Air Toxics Hot Spots Program

Isoprene

Cancer Inhalation Unit Risk Factor

Technical Support Document for
Cancer Potency Factors
Appendix B

January 2025

Air and Site Assessment and Climate Indicators Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

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Technical Support Document for Cancer Potency Factors Appendix B

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List of Abbreviations

BD	1,3-butadiene
BMD	Benchmark Dose
BMDL	95% lower confidence limit for the Benchmark Dose
BMDS	Benchmark Dose Modeling Software
BMR	Benchmark Response
$BR_{a \text{ or } h}$	Breathing Rate (animal or human)
$BW_{a \text{ or } h}$	Body weight (animal or human)
CARB	California Air Resources Board, The
cDNA	Complementary deoxyribonucleic acid
cEH	Cytosolic epoxide hydrolase
CEIDARS	California Emissions Inventory Development and Reporting System
$CSF_{a \text{ or } h}$	Cancer Slope Factor (animal or human)
CYP	Cytochrome P450 enzyme
CYP2A6	Cytochrome P450 2A6 isoenzyme
CYP2B6	Cytochrome P450 2B6 isoenzyme
CYP2D6	Cytochrome P450 2D6 isoenzyme
CYP2E1	Cytochrome P450 2E1 isoenzyme
°C	Degrees Celsius
DNA	Deoxyribonucleic acid
ECHA	European Chemicals Agency, The
EH	Epoxide hydrolase
GST	Glutathione-S-transferase
IARC	International Agency for Research on Cancer, The
IUR	Inhalation Unit Risk Factor (from OEHHA)
LADD	Lifetime average daily dose
LEC_{10}	95% lower confidence limit on the effective concentration corresponding to 10% extra risk
mEH	Microsomal epoxide hydrolase
mg/kg-d	Milligrams per kilogram of body weight per day
mg/m^3	Milligrams per cubic meter
MLE	Maximum likelihood estimate
MOA	Mode of action
mRNA	Messenger ribonucleic acid
µg/L	Micrograms per liter
$\mu g/m^3$	Micrograms per cubic meter
µmol/hr	Micromoles per hour
µmol/kg-d	Micromoles per kilogram BW per day
µmol/L	Micromoles per liter
n	Number
ND	Not determined
NIEHS	National Institute of Environmental Health Sciences, The
Nmol/L	Nanomoles per liter
NRC	National Research Council, The
NT	Not tested
NTP	National Toxicology Program, The
OEHHA	Office of Environmental Health Hazard Assessment, The
$(hour)^{-1}$	Per hour
$(\mu g/m^3)^{-1}$	Per microgram per cubic meter
$(mg/kg\text{-}d)^{-1}$	Per milligram per kilogram of body weight per day
$(ppb)^{-1}$	Per part per billion
PBPK	Physiologically-based pharmacokinetic or toxicokinetic
PK	Pharmacokinetic
POD	Point of departure
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
SD	Standard deviation
TCEQ	Texas Commission on Environmental Quality, The
TRI	Toxics Release Inventory
TSD	Technical Support Document
URF	Unit Risk Factor (from TCEQ)
US EPA	United States Environmental Protection Agency, The
VOC	Volatile Organic Compound

Preface

The Office of Environmental Health Hazard Assessment (OEHHA) is legislatively mandated to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code section 44360(b)(2)). In response to this statutory requirement, OEHHA developed a Technical Support Document (TSD) that describes the methodology for deriving inhalation unit risk factors (IURs) and cancer slope factors (CSFs) for carcinogenic Hot Spots air pollutants. The methodology in the TSD explicitly considers possible differential effects on the health of infants, children, and other sensitive subpopulations under the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Chapter 731, Statutes of 1999, Health and Safety Code Sections 39669.5 et seq.), including procedures for evaluating increased susceptibility to carcinogens.

The IUR defines the excess cancer risk associated with continuous inhalation exposure to a given carcinogen at 1 microgram per cubic meter ($\mu g/m^3$) over a lifetime. The CSF estimates excess lifetime cancer risk associated with exposure at 1 milligram per kilogram of body weight per day (mg/kg-d). In the Hot Spots Program, the IUR and CSF are used for calculating cancer risks from chemical exposures above the background levels.

CARB requested that OEHHA derive an IUR for isoprene (CARB, 2024; CARB 2013), due to its presence in biogas emissions and in the air of residential areas near oil and gas operations. The similarity in chemical structure to 1,3-butadiene, a known human carcinogen, was also a motivating factor for deriving an IUR specific for isoprene.

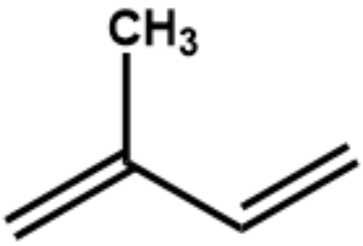
The current document summarizes the carcinogenicity data supporting OEHHA's derivation of an isoprene IUR under the Air Toxics Hot Spots Program. Isoprene is listed as a chemical known to cause cancer in California's Proposition 65 Program. Isoprene is also "presumed" by the European Chemicals Agency (ECHA) to cause cancer to humans (Group 1B), classified by the International Agency for Research on Cancer (IARC) as "possibly carcinogenic to humans" (Group 2B), and "reasonably anticipated to be a human carcinogen" by the United States National Toxicology Program (NTP).

The literature summarized and referenced in the present document covers the relevant publicly available reports and original peer-reviewed research articles on isoprene through July 2024 (See Appendix A). Individual reports summarized herein were primarily those that would be useful for deriving or supporting an IUR for isoprene, including experimental animal carcinogenicity and genetic toxicity studies.

Key isoprene studies investigating human exposure, toxicokinetics, and mechanisms of carcinogenicity were also summarized in this document.

ISOPRENE

Chemical Abstracts Service Registry Number: 78-79-5



I. PHYSICAL AND CHEMICAL PROPERTIES

(NOAA, 1999; NCBI, 2023)

Molecular formula:	C_5H_8
Molecular weight:	68.12 grams per mole
Synonym:	2-methyl-1,3-butadiene; isopentadiene
Description:	Colorless liquid with a mild, petroleum-like odor
Relative gas density:	2.35 (air = 1)
Specific gravity	0.681 @ 20°C (liquid)
Boiling point:	34°C
Melting point:	-145.95°C
Vapor pressure:	550 Torr at 25°C
Solubility:	Miscible with ethanol, ethyl ether, acetone, and benzene; "very poor" solubility in water (642 milligrams per liter at 25°C)
Conversion factor:	1 part per billion (ppb) = 2.79 micrograms per cubic meter ($\mu g/m^3$)

II. HEALTH ASSESSMENT VALUES

Inhalation Unit Risk Factor (IUR):	5.4×10^{-6} per microgram per cubic meter $(\mu g/m^3)^{-1}$; 1.5×10^{-5} per part per billion $(ppb)^{-1}$
Cancer Slope Factor (CSF):	1.9×10^{-2} per milligram per kilogram of body weight per day $(mg/kg\text{-}d)^{-1}$

III. OCCURRENCE AND MAJOR USES

Isoprene is a by-product of the thermal cracking of naphtha and is used mainly to make synthetic isoprene rubber, which is used mainly in the manufacture of vehicle tires but also in the manufacture of footwear (IARC, 1994). Isoprene is also used to

produce butyl rubber for manufactured goods such as hoses and liners in tubeless tires. In addition, the manufacture of styrene-isoprene-styrene polymers is used to make thermoplastic rubber and pressure-sensitive or thermosetting adhesives.

