	44.1 ± 0.6*
Hemoglobin (g/dL)	14.2 ± 0.2	14.5 ± 0.2	14.2 ± 0.3	14.5 ± 0.1	14.7 ± 0.1	14.7 ± 0.2*
Erythrocytes ($10^6/\mu$L)	7.39 ± 0.13	7.62 ± 0.13	7.38 ± 0.15	7.62 ± 0.08	7.51 ± 0.15	7.63 ± 0.15
Reticulocytes ($10^6/\mu$L)	0.31 ± 0.03	0.36 ± 0.04	0.32 ± 0.05	0.34 ± 0.03	0.40 ± 0.02	0.34 ± 0.03
Mean cell volume (fL)	57.2 ± 0.5	57.4 ± 0.6	57.6 ± 1.1	57.0 ± 0.7	58.5 ± 0.9	57.9 ± 0.5
Mean cell hemoglobin (pg)	19.2 ± 0.2	19.1 ± 0.2	19.2 ± 0.3	19.0 ± 0.3	19.6 ± 0.3	19.3 ± 0.2
Mean cell hemoglobin concentration (g/dL)	33.5 ± 0.2	33.2 ± 0.2	33.5 ± 0.2	33.3 ± 0.2	33.5 ± 0.2	33.4 ± 0.2
Platelets ($10^3/\mu$L)	788.5 ± 23.2	837.9 ± 32.9	736.4 ± 41.9	722.5 ± 44.0	868.5 ± 27.8	843.2 ± 27.9
Leukocytes ($10^3/\mu$L)	5.80 ± 0.32	5.98 ± 0.33	5.53 ± 0.33	6.50 ± 0.23	5.54 ± 0.27	6.03 ± 0.45
Segmented neutrophils ($10^3/\mu$L)	0.75 ± 0.11	0.91 ± 0.15	0.66 ± 0.13	0.88 ± 0.09	0.97 ± 0.15	0.73 ± 0.08
Lymphocytes ($10^3/\mu$L)	4.85 ± 0.31	4.72 ± 0.38	4.67 ± 0.33	5.28 ± 0.22	4.35 ± 0.22	4.99 ± 0.42
Monocytes ($10^3/\mu$L)	0.18 ± 0.04	0.31 ± 0.06	0.19 ± 0.05	0.31 ± 0.07	0.20 ± 0.04	0.27 ± 0.05
Eosinophils ($10^3/\mu$L)	0.03 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.02
Clinical Chemistry						
n	10	10	9	10	10	10
Urea nitrogen (mg/dL)	22.9 ± 0.8	25.7 ± 0.9	24.2 ± 1.1	24.1 ± 0.7	26.5 ± 1.3	23.0 ± 0.9
Creatinine (mg/dL)	1.05 ± 0.04	0.96 ± 0.05	0.97 ± 0.04	0.94 ± 0.06	1.08 ± 0.05	1.00 ± 0.04
Alanine aminotransferase (IU/L)	41 ± 2	43 ± 2	41 ± 2	45 ± 3	47 ± 3	41 ± 1
Glutamate dehydrogenase (IU/L)	9.2 ± 1.7	7.9 ± 1.5	8.4 ± 1.2	5.9 ± 1.0	9.8 ± 1.7	6.3 ± 0.8
Sorbitol dehydrogenase (IU/L)	6 ± 1	5 ± 1	6 ± 1	7 ± 1	6 ± 1	6 ± 0
Urinalysis						
n	9	10	10	10	10	10
Creatinine (mg/dL)	25.78 ± 3.19	30.73 ± 3.86	30.14 ± 2.26	21.33 ± 4.20	32.65 ± 4.08	24.65 ± 3.61
Glucose (μg/mg creatinine)	166.5 ± 11.1	181.9 ± 10.9	185.3 ± 14.8	194.2 ± 8.5	172.5 ± 14.2	275.1 ± 86.3
Protein (μg/mg creatinine)	725.2 ± 61.6	603.5 ± 74.7	489.8 ± 70.5*	519.6 ± 50.0	652.9 ± 80.1	592.7 ± 65.9[2]
Alkaline phosphatase (μU/mg creatinine)	148 ± 25	99 ± 28	80 ± 28	56 ± 20*	93 ± 25	83 ± 22[2]
Aspartate aminotransferase (mU/mg creatinine)	162 ± 27	112 ± 32	115 ± 34	70 ± 24	146 ± 32	98 ± 21
Volume (mL/16 hr)	8.5 ± 1.4	7.9 ± 1.4	6.1 ± 0.6	11.4 ± 1.4	7.0 ± 1.3	9.2 ± 1.6
Specific gravity	1.013 ± 0.001	1.016 ± 0.002	1.017 ± 0.001	1.012 ± 0.002	1.017 ± 0.002	1.014 ± 0.002

TABLE D1 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 2-Week Inhalation Study of Isoprene (continued)

	0 ppm	438 ppm	875 ppm	1,750 ppm	3,500 ppm	7,000 ppm
FEMALE						
Hematology						
n	10	10	10	10	10	10
Hematocrit (%)	44.9 ± 0.7	46.4 ± 0.7	45.3 ± 0.8	44.5 ± 0.8	45.3 ± 0.5	44.6 ± 0.5
Hemoglobin (g/dL)	15.0 ± 0.2	15.4 ± 0.2	15.3 ± 0.3	14.8 ± 0.3	15.0 ± 0.1	14.9 ± 0.2
Erythrocytes ($10^6/\mu$L)	7.93 ± 0.18	8.16 ± 0.17	7.83 ± 0.20	7.76 ± 0.16	7.89 ± 0.10	7.75 ± 0.19
Reticulocytes ($10^6/\mu$L)	0.25 ± 0.03	0.27 ± 0.02	0.27 ± 0.05	0.27 ± 0.02	0.28 ± 0.02	0.27 ± 0.03
Mean cell volume (fL)	56.7 ± 0.6	57.0 ± 0.7	58.0 ± 0.9	57.3 ± 0.7	57.5 ± 0.6	57.7 ± 0.9
Mean cell hemoglobin (pg)	19.0 ± 0.3	18.9 ± 0.2	19.5 ± 0.3	19.1 ± 0.2	19.0 ± 0.2	19.2 ± 0.3
Mean cell hemoglobin concentration (g/dL)	33.4 ± 0.2	33.1 ± 0.2	33.7 ± 0.2	33.3 ± 0.2	33.0 ± 0.2	33.3 ± 0.1
Platelets ($10^3/\mu$L)	801.5 ± 41.9	767.9 ± 25.9	722.6 ± 53.1	766.5 ± 35.4	778.1 ± 26.1	821.7 ± 55.6
Leukocytes ($10^3/\mu$L)	6.82 ± 0.48	5.02 ± 0.20*	5.46 ± 0.53	5.86 ± 0.61	6.01 ± 0.22	5.41 ± 0.33
Segmented neutrophils ($10^3/\mu$L)	0.62 ± 0.08	0.40 ± 0.05	0.60 ± 0.09	0.46 ± 0.05	0.73 ± 0.08	0.70 ± 0.08
Lymphocytes ($10^3/\mu$L)	6.04 ± 0.43	4.52 ± 0.17	4.70 ± 0.48	5.29 ± 0.58	5.06 ± 0.17	4.15 ± 0.55
Monocytes ($10^3/\mu$L)	0.15 ± 0.03	0.09 ± 0.03	0.15 ± 0.03	0.10 ± 0.01	0.20 ± 0.04	0.13 ± 0.05
Eosinophils ($10^3/\mu$L)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00
Clinical Chemistry						
n	10	10	8	10	10	10
Urea nitrogen (mg/dL)	20.9 ± 0.8	20.8 ± 1.2	20.9 ± 1.0	22.9 ± 1.2	20.4 ± 1.1	21.0 ± 1.3
Creatinine (mg/dL)	0.84 ± 0.06	0.78 ± 0.05	0.93 ± 0.06	0.81 ± 0.06	0.90 ± 0.06	0.71 ± 0.08
Alanine aminotransferase (IU/L)	37 ± 2	33 ± 1	39 ± 1	38 ± 2	36 ± 2	39 ± 1
Glutamate dehydrogenase (IU/L)	9.3 ± 1.0	7.5 ± 0.8	7.0 ± 1.0	7.3 ± 1.5	9.1 ± 1.2	7.5 ± 0.9
Sorbitol dehydrogenase (IU/L)	8 ± 1	9 ± 0	9 ± 1	8 ± 0	8 ± 1	8 ± 0
Urinalysis						
n	10	10	9	10	10	10
Creatinine (mg/dL)	17.46 ± 2.27	20.70 ± 2.70	29.44 ± 4.85	21.70 ± 1.67	21.60 ± 3.25	18.90 ± 2.60
Glucose (μg/mg creatinine)	191.0 ± 6.9	205.7 ± 7.9	227.9 ± 29.0	210.7 ± 11.3	186.6 ± 5.9	205.0 ± 8.8
Protein (μg/mg creatinine)	465.7 ± 43.8	503.9 ± 58.0	679.0 ± 85.8	634.6 ± 88.8	500.8 ± 70.5	453.1 ± 55.2
Alkaline phosphatase (μU/mg creatinine)	51 ± 20	67 ± 25	73 ± 23	101 ± 27	48 ± 17	40 ± 19
Aspartate aminotransferase (mU/mg creatinine)	93 ± 32	181 ± 62	139 ± 48	194 ± 56	97 ± 39	79 ± 25
Volume (mL/16 hr)	10.9 ± 1.6	8.3 ± 1.0	5.4 ± 1.5*	7.3 ± 0.7	8.8 ± 1.2	9.9 ± 1.3
Specific gravity	1.010 ± 0.001	1.012 ± 0.002	1.024 ± 0.006*	1.013 ± 0.001	1.012 ± 0.002	1.011 ± 0.002

