NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for ISOPRENE

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NTP Report on Carcinogens Listing for Isoprene

Carcinogenicity

Isoprene is *reasonably anticipated to be a human carcinogen* based on evidence of benign and malignant tumor formation at multiple organ sites in multiple species of experimental animals (Melnick et al., 1994; NTP, 1995; NTP, 1997 draft; Placke et al., 1996). Inhalation exposure of mice to isoprene vapors induced increased incidences of neoplasms of the lung, liver, Harderian gland, forestomach, hematopoietic system, and circulatory system. Inhalation exposure of rats to isoprene vapors induced increased incidences of neoplasms of the mammary gland, kidney, and testis.

No studies on the potential carcinogenicity of isoprene in humans were identified.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Isoprene is the 2-methyl analog of 1,3-butadiene, an industrial chemical that has been identified as an animal and human carcinogen. Isoprene and butadiene are metabolized to monoepoxide and diepoxide intermediates by liver microsomal cytochrome P450-dependent monooxygenases from several species, including humans. Detoxification of these intermediates may occur by hydrolysis catalyzed by epoxide hydrolase or conjugation with glutathione catalyzed by glutathione-*S*-transferase. The diepoxide intermediates of isoprene and butadiene are mutagenic in *Salmonella typhimurium* whereas the parent compounds are inactive (Gervasi et al., 1985). In mice, isoprene and 1,3-butadiene induced sister chromatid exchanges in bone marrow cells and increased the frequency of micronucleated erythrocytes in peripheral blood (Tice et al., 1987; cited by NTP, 1997 draft; Tice et al., 1988). Common sites of neoplasm induction by isoprene and butadiene include the mammary gland and testis in rats, and the liver, lung, Harderian gland, forestomach, and circulatory system in mice (NTP, 1997 draft). Lung and Harderian gland neoplasms induced by isoprene in mice had a high frequency of unique K-*ras* mutations (A to T transversions at codon 61) (Hong et al., 1997).

No data are available that would suggest that mechanisms thought to account for tumor induction by isoprene in experimental animals would not also operate in humans.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or

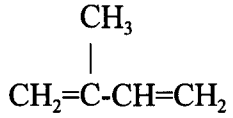
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 CHEMICAL PROPERTIES

Isoprene
[78-79-5]



1.1 Chemical Identification

Isoprene (C_5H_8, mol. wt. = 68.1) is also called:

Isopentadiene
β-Methylbivinyl
2-Methylbutadiene
2-Methyl-1,3-butadiene
2-Methyldivinyl
2-Methylerythrene

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	Colorless	Budavari (1996)
Physical State	Liquid	Budavari (1996)
Melting Point, °C	-145.95	Budavari (1996)
Boiling Point, °C	34.067	Budavari (1996)
Density at 20 °C	0.6805	Budavari (1996)
Solubility:		
Water at 20 °C	Insoluble in water	
Organic Solvents	Miscible with ethanol or diethyl ether	Budavari (1996)
Partition Coefficients:		
Log octanol/water	2.42	Chem. Inspect. Test. Inst. (1992; cited by HSDB, 1997)
Vapor pressure at 25 °C	550 mm Hg	Zwolinski et al. (1971; cited by HSDB, 1997)
Conversion factor	1 ppm = 2.79 mg/m^3 at 25 °C and 760 mm Hg	Clayton and Clayton (1981-1982; cited by HSDB, 1997)

Isoprene is a highly flammable liquid. It has a flash point of -48 °C and is easily ignited by heat, sparks, or flames (U.S. DOT, 1996; cited by HSDB, 1997; Saltman, 1985). Vapors may form highly explosive mixtures with air and may polymerize explosively when heated. It is highly reactive, with reactions similar to those of 1,3-butadiene. In the absence of inhibitors, isoprene forms peroxides upon air exposure (Saltman, 1985).

Isoprene is one of the major photochemically reactive hydrocarbons emitted by numerous plant species (Bowling et al., 1998). The large quantities of non-methane hydrocarbons (NMHCs) emitted by vegetation, especially in tropical and subtropical regions, influence atmospheric processes. Isoprene and other highly reactive natural alkenes can serve as precursors to formation of photochemical oxidants that contribute to regional-scale air pollution (Hoffman et al., 1996). Isoprene, the monoterpenes, and other unsaturated hydrocarbons react with hydroxyl radicals (HO·) and tropospheric ozone (O_3) and may act as photochemical smog precursors. Since they compete directly with methane for hydroxyl radicals, they may indirectly affect the global warming trend (Loreto, 1997). Condensable oxidation products undergo gas-to-particle conversion, forming tropospheric organic particulates (Hoffman et al., 1996). However, Altschuller (1983) stated that the organic aerosols of the eastern United States formed from biogenic hydrocarbons do not contribute "a significant fraction" to urban and rural fine particulate aerosol concentrations.

The lifetime of atmospheric isoprene has been variously estimated to be 1.3 to 34.0 hours and 1 to 2 hours based on its rates of reactions with ozone and hydroxyl radicals. Some monoterpene species are more reactive with lifetimes less than 0.5 h (Altschuller, 1983; Guenther et al., 1995).

In sunlight, ultraviolet irradiation of isoprene, other biogenic NMHCs, and anthropogenic hydrocarbons in the presence of atmospheric nitrogen oxides (NO_x) gives numerous reaction products, including acetaldehyde, acetone, carbon dioxide, carbon monoxide, formaldehyde, formic acid, and peroxyacetyl nitrates (PAN). These products plus methacrolein and methyl vinyl ketone, which are apparently specific to isoprene, represent 30 to 73% of the carbon content of the reacted isoprene (Altschuller, 1983).

2.0 HUMAN EXPOSURE

2.1 Production

Isoprene is recovered from C_5 streams as a by-product of thermal cracking of naphtha or gas oil. The isoprene yield is about 2 to 5% of the ethylene yield (Saltman, 1985). U.S. demand for isoprene grew 6.5% annually from 1985 to 1992 (Chem. Mark. Rep., 1994; Chem. Week, 1994). In 1994, isoprene production in the United States was approximately 619 million pounds (281,000 Mg [metric tons]) (USITC, 1995), an increase of almost 29% over production in 1992 (USITC, 1994). Estimated isoprene production capacity for 8 facilities was 598 million pounds in 1996, based on estimates of isoprene content of product stream available from ethylene production via heavy liquids (SRI Int., 1997).

2.2 Use

About 95% of isoprene production is used to produce *cis*-1,4-polyisoprene; 2%, to produce butyl rubber (isobutene-isoprene copolymer); and 3%, to produce thermoplastic, elastomeric co-block (SIS) polymers (Saltman, 1985; Taalman, 1996).

2.3 Exposure

2.3.1 Endogenous

Isoprene is formed endogenously in humans; concentrations in blood range from 15 to 70 nmol/L (1.0 to 4.8 μg/L) (Cailleux et al., 1992; cited by NTP, 1997 draft). Humans produce isoprene endogenously at a rate of 0.15 μmol/kg/h (Taalman, 1996) [about 17 mg/day for a 150-lb (70-kg) person]. [Endogenous production rates reported for rats and mice are 1.9 and 0.4 μmol/kg/h, respectively (Peter et al., 1987; cited by Taalman, 1996).] The availability and distribution of endogenous isoprene is partially controlled by the activated precursor isopentenyl pyrophosphate for the synthesis of biomolecules that contain isoprene units (NTP, 1997 draft).

Isoprene was the major hydrocarbon (up to 70%) in the air exhaled by all but one of 30 volunteers after they had breathed purified air for 10 min. The quantity exhaled per day per individual was reported as 2 to 4 mg and did not vary with age, sex, ethnicity, diet, life style, or fasting or nonfasting state. However, individuals showed day-to-day variations (Gelmont et al., 1981). The exhalation rate fell within the range of others reported—40 to 250 μg/h or 0.96 to 6.0 mg/day for nonsmokers and 15 to 390 μg/h or 0.36 to 9.36 mg/day for smokers (Clayton and Clayton, 1981-1982; cited by HSDB, 1997).

Breath concentrations in nonsmokers exhaling 1 to 6 mg isoprene/day can be estimated to be 0.05 to 0.4 mg/m^3 based on the assumption that an adult breathes about 15 to 20 m^3 air per day. Somewhat higher values of 10 to 30 nmol/L or 0.68 to 2.0 mg/m^3 were reported by Cailleux and Allain (1989; cited by IARC, 1994). These estimates are comparable to more recent isoprene determinations in human breath. For example, baseline isoprene concentrations in alveolar breath of nonsmokers and smokers were 7.2 ± 0.5 and 6.2 ± 0.4 nmol/L (0.49 and 0.42 mg/m^3), respectively, in the experiments of Euler et al. (1996) further described below. The isoprene concentration in the air breathed by the subjects was 0.18 ± 0.03 nmol/L (0.012 mg/m^3). [In contrast to humans, Gelmont et al. (1981) reported that "significant amounts" of isoprene were not found in the breath of mice, guinea pigs, chickens, rabbit, and dog. Nursing rats and weanling rats eating sour cream and cottage cheese exhaled isoprene, but amounts decreased within 6 days of weaning.]

Analysis of the air of a parked car occupied by one person found that isoprene at 0.020 mg/m^3 was the major volatile non-methane hydrocarbon (NMHC) when the car was not in a traffic-polluted place. The isoprene level was much lower in an unoccupied car, indicating that isoprene originates mainly from expired air (Bjorkvist et al., 1997).

As discussed below, ambient air concentrations of isoprene are generally less than about 10 ppb C or about 0.03 mg isoprene/m^3, an order of magnitude less than the reported breath concentrations given above. Based on estimated human intake of 15 to 20 m^3 air per day, ambient air would contribute less than 0.45 to 0.6 mg isoprene/day to daily exposure. The estimate of 17 mg/day given above for human endogenous production of isoprene is about 30- to 40-fold higher than the contribution from ambient air (calculations by ILS Inc.). Yet recently, Phillips et al. (1994), presented evidence that isoprene is not produced endogenously. The publication did not give the actual isoprene concentrations in breath of 12 volunteers and the air they breathed, but it showed graphically that the breath concentration was lower than that in the ambient air. They concluded that isoprene must come from an exogenous source and is then catabolized or excreted by a pathway other than the lungs (such as the kidneys or the liver).

2.3.2 Occupational

NIOSH collected data on potential exposure to isoprene in the National Occupational Hazard Survey (NOHS) from 1972 to 1974 (NIOSH, 1976) and in the National Occupational Exposure Survey (NOES) from 1981 to 1983 (NIOSH, 1990). These data are presented in Tables 2-2 and 2-3. The first survey (NIOSH, 1976) indicated that 58,000 employees in over 30 different industries were potentially exposed to isoprene. The more limited later survey of six industries showed that approximately 3,700 workers were potentially exposed to isoprene between 1981 and 1983 (NIOSH, 1990).

