

# Inhalation Toxicology of Isoprene in F344 Rats and B6C3F<sub>1</sub> Mice Following Two-Week Exposures

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Isoprene (2-methyl-1,3-butadiene) was selected for toxicologic evaluations because of its structural similarity to 1,3-butadiene, a potent rodent carcinogen. Two-week inhalation toxicology studies of isoprene were conducted in F344 rats and B6C3F<sub>1</sub> mice at exposure concentrations of 0, 438, 875, 1750, 3500, or 7000 ppm. For rats, there were no chemically related changes in survival, body weight gain, clinical signs, hematologic or clinical chemistry parameters, or gross or microscopic lesions. Exposure of mice to isoprene did not produce mortalities and only caused a decrease in body weight gain for male mice in the 7000 ppm exposure group; however, hematologic changes and microscopic lesions including testicular atrophy, olfactory epithelial degeneration, and forestomach epithelial hyperplasia were observed in isoprene-exposed mice. Similar toxicologic effects have been previously observed in B6C3F<sub>1</sub> mice exposed to 1,3-butadiene. A species difference in susceptibility between rats and mice exposed to isoprene was evident in these short-term exposure studies.

## Introduction

Isoprene (2-methyl-1,3-butadiene; CAS No. 78-79-5) was selected for toxicologic evaluations because of its structural similarity to 1,3-butadiene. The latter compound produced one of the most unusual responses in a long-term study by the National Toxicology Program (1,2): a high rate of early mortality due to fatal tumors in B6C3F<sub>1</sub> mice exposed to 625 or 1250 ppm of 1,3-butadiene, seven primary sites of neoplastic development, and induction of uncommon tumors such as hemangiosarcoma of the heart. To investigate the influence of the 2-methyl substituent on the toxicologic profile of 1,3-butadiene, inhalation toxicology studies of isoprene were initiated.

Isoprene differs physically from butadiene in that it is a liquid at room temperature (boiling point: 34°C), whereas butadiene is a gas (boiling point: -4.4°C). Isoprene is a high volatile material, having a vapor pressure of 493 mm Hg at 20°C (3). The annual production volume of isoprene in the United States is about 140 million pounds; this level is about 6% of the U.S. production volume of 1,3-butadiene (4). Isoprene is produced

principally for the synthesis of *cis*-1,4-polyisoprene (5), which is used in the manufacture of rubber tires, automotive parts, gaskets, footwear, adhesives, and flooring.

Few toxicology studies have been published on isoprene. The LC<sub>50</sub> value for isoprene was reported to be about 50,000 ppm in mice after 2 hr of exposure, and 64,500 ppm in rats after 4 hr of exposure (6). Inhalation exposure of B6C3F<sub>1</sub> mice to 438, 1750, or 7,000 ppm isoprene, 6 hr/day, 5 days/week for 2 weeks, induced increases in the frequency of sister chromatid exchanges in bone marrow cells and in the levels of micronucleated erythrocytes in peripheral blood at all exposure levels (7). Isoprene is metabolized to the monoepoxides 3,4-epoxy-3-methyl-1-butene and 3,4-epoxy-2-methyl-1-butene and to isoprene diepoxide (2-methyl-1,2,3,4-diepoxybutane) by liver microsomal monooxygenases of mice, rats, rabbits, and hamsters (8,9). Isoprene diepoxide was mutagenic to *Salmonella typhimurium* strains TA98 and TA100 (10). The maximal rate of metabolism of isoprene in B6C3F<sub>1</sub> mice is about three times greater than that in Wistar rats (11). Isoprene has been identified as the major endogenous hydrocarbon of human breath (12,13), exhaled at a rate of approximately 28 nmole/L. Isoprene is produced endogenously by rats and mice at rates of 1.9 and 0.4  $\mu$ mole/hr/kg, respectively (11). This paper gives results of the effects of inhalation exposure of F344 rats and B6C3F<sub>1</sub> mice to isoprene vapors.