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

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
MALE						
Hematology						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 4	42.2 ± 0.6	41.8 ± 0.3	41.8 ± 0.4	41.6 ± 0.4	41.0 ± 0.4	42.4 ± 0.4
Day 24	44.7 ± 0.3	44.5 ± 0.3	44.4 ± 0.3	45.0 ± 0.3	44.8 ± 0.3	44.4 ± 0.3
Week 13	42.2 ± 0.2	42.2 ± 0.2	42.8 ± 0.3	42.6 ± 0.3	43.0 ± 0.3	42.3 ± 0.6
Hemoglobin (g/dL)						
Day 4	14.9 ± 0.2	14.7 ± 0.1	14.8 ± 0.1	14.8 ± 0.1	14.5 ± 0.1	15.1 ± 0.1
Day 24	16.0 ± 0.1	16.1 ± 0.1	16.1 ± 0.1	16.3 ± 0.1	16.1 ± 0.1	15.9 ± 0.1
Week 13	15.6 ± 0.1	15.6 ± 0.1	15.8 ± 0.1	15.7 ± 0.2	16.0 ± 0.1*	15.8 ± 0.1
Erythrocytes ($10^6/\mu$L)						
Day 4	8.59 ± 0.15	8.49 ± 0.11	8.50 ± 0.10	8.44 ± 0.12	8.36 ± 0.08	8.65 ± 0.08
Day 24	9.33 ± 0.08	9.31 ± 0.08	9.26 ± 0.08	9.36 ± 0.06	9.34 ± 0.05	9.18 ± 0.07
Week 13	9.58 ± 0.06	9.53 ± 0.06	9.71 ± 0.05	9.56 ± 0.08	9.67 ± 0.06	9.45 ± 0.13
Reticulocytes ($10^6/\mu$L)						
Day 4	0.28 ± 0.02	0.36 ± 0.03	0.36 ± 0.02	0.32 ± 0.02	0.40 ± 0.03*	0.30 ± 0.02
Day 24	0.22 ± 0.02	0.23 ± 0.02	0.25 ± 0.02	0.23 ± 0.01	0.20 ± 0.01	0.30 ± 0.03
Week 13	0.14 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.01	0.12 ± 0.02	0.15 ± 0.02
Nucleated erythrocytes ($10^3/\mu$L)						
Day 4	0.03 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.04 ± 0.02	0.08 ± 0.04	0.03 ± 0.01
Day 24	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
Week 13	0.08 ± 0.02	0.05 ± 0.01	0.02 ± 0.01	0.06 ± 0.02	0.02 ± 0.01	0.06 ± 0.02
Mean cell volume (fL)						
Day 4	49.2 ± 0.3	49.3 ± 0.4	49.2 ± 0.2	49.4 ± 0.3	48.9 ± 0.2	49.0 ± 0.3
Day 24	47.9 ± 0.1	47.8 ± 0.2	47.8 ± 0.2	48.0 ± 0.3	47.9 ± 0.2	48.1 ± 0.2
Week 13	44.1 ± 0.1	44.3 ± 0.2	44.0 ± 0.2	44.4 ± 0.2	44.5 ± 0.2	44.7 ± 0.2*
Mean cell hemoglobin (pg)						
Day 4	17.4 ± 0.1	17.3 ± 0.1	17.5 ± 0.1	17.6 ± 0.1	17.4 ± 0.1	17.5 ± 0.1
Day 24	17.2 ± 0.1	17.3 ± 0.1	17.4 ± 0.1	17.4 ± 0.1	17.3 ± 0.1	17.4 ± 0.1
Week 13	16.3 ± 0.0	16.4 ± 0.1	16.3 ± 0.1	16.4 ± 0.1	16.5 ± 0.1	16.7 ± 0.3
Mean cell hemoglobin concentration (g/dL)						
Day 4	35.2 ± 0.1	35.2 ± 0.1	35.5 ± 0.2	35.6 ± 0.1*	35.5 ± 0.1*	35.7 ± 0.1**
Day 24	35.9 ± 0.1	36.1 ± 0.1	36.2 ± 0.1	36.2 ± 0.1	36.0 ± 0.1	35.9 ± 0.2
Week 13	37.0 ± 0.1	37.0 ± 0.1	36.9 ± 0.1	36.8 ± 0.1	37.2 ± 0.3	37.3 ± 0.7
Platelets ($10^3/\mu$L)						
Day 4	742.2 ± 21.1	767.2 ± 7.6	783.0 ± 14.7	788.1 ± 22.5	794.8 ± 15.7	772.7 ± 20.8
Day 24	588.3 ± 8.3	585.2 ± 7.7	578.1 ± 10.8	590.5 ± 11.6	597.6 ± 11.1	587.0 ± 18.9
Week 13	518.8 ± 7.5	490.3 ± 8.8*	511.5 ± 4.8	479.6 ± 11.8**	505.9 ± 5.6	492.6 ± 4.7*
Leukocytes ($10^3/\mu$L)						
Day 4	8.67 ± 0.41	8.79 ± 0.41	9.62 ± 0.32	9.55 ± 0.51	9.63 ± 0.26	8.44 ± 0.46
Day 24	5.08 ± 0.20	4.69 ± 0.23	5.26 ± 0.29	5.26 ± 0.30	4.82 ± 0.18	5.64 ± 0.32
Week 13	5.98 ± 0.23	5.86 ± 0.20	6.20 ± 0.32	6.09 ± 0.18	5.68 ± 0.22	5.88 ± 0.25
Segmented neutrophils ($10^3/\mu$L)						
Day 4	1.00 ± 0.11	0.81 ± 0.07	1.11 ± 0.12	1.01 ± 0.06	1.00 ± 0.10	1.07 ± 0.09
Day 24	0.63 ± 0.05	0.58 ± 0.07	0.75 ± 0.07	0.69 ± 0.08	0.64 ± 0.03	0.76 ± 0.07
Week 13	1.18 ± 0.11	0.86 ± 0.08*	0.93 ± 0.12	0.93 ± 0.13	0.94 ± 0.05	0.77 ± 0.09**

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
MALE (continued)						
Hematology (continued)						
Lymphocytes ($10^3/\mu L$)						
Day 4	7.40 ± 0.38	7.72 ± 0.42	8.28 ± 0.27	8.19 ± 0.45	8.32 ± 0.31	7.17 ± 0.41
Day 24	4.25 ± 0.20	3.92 ± 0.27	4.34 ± 0.25	4.34 ± 0.29	3.95 ± 0.19	4.69 ± 0.29
Week 13	4.53 ± 0.21	4.71 ± 0.19	5.04 ± 0.26	4.94 ± 0.12	4.46 ± 0.21	4.89 ± 0.23
Monocytes ($10^3/\mu L$)						
Day 4	0.23 ± 0.05	0.23 ± 0.04	0.21 ± 0.06	0.29 ± 0.07	0.25 ± 0.06	0.19 ± 0.04
Day 24	0.18 ± 0.03	0.17 ± 0.04	0.10 ± 0.02	0.19 ± 0.04	0.18 ± 0.02	0.18 ± 0.04
Week 13	0.21 ± 0.05	0.19 ± 0.03	0.18 ± 0.03	0.18 ± 0.03	0.19 ± 0.03	0.20 ± 0.05
Eosinophils ($10^3/\mu L$)						
Day 4	0.04 ± 0.01	0.03 ± 0.02	0.02 ± 0.02	0.06 ± 0.04	0.06 ± 0.03	0.02 ± 0.02
Day 24	0.02 ± 0.01	0.03 ± 0.01	0.07 ± 0.02	0.04 ± 0.02	0.05 ± 0.01	0.01 ± 0.01
Week 13	0.05 ± 0.02	0.09 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.09 ± 0.02	0.03 ± 0.01
Total bone marrow cellularity (10^6/femur)						
Day 24	102.7 ± 5.1	98.9 ± 4.9	96.7 ± 7.4[2]	102.0 ± 6.2[2]	105.4 ± 4.0	94.9 ± 6.5
Week 13	117.5 ± 4.4	119.6 ± 5.4	116.5 ± 5.0	129.5 ± 5.7	127.5 ± 12.7	116.3 ± 4.5
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	21.3 ± 0.5	18.8 ± 0.5	20.8 ± 1.0	20.8 ± 0.8	22.6 ± 0.8	18.1 ± 0.7*
Creatinine (mg/dL)	0.69 ± 0.03	0.68 ± 0.02	0.73 ± 0.02	0.74 ± 0.05	0.73 ± 0.02	0.70 ± 0.02
Alanine aminotransferase (IU/L)	43 ± 2	48 ± 3	55 ± 5	50 ± 3	57 ± 5*	43 ± 2
Glutamate dehydrogenase (IU/L)	4.9 ± 1.0	3.0 ± 0.3	3.9 ± 0.7	4.2 ± 0.5	4.1 ± 0.8	3.4 ± 0.4
Sorbitol dehydrogenase (IU/L)	11 ± 1	14 ± 1	15 ± 2	14 ± 1	15 ± 1	13 ± 1
Urinalysis						
n	10	10	10	10	10	10
Creatinine (mg/16 hr)	8.41 ± 0.34	8.40 ± 0.18	8.59 ± 0.29	8.41 ± 0.32	8.82 ± 0.37	8.01 ± 0.46
Glucose (μg/mg creatinine)	110.5 ± 4.3	119.7 ± 5.1	119.1 ± 3.8	110.3 ± 1.5	121.6 ± 4.4*	130.6 ± 4.9**
Protein (μg/mg creatinine)	1,065.0 ± 46.0	889.5 ± 55.4	919.5 ± 45.8	818.1 ± 53.6*	886.4 ± 54.9	852.7 ± 89.4
Alkaline phosphatase (mU/mg creatinine)	143 ± 18	129 ± 13	136 ± 13	112 ± 7	134 ± 7	139 ± 15
Aspartate aminotransferase (mU/mg creatinine)	17 ± 2	15 ± 2	14 ± 1	13 ± 1	18 ± 3	16 ± 1
Volume (mL/16 hr)	12.8 ± 1.4	11.0 ± 0.8	11.7 ± 1.5	9.5 ± 1.0	13.7 ± 1.9	13.3 ± 2.2
Specific gravity	1.018 ± 0.002	1.020 ± 0.001	1.024 ± 0.004	1.026 ± 0.003	1.021 ± 0.002	1.021 ± 0.002

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
FEMALE						
Hematology						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 4	44.1 ± 0.4	44.4 ± 0.5	44.2 ± 0.5	43.9 ± 0.4	44.5 ± 0.6	44.5 ± 0.3
Day 24	45.2 ± 0.3	44.7 ± 0.2	45.3 ± 0.3	45.6 ± 0.3	44.7 ± 0.4	46.1 ± 0.3
Week 13	42.1 ± 0.5	43.4 ± 0.4*	43.3 ± 0.4	43.6 ± 0.2*	43.5 ± 0.2*	43.7 ± 0.3**
Hemoglobin (g/dL)						
Day 4	15.5 ± 0.2	15.6 ± 0.2	15.6 ± 0.2	15.5 ± 0.1	15.7 ± 0.2	15.8 ± 0.1
Day 24	16.4 ± 0.1	16.4 ± 0.1	16.4 ± 0.1	16.6 ± 0.1	16.1 ± 0.4	16.8 ± 0.1*
Week 13	15.6 ± 0.2	16.1 ± 0.1	16.2 ± 0.1*	16.3 ± 0.1**	16.2 ± 0.1**	16.3 ± 0.1**
Erythrocytes ($10^6/\mu$L)						
Day 4	8.96 ± 0.09	9.06 ± 0.13	8.95 ± 0.11	8.95 ± 0.09	9.07 ± 0.12	9.10 ± 0.07
Day 24	9.37 ± 0.07	9.32 ± 0.06	9.34 ± 0.06	9.44 ± 0.07	9.26 ± 0.09	9.55 ± 0.07
Week 13	9.06 ± 0.10	9.31 ± 0.08*	9.30 ± 0.07*	9.37 ± 0.05**	9.32 ± 0.03**	9.36 ± 0.05**
Reticulocytes ($10^6/\mu$L)						
Day 4	0.17 ± 0.02	0.15 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.19 ± 0.02	0.18 ± 0.02
Day 24	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.02	0.12 ± 0.02	0.18 ± 0.02*	0.15 ± 0.02
Week 13	0.12 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.15 ± 0.01
Nucleated erythrocytes ($10^3/\mu$L)						
Day 4	0.04 ± 0.03	0.00 ± 0.00	0.03 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.01 ± 0.01
Day 24	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.04 ± 0.02
Week 13	0.06 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.02 ± 0.01
Mean cell volume (fL)						
Day 4	49.2 ± 0.2	48.9 ± 0.2	49.4 ± 0.3	49.1 ± 0.2	49.1 ± 0.2	48.8 ± 0.1
Day 24	48.3 ± 0.2	48.0 ± 0.2	48.4 ± 0.2	48.3 ± 0.2	48.3 ± 0.2	48.2 ± 0.1
Week 13	46.4 ± 0.2	46.5 ± 0.2	46.6 ± 0.2	46.6 ± 0.2	46.9 ± 0.1*	46.8 ± 0.1*
Mean cell hemoglobin (pg)						
Day 4	17.3 ± 0.1	17.3 ± 0.1	17.4 ± 0.1	17.4 ± 0.1	17.3 ± 0.0	17.4 ± 0.0
Day 24	17.5 ± 0.0	17.6 ± 0.1	17.6 ± 0.1	17.5 ± 0.0	17.3 ± 0.3	17.6 ± 0.0
Week 13	17.2 ± 0.1	17.3 ± 0.1	17.4 ± 0.1*	17.4 ± 0.1	17.4 ± 0.1*	17.5 ± 0.1*
Mean cell hemoglobin concentration (g/dL)						
Day 4	35.1 ± 0.1	35.2 ± 0.1	35.2 ± 0.1	35.4 ± 0.1	35.3 ± 0.1	35.5 ± 0.1*
Day 24	36.2 ± 0.1	36.6 ± 0.1	36.2 ± 0.2	36.4 ± 0.1	35.9 ± 0.6	36.5 ± 0.1
Week 13	37.1 ± 0.1	37.0 ± 0.1	37.5 ± 0.1*	37.3 ± 0.1	37.3 ± 0.1	37.4 ± 0.1
Platelets ($10^3/\mu$L)						
Day 4	683.1 ± 19.2	693.7 ± 39.0	742.0 ± 20.0	717.5 ± 14.7	666.5 ± 26.4	662.6 ± 15.8
Day 24	586.2 ± 6.5	582.9 ± 8.9	579.5 ± 7.9	601.8 ± 9.0	561.5 ± 14.7	547.9 ± 12.1*
Week 13	527.7 ± 18.3	510.1 ± 9.8	513.8 ± 10.2	511.8 ± 14.3	506.5 ± 12.0	511.2 ± 8.7
Leukocytes ($10^3/\mu$L)						
Day 4	8.95 ± 0.33	9.17 ± 0.37	10.56 ± 0.42*	10.41 ± 0.32*	10.64 ± 0.32**	10.48 ± 0.17**
Day 24	4.96 ± 0.13	4.46 ± 0.18	4.69 ± 0.18	4.55 ± 0.32	4.57 ± 0.20	4.61 ± 0.48
Week 13	5.34 ± 0.29	4.41 ± 0.23	4.76 ± 0.27	4.96 ± 0.15	4.29 ± 0.31	4.82 ± 0.43
Segmented neutrophils ($10^3/\mu$L)						
Day 4	0.72 ± 0.13	0.64 ± 0.10	0.97 ± 0.13	1.00 ± 0.12	1.08 ± 0.09*	1.09 ± 0.17
Day 24	0.52 ± 0.05	0.38 ± 0.05	0.41 ± 0.07	0.43 ± 0.07	0.43 ± 0.06	0.52 ± 0.08
Week 13	1.09 ± 0.11	0.69 ± 0.06**	0.64 ± 0.06**	0.73 ± 0.06**	0.59 ± 0.04**	0.71 ± 0.10**