TABLE 2-1. POTENTIAL INDUSTRIAL ISOPRENE EXPOSURES ESTIMATED IN THE 1972-1974 NATIONAL OCCUPATIONAL HAZARD SURVEY (NOHS) (NIOSH, 1976)

SIC CODE	DESCRIPTION	PLANTS	TOTAL EMPLOYEES	FEMALE EMPLOYEES
1511	GENERAL BUILDING CONTRACTORS	40	200	
1941	SIGHTING AND FIRE CONTROL EQUIPMENT	13	26	
2281	YARN MILLS, EXCEPT WOOL	14	14	
2329	MEN'S AND BOYS' CLOTHING, NEC	222	444	
2621	PAPER MILLS, EXCEPT BUILDING PAPER	6	1,051	
2651	FOLDING PAPERBOARD BOXES	94	5,280	
2751	COMMERCIAL PRINTING, EX LITHOGRAPIC	113	2,262	
2818	INDUSTRIAL ORGANIC CHEMICALS, NEC	68	270	
2822	SYNTHETIC RUBBER	20	6,316	
2851	PAINTS AND ALLIED PRODUCTS	47	3,111	
2893	PRINTING INK	23	46	
2992	LUBRICATING OILS AND GREASES	18	234	
3021	RUBBER FOOTWEAR	8	8	
3069	FABRICATED RUBBER PRODUCTS, NEC	33	606	
3291	ABRASIVE PRODUCTS	31	778	
3411	METAL CANS	10	20	
3481	MISC. FABRICATED WIRE PRODUCTS	35	71	
3531	CONSTRUCTION MACHINERY	56	56	
3564	BLOWERS AND FANS	23	69	
3622	INDUSTRIAL CONTROLS	27	108	
3632	HOUSEHOLD REFRIGERATORS AND FREEZERS	24	353	
3651	RADIO AND TV RECEIVING SETS	38	943	
3662	RADIO AND TV COMMUNICATION EQUIPMENT	7	28	
3731	SHIP BUILDING AND REPAIRING	20	490	
3742	RAILROAD AND STREET CARS	20	39	
3821	MECHANICAL MEASURING DEVICES	14	1,571	
4131	INTERCITY BUS LINES	25	422	
4511	CERTIFICATED AIR TRANSPORTATION	24	24	
4832	RADIO BROADCASTING	40	356	
4924	NATURAL GAS DISTRIBUTION	499	2,118	
5092	PETROLEUM AND PETROLEUM PRODUCTS	164	14,596	
5311	DEPARTMENT STORES	57	342	
5321	MAIL ORDER HOUSES	22	22	
7929	ENTERTAINERS' & ENTERTAINMENT GROUPS	57	1,140	
7946	AMUSEMENT PARKS	18	36	
8061	HOSPITALS	349	14,650	
TOTAL		2,278	58,102	

TABLE 2-2. POTENTIAL INDUSTRIAL ISOPRENE EXPOSURES ESTIMATED IN THE 1981-1983 NATIONAL OCCUPATIONAL EXPOSURE SURVEY (NOES) (NIOSH, 1990)

SIC CODE	DESCRIPTION	PLANTS	TOTAL EMPLOYEES	FEMALE EMPLOYEES
2399	FABRICATED TEXTILE PRODUCTS, NEC	73	146	
2819	INDUSTRIAL INORGANIC CHEMICALS, NEC	3	119	
2822	SYNTHETIC RUBBER	15	925	108
2841	SOAP AND OTHER DETERGENTS	7	1,557	276
2911	PETROLEUM REFINING	7	105	7
7391	RESEARCH & DEVELOPMENT LABORATORIES	17	851	187
TOTAL		123	3,703	578

2.3.3 Environmental

2.3.3.1 Global Biogenic Emissions

Isoprene is emitted from plants and trees and is widely present in the environment at low concentrations (Taalman, 1996). Globally, 90% of total emissions of NMHC volatile organic compounds (VOCs) are from natural sources, primarily vegetation and forest fires. NMHC VOCs, however, constitute much smaller fractions of VOCs in industrialized areas (e.g., 16% in France), with automobiles and industrial emissions constituting the major sources of anthropogenic emissions (Dueso, 1997).

Guenther et al. (1995) estimated that the tropics emit 40 to 50% of the 503 Tg (million metric tons) [calculated as carbon] of isoprene emitted globally per year from natural sources. Other estimates have been as high as 70 to 80%. According to the authors' estimates, isoprene emissions represent 43.7% of total global natural VOC emissions. Two other recent estimates of isoprene's contribution to total global VOCs from natural sources were approximately 51%. Estimates from seven other publications from 1979 to 1992 for total global natural isoprene emissions ranged from 175 to 452 Tg C/yr (Guenther et al., 1995).

2.3.3.2 U.S. Biogenic Emissions

The chief concern in the United States with natural or biogenic emissions of NMHCs has been secondary product formation leading to the production of ozone, aerosols, and other species of concern (Altschuller, 1983). The atmospheric reactions of ambient hydrocarbons during the summer months with airborne nitrogen oxides in some areas in the United States generate enough ozone to violate the National Ambient Air Quality Standard. Even in industrialized areas such as Atlanta, GA, biogenic emissions may constitute 50% of the total atmospheric hydrocarbons in the ambient air. Considerable effort has been made to understand the contribution of isoprene since States must develop control strategies for anthropogenic emissions from stationary and mobile sources to meet the air quality standards. The Biogenic Emission Inventory Models BEIS and BEIS2 in conjunction with the Urban Airshed Model may underestimate isoprene emissions (Chang et al., 1996). An hourly biogenic emissions inventory method (PC-BEIS) is used in the U.S. EPA's regional-scale air quality models (Lamb et al., 1993).

Various techniques for predicting emissions of biogenic hydrocarbons have given substantially different estimates. The techniques must extrapolate between different locations, times, and species variations (Altschuller, 1983). Reclassifications of many landscapes from deciduous or coniferous to mixed forests, woodlands, and other categories affect the comparability with older estimates (Guenther et al., 1994). An early U.S. biogenic emissions inventory developed by Zimmerman (1979; cited by Altschuller, 1983) estimated that annual isoprene emissions in the contiguous United States totaled 15 Mg (metric tons) compared to 50 Mg of monoterpenes and 27 Mg anthropogenic emissions. Vehicular emissions represented 44% of the total anthropogenic emissions in 1977. Almost 25% of isoprene emissions and 22% of monoterpene emissions were estimated for the month of July. More recent inventories are orders of magnitude higher.

Emission rate factors for foliar emissions of isoprene, monoterpenes, and other VOCs (< 0.1 to 70, < 0.1 to 3, and < 0.5 to 5 μg C per gram per hour, respectively) at a leaf temperature of 30 °C were recently estimated for 49 tree genera and extrapolated to the United States using land

cover composition estimates. Some species of oak (*Quercus*), *Eucalyptus*, and aspen (*Populus*) emit isoprene at even higher rates. Different woodland types have emission rate factors ranging from 0.8 to 11 mg C/m^2/h. The average total biogenic emission rate factor for total VOCs in the United States is 5.1 mg C/m^2/h. Isoprene concentrations in biogenic emissions range from 8% to 91% of total VOCs, with a 58% average. The average emissions of monoterpenes and other VOCs constitute 18% and 24%, respectively, of the total VOCs. Since isoprene biosynthesis is associated with photosynthesis, isoprene emissions are negligible at night (Guenther et al., 1994).

Mixed forests containing oaks (west coast, southeast, Appalachian mountains) and aspen (Rocky Mountains) have high isoprene emission factors. Spruce forests in the north central and northeast United States have moderate isoprene and high monoterpene emission factors. Pine-dominated forests in the west have low isoprene and high monoterpene emissions. For example, isoprene biogenic emissions from high isoprene emission sources such as the forests of the Appalachian and Ozark mountains are 5.5 to 8 mg/m^2/h (Guenther et al., 1994).

The southern areas of the United States (EPA regions 4 [the southeastern states] and 6 [AK, LA, OK, and TX]) have the highest biogenic emissions. Of EPA regions 3-10, Regions 4 and 6 contributed 18% and 23%, respectively, of the total biogenic emissions. Summertime isoprene emissions are highest in each region and account for more than 50% of annual biogenic emissions. Highest emission rates of 2439 μg/m^2/h occur in Region 6 in July and August. Land area contributors to the total annual U.S. biogenic emissions of 5.9 Tg isoprene are oak forests (2.31 Tg), other deciduous forests (1.01 Tg), coniferous forests (0.61 Tg), scrub lands (1.17 Tg) grass lands (0.49 Tg), crop lands (0.2 Tg), inland waters (0.02 Tg), and urban areas (0.08 Tg). In this inventory based on a simple forest canopy model, isoprene emissions represented 20% of total biogenic hydrocarbon emissions. The authors noted that the uncertainty in these estimates is relatively large (Lamb et al., 1993).

2.3.3.3 U.S. Anthropogenic Emissions

Sources of anthropogenic releases of isoprene to the atmosphere include ethylene production by cracking naphtha, wood pulping, oil fires, wood-burning stoves and fireplaces, other biomass combustion, tobacco smoking (3,100 μg/cigarette), gasoline, and exhaust of turbines and automobiles (HSDB, 1997).

2.3.3.4 Ambient Air

Analysis of ambient air samples from Raleigh, NC (in EPA Region 4), during August 1993, revealed that the isoprene contribution accounted for 4% of total NMHC emissions from all anthropogenic and biogenic sources. This fraction was similar to that reported in the emission inventory for North Carolina (6%) (Lawrimore and Aneja, 1997).

Atmospheric isoprene concentrations determined at sites in the Appalachian Mountains of northwestern North Carolina were wide-ranging (Seila et al., 1984), but consistent with the urban estimates. Ambient air samples taken over 13 months (September 1981 to October 1982) showed that isoprene comprised 0.16-9.8% of the total NMHCs. Essentially no isoprene was identified in meteorological and gas samples collected during the winter at four rural southeastern U.S. sites, but concentrations averaged from 9.8 to 21.15 ppb C (about 0.03 to 0.06 mg/m^3) during the summer (Hagerman et al., 1997). These measurements contrasted with high

levels of several other low-molecular-weight NMHCs, which are at a maximum in the winter and decline in the summer.

Arnts and Meeks (1980) estimated that less than 10% of NMHCs in rural and remote areas is biogenic. Sites of measurements were Tulsa, OK, the Great Smoky Mountains in Tennessee, and Rio Blanco County, CO. Several other studies reviewed by Altschuller (1983) reported that the ambient air concentrations of isoprene plus monoterpenes (1 to 7 ppb C; about 0.003 to 0.02 mg/m^3) accounted for only 1% to 9% of total NMHCs. During stagnation conditions, biogenic hydrocarbons may contribute more to total atmospheric hydrocarbons.

Isoprene plus monoterpene concentrations within forest canopies range from 10 to 100 ppb C, but are usually less than 10 ppb C (< 0.03 mg/m^3 calculated as isoprene). Occasionally, concentrations > 100 ppb C have been reported near isoprene sources on a hot summer day and during periods of leaf drop. The much lower fraction of isoprene and monoterpenes in ambient air concentrations compared to biogenic emission inventories is apparently not due to the short lives of these reactive species or losses during sampling, storage, and analysis. Possibly, the biogenic emission inventories overestimate biogenic emissions and non-urban anthropogenic emissions inventories are underestimates (Altschuller, 1983).