Emitted in large amounts by vegetation, particularly mosses, ferns, and trees (Sharkey and Yeh, 2001), isoprene is found at low concentrations in ambient air. California's biogenic isoprene emissions (i.e., those from vegetation and soil microbes) are estimated to be 1636 tons per day (CARB, 2023). Isoprene air concentrations in the United States (US) have been reported in the range of 0.2 to 4.2 ppb (0.6 to 12 μg/m^3; NTP, 2021). Isoprene is also present in some foods, such as roasted coffee and orange oil, and is produced endogenously in (and emitted by) mammals. In addition to synthetic rubber production, other anthropogenic isoprene sources include rubber abrasion, biomass combustion, wood pulping, tobacco smoking, and exhaust from turbines and automobiles. Wildfires and smoke plume composition are other sources of isoprene exposure (Simmons et al., 2022).

Isoprene is the largest source of volatile non-methane hydrocarbons emitted into Earth's atmosphere. It comprises 50% of the total non-methane hydrocarbon emissions from the biosphere (Loreto and Sharkey, 1993). Global isoprene emissions range from 1.5 to 2.2 million tons of isoprene per day (Guenther et al., 2006), contributing to one-third of the total volatile organic compound (VOC) emissions (Kiendler-Scharr et al., 2009). Per US EPA's Toxics Release Inventory (TRI) database, for the year 2021 (the most recent TRI data available), a total of 187,880 pounds of on-site disposal or other releases were reported for isoprene (US EPA, 2023). The TRI program comprises chemical releases and pollution prevention activities reported by industrial and federal facilities.

Estimated anthropogenic isoprene emissions in California in 2017 were 186 tons per year (approximately 0.5 tons per day), primarily from mobile sources, as off-road equipment, on-road emissions, and recreational boats accounted for about 31%, 29%, and 28% of the total anthropogenic isoprene emissions, respectively (CARB, 2019). The California Emissions Inventory Development and Reporting System (CEIDARS) contains statewide emissions data for all reported point sources and lists 12 facilities (stationary sources) in California that emit isoprene.

Liu et al. (2022) measured the composition and reactivity of VOCs, including isoprene, in the South Coast Air Basin and San Joaquin Valley of California in the summer of 2019. The average and maximum isoprene concentrations were 178 and 651 parts per trillion (ppt; 0.5 and 1.8 μg/m^3), respectively, for the South Coast Air Basin and 36 and 298 ppt (0.1 and 0.8 μg/m^3), respectively, for the San Joaquin Valley. Wernis et al. (2022) looked at major sources of pollution in Livermore, CA,

over 10 days. Several volatile and semi-volatile compounds, including isoprene, were identified. The mean isoprene concentration measured in the study was 68 ppt (0.19 µg/m^3), with peaks in the early morning and early evening. Isoprene was found to correlate with benzene and several other gasoline markers, providing support for attributing these isoprene emissions to anthropogenic sources. Other investigators have reported correlations between isoprene and pollutants of known vehicle traffic origin (Reimann et al., 2000; Borbon et al., 2001; Lee and Wang, 2006; Hellen et al., 2012).

Endogenous Isoprene Production

Isoprene is endogenously produced in humans at an estimated rate of 0.34 micromoles per kilogram of body weight per hour (Filser et al.,1996; Hurst, 2007) and is a major VOC found in human breath. The primary site of production in the body is muscle tissue (Mochalski et al., 2023). Isoprene in exhaled breath of humans is thought to result predominantly from conversion of isopentenyl diphosphate to dimethylallyl pyrophosphate in skeletal-myocellular peroxisomes as part of muscular lipolytic cholesterol metabolism (Sukul et al., 2023). Isoprene is also generated during lipolytic cholesterol metabolism in the endoplasmic reticulum of hepatocytes but is largely metabolized within the liver before reaching the bloodstream.

For adults at rest, steady-state isoprene concentrations in end-tidal breath are 70 to 133 ppb (195 to 371 µg/m^3) by volume for the 25th to 75th quantile range (Mochalski et al., 2023). Mean (± standard deviation; SD) breath levels are lower in young children [28 ± 24 ppb (78 ± 67 µg/m^3), age 7 to 10 years] compared to adults but increase with increasing age of the child (Smith et al., 2010). Very low or undetectable isoprene levels in the exhaled breath of newborn infants have been reported (Nelson et al., 1998). Lower breath levels in children and infants are correlated with lower muscle mass compared to adults (Mochalski et al., 2023). Mean ± SD blood levels of isoprene in adults were measured by Cailleux et al. (1992) at 37 ± 25 nanomoles per liter (nmol/L). Blood levels of isoprene in other animals, such as rats, rabbits, pigs, and dogs, were more than 30 times lower compared to humans (< 1 nmol/L)[1]. Pigs have low blood levels of isoprene compared to humans and undetectable levels of isoprene in breath (Miekisch et al., 2001; Sukul et al., 2023).

[1] An early study by Peter et al. (1987) reported higher rates of endogenous isoprene in mice and rats. However, this finding was called into question by Filser et al. (1996), who reevaluated the data and concluded that the chemical being measured by Peter et al. was acetone.

Isoprene is likely produced in peripheral tissues and liver but not in the muscle tissue of pigs.

IV. CARCINOGENICITY

Isoprene has been listed as a chemical known to cause cancer in California's Proposition 65 Program since 1996 (OEHHA, 1996). This listing was based upon the classification of isoprene as "possibly carcinogenic to humans" (a 2B carcinogen) by the International Agency for Research on Cancer (IARC, 1994). Since then, isoprene has been recognized as "reasonably anticipated to be a human carcinogen" by the National Toxicology Program (NTP, 2021) and "presumed to be carcinogenic in humans" (a 1B carcinogen) by the European Chemicals Agency (ECHA, 2023)[2]. These designations were based on increased tumor formation at multiple organ sites in rodents exposed to isoprene via inhalation. No human epidemiological studies on the carcinogenicity of isoprene were found in the literature by OEHHA, IARC (1999), NTP (2021), or ECHA (2023).

Rodent Carcinogenicity Studies

Three reports (NTP, 1995; Placke et al., 1996; NTP, 1999) with several studies were reviewed to characterize the carcinogenicity of isoprene in rats and mice by inhalation exposure. Statistical analysis of tumor incidence data was performed by OEHHA using the exact conditional Cochran-Armitage test for linear trend (i.e., exact trend test) and the one-tailed Fisher's exact test for pairwise comparisons as recommended for carcinogen risk assessment (US EPA, 2005). A one-tailed test was used by OEHHA to determine if there is an increase in tumor incidences in the treated groups compared to controls (i.e., the null hypothesis is that there is no statistically significant increase in tumor incidence between the control group and an isoprene-exposed group).

NTP (1995)

In the 1995 one-year, stop-exposure study by NTP, male F344/N rats and male $B6C3F_1$ mice were exposed to isoprene for six hours per day, five days per week for six months [number (n) = 30/species/exposure group]. In addition to the control [0 parts per million (ppm), 0 mg/m^3], five isoprene concentrations were tested up to

[2] ECHA is the agency responsible for implementing the European Union's chemicals legislation (e.g., the Registration, Evaluation, Authorisation and Restriction of Chemicals regulation) to protect human health and the environment.

7000 ppm (19,530 mg/m^3). Tumor incidence was observed following an additional six-month follow-up period. Marginally increased incidences of testicular adenomas were observed in isoprene-exposed male rats (Table 1a), and statistically significant increases in liver, lung, forestomach, and Harderian gland[3] tumors were found in isoprene-exposed male mice (Table 1b) compared to controls. In the tables mentioned above, the numerator represents the number of tumor-bearing animals; the denominator represents the number of animals examined.