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## Materials and Methods

### Chemical and Exposure System

Isoprene, containing about 50 ppm of the peroxide inhibitor *t*-butyl catechol, was obtained from the Good-year Tire and Rubber Company. The lots of isoprene used in these studies had purities greater than 99%; the largest impurity identified (approximately 0.2%) was limonene, a dimer of isoprene. Isoprene vapors were generated in a Buchi Rotavapor system at 65°C, carried in nitrogen through a chilled water condenser to a distribution manifold, and then separately metered to each exposure chamber. Chamber concentrations of isoprene were regulated by adjusting the metering valves which controlled individual delivery lines from the distribution manifold and by adjusting the pressure of the compressed air in which the isoprene vapors were diluted prior to entry into the exposure chambers. Concentrations of isoprene in the chambers were measured continuously during the exposures with a Hewlett-Packard 5840 gas chromatograph equipped with a flame ionization detector (oven temperature: 120°C; GC column: 12" × 1/8" nickel column packed with 1% SP-1000 on 60/80 Carboxpack B; carrier gas: nitrogen). The daily mean concentrations of isoprene for all chambers were within 2% of the target concentrations.

### Animal Maintenance

Four-week-old F344 rats and B6C3F<sub>1</sub> mice of each sex were purchased from Simonsen Laboratories (Gilroy, CA) and quarantined for 10 to 13 days prior to the start of the study. Stainless steel Hazelton 2000 chambers (total volume 2.3 m<sup>3</sup>) were used, and animals were exposed in individual wire mesh cage units. The floor area of the mouse cage is 106 cm<sup>2</sup> and the rat cage is 270 cm<sup>2</sup>. Chamber environment was maintained at 75 ± 3°F and 55 ± 15% relative humidity, with chamber air flow at 15 changes/hr. Fresh softened tap water and NIH-07 diet were available *ad libitum*, except during the exposure periods when the feed was removed.

### Treatment Groups

Groups of 20 rats of each sex and 20 mice of each sex were exposed to chamber atmospheres of 0 (control), 438, 875, 1750, 3500, or 7000 ppm isoprene for 6 hr + T90 per day, 5 days per week for 2 weeks. The time interval required to reach 90% of the target concentration, T90, was about 12 min. Animals were observed twice daily for moribundity and mortality. Body weight measurements and clinical observations were made weekly. Ten animals per group were used for clinical pathology evaluations after 4 to 5 exposures; the remaining 10 animals per group were used for histopathology at the end of the study. The exposure schedule ensured that there were at least 2 consecutive exposure days prior to terminal sacrifice; within 24 hr of the last exposure, all surviving animals were killed by CO<sub>2</sub> asphyxiation and immediately necropsied.

### Clinical Pathology

Clinical pathology evaluations for rats after 4 consecutive exposure days or mice after 5 consecutive exposures included hematology (erythrocyte, leukocyte, and platelet counts, leukocyte differential counts, hemoglobin concentration, volume of packed red cells, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and reticulocyte count), serum clinical chemistry (urea nitrogen, creatinine, sorbitol dehydrogenase, glutamic pyruvic transaminase, and glutamate dehydrogenase), and urinalysis (rats only) of 16-hr urine samples collected over ice (volume, pH, appearance, specific gravity, protein, glucose, urea, creatinine, alkaline phosphatase, and glutamic oxaloacetic transaminase). Blood samples were collected from the supraorbital sinus of CO<sub>2</sub>-anesthetized animals into tubes either containing EDTA or free of EDTA. Hematologic indices were measured with an Ortho ELT-8/ds hematology analyzer, while serum and urine chemistries were performed with an Abbott VP chemistry analyzer using Abbott VP methodologies. Urine protein concentration was determined by the Coomassie blue reaction (14).

### Histopathology

Tissue samples that were preserved in 10% neutral buffered formalin were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically from control and high-dose groups: gross lesions, liver, lungs and mainstem bronchi, nasal cavity and turbinates, trachea, larynx, tracheobronchial lymph nodes, heart, brain, thymus, spleen, kidneys, and testes/epididymis. Target organs and gross lesions were examined in lower dose groups until an apparent no-observed-effect level was found.

### Statistics

Mean body weights, organ weights, organ weight/body weight ratios, and clinical pathology results of treated groups were compared to those of control groups using the Dunnett's *t*-test. The minimum level of probability accepted for significance was  $p < 0.05$ .

