Isoprene, an Endogenous Hydrocarbon and Industrial Chemical, Induces Multiple Organ Neoplasia in Rodents after 26 Weeks of Inhalation Exposure

Ronald L. Melnick, Robert C. Sills, Joseph H. Roycroft, Billy J. Chou, Harvey A. Ragan, and Rodney A. Miller

ABSTRACT

Isoprene, the 2-methyl analogue of 1,3-butadiene, is a high production chemical used largely in the manufacture of synthetic rubber and is the major endogenous hydrocarbon exhaled in human breath. Thirteen-week inhalation toxicology studies of isoprene were conducted in male and female F344 rats and B6C3F1 mice at exposure concentrations of 0, 70, 220, 700, 2200, and 7000 ppm (6 h/day; 5 days/week). In addition, 26-week inhalation studies at the same exposure levels, followed by a 26-week recovery period, were conducted in male rats and mice. The 13-week exposures produced no discernible exposure-related toxic effects in rats. Intestinal cell hyperplasia of the testis was observed in all male rats in the 7000 ppm group after 26 weeks of exposure; following the 26-week recovery period the only effect in rats was a marginal increase in benign testicular interstitial cell tumors. In mice, isoprene induced toxic and carcinogenic effects at multiple organ sites. Following the 26-week exposure and 26-week recovery periods, incidences of neoplastic lesions in the liver, lung, forestomach, and harderian gland were significantly increased. Neoplastic effects were observed at 700 ppm and higher exposures. Non-neoplastic lesions in mice exposed to isoprene included spinal cord degeneration, testicular atrophy, degeneration of the olfactory epithelium, and epithelial hyperplasia of the forestomach. A partial hindlimb paralysis and a nonresponsive macrocytic anemia were also seen in mice. Most of the toxic and carcinogenic effects caused by isoprene, as well as the species’ difference in response, had been observed after inhalation exposures to 1,3-butadiene.

INTRODUCTION

Isoprene (2-methyl-1,3-butadiene) is a colorless, volatile, and flammable liquid (boiling point: 34.1°C; vapor pressure: 493 mm Hg at 20°C) (1, 2). Approximately 350 million pounds of isoprene are produced annually in the United States (3), and more than 95% of industrial isoprene is used in the preparation of cis-1,4-polyisoprene elastomers for the manufacture of rubber tires, automotive parts, gaskets, footwear, adhesives, and flooring (1).

Isoprene has also been detected in tobacco smoke, and it is the monomeric unit of natural rubber and naturally occurring terpenes and steroids. Isoprene was identified as the major endogenous hydrocarbon of human breath (4, 5); exhalation of isoprene by human subjects was estimated to be 2–4 mg/day (5). In mice and rats, isoprene is produced at rates of approximately 0.4 and 1.9 μmol/h/kg, respectively (6).

Isoprene was examined for potential toxicological and carcinogenic effects because of its large annual production with potential human exposure and because of its structural similarity to BD, a multiple-organ carcinogen in Sprague-Dawley rats (7) and in B6C3F1 mice (8–11). Results of a 2-week inhalation study of isoprene in F344 rats and B6C3F, mice (12), plus published studies on BD, provide evidence of a structure/activity relationship between isoprene and BD. Both compounds cause degenerative changes, testicular atrophy, and forestomach epithelial hyperplasia in mice (12, 14); and both compounds produce significant increases in sister chromatid exchanges in bone marrow cells and in the frequency of micronucleated erythrocytes in peripheral blood of mice (15, 16). Furthermore, in all species studied, both compounds are metabolized to monooxypoxide and dioxyoxide intermediates by liver microsomal cytochrome P-450-dependent monoxygenase (17–20), and the diepoxide intermediates of both compounds are mutagenic in Salmonella typhimurium whereas the parent compounds are inactive (21, 22). Unlike BD, isoprene did not cause chromosomal aberrations in bone marrow cells of mice (15, 16), and the monooxypoxide intermediates of isoprene biotransformation are not mutagenic to S. typhimurium, whereas the primary monooxypioxide intermediate of BD biotransformation is mutagenic to Salmonella (21). Because lethal thymic lymphomas occurred as early as week 23 in mice exposed to BD (9), it was expected that if isoprene acts similarly to BD, a 26-week exposure to isoprene followed by a 26-week recovery period would also produce this carcinogenic response.