[3] The Harderian glands in mice do not have a human counterpart (Albert et al., 1986). They are pigmented lacrimal glands located posterior to the ocular globes and are found in rodents and some other mammals. The glands release a lipid- and porphyrin-rich material that lubricates the eyes and eyelids. Concordance of site or tumor type between animal models and humans is not assumed or required (OEHHA, 2009; US EPA 2005). Agents observed to produce tumors in both humans and animals have produced tumors either at the same site or different sites. The overarching principle is that tumor induction (at any site) in animals is assumed to indicate the ability of an agent to cause tumors in humans.

Table 1a: Incidence of primary tumors in male rats exposed by inhalation to isoprene for six months, followed by a six-month recovery period (NTP, 1995).

Rat Cancer Endpoint	Cancer Incidence by Isoprene Concentration						Trend test *p*-value[a]
	0 ppm, 0 mg/m^3	70 ppm, 195 mg/m^3	220 ppm, 614 mg/m^3	700 ppm, 1953 mg/m^3	2200 ppm, 6138 mg/m^3	7000 ppm, 19,530 mg/m^3	
Testes: Adenoma	3/30	3/30	4/30	7/30	8/29	9/30	0.021

[(a)] The Cochran-Armitage trend test was conducted by the National Toxicology Program (NTP).

Table 1b. Incidence of primary tumors in male mice exposed by inhalation to isoprene for six months, followed by a six-month recovery period (NTP, 1995).

Mouse Cancer Endpoint	Cancer Incidence by Isoprene Concentration						Trend test *p*-value[a]
	0 ppm, 0 mg/m^3	70 ppm, 195 mg/m^3	220 ppm, 614 mg/m^3	700 ppm, 1953 mg/m^3	2200 ppm, 6138 mg/m^3	7000 ppm, 19,530 mg/m^3	
Liver: Adenoma	4/30	2/30	6/29	15/30**	18/30**	16/28**	<0.001
Liver: Carcinoma	4/30	1/30	3/29	5/30	4/30	9/28*	<0.001
Liver: Adenoma or Carcinoma	7/30	3/30	7/29	15/30*	18/30**	17/28**	<0.001
Lung: Adenoma	2/30	2/30	1/29	4/30	10/30*	8/28*	<0.001
Lung: Carcinoma	0/30	0/30	0/29	1/30	1/30	3/28	0.003
Lung: Adenoma or Carcinoma	2/30	2/30	1/29	5/30	10/30*	9/28*	<0.001
Forestomach: Squamous Cell Papilloma	0/30	0/30	0/30	1/30	2/30	5/30	0.001
Forestomach: Squamous Cell Carcinoma	0/30	0/30	0/30	0/30	2/30	1/30	0.159
Forestomach: Squamous Cell Papilloma or Carcinoma	0/30	0/30	0/30	1/30	4/30	6/30*	<0.001
Harderian Gland: Adenoma	2/30	6/30	4/30	14/30**	13/30**	12/30**	<0.001

Abbreviations: * *p*-value < 0.05, ** *p*-value < 0.01 by Fisher's exact test as reported by the National Toxicology Program (NTP,1995) in Table B5; mg/m^3 – milligrams per cubic meter; ppm – parts per million

[(a)] Logistic regression trend test performed by NTP.

Tumor incidence data for liver adenoma and carcinoma, lung bronchiolar/alveolar adenoma and carcinoma, and forestomach squamous cell papilloma and carcinoma are presented separately and combined in Table 1b. The rationale and guidelines for combining certain neoplasms and sites are discussed by Brix et al. (2010) and McConnell et al. (1986). This guidance is used by US EPA (2005) and OEHHA (2009) for carcinogen risk assessment. The recommendation is that benign and malignant neoplasms of the same cell origin be analyzed separately and in combination. Likewise, neoplasms with the same histogenesis but showing different morphologic and cellular features should be analyzed separately and in combination.

Placke et al. (1996)

The statistically and/or biologically significant tumor incidences from the second inhalation study (Placke et al., 1996), conducted with $B6C3F_1$ mice, are presented in Tables 2a and 2b for males and females, respectively. The primary exposure protocol in this study was eight hours per day, five days per week, over an 80-week exposure period, with a total study time of 105 weeks. Groups of male and female mice (n = 50/sex/group) were exposed to isoprene concentrations of 0, 10, 70, 280, 700, or 2200 ppm (0, 28, 195, 781, 1953, or 6138 mg/m^3), with females excluded from the three highest exposures. The exposures included a 7-minute ramp-up time to reach 90% of the target exposure concentration, resulting in a total exposure time of 8.12 hours on exposure days. Several additional exposure schedules were implemented to examine the effect of exposure intensity on carcinogenic potency. These included exposure periods of 20 or 40 weeks and daily exposures for four (instead of eight) hours. Results from the 20- and 40-week exposure studies are not summarized in the present document.

Due to decreased survival in the 280-, 700-, and 2200-ppm (781-, 1953-, and 6138-mg/m^3) male mice relative to controls, necropsy was performed at 96 weeks for these three exposure groups rather than 105 weeks. Life tables and appearance-of-first-tumor information were not presented in the report. However, the authors reported that by week 95, male mice in the three highest exposure groups had near or below 50% survival rates. The high mortality of these male mice was associated with a greater number of tumors than controls. Survival in the males exposed to ≤ 70 ppm (≤ 195 mg/m^3) remained generally above 60% through week 105. No effects on the survival of isoprene-exposed female mouse groups were noted.

In the primary exposure protocol, significant increases in liver, lung (alveolar/bronchiolar), and Harderian gland tumors were observed in isoprene-exposed male mice compared to their control counterparts (Table 2a). These findings were consistent with the tumor sites observed in the NTP (1995) stop-exposure

study. For lung adenomas, a significantly lower number of neoplasms was observed in the 70-ppm (≤ 195-mg/m^3) group as compared to both concurrent and historical controls. Historical control incidence data were not available for the lab that conducted the Placke study. Although not directly comparable, the historical control incidence for lung adenomas in male mice from time-matched NTP inhalation carcinogenicity studies was 21.2% (NTP, 2023). While the control animals in the Placke et al. (1996) study had a 22% incidence of lung adenomas, the 70-ppm (195-mg/m^3) exposure group had only an 8% incidence.

Forestomach squamous cell papillomas and squamous cell carcinomas were found in some male mice at 280 ppm (781 mg/m^3) or greater, with a statistically significant trend observed for squamous cell carcinomas. However, statistically significant pairwise increases in the incidences of these tumors were not observed compared to control mice. Non-statistically significant increases in histiocytic sarcomas were also reported by Placke et al. (1996). Combined incidence data were not provided for tumor types in which both adenomas and carcinomas were observed. Thus, it is unknown to OEHHA which animals had adenomas and/or carcinomas for specific tumor types.

Table 2a. Incidence of primary tumors in male mice exposed to isoprene by inhalation for 80 weeks (Placke et al., 1996).