TABLE D2 Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
FEMALE (continued)						
Hematology (continued)						
Lymphocytes ($10^3/\mu$L)						
Day 4	8.03 ± 0.38	8.35 ± 0.35	9.29 ± 0.36*	9.19 ± 0.27*	9.29 ± 0.27*	9.11 ± 0.25*
Day 24	4.28 ± 0.14	3.92 ± 0.16	4.12 ± 0.20	3.90 ± 0.26	4.02 ± 0.16	3.91 ± 0.42
Week 13	4.07 ± 0.20	3.55 ± 0.20	3.98 ± 0.27	4.01 ± 0.16	3.55 ± 0.32	3.91 ± 0.37
Monocytes ($10^3/\mu$L)						
Day 4	0.15 ± 0.04	0.14 ± 0.04	0.23 ± 0.06	0.16 ± 0.03	0.17 ± 0.04	0.24 ± 0.05
Day 24	0.12 ± 0.03	0.13 ± 0.02	0.13 ± 0.03	0.17 ± 0.02	0.10 ± 0.02	0.10 ± 0.02
Week 13	0.16 ± 0.03	0.11 ± 0.02	0.12 ± 0.02	0.18 ± 0.02	0.10 ± 0.02	0.16 ± 0.02
Eosinophils ($10^3/\mu$L)						
Day 4	0.05 ± 0.02	0.04 ± 0.02	0.07 ± 0.03	0.06 ± 0.02	0.10 ± 0.03	0.04 ± 0.02
Day 24	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.07 ± 0.02
Week 13	0.02 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Total bone marrow cellularity (10^6/femur)						
Day 24	87.0 ± 3.0	83.3 ± 2.3	79.2 ± 2.5	85.2 ± 3.3[2]	77.8 ± 4.0	78.2 ± 4.6
Week 13	82.9 ± 3.5	74.3 ± 1.9	71.7 ± 3.4	79.1 ± 1.5	85.5 ± 9.7	77.2 ± 3.0
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	21.5 ± 0.6	22.4 ± 1.0	21.3 ± 0.9	19.9 ± 1.0	20.3 ± 0.9	18.1 ± 0.8**
Creatinine (mg/dL)	0.71 ± 0.02	0.75 ± 0.03	0.69 ± 0.02	0.73 ± 0.03	0.69 ± 0.02	0.71 ± 0.02
Alanine aminotransferase (IU/L)	47 ± 4	44 ± 4	54 ± 4	46 ± 4	48 ± 5	45 ± 3
Glutamate dehydrogenase (IU/L)	3.5 ± 0.3	3.2 ± 0.3	3.1 ± 0.4	3.2 ± 0.4	3.1 ± 0.3	2.5 ± 0.3*
Sorbitol dehydrogenase (IU/L)	12 ± 1	11 ± 1	11 ± 1	12 ± 1	11 ± 1	11 ± 1
Urinalysis						
n	10	10	10	10	10	10
Creatinine (mg/16 hr)	4.13 ± 0.24	4.32 ± 0.25	4.35 ± 0.14	4.43 ± 0.13	4.29 ± 0.22	4.14 ± 0.19
Glucose (μg/mg creatinine)	118.4 ± 4.7	120.9 ± 3.2	118.6 ± 2.8	118.5 ± 5.9	119.5 ± 5.4	116.8 ± 1.8
Protein (μg/mg creatinine)	172.2 ± 13.3	144.4 ± 8.6	157.1 ± 5.2	151.3 ± 6.6	139.5 ± 9.2	135.4 ± 5.2*
Alkaline phosphatase (mU/mg creatinine)	132 ± 10	144 ± 11	142 ± 17	140 ± 13	115 ± 8	135 ± 11
Aspartate aminotransferase (mU/mg creatinine)	11 ± 3	9 ± 0	11 ± 1	8 ± 1	8 ± 1	8 ± 2
Volume (mL/16 hr)	8.2 ± 1.0[2]	8.0 ± 1.0	8.2 ± 0.9	9.1 ± 1.3	8.6 ± 0.8	8.4 ± 0.9
Specific gravity	1.017 ± 0.002	1.020 ± 0.002	1.019 ± 0.002	1.019 ± 0.002	1.019 ± 0.001	1.018 ± 0.001

[1] Data are given as mean ± standard error. Urine samples were collected during Week 12 of the study.
[2] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE D3 Hematology Data for Male F344/N Rats After 6 Months of Exposure in the Stop-Exposure Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
n	10	10	10	10	10	10
Hematocrit (%)	48.3 ± 0.4	47.1 ± 0.5	47.1 ± 0.3	47.9 ± 0.4	48.3 ± 0.4	47.8 ± 0.3
Hemoglobin (g/dL)	16.1 ± 0.1	15.8 ± 0.1	15.8 ± 0.1	16.0 ± 0.1	16.1 ± 0.1	15.9 ± 0.1
Erythrocytes ($10^6/\mu$L)	9.37 ± 0.07	9.19 ± 0.07	9.26 ± 0.04	9.30 ± 0.03	9.34 ± 0.07	9.24 ± 0.08
Reticulocytes ($10^6/\mu$L)	0.12 ± 0.01	0.15 ± 0.02	0.12 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.13 ± 0.01
Nucleated erythrocytes ($10^3/\mu$L)	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.02
Mean cell volume (fL)	51.4 ± 0.2	51.0 ± 0.2	50.9 ± 0.2	51.6 ± 0.2	51.8 ± 0.2	51.5 ± 0.3
Mean cell hemoglobin (pg)	17.2 ± 0.0	17.2 ± 0.0	17.1 ± 0.0	17.2 ± 0.1	17.2 ± 0.1	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.1	33.7 ± 0.2	33.6 ± 0.1	33.4 ± 0.1	33.3 ± 0.1	33.4 ± 0.1
Platelets ($10^3/\mu$L)	538.5 ± 9.1	539.1 ± 10.7	543.1 ± 9.6	522.1 ± 14.0	533.4 ± 13.4	538.8 ± 10.1
Leukocytes ($10^3/\mu$L)	4.86 ± 0.24	4.68 ± 0.17	5.64 ± 0.42	5.03 ± 0.30	5.59 ± 0.39	5.17 ± 0.22
Segmented neutrophils ($10^3/\mu$L)	0.91 ± 0.18	0.90 ± 0.07	1.11 ± 0.15	0.87 ± 0.13	1.12 ± 0.15	0.86 ± 0.14
Lymphocytes ($10^3/\mu$L)	3.85 ± 0.24	3.56 ± 0.15	4.39 ± 0.36	4.01 ± 0.31	4.24 ± 0.30	4.20 ± 0.16
Monocytes ($10^3/\mu$L)	0.10 ± 0.02	0.19 ± 0.03	0.13 ± 0.03	0.09 ± 0.03	0.20 ± 0.04	0.10 ± 0.03
Eosinophils ($10^3/\mu$L)	0.01 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.05 ± 0.02	0.03 ± 0.02	0.02 ± 0.01

[1] Data are given as mean ± standard error.

TABLE D4 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 2-Week Inhalation Study of Isoprene[1]

	0 ppm	438 ppm	875 ppm	1,750 ppm	3,500 ppm	7,000 ppm
MALE						
n	10	9	10	10	10	10
Hematology						
Hematocrit (%)	48.9 ± 0.6	44.9 ± 0.4**	45.2 ± 0.4**	43.2 ± 1.5**	44.6 ± 0.2**	44.7 ± 0.4**
Hemoglobin (g/dL)	16.4 ± 0.2	15.1 ± 0.1**	15.2 ± 0.1**	14.6 ± 0.5**	15.0 ± 0.1**	15.0 ± 0.1**
Erythrocytes ($10^6/\mu$L)	9.90 ± 0.11	9.11 ± 0.11**	9.19 ± 0.08**	8.76 ± 0.32**	9.02 ± 0.04**	9.19 ± 0.06**
Reticulocytes ($10^6/\mu$L)	0.11 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.04 ± 0.01**
Howell-Jolly bodies ($10^3/\mu$L)	12.9 ± 3.7	19.2 ± 4.9	26.8 ± 3.0	23.0 ± 4.3	14.5 ± 2.4	24.7 ± 4.0
Mean cell volume (fL)	49.4 ± 0.4	49.3 ± 0.5	49.3 ± 0.4	49.4 ± 0.3	49.5 ± 0.3	48.7 ± 0.5
Mean cell hemoglobin (pg)	16.6 ± 0.1	16.6 ± 0.1	16.5 ± 0.1	16.7 ± 0.2	16.6 ± 0.1	16.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.5 ± 0.1	33.6 ± 0.2	33.7 ± 0.1	33.8 ± 0.2	33.5 ± 0.1	33.5 ± 0.2
Platelets ($10^3/\mu$L)	1,002 ± 32	1,059 ± 34	1,143 ± 43*	1,179 ± 28**	1,146 ± 41**	1,227 ± 31**
Leukocytes ($10^3/\mu$L)	4.93 ± 0.66	4.82 ± 0.60	4.40 ± 0.40	4.48 ± 0.38	4.20 ± 0.31	4.04 ± 0.22
Segmented neutrophils ($10^3/\mu$L)	0.52 ± 0.05	0.86 ± 0.15	0.76 ± 0.13	0.69 ± 0.08	0.95 ± 0.18	0.81 ± 0.09*
Lymphocytes ($10^3/\mu$L)	4.23 ± 0.58	3.80 ± 0.52	3.41 ± 0.34	3.66 ± 0.35	3.01 ± 0.22	3.03 ± 0.13
Monocytes ($10^3/\mu$L)	0.18 ± 0.06	0.14 ± 0.03	0.22 ± 0.06	0.11 ± 0.02	0.17 ± 0.03	0.18 ± 0.03
Eosinophils ($10^3/\mu$L)	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Clinical Chemistry						
Urea nitrogen (mg/dL)	27.0 ± 2.0	26.4 ± 2.8	25.7 ± 2.5	23.1 ± 1.8	24.8 ± 1.4	22.4 ± 1.3
Creatinine (mg/dL)	0.55 ± 0.04	0.48 ± 0.02	0.51 ± 0.02	0.49 ± 0.02	0.48 ± 0.01	0.48 ± 0.02
Alanine aminotransferase (IU/L)	119 ± 18	134 ± 26	86 ± 12	206 ± 82	102 ± 15	81 ± 12
Glutamate dehydrogenase (IU/L)	7.6 ± 0.6	6.3 ± 0.4*	6.1 ± 1.0*	6.4 ± 0.9	6.6 ± 0.6	5.4 ± 0.3**
Sorbitol dehydrogenase (IU/L)	34 ± 1	29 ± 1**	29 ± 1*	29 ± 1**	31 ± 2*	30 ± 2*
FEMALE						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	48.1 ± 0.2	45.6 ± 0.3**	45.5 ± 0.2**	45.3 ± 0.3**	45.9 ± 0.5**	45.2 ± 0.4**
Hemoglobin (g/dL)	16.4 ± 0.1	15.6 ± 0.1**	15.4 ± 0.1**	15.5 ± 0.1**	15.7 ± 0.2**	15.4 ± 0.1**
Erythrocytes ($10^6/\mu$L)	9.74 ± 0.06	9.20 ± 0.09**	9.04 ± 0.06**	9.20 ± 0.10**	9.24 ± 0.10**	9.06 ± 0.11**
Reticulocytes ($10^6/\mu$L)	0.07 ± 0.01	0.04 ± 0.01	0.04 ± 0.01**	0.03 ± 0.01**	0.04 ± 0.01*	0.03 ± 0.01**
Howell-Jolly bodies ($10^3/\mu$L)	18.5 ± 4.7	19.3 ± 6.3	20.8 ± 2.0	22.1 ± 4.4	23.2 ± 5.6	18.8 ± 4.2
Mean cell volume (fL)	49.6 ± 0.3	49.6 ± 0.4	50.4 ± 0.4	49.1 ± 0.4	49.7 ± 0.3	49.9 ± 0.4
Mean cell hemoglobin (pg)	16.8 ± 0.1	16.9 ± 0.1	17.1 ± 0.1	16.8 ± 0.1	17.0 ± 0.1	17.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.1 ± 0.1	34.2 ± 0.1	33.9 ± 0.1	34.2 ± 0.1	34.1 ± 0.1	34.2 ± 0.2
Platelets ($10^3/\mu$L)	883.4 ± 15.3	988.7 ± 26.0**	984.8 ± 17.6**	976.3 ± 20.9**	1,021.0 ± 28.2**	1,001.5 ± 15.2**
Leukocytes ($10^3/\mu$L)	3.52 ± 0.41	4.43 ± 0.14*	4.08 ± 0.38	3.44 ± 0.19	3.55 ± 0.25	3.61 ± 0.32
Segmented neutrophils ($10^3/\mu$L)	0.42 ± 0.07	0.65 ± 0.09	0.40 ± 0.08[2]	0.43 ± 0.03	0.42 ± 0.05	0.50 ± 0.05
Lymphocytes ($10^3/\mu$L)	2.96 ± 0.38	3.58 ± 0.15	3.11 ± 0.36	2.87 ± 0.20	3.01 ± 0.24	2.96 ± 0.33
Monocytes ($10^3/\mu$L)	0.13 ± 0.02	0.19 ± 0.02	0.23 ± 0.06	0.14 ± 0.03	0.10 ± 0.01	0.14 ± 0.02
Eosinophils ($10^3/\mu$L)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01