### Results

There were no deaths of F344 rats exposed to isoprene vapors for 2 weeks at exposure concentrations ranging from 438 to 7000 ppm. Slight differences in final mean body weight or mean body weight gain were not significant at any of the exposure levels when compared to those of control rats (Table 1). There were also no significant differences in mean organ weights (thymus, kidney, heart, lung, spleen, brain, liver, or testis) or organ weight/body weight ratios of rats exposed to isoprene compared to those of controls. In addition, there were no clinical pathology changes or gross or

**Table 1. Final mean body weight and body weight gain for F344 rats exposed to isoprene by inhalation for 2 weeks.**

Exposure concentration, ppm	Males <sup>a</sup>		Females <sup>b</sup>	
	Final body weight, g	Mean body weight gain, g	Final body weight, g	Mean body weight gain, g
0	160 ± 12	76.7 ± 6.3	121 ± 8	43.2 ± 2.9
438	158 ± 15	73.6 ± 8.5	117 ± 10	38.6 ± 10.0
875	152 ± 18	70.5 ± 11.6	122 ± 6	44.2 ± 2.1
1750	152 ± 15	70.5 ± 9.8	119 ± 8	42.3 ± 3.5
3500	151 ± 11	71.6 ± 7.5	119 ± 5	45.8 ± 1.9
7000	150 ± 17	68.2 ± 8.8	119 ± 6	43.3 ± 3.6

<sup>a</sup>Initial mean body weight of male rat groups was 82.1 ± 1.9 g.<sup>b</sup>Initial mean body weight of female rat groups was 76.6 ± 2.1 g.**Table 2. Final mean body weight and body weight gain for B6C3F<sub>1</sub> mice exposed to isoprene by inhalation for 2 weeks.**

Exposure concentration, ppm	Males <sup>a</sup>		Females <sup>b</sup>	
	Final body weight, g	Mean body weight gain, g	Final body weight, g	Mean body weight gain, g
0	28.7 ± 1.8	5.0 ± 1.9	22.6 ± 0.9	2.1 ± 0.6
438	27.1 ± 1.2*	3.9 ± 1.1	22.6 ± 0.9	2.8 ± 1.1
875	26.4 ± 1.2*	3.7 ± 0.6	23.0 ± 0.6	3.4 ± 0.8*
1750	27.2 ± 1.2*	4.2 ± 0.9	22.5 ± 1.1	2.7 ± 0.8
3500	26.7 ± 1.2*	4.0 ± 0.8	22.9 ± 0.8	3.4 ± 0.6*
7000	24.3 ± 1.2*	1.7 ± 0.9*	22.3 ± 1.0	2.5 ± 0.7

<sup>a</sup>Initial mean body weight of male mouse groups was 23.0 ± 0.4 g.<sup>b</sup>Initial mean body weight of female mouse groups was 19.9 ± 0.4 g.\*Different from control (0 ppm), *p* < 0.05.**Table 3. Mean organ weight/body weight ratios (×1000) for male B6C3F<sub>1</sub> mice exposed to isoprene by inhalation for 2 weeks.**

Exposure concentration, ppm	Liver	Thymus	Spleen	Right testis
0	50.3 ± 0.21	1.62 ± 0.024	2.7 ± 0.3	3.61 ± 0.33
438	56.0 ± 2.0*	1.43 ± 0.18	2.5 ± 0.2	3.49 ± 0.25
875	59.4 ± 2.8*	0.99 ± 0.25*	2.3 ± 0.2*	3.15 ± 0.17*
1750	59.7 ± 2.0*	1.10 ± 0.29*	2.3 ± 0.2*	3.08 ± 0.23*
3500	62.3 ± 4.4*	0.90 ± 0.12*	2.3 ± 0.1*	3.10 ± 0.15*
7000	67.3 ± 3.1*	0.60 ± 0.19*	2.0 ± 0.1*	2.84 ± 0.16*

\*Different from control (0 ppm), *p* < 0.05.

histopathological lesions that could be attributed to exposure to isoprene.

There were no deaths of B6C3F<sub>1</sub> mice exposed to isoprene for 2 weeks. A 15% reduction in body weight was observed in the 7000 ppm group of male mice (Table 2). Exposure to isoprene did not cause reduction in mean body weight gain of female mice. Dose-related increases in mean liver weight/body weight ratios and decreases in relative thymus, spleen, and testis weights were observed in mice exposed to isoprene compared to controls. Organ weight changes were observed in both male and female mice; results for males are shown in Table 3.