Male Mouse Cancer Endpoint	Cancer Incidence by Isoprene Concentration						Trend test *p*-value[a]
	0 ppm, 0 mg/m^3	10 ppm, 27.9 mg/m^3	70 ppm, 195 mg/m^3	280 ppm, 781 mg/m^3	700 ppm, 1953 mg/m^3	2200 ppm, 6138 mg/m^3	
Liver: Adenoma	11/50	12/50	15/50	24/50**	27/48**	30/50**	<0.0001
Liver: Carcinoma	9/50	6/50	9/50	16/50	17/48*	16/50	0.0167
Lung: Adenoma	11/50	16/50	4/50[b]	13/50	23/50**	30/50**	<0.0001
Lung: Carcinoma	0/50	1/50	2/50	1/50	7/50**	7/50**	0.0011
Forestomach: Squamous Papilloma	0/50	0/48	0/50	0/50	1/47	1/50	0.0824
Forestomach: Squamous Carcinoma	0/50	0/48	0/50	1/50	0/47	3/50	0.0069
Harderian Gland: Adenoma	4/47	4/49	9/50	17/50**	26/49**	35/50**	<0.0001
Harderian Gland: Carcinoma	0/47	0/49	0/50	1/50	3/49	2/50	0.0537
Histiocytic Sarcoma	0/50	2/50	2/50	4/50	2/50	2/50	0.3916

Abbreviations: * $p < 0.05$, ** $p < 0.01$ by one-tailed Fisher's exact test conducted by OEHHA; mg/m^3 – milligrams per cubic meter; ppm – parts per million.

(a) The exact trend test conducted by OEHHA.

(b) Pairwise comparison of lung alveolar/bronchiolar adenomas of the 70 ppm (195 mg/m^3) group was statistically significantly lower ($p < 0.05$) compared to the control group.

In addition to the tumors shown in Table 2a, cardiac hemangiosarcomas were found in one 280-ppm male, two 700-ppm males, and one 2200-ppm male (781, 1953, and 6138 mg/m^3, respectively). The authors stated that these tumors are rare in male mice, as historical control B6C3F$_1$ mice from previous 2-year inhalation studies have not developed this tumor.

In female mice, exposure-related increases in spleen, pituitary gland, and Harderian gland neoplasms were found (Table 2b).

Table 2b. Incidence of primary tumors in female mice exposed to isoprene by inhalation for 80 weeks (Placke et al., 1996)[a].

Female Mouse Cancer Endpoint	Cancer Incidence by Isoprene Concentration			Trend test *p*-value[b]
	0 ppm, 0 mg/m^3	10 ppm, 27.9 mg/m^3	70 ppm, 195 mg/m^3	
Harderian Gland: Adenoma[c]	2/49	3/49	8/49*	0.0173
Spleen: Hemangiosarcoma	1/50	1/49	4/50	0.0773
Pituitary Gland: Adenoma[c]	1/49	6/46*	9/49**	0.0149

Abbreviations: mg/m^3 – milligrams per cubic meter; ppm – parts per million.

(a) Statistical comparisons of cancer incidence in the control and isoprene-exposed groups are based on one-tailed Fisher's exact tests; * $p < 0.05$, ** $p < 0.01$.

(b) The exact trend test was conducted by OEHHA.

(c) No carcinomas of this tumor type were found in female mice.

The incidence of spleen hemangiosarcomas was reported by Placke et al. (1996) to be exposure-related, given historical control data from NTP carcinogenicity inhalation studies showing the tumors are rare (mean = 0.61%, 4 of 654 mice). In contrast, the authors noted that the mean incidences of Harderian and pituitary gland adenomas in NTP's historical controls were higher and more variable at 22/662 (range: 0% to 16%) and 127/659 (range: 2% to 44%), respectively. The percent incidence of Harderian and pituitary gland adenomas in high-exposure (70-ppm; 195-mg/m^3) female mice in Table 2b were 16.3% and 18.3%, respectively, suggesting to the

authors that these tumors may not be exposure-related. While OEHHA considers concurrent control animal data the most appropriate comparison when evaluating tumor incidence data (IARC, 2019), we note that the more appropriate historical control data would come from the same laboratory as that in which the Placke et al. studies were conducted, using female $B6C3F_1$ mice that were from the same supplier, fed the same diet, and housed under the same conditions as the Placke et al. studies. Therefore, the significantly increased incidences of Harderian and pituitary gland adenomas compared to concurrent controls were considered exposure-related by OEHHA.

The lack of a statistically significant increase in spleen hemangiosarcomas compared to concurrent controls ($p = 0.18$ by Fisher's exact test) and a lack of a statistically significant trend ($p > 0.05$ by exact trend test) led OEHHA to exclude this tumor in the dose-response assessment, as it was not expected to contribute significantly to the overall cancer potency. However, this tumor was considered by OEHHA to be a treatment-related finding.

NTP (1999)

The focus of the third report, conducted by NTP (1999), was two-year inhalation bioassays in male and female F344/N rats (n = 50/sex/exposure group). Male and female rats were exposed to isoprene at 0, 220, 700, or 7000 ppm (0, 614, 1953, or 19,530 mg/m^3) six hours/day, five days/week for 104 weeks. The exposures included a 12-minute ramp-up time to reach 90% of the target exposure concentration. Therefore, the total exposure time on exposure days was 6.2 hours. Male and female survival and body weight (BW) were unaffected by isoprene during the two-year exposures.

The statistically significant and/or biologically noteworthy tumor incidences in male and female rats are shown in Table 3. In male rats, "clear evidence of carcinogenic activity" was found based upon increased incidences of renal tubule, mammary gland, and testicular interstitial cell neoplasms. Exposure-dependent increases in renal tubule adenomas and adenomas or carcinomas (combined) were observed with single-section examinations of the kidneys. The incidence of tubule adenomas was increased in the 7000-ppm (19,530-mg/m^3) group compared to the concurrent control group ($p < 0.05$) and was above the historical control incidence range (0% to 4%). Extended evaluations using step sectioning (8 sections per kidney) resulted in an increased incidence of renal tubule adenomas in the 700- and 7000-ppm (1953- and 19,530-mg/m^3) exposure groups compared to the control group ($p < 0.05$ and $p < 0.01$, respectively). Histopathologic changes associated with male-rat-specific alpha

2μ-globulin protein droplet accumulation were not observed in the isoprene-exposed males.

There were significantly increased incidences of mammary gland fibroadenomas and multiple fibroadenomas in 7000-ppm (19,530-mg/m^3) males compared to the control group (Table 3; multiple fibroadenoma data not shown). The increase in mammary gland fibroadenomas was exposure-dependent and above the historical control range (0% to 6%) in all isoprene-exposed groups. Mammary gland carcinomas were observed in one male rat in each of the 220- and 700-ppm (614- and 1953-mg/m^3) groups and two animals in the 7000-ppm (19,530-mg/m^3) group. The incidence of mammary gland carcinomas did not reach statistical significance in any of the isoprene-exposed groups but is rare in control male rats (Historical incidence: 1 in 905 controls; range 0% to 2%). NTP considered the presence of these carcinomas to be treatment related. Mammary gland fibroadenomas can arise from adenomas and can progress to adenocarcinomas (McConnell et al. 1986; Eighmy et al. 2018). Thus, these mammary gland tumors are shown separately and combined in Table 3.

An exposure-dependent increase in interstitial cell adenomas of the testis was also observed in the male rats. Incidences of these tumors in the 700- and 7000-ppm (1953- and 19,530-mg/m^3) groups were significantly increased compared to the control group ($p < 0.05$ and $p < 0.01$, respectively). The historical control range (46% to 83%) for testicular interstitial cell adenomas was also surpassed in the 700- and 7000-ppm (1953- and 19,530-mg/m^3) groups.