A comparison of toxicological and carcinogenic effects caused by these epoxide-forming chemicals is important because BD is a potent carcinogen in laboratory animals (7–11), and epidemiological studies have consistently found excess mortality for lymphatic and hematopoietic cancers in workers exposed to BD (23–26). The International Agency for Research on Cancer categorizes BD as probably carcinogenic to humans (27). Based on the carcinogenic effects of BD in laboratory animals and in humans, the United States Occupational Safety and Health Administration has proposed lowering the occupational standard for this chemical from 1000 to 2 ppm (28); meanwhile, there is no Occupational Safety and Health Administration standard or threshold limit value for isoprene.

MATERIALS AND METHODS

Exposure System. Isoprene with >99% purity, containing ~50 ppm of the peroxide inhibitor, t-butyl catechol, was obtained from Goodyear Tire and Rubber Company (Akron, OH). Concentrations of isoprene dimers in the bulk liquid were determined by gas chromatography and found to be <0.3%. Isoprene vapors were generated in a Buchi Rotavapor system (model EL-131S) at 50°C, carried in nitrogen through a chilled-water condenser to a distribution manifold, and then separately metered to each Hazleton 2000 exposure chamber (Lab Products, Inc., Aberdeen, MD). 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. The daily mean concentrations of isoprene for all chambers were between 99 and 100% of the target concentrations.

Animal Maintenance. Four- to six-week-old F344/N rats and B6C3F mice were obtained from Taconic Farms (Germantown, NY) and quarantined for 11–13 days prior to the start of the study. Animals were housed in individual wire mesh cage units within the exposure chambers. During the recovery period, animals were housed in chamber cage units stored on open racks. Animal rooms and chambers were maintained at 75 ± 3°C and 55 ± 15% relative humidity with ~15 air changes/h. City water (Richland, WA) and NIH-07 diet were available ad libitum, except during the exposure periods when the feed was removed.
Carcinogenicity of Isoprene

Exposure Regimen. In the 13-week studies, groups of 10 rats of each sex and 10 mice of each sex were exposed to isoprene vapors by whole-body exposures at target concentrations of 0, 70, 220, 700, or 7000 ppm for 6 h and 24 h (time to reach 90% of the target concentration, ~12 min) per day, 5 days/week for 13 weeks (excluding weekends and holidays). Additional rats and mice were used in special clinical pathology studies. At the end of the 13-week exposures, all rats and mice were euthanized and evaluated histopathologically. In the 26-week exposure study, groups of 40 male rats and 40 male mice were exposed to the same concentrations of isoprene as were the animals in the 13-week studies. At the end of the 26-week exposure period, 10 rats and 10 mice/exposure group were euthanized and evaluated. The remaining animals were allowed to recover for an additional 26 weeks without exposure to isoprene at which time they were euthanized and evaluated.

Clinical Pathology. Blood samples were collected from 10 special study rats and mice per sex per exposure group on days 4 and 24, and from animals euthanized after the 13- and 26-week exposures. Blood samples were collected from the retroorbital sinus of CO2-anesthetized animals into tubes either containing EDTA (for hematomical analyses) or devoid of anticoagulant (for clinical chemistry evaluations). The latter samples were allowed to clot, and serum samples were collected after centrifugation. Hematology was performed with an Ortho ELT-8/ds hematology analyzer. The parameters evaluated included erythrocyte count, hemoglobin concentration, hematocrit, reticulocyte count, mean cell volume, mean cell hemoglobin, and platelet and leukocyte counts. Blood smears were stained with Wright-Giemsa for leukocyte differential counts. Serum clinical determinations were made with an Abbott VP chemistry analyzer and included urea nitrogen, creatinine, alanine aminotransferase, glutamate dehydrogenase, and sorbitol dehydrogenase. Urine samples were collected during week 12 from rats held overnight in individual metabolism cages. Abbott VP methodologies were used to measure glucose, creatinine, alkaline phosphatase, and aspartate aminotransferase concentrations.

Sperm Motility and Vaginal Cytology Evaluations. Sperm motility and vaginal cytology evaluations were performed on all rats and mice exposed to 0, 70, 700, or 7000 ppm of isoprene in the 13-week study according to published methods (29).

Forelimb/Hindlimb Grip Strength. Groups of 20 mice from the control group and each of the exposure groups were evaluated for forelimb and hindlimb grip strength (30) at the end of the 26-week exposure period. Ten mice/group were euthanized, and the remaining mice were evaluated at 2 days, and at 1-, 3-, and 6-months postexposure.