TABLE D4 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 2-Week Inhalation Study of Isoprene (continued)

	0 ppm	438 ppm	875 ppm	1,750 ppm	3,500 ppm	7,000 ppm
FEMALE (continued)						
Clinical Chemistry						
Urea nitrogen (mg/dL)	26.4 ± 2.1	21.0 ± 1.5	22.5 ± 1.8	25.9 ± 2.2	23.8 ± 1.5	25.3 ± 1.8
Creatinine (mg/dL)	0.51 ± 0.03[2]	0.46 ± 0.02	0.47 ± 0.02	0.47 ± 0.02	0.44 ± 0.02	0.43 ± 0.02*
Alanine aminotransferase (IU/L)	88 ± 24	48 ± 5	57 ± 9	67 ± 14	98 ± 29	75 ± 14
Glutamate dehydrogenase (IU/L)	5.7 ± 0.8	4.1 ± 0.2	4.3 ± 0.2[2]	5.0 ± 0.6	5.6 ± 0.5	5.2 ± 0.7
Sorbitol dehydrogenase (IU/L)	28 ± 1	25 ± 1	27 ± 1	29 ± 3	27 ± 1	29 ± 1

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

TABLE D5 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
MALE						
Hematology						
n						
Day 4	10	10	10	10	10	10
Day 24	10	10	7	10	10	9
Week 13	10	10	10	10	10	10
Hematocrit (%)						
Day 4	47.5 ± 0.3	47.3 ± 0.5	45.8 ± 0.4*	43.9 ± 0.3**	44.1 ± 0.4**	43.1 ± 0.5**
Day 24	48.9 ± 0.3	49.1 ± 0.2	49.0 ± 0.4	45.9 ± 0.5**	46.2 ± 0.3**	43.8 ± 0.4**
Week 13	48.3 ± 0.4	49.1 ± 0.3	48.3 ± 0.4	44.9 ± 0.4**	45.4 ± 0.3**	42.2 ± 0.3**
Hemoglobin (g/dL)						
Day 4	16.1 ± 0.1	16.0 ± 0.1	15.4 ± 0.2**	14.9 ± 0.1**	14.9 ± 0.1**	14.7 ± 0.2**
Day 24	16.7 ± 0.1	16.8 ± 0.1	16.6 ± 0.1	15.7 ± 0.1**	15.7 ± 0.1**	15.0 ± 0.1**
Week 13	16.8 ± 0.1	17.0 ± 0.1	16.7 ± 0.1	15.7 ± 0.1**	15.7 ± 0.1**	14.6 ± 0.1**
Erythrocytes ($10^6/\mu$L)						
Day 4	10.16 ± 0.07	9.94 ± 0.11	9.63 ± 0.13**	9.25 ± 0.07**	9.21 ± 0.05**	9.09 ± 0.11**
Day 24	10.47 ± 0.08	10.46 ± 0.06	10.37 ± 0.08	9.39 ± 0.09**	9.54 ± 0.06**	8.92 ± 0.07**
Week 13	10.81 ± 0.06	10.80 ± 0.06	10.65 ± 0.06	9.76 ± 0.04**	9.72 ± 0.05**	8.80 ± 0.09**
Reticulocytes ($10^6/\mu$L)						
Day 4	0.29 ± 0.02	0.32 ± 0.03	0.38 ± 0.03	0.18 ± 0.02*	0.17 ± 0.03*	0.11 ± 0.02**
Day 24	0.20 ± 0.03	0.23 ± 0.03	0.14 ± 0.03	0.16 ± 0.02	0.13 ± 0.02	0.08 ± 0.01**[2]
Week 13	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.12 ± 0.02	0.13 ± 0.01	0.13 ± 0.02
Howell-Jolly bodies ($10^3/\mu$L)						
Day 4	18.2 ± 4.5	24.0 ± 7.3	17.3 ± 5.2	14.8 ± 3.5	23.1 ± 6.1	18.9 ± 4.3
Day 24	18.8 ± 3.0	14.6 ± 2.3	29.8 ± 7.0	36.5 ± 6.1*	46.8 ± 8.8*	66.3 ± 12.2**[2]
Week 13	5.4 ± 2.4	5.4 ± 2.4	9.5 ± 2.9	19.5 ± 5.6	29.1 ± 6.6**	23.0 ± 4.5**
Mean cell volume (fL)						
Day 4	46.8 ± 0.2	47.5 ± 0.4	47.5 ± 0.4	47.4 ± 0.4	47.8 ± 0.3	47.4 ± 0.3
Day 24	46.7 ± 0.2	47.0 ± 0.2	47.3 ± 0.2*	49.1 ± 0.2**	48.5 ± 0.2**	48.9 ± 0.3**
Week 13	44.6 ± 0.3	45.4 ± 0.3	45.2 ± 0.3	45.9 ± 0.4**	46.8 ± 0.2**	47.9 ± 0.4**
Mean cell hemoglobin (pg)						
Day 4	15.9 ± 0.1	16.0 ± 0.1	16.0 ± 0.2	16.1 ± 0.1	16.2 ± 0.1*	16.2 ± 0.1*
Day 24	16.0 ± 0.1	16.0 ± 0.1	16.0 ± 0.1	16.7 ± 0.1**	16.5 ± 0.1**	16.8 ± 0.1**
Week 13	15.6 ± 0.1	15.8 ± 0.1	15.7 ± 0.1	16.1 ± 0.1**	16.2 ± 0.1**	16.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.9 ± 0.1	33.8 ± 0.2	33.6 ± 0.2	33.9 ± 0.1	33.7 ± 0.2	34.1 ± 0.1
Day 24	34.2 ± 0.1	34.1 ± 0.1	33.8 ± 0.3	34.1 ± 0.2	34.0 ± 0.2	34.3 ± 0.1
Week 13	34.9 ± 0.2	34.7 ± 0.1	34.7 ± 0.1	34.9 ± 0.1	34.6 ± 0.1	34.5 ± 0.1
Platelets ($10^3/\mu$L)						
Day 4	846.5 ± 20.0	880.4 ± 27.1	875.7 ± 15.6	865.3 ± 24.5	860.8 ± 20.7	850.0 ± 35.3
Day 24	792.1 ± 18.1	808.5 ± 23.1	828.9 ± 19.2	865.7 ± 21.1*[3]	813.1 ± 20.0	870.3 ± 32.0*
Week 13	893.2 ± 20.3	843.6 ± 25.7	875.7 ± 30.0	965.9 ± 14.0*	966.8 ± 12.9*	1118.5 ± 18.3**
Leukocytes ($10^3/\mu$L)						
Day 4	6.25 ± 0.45	5.54 ± 0.28	5.24 ± 0.28	4.95 ± 0.38*	5.34 ± 0.29	4.34 ± 0.33**
Day 24	2.54 ± 0.28	2.72 ± 0.30	2.74 ± 0.42	2.84 ± 0.30	2.66 ± 0.21	1.96 ± 0.16[2]
Week 13	4.33 ± 0.46	3.78 ± 0.37	3.33 ± 0.30	3.42 ± 0.40	3.20 ± 0.38	2.44 ± 0.26**

TABLE D5 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
MALE (continued)						
Hematology (continued)						
Segmented neutrophils ($10^3/\mu$L)						
Day 4	1.15 ± 0.19	0.91 ± 0.08	0.76 ± 0.12	0.53 ± 0.04**	0.62 ± 0.09**	0.45 ± 0.05**
Day 24	0.33 ± 0.06	0.45 ± 0.14	0.43 ± 0.09	0.38 ± 0.04	0.32 ± 0.05	0.32 ± 0.06[2]
Week 13	1.01 ± 0.15	0.78 ± 0.10	0.62 ± 0.08	0.78 ± 0.12	0.76 ± 0.12	0.67 ± 0.09
Lymphocytes ($10^3/\mu$L)						
Day 4	4.78 ± 0.34	4.32 ± 0.29	4.17 ± 0.21	4.16 ± 0.35	4.43 ± 0.26	3.65 ± 0.29*
Day 24	2.15 ± 0.22	2.17 ± 0.18	2.22 ± 0.32	2.39 ± 0.27	2.26 ± 0.18	1.59 ± 0.11[2]
Week 13	3.06 ± 0.33	2.85 ± 0.29	2.55 ± 0.21	2.50 ± 0.29	2.27 ± 0.27	1.64 ± 0.15**
Monocytes ($10^3/\mu$L)						
Day 4	0.29 ± 0.04	0.27 ± 0.02	0.29 ± 0.04	0.25 ± 0.05	0.27 ± 0.05	0.23 ± 0.03
Day 24	0.06 ± 0.02	0.10 ± 0.02	0.09 ± 0.03	0.07 ± 0.01	0.08 ± 0.02	0.05 ± 0.01[2]
Week 13	0.24 ± 0.05	0.12 ± 0.03	0.15 ± 0.03	0.10 ± 0.03	0.16 ± 0.03	0.12 ± 0.03
Eosinophils ($10^3/\mu$L)						
Day 4	0.03 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
Day 24	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[2]
Week 13	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Total bone marrow cellularity (10^6/femur)						
Day 24	21.5 ± 1.4	22.2 ± 1.6[3]	19.7 ± 1.3	18.5 ± 1.2[3]	18.6 ± 0.9	14.9 ± 1.1**
Week 13	22.5 ± 2.6	20.9 ± 1.7	19.9 ± 1.7	18.1 ± 2.2	20.2 ± 1.2	18.1 ± 1.2
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	23.8 ± 1.1	25.1 ± 1.8	25.2 ± 1.8	20.2 ± 1.6	19.6 ± 1.1	20.5 ± 1.1
Creatinine (mg/dL)	0.51 ± 0.06	0.57 ± 0.03	0.56 ± 0.03	0.48 ± 0.03	0.49 ± 0.02	0.55 ± 0.02
Alanine aminotransferase (IU/L)	49 ± 5	39 ± 4	39 ± 4[3]	40 ± 4	43 ± 3	39 ± 2
Glutamate dehydrogenase (IU/L)	3.2 ± 0.2[3]	3.1 ± 0.2	3.5 ± 0.4	3.2 ± 0.3	3.1 ± 0.2	2.9 ± 0.3*
Sorbitol dehydrogenase (IU/L)	31 ± 1	33 ± 2	29 ± 2	30 ± 1	32 ± 3	26 ± 1**