2.3.4 Food and Tobacco

Foods of plant origin would be expected to be a source of daily exposure to isoprene since it is emitted by agricultural crops and is the basic structural unit in countless natural products found in foods such as terpenes and vitamins A and K (IARC, 1994). Its occurrence has been reported in the essential oil of oranges, in the fruit of hops, and in the root of carrots (Duke, 1992).

Isoprene was found to be the major component of hydrocarbons in indoor air polluted by tobacco smoke (16.7%) and in sidestream smoke (29.2%) (Barrefors and Petersson, 1993). Although baseline isoprene concentrations were the same in smokers and nonsmokers, 5 min after smoking, the concentration of isoprene in smokers' breath increased by 86% ± 26% ($P < 0.001$)—from 6.5 ± 0.84 (0.440 mg/m^3) to 10.3 ± 1.1 nmol/L (0.701 mg/m^3). The isoprene concentration declined to baseline within 15 min. The mainstream smoke concentration was 34,086 ± 3,238 nmol/L (2320 mg/m^3) (Euler et al., 1996).

2.4 Regulations

EPA regulates isoprene under the Clean Air Act (CAA) and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Under the CAA, it is listed as a regulated flammable substance with a threshold quantity for accidental release prevention of 10,000 lb. Under the latter act, a final reportable quantity (RQ) of 100 lb (45.4 kg) for the compound has been established. FDA regulates isoprene as an indirect food additive in paper and paperboard components, in polymers, and in adjuvants, production aids, and sanitizers. OSHA regulates isoprene under the Hazard Communication Standard and as a hazardous chemical in laboratories.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 60—PART 60—STANDARDS OF PERFORMANCE FOR NEW STATIONARY SOURCES. Promulgated: 36 FR 24877, 12/23/71. U.S. Code: 42 U.S.C. 7401, 7411, 7413, 7414, 7416, 7601 and 7602.	
	40 CFR 60—Subpart VV—Standards of Performance for Equipment Leaks of VOC in the Synthetic Organic Chemicals Manufacturing Industry. Promulgated: 48 FR 48335, 10/18/83.	This subpart applies to affected facilities in the synthetic organic chemicals manufacturing industry.
	40 CFR 60.489—Sec. 60.489 List of chemicals produced by affected facilities.	Isoprene, produced as an intermediate or final product by process units covered under this subpart, is included in this list.
	40 CFR 60—Subpart NNN—Standards of Performance for Volatile Organic Compound (VOC) Emissions From Synthetic Organic Chemical Manufacturing Industry (SOCMI) Distillation Operations. Promulgated: 55 FR 26942, 06/29/90.	This subpart applies to each affected facility designated in paragraph (b) of this section that is part of a process unit that produces any of the chemicals listed in section 60.667 as a product, co-product, by-product, or intermediate.
	40 CFR 60.667—Sec. 60.667 Chemicals affected by subpart NNN. Promulgated: 55 FR 26942, 06/29/90, as amended at 60 FR 58237 and 58238, 11/27/95.	Isoprene is included in this section.
	40 CFR 60—Subpart RRR—Standards of Performance for Volatile Organic Compound Emissions From Synthetic Organic Chemical Manufacturing Industry (SOCMI) Reactor Processes. Promulgated: 58 FR 45962, 08/31/93.	This subpart applies to each affected facility designated in paragraph (b) of this section that is part of a process unit that produces any of the chemicals listed in section 60.707 as a product, co-product, by-product, or intermediate.
	40 CFR 60.707—Sec. 60.707 Chemicals affected by subpart RRR. Promulgated: 58 FR 45962, 08/31/93, as amended at 60 FR 58238, 11/27/95.	Isoprene is included in this section.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 68—PART 68—CHEMICAL ACCIDENT PREVENTION PROVISION. Promulgated: 59 FR 4493, 01/31/94. U.S. Code: 42 U.S.C. 7412(r), 7601(a)(1), 7661-7661f.	This part sets forth the list of regulated substances and thresholds, the petition process for adding or deleting substances to the list of regulated substances, the requirements for owners or operators of stationary sources concerning the prevention of accidental releases, and the State accidental release prevention programs approved under section 112(r) of the CAA.
	40 CFR 68—Subpart F—Regulated Substances for Accidental Release Prevention.	This subpart designates substances to be listed under section 112(r)(3), (4), and (5) of the CAA, as amended, identifies their threshold quantities, and establishes the requirements for petitioning to add or delete substances from the list.
	40 CFR 68.130—Sec. 68.130 List of substances.	Isoprene is listed as a regulated flammable substance; its threshold quantity for accidental release prevention is 10,000 lb.
	40 CFR 116—PART 116—DESIGNATION OF HAZARDOUS SUBSTANCES. Promulgated: 43 FR 10474, 03/13/78. U.S. Code: 33 U.S.C. 1251 et seq.	This regulation designates hazardous substances under section 311(b)(2)(A) of the FWPCA and applies to discharges of substances designated in Table 116.4.
	40 CFR 116.4—Sec. 116.4 Designation of hazardous substances. Promulgated: 43 FR 10474, 03/13/78 through 54 FR 33482, 08/14/89.	Isoprene is included in Table 116.4.
	40 CFR 117—PART 117—DETERMINATION OF REPORTABLE QUANTITIES FOR HAZARDOUS SUBSTANCES. Promulgated: 44 FR 50776, 08/29/79. U.S. Code: 33 U.S.C. 1251 et seq.	
	40 CFR 117.3—Sec. 117.3 Determination of reportable quantities. Promulgated: 50 FR 13513, 04/04/85 through 60 FR 30937, 06/12/95.	The reportable quantity established for isoprene is 100 lb (45.4 kg).

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 122—PART 122—EPA ADMINISTERED PERMIT PROGRAMS: THE NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM. Promulgated: 48 FR 14153, 04/01/83. U.S. Code: 33 U.S.C. 1251 et seq.	
	40 CFR 122—Subpart D—Transfer, Modification, Revocation and Reissuance, and Termination of Permits.	Isoprene is a hazardous substance required to be identified by existing dischargers if expected to be present (Appendix D).
	40 CFR 302—PART 302—DESIGNATION, REPORTABL QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Code: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	This part designates under section 102(a) of the CERCLA of 1980 those substances in the statutes referred to in section 101(14), identifies reportable quantities for these substances, and sets forth the notification requirements for releases of these substances. This regulation also sets forth reportable quantities for hazardous substances designated under section 311(b)(2)(A) of the CWA.
	40 CFR 302.4—Sec. 302.4 Designation of hazardous substances. Promulgated: 54 FR 33449, 08/14/89.	The statutory reportable quantity (RQ) is 1000 lb. The final RQ is 100 lb (45.4 kg).
	40 CFR 414—PART 414—ORGANIC CHEMICALS, PLASTICS, AND SYNTHETIC FIBERS. Promulgated: 52 FR 42568, 11/05/87. U.S. Codes: 33 U.S.C. 1311, 1314, 1316, 1317, and 1361.	This part applies to process wastewater discharges from all establishments or portions of establishments that manufacture the organic chemicals, plastics, and synthetic fibers (OCPSF) products or product groups covered by subparts B through H of this part.
	40 CFR 414—Subpart G—Bulk Organic Chemicals.	
	40 CFR 414.70—Sec. 414.70 Applicability; description of the bulk organic chemicals subcategory. Promulgated: 52 FR 42568, 11/05/87, as amended at 57 FR 41844, 09/11/92.	The provisions of this subpart are applicable to the process wastewater discharges resulting from the manufacture of isoprene.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 176—PART 176—INDIRECT FOOD ADDITIVES: PAPER AND PAPERBOARD COMPONENTS. Promulgated: 42 FR 14554, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 346, 348, 379e.	
	21 CFR 176—Subpart B—Substances for Use Only as Components of Paper and Paperboard.	
	21 CFR 176.180—Sec. 176.180 Components of paper and paperboard in contact with dry food. Promulgated: 42 FR 14554, 03/15/77.	Isoprene may be safely used as a component of the uncoated or coated food-contact surface of paper and paperboard intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding dry food. The substance is to be used in amounts not to exceed that required to accomplish its intended physical or technical effect, and so used as to accomplish no effect in food other than that ordinarily accomplished by packaging.
	21 CFR 177—PART 177—INDIRECT FOOD ADDITIVES: POLYMERS. Promulgated: 42 FR 14572, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, and 379e.	
	21 CFR 177—Subpart B—Substances for Use as Basic Components of Single and Repeated Use Food Contact Surfaces.	
	21 CFR 177.1420—Sec. 177.1420 Isobutylene polymers.	Isobutylene polymers may be safely used as components of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, provided that isobutylene-isoprene copolymers produced by the copolymerization of isobutylene contain not more than 3 molar percent of isoprene.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
FDA	21 CFR 178—PART 178—INDIRECT FOOD ADDITIVES: ADJUVANTS, PRODUCTION AIDS, AND SANITIZERS. Promulgated: 42 FR 14609, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, and 379e. 21 CFR 178—Subpart D—Certain Adjuvants and Production Aids. 21 CFR 178.3850—Sec. 178.3850 Reinforced wax. Promulgated: 42 FR 14609, 03/15/77, as amended at 47 FR 1288, 01/12/82.	The substances and optional adjuvant substances employed in the production of or added to reinforced wax include the copolymer of isobutylene modified with isoprene.
OSHA	29 CFR 1910—PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS. Promulgated: 39 FR 23502, 06/27/74. 29 CFR 1910.1200—Sec. 1910.1200 Hazard Communication. Promulgated: 61 FR 9245, 03/07/96. U.S. Code: 29 U.S.C. 653, 655, and 657; 5 U.S.C. 553. 20 CFR 1910.1450—Sec. 1910.1450 Occupational exposure to hazardous chemicals in laboratories. Promulgated: 61 FR 5508, 02/13/96. OSH Act. Final Rule.	This section requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication Program to include labels, materials safety data sheets, and worker training. As a select carcinogen (IARC Group 2B, possibly carcinogenic to humans, and now NTP-listed as reasonably anticipated to be a human carcinogen), isoprene is included as a chemical hazard in laboratories. Employers are required to provide employee information and training and a Chemical Hygiene Plan.

[a]The regulations in this table have been updated through the 1998 issues of Federal Code of Regulations titles 21, 29, and 40.

3.0 HUMAN STUDIES

Studies Evaluated by IARC (1994) and after IARC (1994)

A March 1998 online literature search of six biomedical databases and current awareness searches through August 1998 did not identify any studies of the effects of exposure to isoprene on humans. None were described by IARC (1994).