In blood samples of mice exposed for 5 consecutive days to isoprene, mean red blood cell counts, hemoglobin concentrations, and volume of packed red cells were reduced in all exposure groups when compared to controls (Table 4). These changes were not dose related nor accompanied by increases in reticulocyte counts or polychromatic erythrocytes. Similar hematologic changes were observed in male and female mice. The lack of exposure-related changes in serum chemistry parameters in mice indicates that the hepatic and renal systems

**Table 4. Hematologic changes in peripheral blood of B6C3F<sub>1</sub> mice exposed to isoprene by inhalation for 5 days.<sup>a</sup>**

Exposure concentration, ppm	RBC × 10 <sup>6</sup> /μL	HGB, g/dL	VPRC, mL/dL
Males			
0	9.90 ± 0.35	16.4 ± 0.5	48.9 ± 1.9
438	9.11 ± 0.32*	15.1 ± 0.4*	44.9 ± 1.2*
875	9.19 ± 0.25*	15.2 ± 0.3*	45.2 ± 1.1*
1750	8.76 ± 1.01*	14.6 ± 1.7*	43.2 ± 4.8*
3500	9.01 ± 0.13*	15.0 ± 0.2*	44.6 ± 0.5*
7000	9.18 ± 0.20*	15.0 ± 0.2*	44.7 ± 1.2*
Females			
0	9.74 ± 0.20	16.4 ± 0.2	48.1 ± 0.7
438	9.20 ± 0.30*	15.6 ± 0.4*	45.6 ± 0.8*
875	9.03 ± 0.19*	15.4 ± 0.3*	45.5 ± 0.8*
1750	9.20 ± 0.30*	15.5 ± 0.3*	45.3 ± 0.8*
3500	9.24 ± 0.32*	15.7 ± 0.5*	45.9 ± 1.5*
7000	9.06 ± 0.35*	15.4 ± 0.4*	45.2 ± 1.3*

<sup>a</sup>Abbreviations: RBC; red blood cell count; HGB; hemoglobin concentration; VPRC; volume of packed red cells.\*Different from control (0 ppm), *p* < 0.05.

were not adversely affected in this species after 5 days of exposure to isoprene.

Inhalation exposure of male mice to isoprene for 2 weeks was associated with microscopic changes in the thymus, testes, nasal cavity, and forestomach; microscopic lesions in the forestomach were also observed in exposed female mice (Table 5). Thymic atrophy in male mice exposed to 7000 ppm isoprene was characterized by a decrease in cellularity of the cortex. Minimal testicular atrophy was observed in mice exposed to 7000 ppm isoprene. This lesion was scattered and 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 highly glycogenated hepatocytes were observed to similar degrees in all dose groups of exposed male mice. Olfactory epithelial degeneration was observed at 1750, 3500, and 7000 ppm isoprene; the

severity of the nasal lesions increased with increasing concentrations of isoprene. Olfactory degeneration (Fig. 1) 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. Atrophy of sensory nerve bundles and submucosal fibrosis were observed in some animals in the 7000-ppm exposure group.

Epithelial hyperplasia of the forestomach was seen in all groups of male and female mice exposed to isoprene. Grossly, these lesions appeared as focal, white, raised or thickened areas at the anterior pole of the forestomach. Microscopically (Fig. 2), these lesions were characterized by focal epithelial cell proliferation resulting in a thickened and occasionally verrucous appearance. Hyperkeratosis and thickening of the muscle layer below the epithelial lesion were also apparent. The severity of the lesion was relatively consistent throughout the expo-

Table 5. Incidence of microscopic lesions in B6C3F<sub>1</sub> mice exposed to isoprene by inhalation for 2 weeks.

Target: lesion	Exposure concentration, ppm					
	0	438	875	1750	3500	7000
<b>Males</b>						
Thymus: atrophy	0/10 <sup>a</sup>	NE <sup>b</sup>	NE	NE	0/10	7/9
Testes: atrophy	0/10	NE	NE	NE	0/10	9/10
Liver: vacuolized cytoplasm	0/10	8/10	9/10	10/10	10/10	10/10
Nose: olfactory epithelial degeneration	0/10	NE	0/10	3/10	6/10	9/10
Forestomach: epithelial hyperplasia	0/10	3/10	5/10	10/10	8/10	9/10
<b>Females</b>						
Forestomach: epithelial hyperplasia	0/10	8/10	7/10	10/10	9/10	9/10

<sup>a</sup>Indicates number of animals with lesions/number of animals examined.