TABLE D5 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
FEMALE						
Hematology						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 4	46.7 ± 0.3	46.1 ± 0.3	45.0 ± 0.4**	44.2 ± 0.3**	43.5 ± 0.3**	43.4 ± 0.4**
Day 24	47.2 ± 0.5	47.3 ± 0.2	46.4 ± 0.2*	44.8 ± 0.3**	44.8 ± 0.3**	43.6 ± 0.3**
Week 13	47.9 ± 0.5	47.2 ± 0.2	47.3 ± 0.4	46.5 ± 0.2**	46.3 ± 0.3**	45.1 ± 0.2**
Hemoglobin (g/dL)						
Day 4	15.8 ± 0.1	15.6 ± 0.1	15.3 ± 0.1**	15.1 ± 0.1**	14.8 ± 0.2**	14.9 ± 0.1**
Day 24	16.2 ± 0.2	16.3 ± 0.1	15.9 ± 0.1*	15.4 ± 0.1**	15.5 ± 0.1**	15.0 ± 0.1**
Week 13	16.6 ± 0.2	16.6 ± 0.1	16.4 ± 0.1	16.0 ± 0.1**	16.0 ± 0.1**	15.7 ± 0.1**
Erythrocytes ($10^6/\mu$L)						
Day 4	9.65 ± 0.09	9.68 ± 0.05	9.43 ± 0.11	9.24 ± 0.09**	9.12 ± 0.12**	9.16 ± 0.11**
Day 24	9.86 ± 0.14	9.88 ± 0.05	9.61 ± 0.06*	9.17 ± 0.07**	9.21 ± 0.07**	9.01 ± 0.08**
Week 13	10.79 ± 0.07	10.54 ± 0.03**	10.40 ± 0.12**	9.96 ± 0.05**	9.96 ± 0.07**	9.61 ± 0.04**
Reticulocytes ($10^6/\mu$L)						
Day 4	0.38 ± 0.03	0.35 ± 0.03	0.36 ± 0.03	0.20 ± 0.03**	0.17 ± 0.03**	0.15 ± 0.02**
Day 24	0.22 ± 0.02[3]	0.23 ± 0.02	0.22 ± 0.02	0.21 ± 0.01	0.19 ± 0.02	0.19 ± 0.02
Week 13	0.14 ± 0.01	0.09 ± 0.01*	0.12 ± 0.02	0.14 ± 0.02	0.13 ± 0.01	0.13 ± 0.01
Howell-Jolly bodies ($10^3/\mu$L)						
Day 4	21.1 ± 3.3	15.5 ± 4.2	18.0 ± 5.6	21.1 ± 5.0	13.9 ± 3.8	11.0 ± 2.3*
Day 24	13.3 ± 3.7[3]	16.7 ± 6.7	26.1 ± 4.4	41.1 ± 6.4*	32.5 ± 7.5	27.0 ± 4.5
Week 13	12.9 ± 3.1	9.4 ± 3.3	12.5 ± 2.1	20.0 ± 5.8	17.9 ± 4.4	35.5 ± 4.3
Mean cell volume (fL)						
Day 4	48.4 ± 0.2	47.6 ± 0.3	47.6 ± 0.3*	47.9 ± 0.3	47.7 ± 0.4	47.5 ± 0.3*
Day 24	47.9 ± 0.3	47.9 ± 0.3	48.4 ± 0.2	48.8 ± 0.3*	48.6 ± 0.2*	48.5 ± 0.2
Week 13	44.4 ± 0.4	44.8 ± 0.3	45.5 ± 0.2*	46.6 ± 0.2**	46.2 ± 0.1**	47.1 ± 0.2**
Mean cell hemoglobin (pg)						
Day 4	16.4 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.3 ± 0.1	16.3 ± 0.1	16.3 ± 0.1
Day 24	16.5 ± 0.1	16.5 ± 0.1	16.5 ± 0.0	16.8 ± 0.1**	16.8 ± 0.0**	16.7 ± 0.1**
Week 13	15.4 ± 0.1	15.7 ± 0.1*	15.7 ± 0.1*	16.0 ± 0.1**	16.1 ± 0.1**	16.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.8 ± 0.0	33.9 ± 0.1	33.9 ± 0.1	34.1 ± 0.1*	34.1 ± 0.1*	34.3 ± 0.1**
Day 24	34.4 ± 0.1	34.4 ± 0.1	34.2 ± 0.1	34.3 ± 0.1	34.5 ± 0.1	34.5 ± 0.1
Week 13	34.7 ± 0.2	35.1 ± 0.1	34.6 ± 0.1	34.4 ± 0.1	34.6 ± 0.1	34.8 ± 0.1
Platelets ($10^3/\mu$L)						
Day 4	809.2 ± 17.6	803.9 ± 14.9	803.3 ± 15.2	853.9 ± 19.4	780.7 ± 13.0	831.4 ± 6.8
Day 24	763.9 ± 17.7	795.2 ± 19.5	780.6 ± 15.4	782.9 ± 9.5	782.9 ± 7.4	754.8 ± 19.6
Week 13	838.8 ± 16.0	860.1 ± 17.2	847.8 ± 12.7	853.9 ± 18.5	851.3 ± 14.6	856.6 ± 16.2
Leukocytes ($10^3/\mu$L)						
Day 4	4.28 ± 0.23	4.66 ± 0.23	4.92 ± 0.21	4.93 ± 0.16	4.73 ± 0.23	4.53 ± 0.16
Day 24	1.66 ± 0.21	1.57 ± 0.15	1.49 ± 0.09	1.71 ± 0.10	1.60 ± 0.17	1.62 ± 0.18
Week 13	4.20 ± 0.54	3.99 ± 0.42	4.05 ± 0.54	3.86 ± 0.36	3.10 ± 0.25	2.65 ± 0.11**
Segmented neutrophils ($10^3/\mu$L)						
Day 4	0.48 ± 0.04	0.65 ± 0.07	0.52 ± 0.09	0.50 ± 0.05	0.38 ± 0.05	0.52 ± 0.06
Day 24	0.21 ± 0.05	0.14 ± 0.02	0.15 ± 0.03	0.20 ± 0.02	0.23 ± 0.04	0.27 ± 0.03
Week 13	0.98 ± 0.38	0.61 ± 0.10	0.87 ± 0.13	0.67 ± 0.11	0.37 ± 0.06*	0.40 ± 0.06*

TABLE D5 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
FEMALE (continued)						
Hematology (continued)						
Lymphocytes ($10^3/\mu$L)						
Day 4	3.48 ± 0.22	3.70 ± 0.22	4.09 ± 0.18	4.15 ± 0.15	4.08 ± 0.23	3.74 ± 0.13
Day 24	1.38 ± 0.15	1.37 ± 0.14	1.31 ± 0.09	1.40 ± 0.08	1.30 ± 0.13	1.27 ± 0.15
Week 13	2.95 ± 0.22	3.20 ± 0.37	2.94 ± 0.40	2.92 ± 0.27	2.57 ± 0.20	2.12 ± 0.09**
Monocytes ($10^3/\mu$L)						
Day 4	0.31 ± 0.02	0.29 ± 0.03	0.26 ± 0.06	0.25 ± 0.04	0.23 ± 0.03	0.23 ± 0.04
Day 24	0.06 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.02
Week 13	0.26 ± 0.08	0.16 ± 0.02	0.22 ± 0.04	0.24 ± 0.06	0.14 ± 0.02	0.12 ± 0.02
Eosinophils ($10^3/\mu$L)						
Day 4	0.01 ± 0.01	0.02 ± 0.01	0.06 ± 0.02*	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.01
Day 24	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.03 ± 0.01*	0.01 ± 0.01	0.01 ± 0.01
Week 13	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
Total bone marrow cellularity (10^6/femur)						
Day 24	19.5 ± 1.3[2]	20.6 ± 1.1	19.0 ± 1.3[3]	18.4 ± 1.0[3]	19.6 ± 1.1	18.1 ± 1.0[2]
Week 13	18.4 ± 1.6[3]	18.2 ± 1.4	20.3 ± 1.4	18.0 ± 0.9	16.2 ± 1.4	17.6 ± 1.5
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	20.7 ± 0.7	23.1 ± 1.0	20.5 ± 1.2	21.8 ± 0.9	21.4 ± 1.3	17.2 ± 0.6*
Creatinine (mg/dL)	0.58 ± 0.03	0.43 ± 0.02*	0.45 ± 0.03*	0.54 ± 0.03	0.49 ± 0.03	0.44 ± 0.03*
Alanine aminotransferase (IU/L)	29 ± 2	33 ± 2	35 ± 4	47 ± 10	37 ± 4	40 ± 7
Glutamate dehydrogenase (IU/L)	3.0 ± 0.4	2.9 ± 0.2	3.7 ± 0.4	2.9 ± 0.2	3.0 ± 0.2	3.0 ± 0.2
Sorbitol dehydrogenase (IU/L)	22 ± 1	24 ± 2	23 ± 2	29 ± 4	24 ± 1	25 ± 3

[1] Data are given as mean ± standard error.
[2] n=8.
[3] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE D6 Hematology Data for Male B6C3F$_1$ Mice After 6 Months of Exposure in the Stop-Exposure Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	220 ppm	700 ppm	2,200 ppm	7,000 ppm
n	10	10	10	10	10	10
Manual hematocrit (%)	49.9 ± 0.5	49.7 ± 0.4	48.6 ± 0.8	48.3 ± 0.4	49.2 ± 0.3	46.3 ± 0.6**
Automated hematocrit (%)	50.2 ± 0.6	50.3 ± 0.3	50.0 ± 0.4	48.5 ± 0.6	49.2 ± 0.5	46.1 ± 0.7**
Hemoglobin (g/dL)	16.2 ± 0.2	16.4 ± 0.1	16.2 ± 0.1	15.6 ± 0.1*	15.8 ± 0.1*	14.7 ± 0.2**
Erythrocytes ($10^6/\mu$L)	10.47 ± 0.13	10.38 ± 0.07	10.21 ± 0.10	9.68 ± 0.11**	9.70 ± 0.10**	9.01 ± 0.14**
Reticulocytes ($10^6/\mu$L)	0.11 ± 0.01	0.12 ± 0.02	0.12 ± 0.02	0.10 ± 0.02	0.08 ± 0.01	0.19 ± 0.03
Nucleated erythrocytes ($10^3/\mu$L)	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
Howell-Jolly bodies ($10^3/\mu$L)	13.7 ± 4.8	10.4 ± 3.8	7.1 ± 2.2	22.2 ± 4.8	21.6 ± 3.8	33.0 ± 5.9
Mean cell volume (fL)	48.2 ± 0.2	48.5 ± 0.3	48.8 ± 0.3	50.2 ± 0.3**	50.6 ± 0.3**	51.0 ± 0.2**
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.8 ± 0.1*	15.9 ± 0.1**	16.2 ± 0.1**	16.3 ± 0.1**	16.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.2 ± 0.2	32.5 ± 0.1	32.5 ± 0.1	32.2 ± 0.2	32.1 ± 0.1	31.9 ± 0.1
Platelets ($10^3/\mu$L)	935.4 ± 19.7	954.0 ± 16.4	932.2 ± 17.4	835.9 ± 7.6**	896.4 ± 12.9	977.8 ± 26.0
Leukocytes ($10^3/\mu$L)	2.08 ± 0.30	2.39 ± 0.25	2.40 ± 0.36	2.91 ± 0.49	3.44 ± 0.50*	2.61 ± 0.18
Segmented neutrophils ($10^3/\mu$L)	0.36 ± 0.09	0.26 ± 0.06	0.30 ± 0.08	0.57 ± 0.16	0.74 ± 0.14	0.83 ± 0.12*
Lymphocytes ($10^3/\mu$L)	1.59 ± 0.24	2.02 ± 0.21	1.98 ± 0.29	2.18 ± 0.33	2.53 ± 0.37	1.68 ± 0.21
Monocytes ($10^3/\mu$L)	0.10 ± 0.03	0.09 ± 0.03	0.10 ± 0.02	0.13 ± 0.03	0.17 ± 0.03	0.10 ± 0.02
Eosinophils ($10^3/\mu$L)	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.00	0.03 ± 0.01	0.01 ± 0.01	0.00 ± 0.00

[1] Data are given as mean ± standard error.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

APPENDIX E

Reproductive Tissue Evaluations, Estrous Cycle Characterization, and Teratology Studies

Table E1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Inhalation Study of Isoprene E-2

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Table E3 Summary of Reproductive Tissue Evaluations in Male B6C3F_1 Mice in the 13-Week Inhalation Study of Isoprene E-3

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TABLE E1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Inhalation Study of Isoprene[1]

Study Parameters	0 ppm	70 ppm	700 ppm	7,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	342 ± 4	344 ± 9	342 ± 7	340 ± 4
Left cauda epididymis	0.142 ± 0.003	0.128 ± 0.007	0.127 ± 0.008	0.129 ± 0.004
Left testis	1.44 ± 0.02	1.41 ± 0.02	1.40 ± 0.03	1.44 ± 0.03
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.85 ± 0.29	8.77 ± 0.22	9.21 ± 0.41	8.79 ± 0.43
Spermatid heads (10^7/testis)	12.76 ± 0.48	12.38 ± 0.28	12.82 ± 0.52	12.65 ± 0.55
Spermatid count (mean/10^{-4} mL suspension)	63.80 ± 2.42	61.88 ± 1.42	64.08 ± 2.62	63.23 ± 2.75
Epididymal spermatozoal measurements				
Motility (%)	95.62 ± 0.37	95.77 ± 0.54	94.31 ± 0.42*	93.98 ± 0.93
Concentration (10^6/g cauda epididymal tissue)	564.2 ± 19.5	594.3 ± 38.4	610.1 ± 40.4	547.6 ± 21.4

[1] Data are presented as mean ± standard error. Differences from the control group for necropsy body weights are not significant by Dunnett's test. Differences from the control group for cauda epididymal and testis weights, spermatid measurements, and spermatozoal concentrations are not significant by Dunn's test. Left epididymal weights for male rats were not available due to a technical error at necropsy.