In female rats, significantly increased incidences of mammary gland fibroadenomas were observed in all isoprene-exposed groups compared to controls (Table 3). Female rats with multiple fibroadenomas were also significantly increased ($p < 0.01$) in the two highest isoprene-exposed groups (data not shown). The incidence of mammary gland fibroadenomas in the isoprene-exposed groups ranged from 64% to 70%. This range was above the historical control incidence range of 20% to 54% for female rats. The incidence of mammary gland carcinoma was not increased in isoprene-exposed female rats compared to controls.

Table 3. Incidence of primary tumors in male and female rats exposed by inhalation to isoprene for two years (NTP, 1999)[a].

Sex	Tumor Type	Cancer Incidence by Isoprene Exposure Concentration				Trend test *p*-value[b]
		0 ppm, 0 mg/m^3	220 ppm, 614 mg/m^3	700 ppm, 1953 mg/m^3	7000 ppm, 19,530 mg/m^3	
Male	Kidney: Renal Tubule Adenoma or Carcinoma – single section[c]	0/50	2/50	2/50	6/50*	0.0053
	Kidney: Renal Tubule Adenoma or Carcinoma – Single + step sections (combined)	2/50	4/50	8/50*	15/50*	<0.001
	Mammary Gland: Fibroadenoma	2/50	4/50	6/50	21/50**	<0.0001
	Mammary Gland: Carcinoma	0/50	1/50	1/50	2/50	0.1196
	Mammary Gland: Fibroadenoma or Carcinoma	2/50	5/50	7/50	21/50**	<0.0001
	Testes: Adenoma	33/50	37/50	44/50*	48/50**	<0.0001
Female	Mammary Gland: Fibroadenoma	19/50	35/50**	32/50**	32/50**	0.1582
	Mammary Gland: Carcinoma	4/50	2/50	1/50	3/50	0.4601

Abbreviations: NTP – National Toxicology Program; mg/m^3 – milligrams per cubic meter; ppm – parts per million.

(a) Statistical comparisons of cancer incidence in the control and isoprene-exposed groups are based on one-tailed Fisher's exact tests; * *p*-value < 0.05, ** *p*-value < 0.01.

(b) The exact trend test was conducted by OEHHA.

(c) A single kidney renal tubule carcinoma was found during single sectioning in a 700-ppm (1953-mg/m^3) male rat that also had an adenoma. No further carcinomas were found following step sectioning.

NTP noted that the incidences of mammary gland neoplasms in all exposed groups of female rats were greater than those in the chamber control group and nearly equal at each of the three concentrations studied. This dose response resulted in a non-significant trend (p = 0.16). The supralinear appearance of the tumor incidence data suggested to NTP that lower doses than those used in the study would better characterize the dose response for mammary gland tumors in female rats. Therefore, NTP determined there was "some evidence of carcinogenic activity" of isoprene in female rats due to the increased incidence and multiplicity of mammary gland fibroadenomas.

Several rare brain tumors that have seldom or never occurred in female historical control rats were observed in isoprene-exposed female rats from the NTP (1999) study. These tumors included a benign astrocytoma in a 700-ppm (1953-mg/m^3) rat, a malignant glioma in a 7000-ppm (19,530-mg/m^3) rat, a malignant medulloblastoma in a different 7000-ppm rat, a benign granular cell tumor of the meninges in one 220-ppm (614-mg/m^3) and one 7000-ppm rat, and a sarcoma of the meninges in one 220-ppm and one 7000-ppm rat. However, the lack of 1) an effect on survival, 2) a consistent decrease in the age at which the tumors appeared, 3) a dose-response relationship, and 4) a predominance of any one tumor type, led NTP to conclude that it was uncertain whether these tumors resulted from isoprene exposure.

Epidemiology

Isoprene is the raw material used in several kinds of rubber. Exposure may occur during the manufacture of synthetic rubber and elastomers (i.e., elastic polymers). However, the processes in producing rubber for tires and other rubber products involve hundreds of chemicals, many of which are known or suspected to be carcinogenic, including N-nitrosamines, polycyclic aromatic hydrocarbons, and some solvents and phthalates. IARC (1982; 2012) concluded that “occupational exposures in the rubber-manufacturing industry are carcinogenic to humans (Group 1)”. The IARC Working Group noted the complexity of these occupational exposures “precluded a clear conclusion about an association between cancer mortality and incidence and exposure to particular chemicals (except historically well-known associations between 2-naphthylamine and bladder cancer and benzene and leukaemia)” (IARC, 2012).

In a meta-analysis that included case control and cohort studies of cancer risk in the rubber manufacturing industry up to 2016, an increased risk for bladder cancer, lung cancer, leukemia and larynx cancer was found (Boniol et al., 2017). In a stratified analysis, elevated risks for bladder cancer, lung cancer or leukemia were no longer apparent for workers first employed after 1960 or after 1970. The authors suggested

this result was due to major reductions in rubber dust and fume exposure since the 1950s, resulting in decreased carcinogen exposure (most notably benzene and 1,3-butadiene).

Occupational exposure data specific for isoprene is limited to only two older Russian studies. IARC (2012) summarized the results from Pigolev (1968) and Faustov (1972) that found isoprene concentrations in polymerization and rubber separation shops in Russia averaged 8 to 40 mg/m^3 (2.9 to 14 ppm).

IARC also summarized the non-cancer findings of another Russian study by Mitin (1969) in which the upper respiratory tract effects in isoprene rubber production workers were investigated. Toxic effects noted in these workers were subtrophic and atrophic processes (i. e., atrophied or smaller/weaker tissue) in the upper respiratory tract, catarrhal inflammation (i.e., upper airway inflammation of mucous membranes), and degeneration of the olfactory tract. Duration of employment of the workers showed a positive correlation with the prevalence and degree of toxic effects. The contribution of isoprene exposure was unclear from this report. OEHHA notes that NTP (1999) did not observe injury to olfactory epithelium or other upper respiratory tract tissues in 2-year exposures of male and female rats to isoprene.

Metabolism

Isoprene metabolism in rodents and humans is like that of 1,3-butadiene (BD), an analog of isoprene and listed for cancer by California's Proposition 65 Program. As outlined in Figure 1, it involves enzymatic activation by the cytochrome P450 (CYP) system to various epoxide intermediates[4], followed by enzyme-catalyzed hydrolysis, glutathione conjugation, and further oxidation of the diols formed via hydrolysis (NTP, 1999; Hurst, 2007; NTP, 2021).

Experimental results upon which the metabolic scheme is based include the following.

- Inhalation exposure of male F344 rats to isoprene concentrations of 8 to 8200 ppm (22 to 22,878 mg/m^3) produced mono-epoxides, diols, the diepoxide, and

[4] The two initial mono-epoxide intermediates of isoprene are referred to by different authors as "2-ethenyl-2-methyl oxirane (1,2-epoxy-2-methyl-3-butene) and 2-(1-methylethenyl)-oxirane (3,4-epoxy-2-methyl-l-butene)."

metabolite conjugates in blood, liver, kidney, lung, and other tissues (Dahl et al., 1987).

- Liver microsomes from rodents and humans converted isoprene to its mono-epoxides and the diepoxide and converted the epoxides into diols and glutathione conjugates (Small, 1997; Bogaards et al., 2001; Golding et al., 2003).

- Liver microsomes from male Sprague-Dawley rats converted the isoprene diepoxide into an epoxy-diol, and liver microsomes from phenobarbital- or pyrazole-treated rats converted isoprene diols into epoxy-diols at a slow rate (Chiappe et al., 2000).