<sup>b</sup>NE, not examined microscopically.

**A**

**B**

FIGURE 1. Nasal olfactory epithelial sections from a control male mouse (A) and a 7000-ppm male mouse (B). Note the loss of sensory epithelial thinning of the olfactory epithelium, atrophy of sensory nerve bundles, and submucosal fibrosis in the mouse exposed to isoprene.  $\times 50$ .

A

B

FIGURE 2. Forestomach of a control female mouse (A) and a 7000 ppm female mouse (B). Epithelial hyperplasia and hyperkeratosis were observed in mice exposed to isoprene.  $\times 50$ .

sure groups; a no apparent observable-effect level for this lesion was not achieved at the exposure concentrations used in this study.

## Discussion

Inhalation exposure of F344 rats to isoprene for 2 weeks at concentrations up to 7000 ppm (nearly 50% of the flammable level of this compound) did not affect hematologic and clinical chemistry parameters, body weight gain, or survival, or produce any apparent gross or microscopic lesions. Exposure of B6C3F<sub>1</sub> mice to the same atmospheric concentrations of isoprene did not produce mortality and caused a decrease in body weight gain only in males in the 7000-ppm exposure group. The following treatment-related effects were observed in isoprene-exposed mice: *a*) slight increases in liver weight; *b*) decreases in thymus, spleen, and testis weights; *c*) hematologic changes (reductions in red blood cell counts, hemoglobin concentrations, and volume of packed red cells); and *d*) microscopic lesions including thymus and testicular atrophy, olfactory epithelial degeneration, and forestomach epithelial hyperplasia. Many of the effects of exposure to isoprene have been observed in B6C3F<sub>1</sub> mice exposed to 1,3-butadiene (1,2,15,16). The changes in spleen weights in mice exposed to isoprene were not associated with histopathological alterations in this organ. The significance of the observed changes in the liver of male mice but not female mice, is unclear.

Mean corpuscular volume was increased in B6C3F<sub>1</sub> mice exposed to 625 or 1250 ppm 1,3-butadiene for 6 weeks or longer (15,16); this change was not observed in mice exposed to isoprene for 5 days at concentrations up to 7000 ppm. Whether the absence of this effect was due

to the short exposure period of 5 days will be evaluated in ongoing inhalation studies of isoprene with 13-week and 6-month exposures.

Testicular atrophy and epithelial hyperplasia of the forestomach were prominent effects of inhalation exposure of B6C3F<sub>1</sub> mice to 1,3-butadiene (1,2,15,16). Butadiene-induced hyperplasia of the forestomach may represent an early change in the development of forestomach neoplasms that were increased in incidence in B6C3F<sub>1</sub> mice exposed to 625 or 1250 ppm 1,3-butadiene (1,2,16). Chronic olfactory lesions were predominantly a male effect in mice exposed to 1,3-butadiene (1,2).

The lack of any observable toxicologic effects in F344 rats exposed to isoprene for 2 weeks provides preliminary evidence for a species difference between rats and mice in susceptibility to isoprene. Gage (17) also did not observe any toxic signs or histopathologic changes in rats exposed 6 hr/day for 15 days to 1670 ppm isoprene; the lungs of rats exposed for 6 days to 6000 ppm isoprene were slightly congested.

A structure/activity relationship between isoprene and the rodent carcinogen 1,3-butadiene is evident. Both compounds induce increases in the frequency of sister chromatid exchanges in bone marrow cells and in the levels of micronucleated erythrocytes in peripheral blood of mice (7,18); both compounds can be metabolized to monoepoxide and diepoxide intermediates by liver microsomal monooxygenases (8,9,19,20), the diepoxide intermediates of both compounds are mutagenic in *Salmonella* (10,21); both compounds cause reductions in red blood cell counts, hemoglobin concentrations, and volume of packed red cells in mice; and both compounds produced olfactory epithelial changes, testicular atrophy, and forestomach epithelial hyperplasia in mice. Due to these similarities, 13-week and 6-month inhalation

exposure studies of isoprene at concentrations ranging from 70 to 7000 ppm have been initiated in F344 rats and B6C3F<sub>1</sub> mice to determine whether isoprene acts similar to 1,3-butadiene after longer exposure durations.

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