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

TABLE E2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Inhalation Study of Isoprene[1]

Study Parameters	0 ppm	70 ppm	700 ppm	7,000 ppm
n	10	10	10	10
Necropsy body weight	201 ± 3	208 ± 3	204 ± 3	208 ± 3
Estrous cycle length (days)	4.90 ± 0.07	4.90 ± 0.10	5.05 ± 0.14	5.00 ± 0.07
Estrous stages (% of cycle)				
Diestrus	39.2	40.0	41.7	43.3
Proestrus	20.0	19.2	15.8	16.7
Estrus	25.0	18.3	22.5	20.8
Metestrus	15.8	22.5	19.2	19.2
Uncertain diagnoses (%)	0.0	0.0	0.8	0.0

[1] Necropsy body weights and estrous cycle lengths are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test. By multivariate analysis of variance, exposed groups do not differ significantly from the controls in cycle length or in the relative length of time spent in the estrous stages.

TABLE E3 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene[1]

Study Parameters	0 ppm	70 ppm	700 ppm	7,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	36.4 ± 0.8	36.1 ± 0.7	37.4 ± 0.6	36.7 ± 0.9
Left epididymis	0.043 ± 0.001	0.043 ± 0.002	0.038 ± 0.001**	0.030 ± 0.001**
Left cauda epididymis	0.015 ± 0.001	0.014 ± 0.001	0.013 ± 0.001*	0.009 ± 0.001**
Left testis	0.113 ± 0.003	0.122 ± 0.005	0.107 ± 0.001	0.071 ± 0.003**
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.87 ± 0.57	18.46 ± 0.84	18.67 ± 0.82	17.29 ± 0.69*
Spermatid heads (10^7/testis)	2.25 ± 0.09	2.24 ± 0.09	1.99 ± 0.07*	1.22 ± 0.06**
Spermatid count (mean/10^{-4} mL suspension)	70.43 ± 2.67	69.88 ± 2.94	62.13 ± 2.33*	38.08 ± 2.00**
Epididymal spermatozoal measurements				
Motility (%)	94.38 ± 0.49[2]	92.93 ± 1.26	89.05 ± 1.23**	72.40 ± 2.28**
Concentration (10^6/g cauda epididymal tissue)	1353.4 ± 135[2]	1374.4 ± 83.7	707.3 ± 132**	161.9 ± 29.7**[2]

[1] Data are presented as mean ± standard error. Differences from the control group for necropsy body weights are not significant by Dunnett's test.
[2] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE E4 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene[1]

Study Parameters	0 ppm	70 ppm	700 ppm	7,000 ppm
n	10	10	10	10
Necropsy body weight	34.0 ± 0.7	30.8 ± 0.6**	30.1 ± 0.4**	29.9 ± 0.6**
Estrous cycle length (days)	4.15 ± 0.11	4.05 ± 0.05	4.45 ± 0.14	4.80 ± 0.17**
Estrous stages (% of cycle)				
Diestrus	27.5	36.7	29.2	28.3
Proestrus	24.2	20.0	20.8	20.8
Estrus	30.0	31.7	34.2	35.8
Metestrus	18.3	11.7	15.8	14.2
Uncertain diagnoses (%)	0.0	0.0	0.0	0.8

[1] Necropsy body weights and estrous cycle lengths are presented as mean ± standard error. By multivariate analysis of variance, exposed groups do not differ significantly from controls in the relative length of time spent in the estrous stages.
** Significantly different ($P \leq 0.01$) from the control group by William's test (necropsy body weights) or Shirley's test (estrous cycle length).

TERATOLOGY STUDIES

Materials and Methods

TERATOLOGY STUDIES

To assess the maternal and developmental toxicity of isoprene, teratology studies were performed on Sprague-Dawley rats and CD-1® Swiss mice. Male and female rats and mice used in the studies were obtained from Charles River Breeding Laboratories (Portage, MI). Rats and mice were 12 to 13 weeks old at receipt and were quarantined for 3 to 4 weeks before the start of the studies.

For breeding, two to three females were housed overnight with each male. On the first day of vaginal plug or sperm detection (gestation Day 0), positively mated females were assigned to exposure groups by weight. Breeding was conducted for 4 consecutive nights to obtain 28 to 29 positively mated female rats and 33 positively mated female mice per exposure group. Beginning on gestation Day 6, these animals were exposed to isoprene vapor through whole-body exposure at target concentrations of 0, 280, 1,400, or 7,000 ppm for 6 hours plus T_{90} (12 minutes) per day for 12 days (mice, gestation Days 6-17) or 14 days (rats, gestation Days 6-19); for comparison, 10 virgin rats and mice per group were exposed to isoprene vapor concurrently with the positively mated animals.

During the teratology studies, rats and mice were housed individually in cages within the exposure chambers. Drinking water was available *ad libitum*. NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form was available *ad libitum* except during exposure periods. Rats and mice were observed daily for mortality/morbidity and overt signs of toxicity. Mated female rats were weighed on gestation Days 0, 6, 10, 14, and 17 and at termination (gestation Day 20); mated female mice were weighed on gestation Days 0, 6, 9, 12, and 15 and at termination (gestation Day 18). Virgin female rats and mice were weighed prior to the first exposure, on exposure Days 1, 5, and 10, and at termination.

Virgin and mated females were killed 1 day after the final day of exposure. Mated females were examined grossly for signs of maternal toxicity. Maternal liver, kidney, and gravid uterine weights were recorded. Implantation sites were counted for each gravid uterus, and the position and status of each site were noted; apparently nongravid uteri were stained with ammonium sulfide to detect implantation sites. Resorptions and live and dead fetuses per litter were counted, and placentas were examined and discarded unless abnormal.

Live fetuses were weighed individually and examined for gross defects; fetuses were then killed by lethal injection of sodium pentobarbital and sexed. Half of the fetuses from each litter as well as fetuses with gross abnormalities were examined for visceral defects using methods adapted from Staples (1977). Half of the fetuses were also decapitated; heads were fixed in Bouin's fixative, sectioned, and examined for soft-tissue craniofacial defects. All carcasses were double stained with Alcian Blue and Alizarin Red S and examined for skeletal malformations.

STATISTICAL METHODS

For the teratology studies, means and standard deviations were calculated with SAS statistical software on a VAX 11/780 computer. Mean body weights (as a mean of litter means for fetal data) were analyzed using the SAS General Linear Models (GLM) Procedure (SAS, 1985) with an analysis of variance (ANOVA) model for unbalanced data. Response variables, either body weight or the arcsine transformations of proportional incidence data, were analyzed against the class variable "treatment" in a one-way ANOVA model. A

Tukey's *t*-test (two-tailed) was used to assess statistically significant differences between control and exposed groups. When appropriate, the dose-response relationship was determined by means of an orthogonal trend test (Winer, 1971). In the case of proportional data, the *t*-tests and trend analyses were performed on transformed variables. The litter was used as the basis for analysis of fetal variables.

Results

TERATOLOGY STUDY IN SPRAGUE-DAWLEY RATS

In the teratology study of isoprene in rats, 24 to 26 sperm-positive rats per exposure group were confirmed pregnant (Table 1). No pregnant or virgin rats died during the study, and there were no clinical signs of toxicity. The mean body weights of pregnant and virgin females exposed to 280, 1,400, or 7,000 ppm isoprene were similar to those of the respective controls at all time points during the study (Tables 1 and 2). In addition, the gravid uterine weights, extra-gestational weight gains, absolute and relative liver weights, and absolute kidney weights of exposed dams were not affected by isoprene exposure; however, the relative kidney weight of dams in the 7,000 ppm group was slightly but significantly greater than that of the controls (Table 1).

No statistically significant differences in embryo/fetal parameters such as implantations per dam, resorptions per litter, fetal mortality, and fetal body weights were noted between the control and exposed groups (Table 3). Gestational exposure to isoprene did not significantly increase the overall incidence of fetal malformations or the percent of malformed fetuses per litter (Table 4). Similarly, gestational exposure did not affect the overall incidence of fetuses with variations/reduced ossifications or the overall percent of fetuses per litter with variations/reduced ossifications, although there was an exposure-related increase in the mean percent of fetuses per litter with reduced vertebral ossifications (data not shown).

TERATOLOGY STUDY IN CD-1® SWISS MICE

Twenty-eight to 30 plug-positive mice per exposure group were confirmed pregnant during the teratology study in mice (Table 5). No pregnant or virgin mice died during the study, and there were no clinical signs of toxicity. The mean body weights of virgin mice in the 280, 1,400, and 7,000 ppm groups remained similar to control values throughout the study (Table 6). However, exposure-related decreases were noted for the mean body weights of exposed dams on gestation Days 12, 15, and 18, and the mean body weight of dams in the 7,000 ppm group was significantly lower than that of the control on gestation Days 15 and 18 (Table 5). At necropsy, the gravid uterine weight of dams exposed to 7,000 ppm isoprene was also significantly less than that of the control. There were no statistically significant differences between exposed and control dams for absolute liver and kidney weights; however, the relative liver weight of dams in the 1,400 ppm group and the relative liver and kidney weights of dams in the 7,000 ppm group were significantly greater than those of the control group (Table 5).

In the teratology study in mice, gestational exposure to isoprene did not affect the number of implantations per dam, litters with resorptions, or resorptions per litter (Table 7). In addition, no statistically significant differences in fetal mortality or the number of live fetuses per litter were noted between the control and exposed groups. However, male and female fetal body weights decreased with each increasing exposure concentration, and the body weights of male fetuses in the 1,400 and 7,000 ppm groups and female fetuses in all exposed groups were significantly less than those of the control groups (Table 7).

Gestational exposure to isoprene did not significantly increase the incidence of total fetal malformations or the percent of malformed fetuses per litter (Table 8); the only malformation observed in mouse fetuses was cleft palate, which occurred in 1 fetus each in the 1,400 and 7,000 ppm groups. There were no statistically significant differences between the control and exposed groups in the overall incidence of fetal variations/reduced ossifications. However, the mean percent of fetuses per litter with variations/reduced ossifications increased with increasing exposure and was significantly elevated at the highest exposure level (Table 8); for the most part, this difference could be attributed to an exposure-related increase in the percentage of fetuses per litter with supernumerary ribs (data not shown).

TABLE 1 Maternal Toxicity in Sprague-Dawley Rats Exposed to Isoprene Through Inhalation on Gestation Days 6 to 19[1]

	0 ppm	280 ppm	1,400 ppm	7,000 ppm
Number of sperm-positive females	29	29	29	28
Number pregnant at sacrifice	26 (90%)	25 (89%)	25 (86%)	24 (86%)
Number examined	26	25	25	24
Maternal body weight (g)				
Gestation Day 0	248 ± 23	246 ± 22	248 ± 23	250 ± 20
Gestation Day 6	270 ± 24	266 ± 23	273 ± 26	271 ± 20
Gestation Day 10	282 ± 31	282 ± 24	287 ± 28	276 ± 18
Gestation Day 14	302 ± 29	302 ± 26	308 ± 29	297 ± 19
Gestation Day 17	326 ± 31	324 ± 29	334 ± 33	322 ± 20
Gestation Day 20	359 ± 33	356 ± 33	368 ± 38	352 ± 25
Gravid uterine weight (g)	70.0 ± 11.8	71.3 ± 9.0	72.0 ± 11.5	65.0 ± 12.4
Extra-gestational weight gain (g)	41.6 ± 12.3	39.0 ± 27.3	47.2 ± 14.4	37.4 ± 12.7
Maternal liver weight				
Absolute (g)	14.58 ± 1.30	14.53 ± 2.01	14.85 ± 1.85	14.97 ± 1.72
Relative (% body weight)	4.07 ± 0.24	4.07 ± 0.35	4.04 ± 0.32	4.25 ± 0.35
Maternal kidney weight				
Absolute (g)	1.90 ± 0.18	1.92 ± 0.22	1.99 ± 0.22	1.99 ± 0.21
Relative (% body weight)	0.53 ± 0.04	0.54 ± 0.04	0.54 ± 0.05	0.57 ± 0.05*

[1] Maternal body and organ weights and weight gains are given as mean ± standard deviation.
* Significantly different (P<0.05) from the control group by Tukey's *t*-test.