4.0 EXPERIMENTAL CARCINOGENICITY

4.1 Animal Studies Reviewed by IARC (1994)

IARC (1994) evaluated a draft of an NTP study (Melnick et al., 1994) with male rats and male mice designed to determine if isoprene produces a carcinogenic response after intermediate exposure durations. In this study, groups of 40 male rats (F344/N) and 40 male mice ($B6C3F_1$) were exposed to isoprene by inhalation of 0 (chamber control), 70, 220, 700, 2200, or 7000 ppm for 6 h/day on 5 days/wk for 6 mo followed by a 6-mo recovery period without exposure to isoprene (stop-exposure protocol). Ten animals per group were killed at the end of the exposure period and examined for exposure-related effects.

Rats had an increased incidence and severity of interstitial cell hyperplasia of the testis after 6 mo of exposure to 7000 ppm isoprene. Following the 6-mo recovery period, slightly greater incidences of interstitial cell adenoma of the testes were observed in rats that had been exposed to 700, 2200, or 7000 ppm (3/30 [control], 7/30, 8/29, 9/30, respectively; trend test: $p = 0.02$). Survival was unaffected by isoprene exposure.

The IARC Working Group considered the rat study to be inadequate for assessment of carcinogenicity because of the short duration of the study and the high spontaneous incidence of interstitial cell tumors in this strain of rat at two years.

Mice, after exposure to 700 ppm or higher concentrations of isoprene for 6 mo, had increased incidences of neoplasms at four organ sites when examined after the 6-mo recovery. Compared to the control group, neoplasms in groups exposed to 700, 2200, or 7000 ppm occurred at a significantly greater incidence in the liver (7/30 [control], 15/30, 18/30, 17/28, respectively), lungs (2/30 [control], 5/30, 10/30, 9/28, respectively), forestomach (0/30 [control], 1/30, 4/30, 6/30, respectively), and Harderian gland (2/30 [control], 14/30, 13/30, 12/30, respectively). Incidences of multiple neoplasms and/or malignant neoplasms were also increased in exposed groups compared to controls. Survival was decreased in the highest (7000 ppm) exposure group.

The IARC Working Group concluded that the mouse study provided sufficient evidence in experimental animals for the carcinogenicity of isoprene.

4.2 Studies Post-IARC (1994)

Experimental animals, methods, and cancer incidences are presented in Table 4.1. A chronic inhalation study with mice evaluated three independent variables: concentration, length of daily exposure, and number of weeks of exposure (Placke et al., 1996). Male $B6C3F_1$ mice were exposed to isoprene for 5 days/wk, 8 h/day or 4 h/day, for 20, 40, or 80 wk, so that the concentration x time (duration of exposure) values provided a series of theoretically equivalent exposure hazards. Exposure to isoprene produced increased incidences of neoplasms at multiple organ sites. However, the duration of exposure did not appear to predict tumor risk at any site.

For groups of 50 males, the 8 h/day exposure levels, expressed as ppm-wk, were 0-80, 10-80, 70-40, 70-80, 140-40, 280-20, 280-80, 700-80, 2200-40, and 2200-80. The 4 h/day ppm-wk exposures for two additional groups were 2200-20 and 2200-80. Groups were held for 96 or 105 wk. Significant ($p < 0.05$) increases were observed in the incidence of liver adenoma, liver carcinoma, primary alveolar and bronchiolar adenomas, lung carcinoma, Harderian gland adenomas, and histiocytic sarcoma.

In one part of this study, groups of female mice were exposed to 0, 10, or 70 ppm isoprene 8 h/day for 80 wk only. The 70-ppm group had a significant increase ($p < 0.05$) in the incidence of Harderian gland adenoma (2/49 [control], 8/49) and pituitary adenoma (1/49 [control], 9/49). Based on comparison to historical controls, this increase may have been related to isoprene exposure.

In a rat study, isoprene was found to be carcinogenic to the kidney and testes of males and to the mammary gland of males and females. Groups of 50 male and 50 female F344/N rats were exposed to 0, 220, 700, or 7000 ppm isoprene by inhalation, 6 h/day, 5 days/w for two years (NTP TR 486, 1997 draft). Survival and mean body weights of exposed males and females were similar to those of chamber controls.

In groups of males exposed to 7000 ppm isoprene, there was a significant increase in the incidence of fibroadenoma versus the chamber control groups (2/50 [control], 21/50). The incidence of multiple fibroademona was also increased in this exposure group (1/50 [control], 7/50; $p \leq 0.05$). Although the incidences of fibroadenoma in the male rats exposed to 220 and 700 ppm isoprene were not significantly greater than the chamber control group (2/50 [control], 4/50, 6/50, respectively), they exceeded the historical control ranges for this neoplasm (0-6%). In addition, the finding of mammary gland carcinoma in exposed male rats (0/50 [control], 1/50 [220 ppm], 1/50 [700 ppm], 2/50 [7000 ppm], respectively) is noteworthy because these neoplasms rarely occur in control male rats (1/950). In females exposed to 220, 700, or 7000 ppm isoprene, the incidence of mammary gland fibroadenoma (19/50 [control], 35/50, 32/50, 32/50, respectively) and multiple fibroadenoma (7/50 [control], 12/50, 19/50, 17/50, respectively) was significantly increased.

A significant increase in the incidence of renal tubule adenoma or carcinoma occurred in groups of male rats exposed to 700 or 7000 ppm isoprene (2/50 [control], 6/50, 8/50, respectively). The incidence of renal tubule hyperplasia, thought to represent an early stage in the development of renal carcinoma and adenoma, was also significantly increased in these groups.

Exposure-related increases in the incidence of testicular interstitial cell adenoma in the 700- and 7000-ppm groups (33/50 [control], 44/50, 48/50, respectively) significantly exceeded the high spontaneous incidence of this adenoma in F344/N rats. The significantly increased incidence of bilateral testicular interstitial cell adenoma observed in the 700- and 7000-ppm groups (20/50 [control], 37/50, 48/50, respectively) support the contention that exposure to isoprene increased the incidence of these neoplasms.

A low incidence of rare brain neoplasms including benign astrocytoma, malignant glioma, malignant medulloblastoma, benign granular cell tumor, and meningeal sarcoma, was seen in exposed females and may have been due to isoprene exposure.

Table 4-1. Experimental Carcinogenicity Studies with Isoprene (Post-IARC, 1994)

Age, Strain, Species	No. and Sex Exposed	No. and Sex Controls	Chemical Form and Purity	Dose and Route	Duration of Exposure	Results/Comments	Reference
Mice							
10 wk $B6C3F_1$ mice	eleven groups of 50 males	one group of 50 males	gaseous isoprene ≥ 99% purity	males exposed by inhalation in test chamber to 0, 10, 70, 140, 280, 700, or 2200 ppm	males exposed 4 or 8 h/day, 5 days/wk for 20, 40, or 80 wk The 8 h/day exposure levels, expressed as ppm-wk, were 0-80, 10-80, 70-40, 70-80, 140-40, 280-20, 280-80, 700-80, 2200-40, and 2200-80. The 4 h/day ppm-wk exposures for two additional groups were 2200-20 and 2200-80. Groups were held for 96 or 105 wk.	**NEOPLASM INCIDENCE IN MALES ($p < 0.05$)** Liver adenoma 11/50 [control], 22/50, 18/49, 24/50, 27/48, 28/47, 30/50 at exposures* of 0-80, 140-40, 280-20, 280-80, 700-80, 2200-40, 2200-80 ppm-wk for 8 h/day. 11/50 [control], 22/50, 21/50 at exposures of 0-80, 2200-20 and 2200-80 ppm-wk for 4 h/day Liver carcinoma 9/50 [control], 10/50, 12/49, 16/50, 17/48, 18/47, 16/50 at exposures of 0-80, 140-40, 280-20, 280-80, 700-800, 2200-40, and 2200-80 ppm-wk for 8 h/day. 9/50 [control], 12/50, 15/50 at exposures of 0-80, 2200-20 and 2200-80 for 4 h/day Lung primary alveolar & bronchiolar adenoma 11/50 [control], 23/50, 29/49, 30/50 at exposures of 0-80, 700-80, 2200-40, and 2200-80 for 8 h/day Lung carcinoma 0/50 [control], 7/50, 3/49, 7/50 at exposures of 0-80, 700-80, 2200-40, and 2200-80 for 8 h/day Harderian gland adenoma 4/47 [control], 12/50, 16/49, 17/50, 26/49, 31/49, 35/50 at exposures of 0-80, 140-40, 280-20, 280-80, 700-80, 2200-40, 2200-80 ppm-wk for 8 hr/day 4/47 [control], 19/49 and 28/50 at exposures of 0-80, 2200-20 and 2200-80 ppm-wk for 4 h/day	Placke et al. (1996)

Table 4-1. Experimental Carcinogenicity Studies with Isoprene (Post-IARC, 1994) (Continued)

Age, Strain, Species	No. and Sex Exposed	No. and Sex Controls	Chemical Form and Purity	Dose and Route	Duration of Exposure	Results/Comments	Reference
10 wk B6C3F1 mice	two groups of 50 females	one group of 50 females	gaseous isoprene ≥ 99% purity	females exposed by inhalation in test chamber to 0, 10, or 70 ppm	females exposed 8 h/day, 5 days/wk for 80 wk	**NEOPLASM INCIDENCE IN MALES ($p < 0.05$) (cont'd.)** Histiocytic sarcoma 0/50 [control], 8/50 at exposures of 0-80, 280-20 ppm-wk for 8 h/day 0/50 [control], 7/50 at exposures of 0-80, 2200-80 ppm-wk for 4 h/day **NEOPLASM INCIDENCE IN FEMALES ($p < 0.05$)** Harderian gland adenoma 2/49 [control], 8/49 at exposures of 0-80, 70-80 ppm-wk for 8 h/day Pituitary adenoma 1/49 [control], 9/49 in group at exposures of 0-80, 70 80 ppm Concentration-related reduction in survival - groups exposed to 280-2200 ppm at wk 80 had < 50% survival compared to controls or lower concentration groups, animals necropsied at wk 96	Placke et al. (1996)

Table 4-1. Experimental Carcinogenicity Studies with Isoprene (Post-IARC, 1994) (Continued)

Age, Strain, Species	No. and Sex Exposed	No. and Sex Controls	Chemical Form and Purity	Dose and Route	Duration of Exposure	Results/Comments	Reference
Rats							
6 wk F344/N rats	groups of 50 M and 50 F	50 M; 50 F	isoprene vapor; purity > 99%	220, 700, or 7000 ppm by inhalation	6 h/day, 5 days/wk for 104 wk	**NEOPLASM INCIDENCE IN MALES ($p < 0.05$)** Mammary gland fibroadenoma 2/50 [control], 4/50, 6/50, 21/50 at exposures of 220, 700, or 7000 ppm, respectively Mammary gland fibroadenoma or carcinoma (combined) 2/50 [control], 5/50, 7/50, 21/50 at exposures 220, 700, or 7000 ppm, respectively Mammary gland multiple fibroadenoma 1/50 [control], 7/50 at exposures of 7000 ppm Renal tubule adenoma or carcinoma 2/50 [control], 8/50, 15/50 at exposures of 700 or 7000 ppm, respectively Testicular interstitial cell adenoma 33/50 [control], 37/50, 44/50, 48/50 at exposures of 220, 700, or 7000 ppm, respectively Testicular bilateral interstitial cell adenoma 20/50 [control], 29/50, 37/50, 48/50 at exposures of 220, 700, or 7000 ppm, respectively **NEOPLASM INCIDENCE IN FEMALES ($p < 0.05$)** Mammary gland fibroadenoma 19/50 [control], 35/50, 32/50, 32/50 at exposures of 220, 700, or 7000 ppm, respectively Mammary gland multiple fibroadenoma 7/50 [control], 12/50, 19/50, 17/50 at exposures of 220, 700, or 7000 ppm, respectively	NTP TR 486 (1997 draft)

* #-# = ppm-wk; daily exposure 8 h unless otherwise noted

5.0 GENOTOXICITY

Studies on the genotoxic effects of isoprene have been reviewed by IARC (1994) and NTP (1995, 1997 draft).