- The main urinary metabolites of isoprene in rats were 2-methyl-3-butene-1,2-diol together with its glucuronide and vinyl lactic acid (2-hydroxy-2-methyl-3-butenoic acid) after intraperitoneal injection (Buckley et al., 1999).

Although not indicated in Figure 1, isoprene's metabolites exist as various stereoisomers[5]. Several investigators have looked at the differential rates of formation and reactivity of these stereoisomers *in vitro* and found evidence for metabolic variability among some of them (Chiappe et al., 2000; Golding et al., 2003). Given the limited understanding of isoprene's carcinogenic mechanism of action, a detailed consideration of metabolite stereoisomerism was not necessary for determining the IUR.

[5] A stereoisomer is “any of a group of isomers in which atoms are linked in the same order but differ in their spatial arrangement” (Merriam-Webster, 2023b).

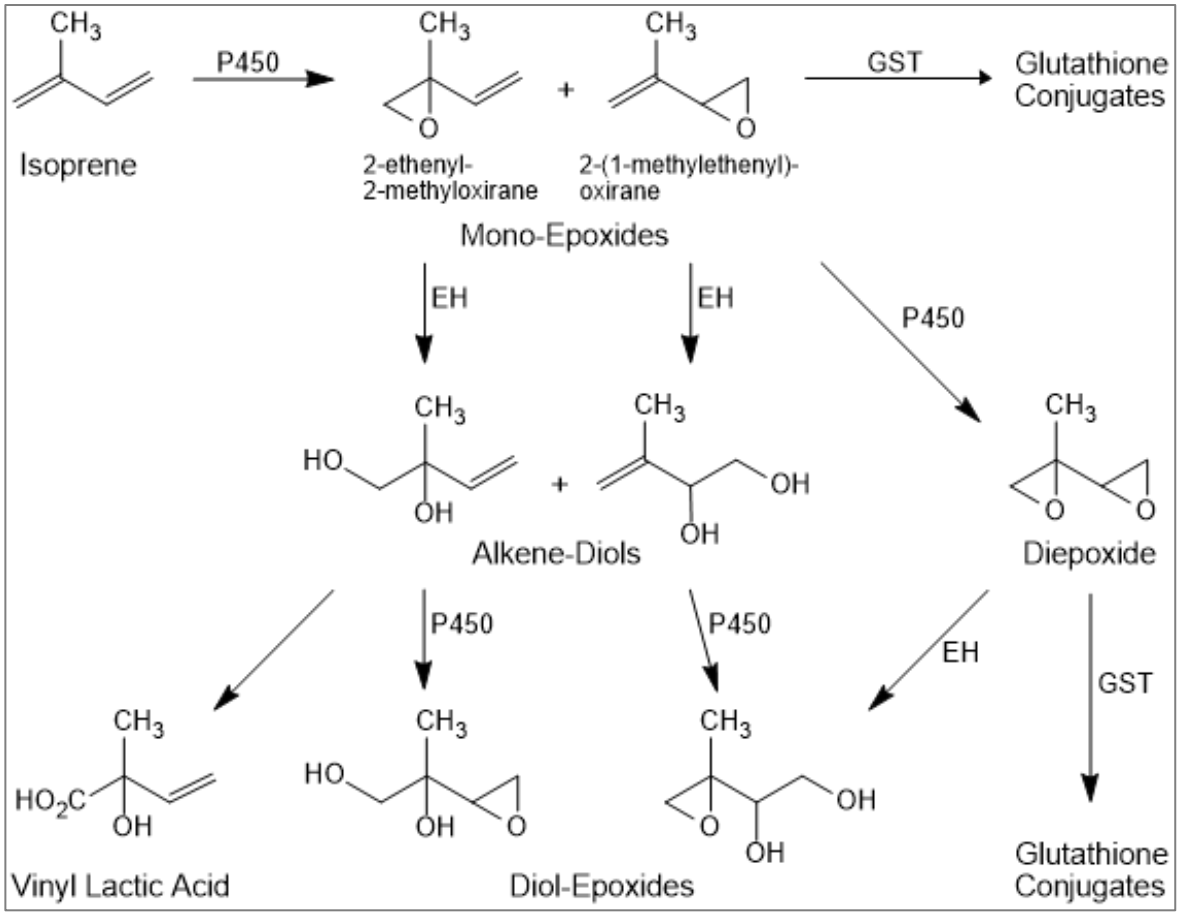


Figure 1. Metabolic Pathways of Isoprene. P450 = Cytochrome P450 enzyme (primarily the CYP2E1 isozyme); GST = Glutathione-S-Transferase enzyme; EH = Epoxide Hydrolase enzyme; Figure adapted from NTP (1999), Chiappe et al. (2000), and Bogaards et al. (2001).

The epoxides of isoprene appear to be produced mainly by the CYP2E1 isoenzyme. Bogaards et al. (1996) used microsomes from complementary deoxyribonucleic acid (cDNA)-transfected human lymphoblastoid cells to test individual CYP isozymes and found that CYP2E1 was able to convert isoprene to its mono-epoxides and diepoxide. In contrast, the other forms were either inactive or—in the case of CYPs 2A6, 2B6, and 2D6—less active, forming smaller quantities of only one epoxide, 2-ethenyl-2-methyloxirane. In human liver microsomes, epoxide formation was significantly correlated only with chlorzoxazone oxidation, with *p*-values of < 0.05 and < 0.01 for correlation coefficients ranging from 0.71 to 0.82. Chlorzoxazone is used as a specific marker of CYP2E1 activity.

CYP2E1 is found mostly in the liver, though small amounts of this isoform are also present in the lungs, kidneys, and small intestines (Pavek & Dvorak, 2008). Studies

that have modeled the pharmacokinetic behavior of inhaled isoprene in animals and humans (e.g., Bogaards et al., 2001; Csanády and Filser, 2001) have assumed that 10% to 13% of CYP450-mediated oxidation occurs outside the liver.

The mono-epoxides and diepoxide of isoprene appear to be deactivated predominantly by hydrolysis via microsomal epoxide hydrolase (mEH). For example, *in vitro* intrinsic clearance values for 2-ethenyl-2-methyloxirane in human liver microsomes were 3582 per hour $(hour)^{-1}$ for mEH hydrolysis but only 25 $(hour)^{-1}$ and 0.11 $(hour)^{-1}$ for cytosolic epoxide hydrolase (cEH)-mediated hydrolysis and glutathione-S-transferase (GST)-mediated conjugation, respectively (Bogaards et al., 2001). Also, the diepoxide was a substrate only of mEH (ibid). Not much information is available on the metabolic deactivation of isoprene's diol-epoxides, but rat-liver mEH was found incapable of hydrolyzing them (Chiappe et al., 2000).

Toxicokinetic studies of isoprene-exposed mice and rats have indicated that metabolic saturation of the oxidative pathway occurs at the higher isoprene exposure concentrations tested in the available rodent carcinogenicity studies. For example, Peter et al. (1990) found that the initial enzymatic oxidation of isoprene follows Michaelis-Menten kinetics with a first-order[6] isoprene-to-epoxide turnover rate up to an exposure concentration of about 300 ppm (837 mg/m^3) and saturation occurring at about 1000 ppm (2790 mg/m^3) in rats and 2000 ppm (5580 mg/m^3) in mice. The studies chosen by OEHHA for the dose-response assessment included several concentrations above 300 ppm (837 mg/m^3).