TABLE 2 Mean Body Weights of Virgin Female Sprague-Dawley Rats Exposed to Isoprene Through Inhalation for 14 Days[1]

	0 ppm	280 ppm	1,400 ppm	7,000 ppm
n	10	10	10	10
Day 1	253 ± 23	251 ± 26	251 ± 22	251 ± 26
Day 5	261 ± 24	264 ± 26	261 ± 23	256 ± 26
Day 10	265 ± 24	273 ± 28	266 ± 21	257 ± 23
Termination	264 ± 24	269 ± 25	269 ± 24	260 ± 27

[1] Data are given as mean ± standard deviation.

TABLE 3 Developmental Toxicity in Sprague-Dawley Rats Following Maternal Exposure to Isoprene Through Inhalation on Gestation Days 6 to 19

	0 ppm	280 ppm	1,400 ppm	7,000 ppm
Number of dams/litters examined	26	25	25	24
Implantations per dam[1]	14.2 ± 2.2	14.6 ± 1.5	14.9 ± 1.9	13.8 ± 2.6
Litters with resorptions	9	10	14	13
Resorptions per litter[1]				
Early	0.2 ± 0.4	0.6 ± 0.9	0.6 ± 0.8	0.7 ± 0.8
Late	0.2 ± 0.4	0.1 ± 0.4	0.2 ± 0.4	0.0 ± 0.0
Total	0.4 ± 0.6	0.6 ± 0.9	0.8 ± 0.8	0.7 ± 0.8
Dead fetuses per litter	0	0	0	0
Live fetuses per litter[1]	13.8 ± 2.4	13.9 ± 1.7	14.1 ± 2.1	13.1 ± 2.6
Average fetal body weight per litter[1] (g)				
Live male fetuses	3.38 ± 0.23	3.45 ± 0.28	3.47 ± 0.24	3.40 ± 0.24
Live female fetuses	3.27 ± 0.24	3.30 ± 0.28	3.29 ± 0.22	3.21 ± 0.22
Live male fetuses per litter[1] (%)	55 ± 15	48 ± 16	46 ± 12	52 ± 13

[1] Data are given as mean ± standard deviation.

TABLE 4 Morphologic Abnormalities Observed in Live Sprague-Dawley Rat Fetuses Following Maternal Exposure to Isoprene Through Inhalation on Gestation Days 6 to 19

	0 ppm	280 ppm	1,400 ppm	7,000 ppm
Total live fetuses examined	359	348	352	315
Total litters examined	26	25	25	24
Malformations				
Fetuses with malformations	2 (0.6%)	1 (0.3%)	2 (0.6%)	0 (0.0%)
Litters with malformations	2 (7.7%)	1 (4.0%)	2 (8.0%)	0 (0.0%)
Malformed fetuses per litter[1] (%)	1.1 ± 4.6	0.3 ± 1.7	0.6 ± 2.0	0.0 ± 0.0
Variations and/or reduced ossifications				
Fetuses with variations and/or reduced ossifications	48 (13.4%)	40 (11.5%)	46 (13.1%)	55 (17.5%)
Litters with variations and/or reduced ossifications	16 (61.5%)	16 (64.0%)	16 (64.0%)	17 (70.8%)
Fetuses with variations and/or reduced ossifications per litter[1] (%)	17.4 ± 24.8	19.7 ± 49.5	16.5 ± 17.7	19.5 ± 24.0

[1] Data are given as mean ± standard deviation.

TABLE 5 Maternal Toxicity in Swiss (CD-1®) Mice Exposed to Isoprene Through Inhalation on Gestation Days 6 to 17[1]

	0 ppm	280 ppm	1,400 ppm	7,000 ppm
Number of plug-positive females	33	33	33	33
Number pregnant at sacrifice	30 (91%)	30 (91%)	28 (85%)	28 (85%)
Number examined	28[2]	29[3]	28	27[3]
Maternal body weight (g)				
Gestation Day 0	28.4 ± 2.4	28.4 ± 1.9	28.2 ± 2.1	28.2 ± 2.1
Gestation Day 6	30.7 ± 2.0	30.5 ± 2.2	30.4 ± 2.2	30.3 ± 1.9
Gestation Day 9	32.4 ± 1.9	32.3 ± 2.3	31.9 ± 2.5	32.2 ± 2.4
Gestation Day 12	37.9 ± 2.4	37.5 ± 3.3	37.3 ± 2.9	36.4 ± 2.6
Gestation Day 15	45.5 ± 3.4	45.4 ± 3.7	44.7 ± 3.3	43.2 ± 2.4*
Gestation Day 18	53.5 ± 4.9	53.1 ± 5.0	52.5 ± 4.2	49.8 ± 4.3*
Gravid uterine weight (g)	19.1 ± 4.4	19.3 ± 2.8	18.1 ± 3.0	15.9 ± 2.2*
Extra-gestational weight gain (g)	5.9 ± 1.9	5.4 ± 2.0	6.2 ± 1.5	5.7 ± 2.8
Maternal liver weight				
Absolute (g)	2.96 ± 0.25	3.01 ± 0.32	3.10 ± 0.29	3.03 ± 0.23
Relative (% body weight)	5.56 ± 0.47	5.68 ± 0.41	5.91 ± 0.52*	6.11 ± 0.53*
Maternal kidney weight				
Absolute (g)	0.47 ± 0.04	0.46 ± 0.05	0.48 ± 0.05	0.50 ± 0.07
Relative (% body weight)	0.88 ± 0.09	0.88 ± 0.08	0.91 ± 0.09	1.01 ± 0.11*

[1] Maternal body and organ weights and weight gains are given as mean ± standard deviation.
[2] One dam in this group was removed from the study because of premature delivery; another dam in this group with two or fewer implants was also removed from the study.
[3] One dam in this group was removed from the study because of premature delivery.
* Significantly different (P<0.05) from the control group by Tukey's *t*-test.

TABLE 6 Mean Body Weights of Virgin Female Swiss (CD-1®) Mice Exposed to Isoprene Through Inhalation for 12 Days[1]

	0 ppm	280 ppm	1,400 ppm	7,000 ppm
n	10	10	10	10
Day 1	27.9 ± 1.5	28.1 ± 1.9	28.1 ± 1.5	28.1 ± 1.6
Day 5	28.4 ± 1.3	27.9 ± 1.9	28.0 ± 1.6	27.2 ± 1.5
Day 10	28.3 ± 1.3	28.3 ± 1.8	28.9 ± 1.3	27.7 ± 1.3
Termination	27.8 ± 2.4	28.6 ± 2.0	29.5 ± 1.9	28.5 ± 1.7

[1] Data are given as mean ± standard deviation.

TABLE 7 Developmental Toxicity in Swiss (CD-1®) Mice following Maternal Exposure to Isoprene Through Inhalation on Gestation Days 6 to 17

	0 ppm	280 ppm	1,400 ppm	7,000 ppm
Number of dams/litters examined	28	29	28	27
Implantations per dam[1]	12.3 ± 2.9	12.8 ± 1.5	12.4 ± 2.1	12.0 ± 1.5
Litters with resorptions	15	15	11	16
Resorptions per litter[1]				
Early	0.5 ± 0.7	0.5 ± 0.7	0.4 ± 0.6	0.7 ± 1.0
Late	0.3 ± 0.5	0.2 ± 0.4	0.2 ± 0.4	0.3 ± 0.6
Total	0.7 ± 0.8	0.7 ± 0.8	0.5 ± 0.8	1.0 ± 1.1
Dead fetuses per litter[1]	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Live fetuses per litter[1]	11.5 ± 3.0	12.0 ± 1.9	11.9 ± 2.2	10.9 ± 1.8
Average fetal body weight per litter[1] (g)				
Live male fetuses	1.37 ± 0.11	1.30 ± 0.10	1.23 ± 0.10*	1.16 ± 0.12*
Live female fetuses	1.32 ± 0.10	1.25 ± 0.10*	1.20 ± 0.10*	1.12 ± 0.13*
Live male fetuses per litter[1] (%)	48 ± 15	49 ± 14	52 ± 13	54 ± 16

[1] Data are given as mean ± standard deviation.
* Significantly different (P<0.05) from the control group by Tukey's *t*-test.

TABLE 8 Morphologic Abnormalities Observed in Live Swiss (CD-1®) Mouse Fetuses Following Maternal Exposure to Isoprene Through Inhalation on Gestation Days 6 to 17

	0 ppm	280 ppm	1,400 ppm	7,000 ppm
Total live fetuses examined	323	349	333	295
Total litters examined	28	29	28	27
Malformations				
Fetuses with malformations	0 (0.0%)	0 (0.0%)	1 (0.3%)	1 (0.3%)
Litters with malformations	0 (0.0%)	0 (0.0%)	1 (3.6%)	1 (3.7%)
Malformed fetuses per litter[1] (%)	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 1.9	0.3 ± 1.6
Variations and/or reduced ossifications				
Fetuses with variations and/or reduced ossifications	72 (22.3%)	88 (25.2%)	116 (34.8%)	119 (40.3%)
Litters with variations and/or reduced ossifications	20 (71.0%)	22 (75.9%)	25 (89.3%)	26 (96.3%)
Fetuses with variations and/or reduced ossifications per litter[1] (%)	24.0 ± 25.6	25.3 ± 27.0	36.4 ± 26.4	41.3 ± 21.8*

[1] Data are given as mean ± standard deviation.
* Significantly different (P<0.05) from the control group by Tukey's *t*-test after arcsine transformation.

APPENDIX F

Genetic Toxicology

Table F1 Mutagenicity of Isoprene in *Salmonella typhimurium* F-2

Table F2 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Isoprene F-3

Table F3 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Isoprene F-4

TABLE F1 Mutagenicity of Isoprene in *Salmonella typhimurium* [1]

Strain	Dose (µg/plate)	Revertants/plate [2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
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 [3]	56 ± 9.2 [3]	84 ± 12.2 [3]	85 ± 5.4	90 ± 6.4 [3]	94 ± 6.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control [4]		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 [3]	0 ± 0.0 [3]	5 ± 1.2 [3]	6 ± 1.5	5 ± 1.8 [3]	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 [3]	4 ± 1.0 [3]	4 ± 1.2 [3]	3 ± 0.9	5 ± 0.9 [3]	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 [3]	2 ± 2.3 [3]	17 ± 3.7 [3]	17 ± 1.9	18 ± 1.5 [3]	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

[1] Study performed at SRI International. The detailed protocol and these data are presented in Mortelmans *et al.* (1986); 0 µg/plate dose was the solvent control.

[2] Revertants are presented as mean ± standard error from three plates.

[3] Slight toxicity.

[4] 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 F2 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Isoprene [1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%) [2]
-S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,033	475	0.45	9.5	26.0	
Mitomycin-C	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 [3]
+S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,047	398	0.38	8.0	26.0	
Cyclophosphamide	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

[1] Study performed at Environmental Health Research & Testing, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the protocol is presented by Galloway *et al.* (1987).
[2] Percentage increase in SCEs/chromosome of culture exposed to isoprene relative to those of culture exposed to solvent.
[3] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose.

TABLE F3 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Isoprene [1]

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Trial 1) Harvest time: 12 hours					**Trial 1**) Harvest time: 13 hours				
Summary: Negative					Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	200	1	0.01	0.5		200	4	0.02	1.5
Mitomycin-C					Cyclophosphamide				
0.125	200	54	0.27	21.5	5.0	200	29	0.15	13.5
0.250	50	33	0.66	34.0	7.5	50	23	0.46	42.0
Isoprene					Isoprene				
1,600	200	3	0.02	1.5	1,600	200	4	0.02	2.0
3,000	200	1	0.01	0.5	3,000	200	6	0.03	3.0
5,000	200	3	0.02	1.5	5,000	200	3	0.02	1.5
				P=0.276 [2]					P=0.394

[1] Study performed at Environmental Health Research and Testing, Inc. Abs=aberrations. A detailed presentation of the protocol is presented in Galloway *et al.* (1987).

[2] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose.