5.1 Genotoxicity Studies Reviewed by IARC (1994)

In bacterial systems, isoprene did not induce reverse mutations in *Salmonella typhimurium* strains TA98, TA100, TA1530, TA1535, TA1537 and TA1538 tested in the presence and absence of metabolic activation (de Meester et al., 1981; Mortelmans et al., 1986; both cited by IARC, 1994). The monoepoxide intermediates 3,4-epoxy-2-methyl-1-butene (EPOX-I) and 3,4-epoxy-2-methyl-1-butene (EPOX-II) were negative in TA98 and TA100, whereas the diepoxide intermediate was a potent mutagen in TA100. It should be noted that the lack of mutagenicity of isoprene may be an artifact of the preincubation protocol, which may have resulted in evaporation and/or incomplete biotransformation, and the monoepoxide EPOX-I may have been rapidly hydrolyzed, thus precluding detection of EPOX-I genetic toxicity in *S. typhimurium* (NTP, 1997 draft).

In vivo, isoprene induced a significant increase in sister chromatid exchange (SCE) in bone marrow and micronuclei in erythrocytes of male $B6C3F_1$ mice exposed to isoprene at concentrations of 438, 1750, or 7000 ppm via inhalation for 12 days (6 h/day), but an increase in chromosome aberrations was not observed in the bone marrow of the same mice (Tice et al., 1988).

5.2 Additional Genotoxicity Studies Summarized in NTP (1995, 1997 draft)

In vitro assays of isoprene with cultured Chinese hamster ovary (CHO) cells showed no increase in SCE or chromosomal aberrations, in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix (Galloway et al., 1987; cited by NTP, 1995; NTP, 1997 draft). Isoprene concentrations in these tests may have been reduced by evaporation (NTP, 1997 draft).

In vivo genetic toxicity assays with isoprene gave positive results. Male and female mice exposed to isoprene for 13 weeks had significantly increased frequencies of micronuclei in polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) (Jauhar et al., 1988; cited by NTP, 1997 draft). Male and female rats exposed to isoprene by inhalation for 4 weeks showed no significant increase in the frequency of micronucleated lung fibroblasts (NTP, 1997 draft).

5.3 Genetic Mutations in Isoprene-Induced Tumors

An analysis of *ras* protooncogenes in tumors from mice chronically exposed to isoprene indicated that specific mutations contribute to tumorigenesis (Hong et al., 1997). The tumors were Harderian gland tumors from male $B6C3F_1$ mice given two doses of isoprene (2200 or 7000 ppm) by inhalation (6 h/day; 5 days/wk) for 26 weeks, followed by a 26-week recovery. Mutations in these tumors were compared to mutations in Harderian gland tumors from mice unexposed to isoprene (negative control) and to mutations in 1,3-butadiene-induced Harderian gland tumors (positive control). A higher frequency of *ras* mutations occurred in isoprene-induced tumors than in tumors from the positive or negative controls. Most of the mutations were A→T transversions at K-*ras* codon 61 and C→A transversions at H-*ras* codon 61.

6.0 OTHER RELEVANT DATA

6.1 Absorption, Distribution, Metabolism, and Excretion in Humans

Isoprene is probably an endogenous precursor of cholesterol (Deneris et al., 1984; cited by IARC, 1994). Concentrations were reported to range from 15 to 70 nmol/L in human blood (Cailleux et al., 1992; cited by IARC, 1994) and from 10 to 30 nmol/L (0.68 to 2.0 mg/m^3) in human breath (Cailleux and Allain, 1989; cited by IARC, 1994).

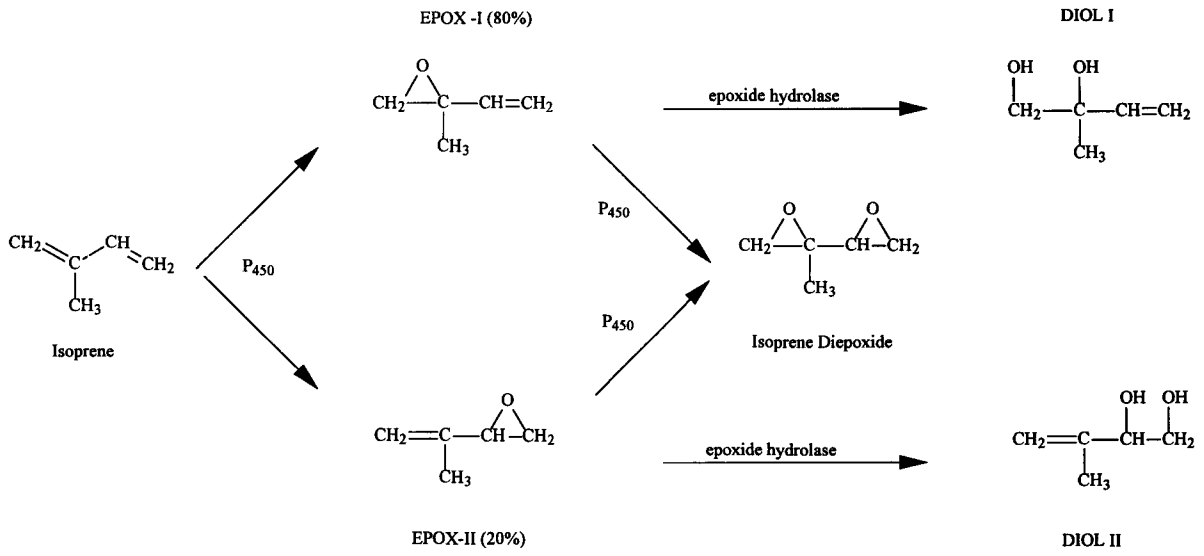
The metabolism of isoprene and two isoprene monoepoxides (Fig. 1) was explored with microsomes derived from cell lines expressing eight human cytochrome P450 enzymes and with liver microsomes from humans, rats, and mice (Bogaards et al., 1996). The single human enzymes were CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4. The enzyme CYP2E1 was associated with the highest formation rate of the two isoprene monoexpoxides and with the formation of the diepoxide from each of the monoepoxide intermediates (Fig. 1). Results from incubation of human liver microsomal preparations were consistent with those obtained from tests with single human enzymes. In the presence of inhibitor, rates of monoepoxide formation were similar in human, mouse, and rat liver microsomes; however, without inhibitor, the total amount of monoepoxides present at the end of the incubation period was highest for mouse liver microsomes.

6.2 Absorption, Distribution, Metabolism, and Excretion in Experimental Systems

The rate of endogenous isoprene production was 1.9 μmol/kg/h in rats and 0.4 μmol/kg/h in mice (Peter et al., 1987; cited by NTP, 1997 draft). Rats exposed 6 hours to radiolabeled isoprene by nose-only inhalation at concentrations of 8, 260, 1480, or 8200 ppm (23, 738, 4200, 23,268 mg/m^3) retained 19, 9, 6, or 5%, respectively (Dahl et al., 1987; cited by IARC, 1994). Mice showed steady-state levels of 25 to 6800 ng/mL (0.0004 to 0.1 μmol/mL) in blood after inhalation exposure to 20 to 2000 ppm isoprene (Bond et al., 1991; cited by IARC, 1994). In rats, most of the inhaled isoprene and metabolites was distributed to liver, blood, and fat (Dahl et al., 1987; cited by IARC, 1994).

Isoprene was metabolized to two monoepoxides (Fig. 6-1) by liver microsomes from mice, rats, rabbits, hamsters, and humans; these metabolites may be hydrolyzed, conjugated with glutathione, or further oxidized to isoprene diepoxide (Del Monte et al., 1985; Gervasi and Longo, 1990; Wistuba et al., 1994; all cited by NTP, 1997 draft; Bogaards et al., 1996). In rats exposed to isoprene via inhalation, volatile metabolites in respiratory tract tissue suggested significant metabolism of isoprene in the respiratory tract (Dahl et al., 1987; cited by IARC, 1994). The maximal metabolic velocity (V_{max}) for diepoxide formation was 6-fold greater in microsomes from mice and Syrian hamsters than the V_{max} in microsomes from rats and rabbits (Longo et al., 1985; cited by IARC, 1994), indicative of significant species differences in metabolism.

Figure 6-1. Metabolism of Isoprene in the Liver of Rabbits, Hamsters, Rats, and Mice.



[Excerpt from NTP, 1997 draft; Original Source: Gervasi and Longo, 1990]
EPOX I = 3,4-epoxy-3-methyl-1-butene; EPOX II = 3,4-epoxy-2-methyl-1-butene

Comparative studies with mammalian *in vitro* systems indicate that there are significant stereochemical and mechanistic differences among species (Small et al., 1997). Enantiomers of the monoexpoxide 2-(1-methylethenyl)oxirane were identified in liver microsome preparations from rats, mice, rabbits, dogs, monkeys, and humans. Rats preferentially formed (*S*)-2-(1-methylethenyl)oxirane compared with the (*R*)-enantiomer, whereas microsomes from dog, monkey, or male human preferentially formed (*R*)-2-(1-methylethenyl)oxirane. Metabolites from isoprene incubated with human female microsomes did not show enantioselectivity.

Studies of the metabolism of isoprene indicate formation of a diepoxide from both monoepoxides (Fig. 1) in all mammalian systems (Small et al., 1997; Wistuba et al., 1994; Bogaards et al., 1996; both cited by NTP, 1997 draft). It should be noted that data were obtained mostly from *in vitro* studies.

About 75% of the retained isoprene radioactivity, delivered to rats as described in the first paragraph of this subsection, was excreted in urine within 66 hours (Dahl et al, 1987; cited by IARC, 1994). The maximal metabolic elimination rates for isoprene inhaled by rats and mice were estimated to be 130 and 400 µmol/kg/h, respectively (Peter et al., 1987; cited by IARC, 1994).

A chemical study of the isoprene monoepoxide EPOX-I reactivity with nucleophiles found a relatively high S_N2 reactivity at C-3, leading to efficient adduct formation (Bleasdale et al., 1996).