Histopathology. Complete necropsies were performed on all animals. The brain, heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed prior to fixation. Tissue samples, preserved in 10% neutral buffered formalin, were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: gross lesions and tissue masses, adrenal glands, brain, esophagus, femur and marrow, gallbladder (mice only), heart, small intestine, large intestine, kidneys, larynx, liver, lungs, lymph nodes (bronchial, mediastinal, mandibular, and mesenteric), mammary gland with adjacent skin, nasal cavity and turbinates, ovaries, pancreas, parathyroid glands, pituitary gland, preputial gland, prostate gland, salivary glands, spinal cord, and sciatic nerve (when neurological signs were present), spleen, forestomach, glandular stomach, testes with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.

Statistical Analyses. Differences in survival were analyzed by life table methods, and incidences of neoplasms and nonneoplastic lesions (the ratio of the number of animals bearing such lesions at a specific anatomical site to the number of animals in which that site was examined), including information on time of death were analyzed by life table tests (31, 32), logistic regression analyses (33), Fisher exact analyses, and Cochran-Armitage trend analyses (34, 35). Clinical chemistry, hematology, and grip strength data were analyzed using the nonparameter multiple comparisons methods of Shirley (36) or Dunn (37).

RESULTS

Effects in Rats. No treatment-related gross or microscopic changes or effects on survival, body weight gain, clinical signs of toxicity, or clinical pathology (hematology, clinical chemistry, and urinalysis) were observed in rats exposed to isoprene for 13 weeks. Following 26 weeks of exposure, the only effect in rats was an increase in the incidence and relative severity of interstitial cell hyperplasia of the testis in the 7000 ppm isoprene group (Table 1). This lesion is generally uncommon in 9-month-old F344 rats; however, the incidence of this spontaneous lesion increases rapidly in untreated controls after 1 year of age (38). Following the 26-week recovery period, the incidence of interstitial cell adenoma was slightly greater in male rats exposed to 700 ppm or higher concentrations of isoprene. The incidence and/or severity of interstitial cell hyperplasia was also slightly greater in exposed groups compared to controls at this time; however, concentration-related differences were not clearly evident because of the high incidence in the control group. Based on the findings of increased hyperplastic lesions at 26 weeks and adenomas following the 26-week recovery, the early development of interstitial cell-proliferative lesions was considered to be exposure-related. No other gross or histopathological lesions in rats were attributed to isoprene exposures.

Hematological and Histopathological Effects in Mice following 13 Weeks of Exposure. At the end of the 13-week exposure to isoprene, there were no concentration-related effects on survival, body weight gain, or clinical signs of toxicity. A mild normocytic, normochromic, nonresponsive anemia (reduced erythrocyte count, hematocrit, and hemoglobin concentration without effect on mean cell volume, reticulocyte numbers, or frequency of polychromatic cells) was observed in male and female mice exposed to isoprene for 3 days; by day 24, the nonresponsive anemia became macrocytic, as evidenced by the increase in mean cell volume values in exposed groups (Table 2). Similar hematological changes were evident after 13 and 26 weeks of exposure to isoprene (results not shown).

Table 1: Testicular lesions in male F344 rats exposed to isoprene for 26 wk followed by a 26-wk recovery

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>0</th>
<th>70</th>
<th>220</th>
<th>700</th>
<th>2200</th>
<th>7000</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 26-wk exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal cell hyperplasia</td>
<td>1/10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/10</td>
<td>3/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
</tr>
<tr>
<td>After 26-wk recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal cell hyperplasia</td>
<td>2/30</td>
<td>3/30</td>
<td>2/30</td>
<td>2/30</td>
<td>2/30</td>
<td>2/30</td>
</tr>
<tr>
<td>Intestinal cell adenoma</td>
<td>3/30</td>
<td>3/30</td>
<td>4/30</td>
<td>7/30</td>
<td>8/30</td>
<td>9/30</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of lesion-bearing animals/number of animals examined.

<sup>b</sup> P < 0.05, significantly different from control group.

<sup>c</sup> Average severity based on the number of animals with lesion: 1, minimal; 2, mild; 3, moderate; and 4, marked.

<sup>d</sup> P < 0.05, significantly different from control group.

<sup>e</sup> Beneath the control incidence is the P-value associated with the Cochran-Armitage trend test. Beneath the incidence values of the exposed groups are the Fisher exact test P values corresponding to pairwise comparisons between the control and the exposed groups.