Overall, the risk-relevant part of isoprene metabolism in humans consists mainly of the activation-deactivation sequence mediated by CYP2E1 and mEH. Isoprene is oxidized by CYP2E1 to its mono-epoxides and diepoxide, and these metabolites are hydrolyzed by mEH to alkene-diols and diol-epoxides. To a lesser extent, epoxidation may be accomplished by other CYP isoforms, such as CYP2D6, and the epoxides may be deactivated by GST-mediated conjugation or cEH-mediated hydrolysis. The diol-epoxides appear to be formed primarily through hydrolysis of the diepoxide, as opposed to CYP450 epoxidation of the alkene-diols.

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[6] Michaelis-Menten kinetics can be defined as “the behavior of an enzyme-catalyzed reaction with a single substrate especially as exhibited by plotting the velocity of the reaction against the concentration of the substrate which yields a hyperbolic curve approaching a horizontal asymptote rather than yielding a straight line as in nonenzymatic reactions” (Merriam-Webster, 2023a). A “first order” rate of a reaction is one that increases in direct proportion to the concentration of enzyme substrate.

Certain subpopulations may be more susceptible to the carcinogenic action of isoprene if enhanced bioactivation due to induction of CYP2E1 occurs. For example, ethanol is the most well-known inducer of CYP2E1 (Hakkola et al., 2020). Tobacco smoking can also induce CYP2E1, probably through exposure to compounds that are known inducers, (e.g., benzene derivatives). In addition, medications such as isoniazid and oral all-*trans* retinoic acid have been shown to induce CYP2E1 (ibid).

Genotoxicity

Studies on the genotoxicity of isoprene have been reviewed by IARC, NTP, and ECHA. These studies were conducted in various *in vitro* and *in vivo* systems, with and without metabolic activation (Table 4).

IARC (1999) noted that there were no data on the genetic and related effects of isoprene on humans. However, in mice exposed via inhalation, "isoprene could induce sister chromatid exchanges and micronuclei in bone-marrow cells."

According to IARC (1994),

> "Neither isoprene nor its primary metabolites, 3,4-epoxy-2-methyl-l-butene and 1,2-epoxy-2-methyl-3-butene, were mutagenic to bacteria. [However,] 2-Methyl-1,2,3,4-diepoxybutane, a metabolite of 3,4-epoxy-2-methyl-1-butene, was mutagenic to *Salmonella typhimurium*" (Table 4).

NTP (1999) reported similarly mixed results, mostly non-mutagenic findings *in vitro* and some signs of genotoxicity *in vivo.* In summarizing the evidence for genotoxicity, NTP stated:

> "Isoprene was not mutagenic in *S. typhimurium* and did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells with or without exogenous metabolic activation; however, in mice, isoprene induced increases in the frequency of sister chromatid exchanges in bone marrow cells and in the frequency of micronucleated erythrocytes in peripheral blood. The cell cycle duration of proliferating bone marrow cells of mice exposed to 7,000 ppm [19,530 mg/m^3] isoprene was significantly lengthened. No increases in the frequency of chromosomal aberrations were observed in bone marrow cells of male mice after 12 days of exposure to isoprene, and lung fibroblasts of male and female rats exposed to isoprene for 4 weeks showed no increase in the frequency of micronuclei."

ECHA (2023) lists isoprene as a Class 2 mutagen. Criteria for Class 2 mutagens include mutations in somatic cells *in vivo* and genotoxicity in somatic cells *in vivo* in

combination with mutagenicity *in vitro*. Structural similarity with a known germ-cell mutagen in combination with mutagenicity *in vitro* can also trigger this classification (ECHA, 2018).

Table 4. Genetic and related effects of isoprene and selected metabolites[a].

Biological endpoint	Cell type or species/strain	Chemical	Description	Exogenous metabolic activation		Reference
				without	with	
Bacterial reverse mutation tests	*Escherichia coli*	Isoprene	WP2 uvr A pKM 101	-	-	ECHA (2023)
	Salmonella enterica serovar Typhimurium	Isoprene	TA98	-	-	de Meester et al. (1981)
			TA100	-	-	
			TA1530	-	-	
			TA1535	-	-	
			TA1538	-	-	
		Isoprene	TA102	-	NT	Kushi et al. (1985 abstract)
			TA104	-	NT	
		Isoprene	TA98	-	-	Mortelmans et al. (1986)
			TA100	-	-	
			TA1535	-	-	
			TA1537	-	-	
		Isoprene	TA98	-	-	ECHA (2023)
			TA100	-	-	
			TA1535	-	-	
			TA1537	-	-	

Abbreviations: minus sign (-) – negative; NT – not tested; plus sign (+) – positive.

[(a)] Data from IARC (1999, Table 2) and NTP (1999, Tables C2 to C7).

Table 4. Genetic and related effects of isoprene and selected metabolites (continued)[a].

Biological endpoint	Cell type or species/strain	Chemical	Description	Exogenous metabolic activation		Reference
				without	with	
Bacterial reverse mutation tests (continued)	*Salmonella enterica* serovar Typhimurium	1,2 Epoxy-2-methylbutene	TA98	-	NT	Gervasi et al. (1985)
			TA100	-	NT	
		3,4-Epoxy-2-methyl-1-butene	TA98	-	NT	Gervasi et al. (1985)
			TA100	-	NT	
		2-Methyl-1,2,3,4-diepoxybutane	TA98	+	NT	Gervasi et al. (1985)
			TA100	+	NT	
Chromosomal damage	Chinese hamster ovary cells	Isoprene	Sister chromatid exchanges	-	-	Galloway et al. (1987)
			Chromosomal aberrations	-	-	
	Mouse peripheral red blood cells (*in vivo*)	Isoprene	Micronuclei after 12-day (6 hours/day) inhalation exposure	+	NT	Tice et al. (1988)
	Mouse bone marrow cells (*in vivo*)	Isoprene	Sister chromatid exchanges after 12-day (6 hours/day) inhalation exposure	+	NT	Tice et al. (1988)

Abbreviations: minus sign (-) – negative; NT – not tested; plus sign (+) – positive.

[(a)] Data from IARC (1999, Table 2) and NTP (1999, Tables C2 to C7).

Table 4. Genetic and related effects of isoprene and selected metabolites (continued)[a].

Biological endpoint	Cell type or species/strain	Chemical	Description	Exogenous metabolic activation		References
				without	with	
Chromosomal damage (continued)	Mouse bone marrow cells (*in vivo*)	Isoprene	Chromosomal aberrations after 12-day (6 hours/day) inhalation exposure	-	NT	Tice et al. (1988)
	Mouse peripheral red blood cells (*in vivo*)	Isoprene	Micronuclei after 13-week inhalation exposure	+	NT	Jauhar et al. (1988)
	Rat lung fibroblasts (*in vivo*)	Isoprene	Micronuclei after 4-week inhalation exposure	-	NT	Khan and Heddle (1991, 1992)
	Mouse peripheral red blood cells (*in vivo*)	Isoprene	Micronuclei after 40- and 80-week inhalation exposures	+	NT	ECHA (2023); Placke et al. (1996)
Covalent binding to hemoglobin	Mouse red blood cells (*in vivo*)	Isoprene	Binding after single intraperitoneal injection exposure	+	NT	Sun et al., (1989)
	Rat red blood cells (*in vivo*)	Isoprene	Binding after single intraperitoneal injection exposure	+	NT	
	Mouse red blood cells (*in vivo*)	Isoprene	Binding after 6-hour inhalation exposure	+	NT	Bond et al. (1991)

Abbreviations: minus sign (-) – negative; NT – not tested; plus sign (+) – positive.

[(a)] Data from IARC (1999, Table 2) and NTP (1999, Tables C2 to C7).