6.3 Structure-Activity Relationships (SAR)

Isoprene is the 2-methyl analogue of 1,3-butadiene, while chloroprene is the 2-chloro analogue of 1,3-butadiene. Chloroprene is also an analogue of vinyl chloride. The three structural analogues of isoprene are animal carcinogens and known (1,3-butadiene, vinyl chloride) or reasonably anticipated to be (chloroprene) human carcinogens.

6.3.1 1,3-Butadiene

Based on the human epidemiological and rodent carcinogenicity data published prior to 1992, IARC (1992) concluded that there was sufficient evidence for the carcinogenicity of 1,3-butadiene in experimental animals and limited evidence for its carcinogenicity in humans. The results of more recent mechanistic and epidemiological studies strengthened the link between occupational exposure to 1,3-butadiene and cancer, with the result that NTP has classified 1,3-butadiene as *known to be a human carcinogen* (NTP, 1999 [Report on Carcinogens, 9th ed]). The increased mortality risk in humans occupationally exposed to 1,3-butadiene is largely for leukemia, lymphosarcoma, and reticulosarcoma (IARC, 1992; West et al., 1995; Ward et al., 1996; Divine and Hartman, 1996; Macaluso et al., 1996; Delzell et al., 1996).

1,3-Butadiene is a multi-species, multi-organ carcinogen (IARC, 1992; NTP, 1993; Melnick et al., 1994). In the NTP inhalation bioassay (NTP, 1993), 1,3-butadiene induced significant increases in the incidence of hemangiosarcomas of the heart, malignant lymphomas, alveolar/bronchiolar adenomas and carcinomas, squamous cell papillomas or carcinomas of the forestomach, Harderian gland adenomas, hepatocellular adenomas or carcinomas, mammary-gland carcinomas, and granulosa-cell tumors of the ovary in mice. In a rat inhalation study, 1,3-butadiene induced an increased incidence of pancreatic exocrine neoplasms and Leydig cell tumors of the testis in males, and uterine stromal sarcomas, Zymbal's gland carcinomas, thyroid follicular-cell neoplasms, and mammary gland neoplasms in females (Owen et al., 1987).

Based on generally positive results in a variety of short-term *in vitro* and *in vivo* genotoxicity studies, 1,3-butadiene would be classified as a genotoxic carcinogen (IARC, 1992; NTP, 1993). 1,3-Butadiene was positive for the induction of gene mutations in *S. typhimurium*, but not in mouse lymphoma cells, in both the presence and absence of metabolic activation. It was negative for the induction of both wing spot and sex-linked recessive lethal mutations in *D. melanogaster*. In mammalian systems *in vitro*, 1,3-butadiene was weakly positive for the induction of SCE in Chinese hamster ovary cells with metabolic activation, while reported as negative and positive for SCE induction in human lymphocytes (conflicting studies) with and without metabolic activation. Of most relevance, 1,3-butadiene was positive *in vivo* for the induction of DNA-DNA and DNA-protein cross-links in liver and lung of mice, but not rats, and in mice for the induction of dominant lethal mutations and sperm abnormalities, SCE and chromosomal damage in bone marrow cells, and micronucleated erythrocytes measured in peripheral blood. In occupationally exposed humans, 1,3-butadiene was positive for the induction of hemoglobin adducts, negative for SCE in lymphocytes, and reported as both positive and negative (conflicting studies) for *hprt* mutations and chromosomal aberrations in lymphocytes.

6.3.2 Chloroprene

Chloroprene is *reasonably anticipated to be a human carcinogen* based on evidence of benign and malignant tumor formation at multiple tissue sites in multiple species of experimental animals (NTP, 1998). Inhalation exposure of rats to chloroprene vapors induced increased incidences of neoplasms of the oral cavity, thyroid gland, and kidney in males and females, neoplasms of the lung in males, and neoplasms of the mammary gland in females. Inhalation exposure of mice to chloroprene vapors induced increased incidences of neoplasms of the lung, circulatory system (hemangiomas and hemangiosarcomas), forestomach, and Harderian gland in males and females, kidney neoplasms in males, and neoplasms of the mammary gland, liver, Zymbal's gland, skin, and mesentery in females.

There is limited evidence for the carcinogenicity of chloroprene in humans. Data from two studies suggest that occupational exposure to chloroprene may increase cancer risk for digestive and lymphatic/hematopoietic tumors (Pell, 1978, cited by IARC 1979a) and for liver, lung, and lymphatic tumors (Li et al., 1989). One case of liver angiosarcoma was reported in a worker with no known occupational exposure to vinyl chloride who had extensive exposure to polychloroprene (which may contain 0.5% chloroprene) (Infante, 1977).

Recent studies on the genotoxicity of chloroprene have been uniformly negative; conflicting results with earlier studies have been attributed to differences in the age and purity of the test samples. Positive mutagenicity results of chloroprene in bacteria (Bartsch et al., 1975; 1979) were considered to be due to cyclic dimers that accumulate in aged samples (Westphal et al., 1994). At the exposure concentrations used in the cancer inhalation studies, chloroprene did not induce sister chromatid exchanges or chromosomal aberrations in mouse bone marrow cells nor did it increase the frequency of micronucleated erythrocytes in peripheral blood (Tice et al., 1988). Oxidation of chloroprene to epoxide intermediates has been postulated to occur based on the detection of alkylated derivatives of the trapping agent 4-(4-nitrobenzyl)pyridine in incubations of chloroprene and mouse liver microsomes (Bartsch et al., 1979). Chloroprene-induced lung and Harderian gland neoplasms in mice had a high frequency of unique K-*ras* mutations (NTP, 1998). Chloroprene induced all of the types of tumors that were induced by 1,3-butadiene in mice except for lymphomas and ovarian neoplasms.

6.3.3 Vinyl Chloride

Based on human epidemiological studies and case reports and rodent carcinogenicity data, IARC (1979b) concluded that there was sufficient evidence for the carcinogenicity of vinyl chloride to humans and experimental animals. IARC (1987) reaffirmed vinyl chloride's evaluation as a human carcinogen, citing several additional epidemiological studies and case reports. IARC (1987) and Green (1990) confirmed a causal association between occupational exposure to vinyl chloride and angiosarcoma of the liver, hepatocellular carcinoma, brain and lung tumors, and malignancy of the hematopoietic and lymphatic system. Some studies (IARC, 1987) indicated a possibility of increased risk of gastric, liver, and gastrointestinal cancer. Green (1990) noted that workers in vinyl chloride manufacturing also experienced increases in tumors of the skin and thyroid, though a causal relationship was not established. One epidemiological study indicated excessive fetal mortality among wives of workers exposed to vinyl chloride, and several others reported increased rates of birth defects in children whose parents lived in communities with vinyl chloride-poly(vinyl chloride) or other chemical processing facilities (IARC, 1979b). Two proportionate mortality studies of deceased workers who had been

involved in plastics fabrication suggested increases in cancer of the digestive system (both sexes) and increases in cancer of the urinary system and breast in women (IARC, 1979b).

Vinyl chloride has been extensively tested in rats, hamsters, and mice via inhalation exposure and oral, subcutaneous, and intraperitoneal administration. Oral administration or inhalation of vinyl chloride induced Zymbal gland tumors in rats and hamsters, nephroblastomas in rats, forestomach papillomas and melanomas in hamsters, and pulmonary and mammary gland tumors in mice (IARC, 1979b; 1987). In all three species, exposure to vinyl chloride induced hemangiosarcoma of the liver (IARC, 1979b; 1987; Green, 1990). Vinyl chloride was carcinogenic in rats exposed prenatally (IARC, 1979b).

Green (1990) observed that vinyl chloride's wide range of effects in many species was characteristic of a genotoxic carcinogen. Workers exposed to vinyl chloride vapor showed induction of chromosomal aberrations in peripheral blood lymphocytes (IARC, 1987). Two additional studies of exposed workers indicated negative results for SCE, while one study indicated a weakly positive response (IARC, 1987). In rodents exposed *in vivo*, vinyl chloride induced chromosomal aberrations, SCE, and micronuclei in bone marrow cells, and alkylated DNA in tissues of mice and rats (IARC, 1987). *In vitro*, vinyl chloride induced unscheduled DNA synthesis in rat hepatocytes, gene mutation in Chinese hamster lung cells, gene conversion in yeast, and DNA damage and mutation in bacteria. It also induced sex-linked recessive lethals in *Drosophila* and was mutagenic in plants and *Schizosaccharomyces pombe* (but not other fungi) (IARC, 1987). Green (1990) suggested that vinyl chloride's carcinogenic activity results from its metabolism by microsomal mixed-function oxidases to chloro-oxirane (chloroethylene oxide) and chloroacetaldehyde, two mutagenic metabolites, and concluded that vinyl chloride is a classical genotoxin causing cancer by somatic mutation.

7.0 MECHANISMS OF CARCINOGENESIS

Isoprene was tested for carcinogenicity because of its structural similarity to 1,3-butadiene, an animal and human carcinogen, and because of the potential for human exposure (NTP, 1997 draft). Bioassays with rats and mice investigated cancer occurrence after 26 weeks of inhalation exposure followed by 26 weeks of recovery and after 2 years of inhalation exposure (see Section 4), respectively. Results indicated that isoprene is clearly carcinogenic in both species. There are several common sites of tumor induction by 1,3-butadiene and isoprene and some sites that are specific for each chemical (Table 7-1).

TABLE 7-1. Summary of Sites of Increased Incidences of Neoplasms in 2-Year Inhalation Studies of Isoprene and 1,3-Butadiene in Male and Female Rats and Mice

	Isoprene	1,3-Butadiene
Rats	Kidney (M) Mammary Gland (M, F) Testis (M)	Brain (M) Mammary Gland (F) Pancreas (M) Testis (M) Thyroid Gland (F) Uterus (F) Zymbal's Gland (F)
Mice	Circulatory System (Heart and Spleen Hemangiosarcoma) (M, F) Forestomach (M) Harderian Gland (M, F) Hematopoietic System (M) Liver (M) Lung (M) Pituitary Gland (F)	Circulatory System (Heart and Spleen Hemangiosarcoma) (M,F) Forestomach (M) Harderian Gland (M, F) Hematopoietic System (M, F) Kidney (M) Liver (M) Lung (M, F) Mammary Gland (F) Ovary (F) Preputial Gland (M)

Adapted from NTP (1997 draft)

As with 1,3-butadiene, reactive alkylating metabolites of isoprene may play a role in carcinogenesis, although other mechanisms must also be considered. Isoprene and 1,3-butadiene are metabolized to mono- and diepoxide intermediates by microsomal cytochrome P450-dependent monooxygenases (CYP2E1) (Del Monte et al., 1985; Longo et al., 1985; Malvoisin et al., 1979; Malvoisin and Roberfroid, 1982; all cited by NTP, 1997 draft; Bogaards et al., 1996). Detoxification of these epoxide intermediates may occur by hydrolysis catalyzed by epoxide hydrolase or conjugation with glutathione catalyzed by glutathione-*S*-transferase. The diepoxide intermediates of both compounds were mutagenic in *S. typhimurium*; however, unlike the monoepoxide intermediates of isoprene, the monoepoxide intermediate of 1,3-butadiene biotransformation was also mutagenic in the *Salmonella* assay (Gervasi et al., 1985; cited by NTP, 1997 draft). Bleasdale et al. (1996) recently reported that EPOX I is highly reactive with amino and thiolate nucleophiles via an S_N2 mechanism. Therefore, the lack of observed mutagenicity of this epoxide in *S. typhimurium* may be an artifact of the incubation protocol and the high spontaneous hydrolysis rate of this reactive compound (NTP, 1997 draft).