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Inhalation exposure of mice to isoprene for 13 weeks produced microscopic changes in the forestomach, nasal cavity, liver, and testis (Table 3). Focal epithelial hyperplasia of the forestomach was observed in male and female mice exposed to 700 ppm or higher concentrations of isoprene. Olfactory epithelial degeneration, characterized by loss of sensory epithelial cells and thinning of the olfactory epithelium, was observed in all mice exposed to 7000 ppm isoprene. Cytoplasmic vacuolization of hepatocytes due to glycogen accumulation was observed in males only. There was no evidence of hepatocellular necrosis from either microscopic or clinical pathology evaluations. Testicular weight was reduced by 35% in the 7000 ppm exposure group (Table 3). The increase in forestomach neoplasms was significantly greater in the 2200 and 7000 ppm exposure groups than in the controls, and the incidence of squamous cell papillomas or carcinomas was increased in these groups. The increase in the forestomach epithelium was observed in most mice in the 700-, 2200-, and 7000 ppm exposure groups, and the incidence of alveolar!bronchiolar neoplasms was significantly greater in the 2200 and 7000 ppm exposure groups than in the control group. The incidence of altered hepatocellular foci was also increased in these groups.

No treatment-related histopathological changes were detected in the lungs of isoprene-exposed mice at the end of the 26-week exposure; however, following the 26-week recovery period, the incidence of alveolar epithelial hyperplasia was increased in the 700 ppm and higher exposure groups and the incidence of alveolar/bronchiolar neoplasms was significantly greater in the 2200 and 7000 ppm exposure groups than it was in controls (Table 4). The increase in lung neoplasms in the 700 ppm group was not statistically significant. Alveolar epithelial hyperplasia, a proliferative lesion that may represent a preneoplastic change in the lung, consisted of a focal increase in the cellularity of the alveolar epithelium with retention of alveolar structure due to the formation of complex, irregular papillary patterns lined by relatively uniform cuboidal or columnar cells. The carcinomas were similar to the adenomas but consisted of heterogeneous cell populations with varying degrees of cellular pleomorphism and atypia. Carcinomas were larger and highly anaplastic, and they often contained areas of hemorrhage or necrosis.

At the end of the 26-week exposure, focal hyperplasia of the forestomach epithelium was observed in most mice in the 700-, 2200-, and 7000 ppm exposure groups, and a squamous cell papilloma was observed in one mouse in the 700 ppm group. No changes were seen in the forestomach of controls. At the end of the 26-week recovery period, the incidence of forestomach hyperplasia was greater in the 700 ppm and higher exposure groups than in the controls, and the incidence of squamous cell papillomas or carcinomas was increased in the 7000 ppm group (Table 4). The increase in forestomach neoplasms
in the 2200 ppm group was not statistically significant. Forestomach epithelial hyperplasia was typically a focal lesion consisting of thickened epithelium forming blunt folds. Papillomas consisted of short stalks and branching papillae with a well-differentiated stratified squamous epithelium overlying a fibrovascular stroma. Carcinomas exhibited invasion of the forestomach mucosa by cords and sheets of anaplastic epithelial cells.

The incidence of harderian gland adenomas was significantly increased in the 700, 2200, and 7000 ppm exposure groups (Table 4). Adenomas were usually larger than hyperplastic lesions and caused compression of the surrounding tissue.

After 26 weeks of exposure to isoprene, mild to minimal olfactory epithelial degeneration in the nasal cavity was observed in all mice in the 7000 ppm exposure group (Table 5). At the end of the 26-week

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**Table 4** Incidence of neoplastic and nonneoplastic lesions in the liver, lung, forestomach, and harderian gland induced by isoprene in male mice after 26 wk of inhalation exposure and 26 wk recovery

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Concentration (ppm)</th>
<th>No. of animals examined</th>
<th>No. of lesion-bearing animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>70</td>
<td>220</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophilic focus</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Eosinophilic focus</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mixed-cell focus</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hepatocellular adenoma&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hepatocellular carcinoma&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Adenoma or carcinoma&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar epithelial hyperplasia&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Alveolar/bronchiolar adenoma&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Alveolar/bronchiolar carcinoma&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adenoma or carcinoma&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Forestomach</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell papilloma&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Papilloma or carcinoma&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>Harderian gland</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Adenoma&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Superscripts by the name of a particular lesion indicate significant trend tests, superscripts by incidence values indicate significance between control and exposed groups.