APPENDIX G

Tissue Glutathione Concentration Analyses

Table G1 Glutathione Levels and Total-Sulfhydryl-to-Glutathione Ratios in the Tissues of F344/N Rats in the 13-Week Inhalation Study of Isoprene G-2

Table G2 Glutathione Levels and Total-Sulfhydryl-to-Glutathione Ratios in the Tissues of B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene G-4

TABLE G1 Glutathione Levels and Total-Sulfhydryl-to-Glutathione Ratios in the Tissues of F344/N Rats in the 13-Week Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	700 ppm	7,000 ppm
MALE				
n	5	5	5	5
Kidney				
Day 1				
Glutathione	1.10 ± 0.14	1.10 ± 0.55	1.14 ± 0.39	0.68 ± 0.11
TSH/Glutathione	12.58 ± 1.88	18.66 ± 4.27	15.68 ± 4.87	20.14 ± 3.39
Week 12				
Glutathione	1.78 ± 0.31	1.16 ± 0.11	1.38 ± 0.23	0.92 ± 0.06**
TSH/Glutathione	6.62 ± 1.24	10.60 ± 0.68*	9.74 ± 1.49	12.36 ± 0.69**
Liver				
Day 1				
Glutathione	2.70 ± 0.34	2.82 ± 0.27	2.52 ± 0.26	2.38 ± 0.15
TSH/Glutathione	5.24 ± 0.44	5.42 ± 0.59	6.20 ± 0.44	5.54 ± 0.33
Week 12				
Glutathione	4.90 ± 0.32	4.20 ± 0.27	4.28 ± 0.30	4.32 ± 0.12
TSH/Glutathione	2.48 ± 0.17	2.86 ± 0.13	2.98 ± 0.35	2.78 ± 0.12
Lung				
Day 1				
Glutathione	0.28 ± 0.16	0.16 ± 0.04	0.40 ± 0.18	0.30 ± 0.09
TSH/Glutathione	47.04 ± 11.11	40.90 ± 6.74	30.38 ± 7.96	36.84 ± 8.81
Week 12				
Glutathione	0.16 ± 0.02	0.20 ± 0.06	0.28 ± 0.11	0.18 ± 0.06
TSH/Glutathione	45.54 ± 6.93	36.52 ± 7.74	30.82 ± 6.52	36.86 ± 5.92
Thymus				
Day 1				
Glutathione	1.66 ± 0.11	1.60 ± 0.08	1.58 ± 0.09	1.54 ± 0.12
TSH/Glutathione	5.16 ± 0.26	5.08 ± 0.22	5.40 ± 0.22	5.38 ± 0.25
Week 12				
Glutathione	1.83 ± 0.17[2]	2.08 ± 0.19	1.90 ± 0.11	1.98 ± 0.07
TSH/Glutathione	4.60 ± 0.35[2]	4.08 ± 0.31	4.46 ± 0.24	4.08 ± 0.18

TABLE G1 Glutathione Levels and Total-Sulfhydryl-to-Glutathione Ratios in the Tissues of F344/N Rats in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	700 ppm	7,000 ppm
FEMALE				
n	5	5	5	5
Kidney				
Day 1				
Glutathione	2.32 ± 0.16	2.98 ± 0.20	2.60 ± 0.31	2.18 ± 0.15
TSH/Glutathione	6.64 ± 0.56	5.20 ± 0.23	5.24 ± 0.98	6.06 ± 0.43
Week 12				
Glutathione	1.88 ± 0.34	1.96 ± 0.25	1.90 ± 0.14	1.72 ± 0.09
TSH/Glutathione	7.44 ± 1.05	6.66 ± 0.64	7.36 ± 0.40	7.78 ± 0.40
Liver				
Day 1				
Glutathione	4.40 ± 0.38	4.46 ± 0.16	3.50 ± 0.36*	3.90 ± 0.25
TSH/Glutathione	3.76 ± 0.26	3.68 ± 0.09	4.44 ± 0.43	4.14 ± 0.13
Week 12				
Glutathione	4.07 ± 0.16	3.06 ± 0.37	3.08 ± 0.35	3.94 ± 0.25
TSH/Glutathione	4.06 ± 0.13	5.38 ± 0.53*	5.76 ± 0.65*	4.34 ± 0.29
Lung				
Day 1				
Glutathione	1.08 ± 0.09	0.78 ± 0.15	0.92 ± 0.06	0.94 ± 0.12
TSH/Glutathione	8.02 ± 0.66	12.66 ± 3.25	8.76 ± 0.50	9.18 ± 1.00
Week 12				
Glutathione	0.50 ± 0.10	0.68 ± 0.04	0.52 ± 0.09	0.62 ± 0.11
TSH/Glutathione	16.74 ± 3.68	10.54 ± 0.75	17.88 ± 6.27	15.90 ± 6.75
Thymus				
Day 1				
Glutathione	2.14 ± 0.14	2.26 ± 0.05	2.44 ± 0.06*	2.34 ± 0.05
TSH/Glutathione	4.30 ± 0.10	4.20 ± 0.08	4.12 ± 0.12	3.96 ± 0.07*
Week 12				
Glutathione	2.44 ± 0.09	2.56 ± 0.08	2.38 ± 0.14	2.70 ± 0.21
TSH/Glutathione	3.64 ± 0.12	3.64 ± 0.09	3.88 ± 0.15	3.38 ± 0.21

[1] Data are presented as mean ± standard error. Glutathione tissue levels are given in µmol/g of organ; TSH/glutathione = the ratio of total sulfhydryl to glutathione in tissues.

[2] n=4.

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE G2 Glutathione Levels and Total-Sulfhydryl-to-Glutathione Ratios in the Tissues of B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene[1]

	0 ppm	70 ppm	700 ppm	7,000 ppm
MALE				
n	5	5	5	5
Kidney				
Day 1				
Glutathione	2.26 ± 0.13	2.42 ± 0.18	2.92 ± 0.23	2.28 ± 0.35
TSH/Glutathione	6.85 ± 0.57[2]	6.38 ± 0.38	5.20 ± 0.30	7.24 ± 1.45
Week 12				
Glutathione	2.78 ± 0.16	2.68 ± 0.18	3.54 ± 0.38	1.86 ± 0.24
TSH/Glutathione	5.12 ± 0.34	5.14 ± 0.17	4.42 ± 0.49	6.68 ± 0.59
Liver				
Day 1				
Glutathione	2.34 ± 0.30	2.76 ± 0.14	3.64 ± 0.14*	2.00 ± 0.24
TSH/Glutathione	5.54 ± 0.60	4.60 ± 0.14	3.44 ± 0.13*	6.60 ± 0.98
Week 12				
Glutathione	3.42 ± 0.15	3.38 ± 0.28	4.38 ± 0.14	1.40 ± 0.15
TSH/Glutathione	2.80 ± 0.10	2.94 ± 0.19	2.46 ± 0.18	7.24 ± 0.57*
Lung				
Day 1				
Glutathione	0.54 ± 0.12	0.68 ± 0.24	0.36 ± 0.04	0.34 ± 0.10
TSH/Glutathione	16.48 ± 3.34	19.44 ± 8.90	16.60 ± 4.82	27.64 ± 9.10
Week 12				
Glutathione	1.18 ± 0.04	1.22 ± 0.02	1.24 ± 0.09	0.70 ± 0.05*
TSH/Glutathione	6.92 ± 0.30	6.84 ± 0.20	6.64 ± 0.37	12.58 ± 1.21*
Thymus				
Day 1				
Glutathione	1.52 ± 0.10	2.68 ± 1.00	0.93 ± 0.21[2]	1.20 ± 0.13
TSH/Glutathione	2.20 ± 0.58	1.70 ± 0.31	5.13 ± 1.76[2]	2.62 ± 0.57
Week 12				
Glutathione	3.12 ± 0.19	3.36 ± 0.15	2.74 ± 0.25	3.38 ± 0.18
TSH/Glutathione	2.82 ± 0.22	1.92 ± 0.26*	2.62 ± 0.35	1.60 ± 0.27**

TABLE G2 Glutathione Levels and Total-Sulfhydryl-to-Glutathione Ratios in the Tissues of B6C3F$_1$ Mice in the 13-Week Inhalation Study of Isoprene (continued)

	0 ppm	70 ppm	700 ppm	7,000 ppm
FEMALE				
n	5	5	5	5
Kidney				
Day 1				
Glutathione	2.60 ± 0.06	2.60 ± 0.09	2.80 ± 0.08	2.70 ± 0.19
TSH/Glutathione	5.70 ± 0.11	5.82 ± 0.15	5.52 ± 0.13	5.78 ± 0.27
Week 12				
Glutathione	2.38 ± 0.12	2.70 ± 0.11	3.74 ± 0.19**	2.40 ± 0.25
TSH/Glutathione	4.84 ± 0.64	4.22 ± 0.54	3.78 ± 0.18	4.04 ± 0.68
Liver				
Day 1				
Glutathione	2.66 ± 0.28	3.15 ± 0.35[2]	4.06 ± 0.11**	4.02 ± 0.32**
TSH/Glutathione	5.44 ± 0.29	4.80 ± 0.32[2]	3.54 ± 0.13**	3.66 ± 0.40**
Week 12				
Glutathione	3.18 ± 0.36	3.28 ± 0.17	4.04 ± 0.20	2.04 ± 0.22
TSH/Glutathione	2.94 ± 0.51	3.26 ± 0.17	2.58 ± 0.14	4.32 ± 0.66
Lung				
Day 1				
Glutathione	1.46 ± 0.21	1.36 ± 0.13	1.32 ± 0.18	0.70 ± 0.10**
TSH/Glutathione	6.14 ± 0.74	6.06 ± 0.75	7.26 ± 1.25	12.08 ± 2.47*
Week 12				
Glutathione	1.44 ± 0.07	1.48 ± 0.04	1.40 ± 0.13	0.68 ± 0.06**
TSH/Glutathione	6.22 ± 0.32	5.68 ± 0.05	5.90 ± 0.58	11.34 ± 0.95*
Thymus				
Day 1				
Glutathione	1.94 ± 0.17	2.12 ± 0.08	1.78 ± 0.17	1.90 ± 0.22
TSH/Glutathione	3.86 ± 0.12	3.82 ± 0.17	3.70 ± 0.34	4.14 ± 0.25
Week 12				
Glutathione	3.50 ± 0.20	3.68 ± 0.84	3.22 ± 0.37	3.10 ± 0.03
TSH/Glutathione	2.44 ± 0.15	2.60 ± 0.37	2.80 ± 0.44	2.52 ± 0.31

[1] Data are presented as mean ± standard error. Glutathione tissue levels are given in µmol/g of organ; TSH/glutathione = the ratio of total sulfhydryl to glutathione in tissues.

[2] n=4.

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
1	Hexachloro-1,3-butadiene	Dosed Feed	91-3120
2	*n*-Hexane	Inhalation	91-3121
3	Acetone	Drinking Water	91-3122
4	1,2-Dichloroethane	Drinking Water, Gavage	91-3123
5	Cobalt Sulfate Heptahydrate	Inhalation	91-3124
6	Pentachlorobenzene	Dosed Feed	91-3125
7	1,2,4,5-Tetrachlorobenzene	Dosed Feed	91-3126
8	D & C Yellow No. 11	Dosed Feed	91-3127
9	*o*-Cresol *m*-Cresol *p*-Cresol	Dosed Feed	92-3128
10	Ethylbenzene	Inhalation	92-3129
11	Antimony Potassium Tartrate	Drinking Water, I.P. Inject.	92-3130
12	Castor Oil	Dosed Feed	92-3131
13	Trinitrofluorenone	Dermal, Dosed Feed	92-3132
14	*p*-Chloro-α,α,α-Trifluorotoluene	Gavage (corn oil, a-CD)	92-3133
15	*t*-Butyl Perbenzoate	Gavage	92-3134
16	Glyphosate	Dosed Feed	92-3135
17	Black Newsprint Ink	Dermal	92-3340
18	Methyl Ethyl Ketone Peroxide	Dermal	92-3341
19	Formic Acid	Inhalation	92-3342
20	Diethanolamine	Drinking Water, Dermal	92-3343
21	2-Hydroxy-4-Methoxybenzophenone	Dosed Feed, Drinking Water	92-3344
22	N, N-Dimethylformamide	Inhalation	93-3345
23	*o*-Nitrotoluene *m*-Nitrotoluene *p*-Nitrotoluene	Dosed Feed	92-3346
24	1,6-Hexanediamine	Inhalation	93-3347
25	Glutaraldehyde	Inhalation	93-3348
26	Ethylene Glycol Ethers	Drinking Water	93-3349
27	Riddelliine	Gavage	94-3350
28	Tetrachlorophthalic Anhydride	Gavage	93-3351
29	Cupric Sulfate	Drinking Water, Dosed Feed	93-3352

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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
32	Methylene Bis(thiocyanate)	Gavage	94-3381
33	2-Chloronitrobenzene 4-Chloronitrobenzene	Inhalation	93-3382
35	Chemical Mixture of 25 Groundwater Contaminants	Drinking Water	93-3384
36	Pesticide/Fertilizer Mixtures	Drinking Water	93-3385
37	Sodium Cyanide	Drinking Water	94-3386
38	Sodium Selenate Sodium Selenite	Drinking Water	94-3387
40	β-Bromo-β-nitrostyrene	Gavage	94-3389