In mice, isoprene and 1,3-butadiene induced SCE in bone marrow cells and increased the frequency of micronucleated erythrocytes in peripheral blood (Tice et al., 1987; cited by NTP, 1997 draft; Tice et al., 1988). Unlike isoprene, 1,3-butadiene also induced chromosomal aberrations in bone marrow cells of mice. Neither compound has been shown to be genotoxic in rats. The above findings, as well as the identification of unique oncogene mutations in

neoplasms of mice exposed to isoprene, are consistent with a genotoxic mechanism of tumor induction.

Activation of K-*ras*, predominantly by an A→T transversion at codon 61, appears to be involved in the induction of Harderian gland neoplasms (Hong et al., 1997).

Evaluations of dose-response relationships for isoprene-induced neoplasms in rats indicated that the carcinogenic effects in the kidney are probably mediated by the epoxide intermediates (NTP, 1997 draft). Carcinogenic effects in the mammary gland of rats may involve a combination of effects from isoprene epoxides and the parent compound with possible hormonal influences.

8.0 REFERENCES

Altschuller, A. 1983. Natural volatile organic substances and their effect on air quality in the United States. Atmos. Environ. 17(11):2131-2165.

Arnts, R. R., and S. A. Meeks. 1980. Biogenic Hydrocarbon Contribution to the Ambient Air of Selected Areas—Tulsa; Great Smoky Mountains; Rio Blanco County, Colorado. Report No. EPA-600/3-80-023. U.S. Environmental Protection Agency, Environmental Sciences Research Laboratory, Research Triangle Park, NC. NTIS Report No. PB80-139066. Abstract from NTIS 80(11):1259.

Barrefors, G., and G. Petersson. 1993. Assessment of ambient volatile hydrocarbons from tobacco smoke and from vehicle emissions. J. Chromatogr. 642(1-2):71-76.

Bartsch, H., C. Malaveille, R. Montesano, and L. Tomatis. 1975. Tissue-mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in *Salmonella typhimurium*. Nature (London) 255:641-643. (Cited by IARC, 1979a)

Bartsch, H., C. Malaveille, A. Barbin, and G. Planche. 1979. Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues: Evidence for oxirane formation by p450-linked microsomal monooxygenases. Arch. Toxicol. 41:249-277. (Cited by IARC, 1979a, as "in press")

Bjorkqvist, S., A. Spetz, O. Ramnas, and G. Peterson. 1997. Isoprene from expired air inside a private car. Sci. Total Environ. 217(1):63-67. Abstract from MEDLINE 1998059990.

Bleasdale, C., R. Small, W. Watson, J. Wilson, and B. Golding. 1996. Studies on the molecular toxicology of buta-1,3-diene and isoprene epoxides. Toxicology 113(1-3):290-293.

Bogaards, J., J. Venekamp, and P. van Bladeren. 1996. The biotransformation of isoprene and the two isoprene monoepoxides by human cytochrome P450 enzymes, compared to mouse and rat liver microsomes. Chem.- Biol. Interact. 102(3):169-182.

Bond, J., W. Bechtold, L. Birnbaum, A. Dahl, M. Medinsky, J. Sun, and R. Henderson. 1991. Disposition of inhaled isoprene in B6C3F1 mice. Toxicol. Appl. Pharmacol. 107: 494-503. (Cited by IARC, 1994)

Bowling, D. R., A. A. Turnipseed, A. C. Delany, D. D. Baldocchi, J. P. Greenberg, and R. K. Monson. 1998. The use of relaxed eddy accumulation to measure biosphere-atmosphere exchange of isoprene and other biological trace gases. Oecologia (Berlin) 116(3):306-315. Abstract from BIOSIS 1998:493516.

Budavari, S. Ed. 1996. Isoprene. In: The Merck Index. 12th ed., Merck & Co., Inc., Whitehall, NJ, pp. 887-888.

Cailleux, A., and P. Allain. 1989. Isoprene and sleep. Life Sci. 44: 1877-1880. (Cited by IARC, 1994)

Cailleux, A., M. Cogney, and P. Allain. 1992. Blood isoprene concentrations in humans and in some animal species. Biochem. Med. Metab. Biol. 47: 157-160. (Cited by NTP, 1997 draft and IARC, 1994)

Chang, M., D. Hartley, C. Cardelino, and W.-L. Chang. 1996. Temporal and spatial distribution of biogenic emissions of isoprene based on an inverse method using ambient isoprene observations from the 1992 Southern Oxidants Study, Atlanta Intensive. School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA. Abstract available at URL http://sosona.eas.gatech.edu/~chang/docs/abstracts/pres3.html; last accessed February 16, 1999.

Chem. Inspect. Test. Inst. 1992. Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. ISBN 4-89074-101-1. Japan Chemical Industry Ecology-Toxicology & Information Center. (Cited by HSDB, 1997)

Chem. Mark. Report. 1994. Report from Houston: Stress on C-5 Supply. (May 30, 1994):45. Full text from PROMT 94:283044.

Chem. Week. 1994. Demand for high-purity isoprene up 6.5% +/1 yr 1985-1994 to 230 mil lbs, 1992, may go up more sharply by 2000. (April 27, 1994): 43. Full text from PROMT 94:230723.

Clayton, G. D., and F. E. Clayton, Eds. 1981-1982. Patty's Industrial Hygiene and Toxicology, Volumes 2A, 2B, 2C: Toxicology, 3rd ed., John Wiley & Sons, New York, p. 3209. (Cited by HSDB, 1997)

Dahl, A., L. Birnbaum, J. Bond, P. Gervasi, and R. Henderson. 1987. The fate of isoprene inhaled by rats: Comparison to butadiene. Toxicol. Appl. Pharmacol. 89: 237-248. (Cited by IARC, 1994)

de Meester, C., M. Mercier, and F. Poncelet. 1981. Mutagenic activity of butadiene, hexachlorobutadiene and isoprene. In: Gut, I., M. Cikrt, and G. L. Plaa, Eds. Industrial and Environmental Xenobiotics. Springer, Berlin, pp. 195-203. (Cited by IARC, 1994).

Del Monte, M., L. Citti, and P. Gervasi. 1985. Isoprene metabolism by liver microsomal mono-oxygenases. Xenobiotica 15: 591-597. (Cited by NTP, 1997 draft)

Delzell, E., N. Sathiakumar, M. Hovinga, M. Macaluso, J. Julian, R. Larson, P. Cole, and D. C. F. Muir. 1996. A follow-up study of synthetic rubber workers. Toxicology 113:182-189.

Deneris, E., R. Stein, and J. Mead. 1984. In vitro biosynthesis of isoprene from mevalonate utilizing a rat liver cytosolic fraction. Biochem. Biophys. Res. Commun. 123: 691-696. (Cited by IARC, 1994)

Divine, B. J., and C. M. Hartman. 1996. Mortality update of butadiene production workers. Toxicology 113:169-181.

Dueso, N. 1997. Pollution by volatile organic compounds: Definitions, sources and solutions. Inf. Chim. 387:76-79. Abstract from Chem. Abstr. 127:8284.

Duke, J. 1992. Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants. CRC Press, Boca Raton, FL. (Cited in the search results for plants containing isoprene in the Phytochemical and Ethnobotanical Databases produced by the U.S. Department of Agriculture, Agricultural Research Service, NGRL, Beltsville Agricultural Research Center, Beltsville, MD. URL: http://sun.ars-grin.gov/~ngrlsb/; last accessed February 18, 1999.)

Euler, H., S. Davé, and H. Guo. 1996. Effect of cigarette smoking on pentane excretion in alveolar breath. Clin. Chem. 42(2):303-308.

Galloway, S., M. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. Margolin, M. Resnick, B. Anderson, and E. Zeiger. 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. Environ. Mol. Mutagen. 10 (Suppl. 10):1-175. (Cited by NTP, 1997 draft and NTP, 1995)

Gelmont, D., R. A. Stein, and J. F. Mead. 1981. Isoprene—the main hydrocarbon in human breath. Biochem. Biophys. Res. Commun. 99:1456-1460.

Gervasi, P., and V. Longo. 1990. Metabolism and mutagenicity of isoprene. Environ. Health Perspect. 86: 85-87. (Cited by NTP, 1997 draft)

Gervasi, P.G., L. Citti, M. Del Monte, V. Longo, and D. Benetti. 1985. Mutagenicity and chemical reactivity of epoxidic intermediates of the isoprene metabolism and other structurally related compounds. Mutat. Res. 156:77-82. (Cited by NTP, 1997 draft)

Green, T. 1990. Chloroethylenes: A mechanistic approach to human risk evaluation. Annu. Rev. Pharmacol. Toxicol. 30:73-90.

Guenther, A., P. Zimmerman, and M. Wildermuth. 1994. Natural volatile organic compound emission rate estimates for U.S. woodland landscapes. Atmos. Environ. 28(6):1197-1210.

Guenther, A., C. Hewitt, D. Erickson, R. Fall, C. Geron, T. Graedel, P. Harley, L. Klinger, et al. 1995. What is the contribution of Amazonia to the global atmospheric budgets of non methane hydrocarbons (NMHC)? Data presented from J. Geophys. Res. 100:8873-8892 by the University of Mainz, Germany, at URL http://www.mpch-mainz.mpg.de/~eustach/intro/voc.htm; last accessed February 16, 1999.

Hagerman, L., V. Aneja, and W. Lonneman. 1997. Characterization of non-methane hydrocarbons in the rural southeast United States. Atmos. Environ. 31(23):4017-4038. Abstract from EMBASE 97321343.

Hoffman, T., J. Kahl, and D. Klackow. 1996. Emission and degradation of isoprene and terpenes: The contribution of vegetation to atmospheric aerosol production. An. Acad. Bras. Cienc. 68(Suppl. 1):251-259. Abstract from TOXLINE 1997:119682.

HSDB (Hazardous Substance Data Bank). 1997. Isoprene profile last updated 07/08/97. Online database produced by the National Library of Medicine.

Hong, H., T. Devereux, R. Melnick, S. Elridge, A. Greenwell, J. Haseman, G. Boorman, and R. Sills. 1997. Both K-*ras* and H-*ras* protooncogene mutations are associated with Harderian gland tumorigenesis in B6C3F1 mice exposed to isoprene for 26 weeks. Carcinogenesis 18(4):783-789.

IARC (International Agency for Research on Cancer). 1979a. Chloroprene and polychloroprene. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. 19(Some Monomers, Plastics and Synthetic Elastomers, and Acrolein):131-151.

IARC (International Agency for Research on Cancer). 1979b. Vinyl chloride and polymers. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. 19(Some Monomers, Plastics and Synthetic Elastomers, and Acrolein):377-438.