<sup>b</sup> *P* ≤ 0.01.

<sup>c</sup> *P* ≤ 0.05.
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Fig. 2. Exposure-response curves for hepatocellular adenomas or carcinomas of the liver (■), adenomas of the hardarian gland (●), alveolar-bronchiolar adenomas or carcinomas of the lung (□), and squamous cell papillomas or carcinomas of the forestomach (▲) in male mice exposed to isoprene for 26 weeks followed by a 26-week recovery period.

recovery period, the incidence of mild to moderate olfactory epithelial degeneration was significantly elevated in the 220 ppm and higher exposure groups.

Exposure-related decreases in testis weight were observed in mice after 13 or 26 weeks of exposure to isoprene but not after the recovery period (results not shown). The incidence of testicular atrophy was also greater in male mice exposed to 7000 ppm isoprene for 26 weeks, but not after the recovery period (Table 5).

Minimal degeneration of the spinal cord white matter was evident in mice exposed to 7000 ppm isoprene for 26 weeks; however, after the 26-week recovery period the incidence of spinal cord degeneration was significantly increased in all exposure groups. Spinal cord degeneration most likely accounted for the hindlimb dysfunction discussed above.

DISCUSSION

The inhalation studies reported here demonstrate that isoprene, the 2-methyl analogue of BD, induces multiple organ neoplasia in mice after only 26 weeks of exposure. Because most neoplastic lesions were observed after the 26-week recovery period, it is evident that preneoplastic lesions resulting from exposure to isoprene persisted and progressed to tumors in the absence of further exposure to this chemical. This response is similar to that previously observed for BD, where multiple organ neoplasia was seen in mice exposed to BD for 13 or 26 weeks and then held for up to 2 years without additional exposure (9). The present studies with isoprene also show that like BD, the carcinogenic outcomes are different in rats than in mice.

Both isoprene and BD are metabolized to mono- and diepoxide intermediates by liver microsomal cytochrome P-450 dependent monoxygenase (17–20), and the metabolic elimination rates of isoprene and BD are 2–3 times greater in mice than in rats (6, 39). In contrast to BD, isoprene can be metabolized to two different monoepoxide intermediates, and only the minor intermediate (20%), 3,4-epoxy-2-methyl-1-butene, was found to be oxidized to isoprene diepoxide (17, 18). The primary metabolite of BD metabolism, 1,2-epoxy-3-butene, can be further oxidized to diepoxybutane. The epoxide intermediates of isoprene and BD metabolism are 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 BD metabolism are mutagenic in Salmonella, whereas the monoepoxide intermediates of isoprene metabolism were inactive (21). Thus, based on differences in their metabolism and in the mutagenicity profiles of their metabolic intermediates, it is expected that exposure to BD would result in a greater body burden of mutagenic epoxides than would exposure to an equivalent concentration of isoprene. BD and isoprene are also genotoxic to mouse bone marrow cells, with effects of BD extending to lower exposure concentrations (15, 16). The epoxide intermediates of BD metabolism are thought to be important in the carcinogenicity of this chemical because they are direct-acting mutagens (21, 22), and because they induce local neoplasms when administered by skin application or by s.c. injection (27).

As a consequence of the numerous similarities between isoprene and BD (structure, species sensitivity, organ toxicity, genetic toxicity, and metabolism), we considered the possibility that isoprene produces carcinogenic effects similar to those of BD. The 26-week exposure duration was selected because 13- and 26-week stop-exposure studies with 625 ppm of BD produced neoplasms in male mice at the same sites as mice in the 2-year exposure study of BD and because high incidences of lymphomas were detected within approximately 23 weeks after the start of exposure to BD (9).

In rats exposed to up to 7000 ppm isoprene for 13 weeks, no toxicological effects were evident. These results were not totally unexpected since no treatment-related changes were reported in Sprague-Dawley rats exposed to BD (1000–8000 ppm; 6 h/day; and 5 days/week) for 13 weeks (40). Interstitial cell hyperplasia of the testis was observed in male rats exposed to 7000 ppm isoprene for 26 weeks, and following the 26-week recovery period, the incidence of benign interstitial cell testicular tumors was marginally greater in this group than in the controls (Table 1). To confirm this finding, a
long-term study in a strain of rat that does not exhibit a high rate of spontaneous testicular interstitial cell lesions would be necessary. In untreated F344 rats, the incidence of interstitial cell proliferative lesions increases rapidly after 1 year of age, and the incidence of testicular interstitial cell adenomas is greater than 80% by 18 months (38). Furthermore, because 2-year exposures were necessary to demonstrate the carcinogenicity of BD in rats (7), an exposure duration greater than 26 weeks would be necessary to more fully evaluate the carcinogenic potential of isoprene in this species.