IARC (International Agency for Research on Cancer). 1987. Chloroprene. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. Supplement 7(Genetic and Related Effects: An Updating of Selected IARC Monographs From Volumes 1-42):373-376.

IARC (International Agency for Research on Cancer). 1992. 1,3-Butadiene. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. 54(Occupational Exposures to Mists and Vapours from Strong Inorganic Acids; and Other Industrial Chemicals):237-285.

IARC (International Agency for Research on Cancer). 1994. Isoprene. IARC Monogr. Eval. Carcinog. Chem. Risks Hum. 60(Some Industrial Chemicals): 215-232.

Infante, P. F. 1977. Mutagenic and carcinogenic risks associated with halogenated olefins. Environ. Health Perspect. 21:251-254.

Jauhar, P., P. Henika, J. MacGregor, C. Wehr, M. Shelby, S. Murphy, and B. Margolin. 1988. 1,3-Butadiene: Induction of micronucleated erythrocytes in the peripheral blood of $B6C3F_1$ mice exposed by inhalation for 13 weeks. Mutat. Res. 209: 171-176. (Cited by NTP, 1997 draft)

Lamb, B., D. Gay, H. Westburg, and T. Pierce. 1993. A biogenic hydrocarbon emission inventory for the U.S.A. using a simple forest canopy model. Atmos. Environ. 27A(11):1673-1690.

Lawrimore, J., and V. Aneja. 1997. A chemical mass balance analysis of nonmethane hydrocarbon emissions in North Carolina. Chemosphere 35(11):2751-2765. Abstract from TOXLINE 1998:9369.

Li, S, Q. Dong, Y. Liu, and Y. Liu. 1989. Epidemiologic study of cancer mortality among chloroprene workers. Biomed. Environ. Sci. 2:141-149.

Longo, V., L. Citti, and P. Gervasi. 1985. Hepatic microsomal metabolism of isoprene in various rodents. Toxicol. Lett. 29:33-37. (Cited by NTP, 1997 draft)

Macaluso, M., R. Larson, E. Delzell, N. Sathiakumar, M. Hovinga, J. Julian, D. Muir, and P. Cole. 1996. Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. Toxicology 113:190-202.

Malvoisin, E., G. Lhoest, F. Poncelet, M. Roberfroid, and M. Mercier. 1979. Identification and quantitation of 1,2-epoxybutene-3 as the primary metabolite of 1,3-butadiene. J. Chromatogr. 178: 419-425. (Cited by NTP, 1997 draft)

Malvoisin, E., and M. Roberfroid. 1982. Hepatic microsomal metabolism of 1,3-butadiene. Xenobiotica 12:137-144. (Cited by NTP, 1997 draft)

Melnick, R., R. Sills, J. Roycroft, B. Chou, H. Ragan, and R. Miller. 1994. Isoprene, an endogenous hydrocarbon and industrial chemical, induces multiple organ neoplasia in rodents after 26 weeks of inhalation exposure. Cancer Res. 54(20):5333-5339.

Mortelmans, K., S. Haworth, T. Lawlor, W. Speck, B. Tainer, and E. Zeiger. 1986. Salmonella metagenicity tests: II. Results from the testing of 270 chemicals. Environ. Mutagen. 8(Suppl. 7):1-119. (Cited by IARC, 1994)

NIOSH (National Institute for Occupational Safety and Health). 1976. National Occupational Hazard Survey (1972-1974). U.S. Department of Health and Human Services, Public Health

Service, Centers for Disease Control, NIOSH, Division of Surveillance, Hazard Evaluations and Field Studies, Surveillance Branch, Hazard Section, Cincinnati, OH.

NIOSH (National Institute for Occupational Safety and Health). 1990. National Occupational Exposure Survey (1981-83) Unpublished provisional data as of 7/1/90. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, NIOSH, Division of Surveillance, Hazard Evaluations and Field Studies, Surveillance Branch, Hazard Section, Cincinnati, OH.

NTP (National Toxicology Program). 1993. Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No. 106-99-0) in B6C3F$_1$ Mice (Inhalation Studies). Technical Report Series No.434. NIH Publication No. 93-3165. U.S. Department of Health and Human Service, National Institutes of Health, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP (National Toxicology Program). 1995. NTP Technical Report on Toxicity Studies of Isoprene (CAS No. 78-79-5) Administered by Inhalation to F344/N Rats and B6C3F$_1$ Mice. Toxicity Report Series No. 31. NIH Publication No. 95-3354. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP (National Toxicology Program). 1997 draft. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Isoprene (CAS No. 78-79-5) in F344/N Rats (Inhalation Studies). Technical Report No. 486. NIH Publication No. 97-3976. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NTP (National Toxicology Program). 1998. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Chloroprene (CAS No. 126-99-8) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). NTP TR 467. NIH Publication No. 98-3957. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Environmental Health Sciences, Research Triangle Park, NC, September 1998.

NTP (National Toxicology Program). 1999. NTP Report on Carcinogens, 9th ed. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC.

Owen, P. E., J. R. Glaister, I. F. Gaunt, and D. H. Pullinger. 1987. Inhalation toxicity studies with 1,3-butadiene. 3. Two year toxicity/carcinogenicity study in rats. Am. Ind. Hyg. Assoc. J. 48:407-413. (Cited by IARC, 1992)

Pell, S. 1978. Mortality in workers exposed to chloroprene. J. Occup. Med. 20:21-29. (Cited by IARC, 1979a)

Peter, H., H. Wiegand, H. Bolt, H. Greim, G. Walter, M. Berg, and J. Filser. 1987. Pharmacokinetics of isoprene in mice and rats. Toxicol. Lett. 36:9-14. (Cited by IARC, 1994, NTP, 1997 draft, and Taalman, 1996)

Phillips, M., J. Greenberg, and J. Awad. 1994. Metabolic and environmental origins of volatile organic compounds in breath. J. Clin. Pathol. 47(11):1052-1053.

Placke, M., L. Griffis, M. Bird, J. Bus, R. Persing, and A. Cox, Jr. 1996. Chronic inhalation oncogenicity study of isoprene in B6C3F1 mice. Toxicology 113:253-262.

Saltman, W. 1985. Isoprene. In: Concise Encyclopedia of Chemical Technology, M. Grayson, Ed., John Wiley & Sons, New York, pp. 674-675.

Seila, R., R. Arnts, and J. Buchanan. 1984. Atmospheric volatile hydrocarbon composition at five remote sites in northwestern North Carolina. EPA-600-D-84-092. NTIS Report No. PB84-177930. Abstract from TOXLINE 1984:54695.

Small, R., B. Golding, and W. Watson. 1997. Species differences in the stereochemistry of the metabolism of isoprene in vitro. Xenobiotica 27(11):1155-1164.

SRI Int. 1997. 1997 Directory of Chemical Producers. United States of America. SRI International, Menlo Park, CA.

Taalman, R. 1996. Isoprene: Background and issues. Toxicology 113(1-3):242-246.

Tice, R., R. Boucher, C. Luke, and M. Shelby. 1987. Comparative cytogenetic analysis of bone marrow damage induced in male B6C3F1 mice by multiple exposures to gaseous 1,3-butadiene. Environ. Mutagen. 9: 235-250. (Cited by NTP, 1997 draft)

Tice, R., R. Boucher, C. Luke, D. Paquette, R. Melnick, and M. Shelby. 1988. Chloroprene and isoprene: Cytogenetic studies in mice. Mutagenesis 3:141-146.

U.S. DOT (U.S. Department of Transportation). 1996. 1996 North American Emergency Response Guidebook. A Guidebook for First Responders During The Initial Phase of a Hazardous Materials/Dangerous Goods Incident. Research and Special Programs Administration, Office of Hazardous Materials Initiatives and Training (DHM-50), Washington, DC, p. G-130P. (Cited by HSDB, 1997)

USITC (U.S. International Trade Commission). 1994. Synthetic Organic Chemicals. United States Production and Sales, 1992. USITC Publication 2720. U.S. International Trade Commission, Washington, DC.

USITC (U.S. International Trade Commission). 1995. Synthetic Organic Chemicals. United States Production and Sales, 1994. USITC Publication 2933. U.S. International Trade Commission, Washington, DC.

Ward, E. M., J. M. Fajen, A. M. Ruder, R. A. Rinsky, W. E. Halperin, and C. A. Fessler-Flesch. 1996. Mortality study of workers employed in 1,3-butadiene production units identified from a large chemical workers cohort. Toxicology 113:157-168.

West, R. R., D. A. Stafford, A. Farrow, and A. Jacobs. 1995. Occupational and environmental exposures and myelodysplasia: A case-control study. Leuk. Res. 19:127-139.

Westphal, G. A., M. Blaszkewicz, M. Leutbecher, A. Müller, E. Hallier, and H. M. Bolt. 1994. Bacterial mutagenicity of 2-chloro-1,3-butadiene (chloroprene) caused by decomposition products. Arch. Toxicol. 68:79-84.

Wistuba, D., K. Weigand, and H. Peter. 1994. Stereoselectivity of in vitro isoprene metabolism. Chem. Res. Toxicol. 7: 336-343. (Cited by NTP, 1997 draft)

Zimmerman, P. R. 1979. Testing for hydrocarbon emissions from vegetation leaf litter and aquatic surfaces, and development of a methodology for compiling biogenic emission inventories. EPA Report 450/4-4-79-004. (Cited by Altschuller, 1983)

Zwolinski, B. J., and R. C. Wilhoit. 1971. Handbook of Vapor Pressures and Heats of Vaporization of Hydrocarbons and Related Compounds. API44-TRC101. Thermodynamics Research Center, College Station, TX. (Cited by HSDB, 1997)

APPENDIX A

Excerpt from IARC Monograph (1994)
Volume 60; pp. 215-232

APPENDIX B

Report on Carcinogens (RoC), 9th Edition
Review Summary

Report on Carcinogens (RoC), 9th Edition
Review Summary

Isoprene

NOMINATION
Review based on results of an NTP Bioassay of Isoprene (1995, 1997) reporting clear evidence of benign and malignant tumor formation at multiple organ sites in multiple species of experimental animals.

DISCUSSION
Isoprene is used in the production of 95% polyisoprene and 2% isoprene-butadiene copolymers and is also the monomeric unit of natural rubber and naturally occurring terpenes and steroids. There is clear evidence of benign and malignant tumor formation at multiple organ sites from inhalation studies of isoprene in multiple species of experimental animals. Isoprene has a close structural relationship to 1,3-butadiene. The recommendations from the three NTP reviews of this nomination are as follows:

Review Committee	Recommendation	Vote
NIEHS (RG1)	list as a reasonably anticipated human carcinogen	6 yes/0 no
NTP EC Working Group (RG2)	list as a reasonably anticipated human carcinogen	6 yes/0 no/1 a*
NTP Board RoC Subcommittee	list as a reasonably anticipated human carcinogen	13 yes/0 no

*a-abstentions

Public Comments Received
A total of 2 public comments were received, both against listing in the RoC in any category.