In mice, isoprene was carcinogenic to the liver, lung, forestomach, and harderian gland. Based on the data reported here and on the carcinogenicity of BD following exposure to 625 ppm for 13 or 26 weeks or exposure to 200 ppm BD for 40 weeks (9, 11), isoprene appears to be less active than BD in inducing neoplastic lesions in mice. The difference between the carcinogenic outcomes for isoprene and BD may be due in part to differences in experimental design. Evaluations of the carcinogenicity of BD were made after 2 years of exposure or at the end of a 2-year period that included 13–52 weeks of exposure followed by an extended recovery period; whereas evaluations of the carcinogenicity of isoprene were made following 26 weeks of exposure plus a 26-week recovery period. Like BD, isoprene is genotoxic to bone marrow cells, suppresses hematopoiesis (16), and induces a nonresponsive, macrocytic anemia in mice (Table 2). However, unlike BD (16), exposure to isoprene did not produce an increase in lymphomas. Because lymphomas were observed as early as week 23 in mice exposed to 625 ppm BD (9), the exposure regimen used in the isoprene studies should have been sufficient to detect a carcinogenic response in the hematopoietic system if isoprene was as active as BD.

As noted above, differences in mutagenicity of the metabolic intermediates may contribute to the dissimilar carcinogenic outcomes for BD and isoprene. Metabolic saturation limits the production of epoxide intermediates at isoprene exposures greater than approximately 1500 ppm (6). Thus, exposures to isoprene may not achieve the same levels of mutagenic epoxides as that resulting from exposure to 625 ppm BD. Metabolic saturation may also account for why the incidences and severities of many toxic and carcinogenic effects of isoprene produced at 700 or 2200 ppm were not very different from those at 7000 ppm. Toxic effects which were more severe or which occurred at a higher incidence in the 7000 ppm exposure group may reflect the involvement of the parent compound.

As with BD, species differences were observed between the toxicological and carcinogenic effects of isoprene in rats and mice. The basis for species differences resulting from exposure to BD are not fully understood. Physiologically based pharmacokinetic models of the uptake, tissue distribution, and metabolism of inhaled BD did not reveal species differences in concentrations of epoxybutene in blood or lung that would be of sufficient magnitude to account for the difference in carcinogenic responses observed between rats and mice (41, 42). Evidently, factors beyond tissue dosimetry of epoxybutene must be crucial in accounting for species differences in BD-induced carcinogenesis. Tissue dosimetry data for isoprene and its epoxide intermediates have not been reported.

N2-guanine alkylation products formed by the reaction of BD-epoxide metabolites with isolated DNA (43) have been detected in liver DNA of mice exposed to BD (44). Furthermore, BD and its epoxide metabolites have been shown to produce transition and transversion mutations as well as a high frequency of frameshift mutations in mice (45). Similar data on DNA adducts and genetic alterations induced by isoprene are needed. Activated K-ras genes with codon 13 mutations (mostly G to C transversions) and allelic losses at loci near several tumor suppressor genes were detected in tumor tissues obtained from mice exposed to BD (46, 47). These genetic changes reflect the potential involvement of activated K-ras oncogenes and inactivated tumor suppressor genes in BD carcinogenesis. Studies on the patterns of mutations in oncogenes and tumor suppressor genes in tumor tissues from mice exposed to isoprene are in progress.

In conclusion, most of the toxic and carcinogenic effects resulting from exposure to isoprene were also caused by inhalation exposure to BD. Isoprene appears to be less active than BD in inducing tumors at low concentrations; this may be due to differences in experimental design (exposure duration) and to differences in body burdens of mutagenic metabolites. Unlike BD, isoprene is produced endogenously; only about 15% of endogenous isoprene is exhaled by rats and about 25% is exhaled by mice. The calculated rates of metabolism of endogenously produced and systemically available isoprene are 1.6 μmol/h/kg in rats and 0.3 μmol/h/kg in mice (6). Based on an estimated absorption of 10%, these rates would be equivalent to isoprene exposures of approximately 0.5 ppm for rats and 0.1 ppm for mice. Thus, endogenously produced isoprene contributes negligibly to the neoplastic effects reported here.

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