In addition to the *in vitro* findings reported by ECHA, IARC, and NTP (Table 4), both isoprene and its mono-epoxide, 2-ethenyl-2-methyloxirane, were shown by Fabiani et al. (2007, 2012) to cause DNA damage in the comet assay using human peripheral-blood mononuclear cells and human leukemia cells with microsomal activation. In a 2014 study using the comet assay with human cell types [normal hepatocytes (L02), hepatocellular carcinoma (HepG2), and leukemia cells (HL60)], Li et al. (2014) found evidence of statistically significant DNA damage in all metabolite-exposed cell lines compared to controls. The most genotoxic metabolite was 2-(1-methylethenyl) oxirane, followed by 2-methyl-2,2'-bioxirane and 2-ethenyl-2-methyloxirane. Isoprene's mono-epoxides [i.e., 2-(1-methylethenyl) oxirane and 2-ethenyl-2-methyloxirane] also showed potential genotoxicity by forming deoxyadenosine adducts *in vitro* (Begemann et al., 2011).

In vivo, Fred et al. (2005) showed intraperitoneal injection of male C57/Black mice with isoprene epoxide (1,2-epoxy-2-methyl-3-butene) increased micronuclei and hemoglobin adduct formation compared to their untreated counterparts.

Mutagenicity tests have not been carried out on the diol-epoxides of isoprene. However, in the case of structurally similar BD, studies in rodents indicate that one or more of BD's diol-epoxides may contribute significantly to BD's genotoxicity. For example, relatively high diol-epoxide concentrations were found in the blood of mice and rats exposed to BD via inhalation (Filser et al., 2007), and DNA adducts of BD diol-epoxides were found in rodent liver, kidney, and lung tissues. Moreover, DNA adducts of BD diol-epoxides accounted for 98 percent of the total alkylated DNA adducts in the lung tissue of mice exposed by inhalation (Koc et al., 1999; Koivisto et al., 1999; Koivisto and Peltonen, 2001; Boogaard et al., 2004). Also, an *in vitro* mutagenicity study found that a particular BD diol-epoxide stereoisomer (2R, 3S) was moderately mutagenic, being 10- to 20-fold more potent than the BD mono-epoxides but 5- to 10-fold less mutagenic than the diepoxide (Meng et al., 2010).

These results provide indirect evidence for the possible importance of diol-epoxides in isoprene's mutagenic mode of action (MOA). As noted above, *in vitro* metabolic studies of isoprene showed that several pathways could yield the diol-epoxides, and the primary deactivation pathway (i.e., mEH-mediated hydrolysis) for isoprene's other epoxides may not be operable in this case.

V. CANCER HAZARD EVALUATION

Evaluations of the carcinogenicity of isoprene undertaken by national and international agencies point towards a similar conclusion, evidence base, and mechanism of carcinogenicity.

- IARC (1999) concluded that isoprene is "possibly carcinogenic to humans" based on inadequate evidence in humans and sufficient evidence in animals. Their conclusion was supported by genotoxic and multiple-organ neoplastic effects in mice.

- Isoprene has been listed in NTP's Report on Carcinogens since 2000 and is "reasonably anticipated to be a human carcinogen" (NTP, 2021). This listing is based upon "clear evidence of carcinogenic activity"[7] in female mice, male mice, and male rats; "some evidence of carcinogenicity"[8] in female rats; and chromosomal effects in mice exposed to isoprene via inhalation.

- ECHA (2023) noted isoprene is "presumed to be carcinogenic to humans" and "suspected to be mutagenic." Isoprene is also recognized in the European Union as carcinogenic.

Isoprene has been listed as a chemical known to cause cancer in California's Proposition 65 Program since 1996 (OEHHA, 1996). The present assessment aligns with the above conclusions of IARC, NTP, and ECHA regarding the carcinogenicity of isoprene.

Isoprene is produced endogenously in humans, with end-tidal breath concentrations of 70 to 133 ppb (195 to 371 μg/m^3) in adults and a lower mean concentration of 28 ppb (78 μg/m^3) in 7–10-year-old children (Mochalski et al., 2023; Smith et al., 2010). Exposure to isoprene in the environment adds to these endogenous levels. Isoprene concentrations in the ambient air range from 0.2 to 4.2 ppb (0.6 to 12 μg/m^3) in the United States (NTP, 2021). Emissions from facilities that use isoprene could result in concentrations higher than these ambient levels, thereby impacting nearby residential and commercial areas. Antiquated occupational exposure data suggested average isoprene concentrations could reach as high as 2900 to 14,000 ppb (8000 to 40,000

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[7] NTP uses five evidential categories of carcinogenic activity to summarize the strength of the evidence observed in their carcinogenesis studies. According to NTP (1999), clear evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from their or other studies of the ability of such tumors to progress to malignancy.

[8] Some evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence (NTP, 1999).

μg/m^3) in polymerization and rubber separation facilities (IARC, 1994). Current occupational exposure information for isoprene is unknown but will be less due to improvements in occupational hygiene since the 1950s. Considering the potential biogenic, anthropogenic, and endogenous exposures to isoprene and its structural similarity to 1,3-butadiene, a known human carcinogen, the derivation of an IUR for isoprene was conducted.

VI. QUANTITATIVE CANCER RISK ASSESSMENT

Since the dose-response information for isoprene was available in studies conducted with mice and rats, OEHHA estimated the cancer potency[9] of isoprene in humans using a workflow that consisted of the following tasks:

1. designating the primary dose-response data set (or sets) to be used in the evaluation; identifying tumor types to be included based on increased rates of tumor formation in isoprene-exposed animals
2. choosing the appropriate dose-response model for the quantitative assessment
3. defining the dose metric to be used in the dose-response model and estimating the lifetime average daily doses (LADDs) of this dose metric
4. adjusting the dose-response data obtained from the primary study to account for intercurrent mortality[10] (for toxicity studies using animals)
5. using the United States Environmental Protection Agency's (US EPA's) Benchmark Dose Software (BMDS) with the adjusted dose-response data to obtain a benchmark dose level [BMDL; the 95th percentile lower confidence level for the Benchmark Dose (BMD)], carrying out a multitumor risk analysis where appropriate

[9] OEHHA's cancer potency estimates are presented as Cancer Slope Factors in units of risk per milligram of chemical per kilogram body weight per day (mg/kg-d)$^{-1}$ and as Inhalation Unit Risk Factors in units of risk per microgram per cubic meter (μg/m^3)$^{-1}$ for external exposure (i.e., exposures above background).

[10] Intercurrent mortality in animal carcinogenicity studies refers to deaths that occur before the end of the study in animals that did not develop the tumor of interest. Specific information on individual animal survival and tumor data may allow for adjustments to the denominator when expressing tumor incidence, to reflect the number of animals that lived long enough to be at risk of developing tumors (i.e., animals that were alive at first occurrence of the tumor of interest).

6. converting the BMDL into the incremental cancer risk in animals per unit of exposure (i.e., cancer slope factor in animals, or CSF_a)
7. applying allometric scaling factors to extrapolate from the CSF_a to a cancer slope factor in humans (CSF_h)
8. converting the CSF_h [in units of $(mg/kg\text{-}d)^{-1}$] into the IUR [in units of $(\mu g/m^3)^{-1}$] that describes the excess cancer risk associated with lifetime inhalation exposure to an isoprene concentration of 1 $\mu g/m^3$

These risk assessment tasks are discussed in more detail in the following sub-sections.

Primary Data Sets for Analysis

The Placke et al. (1996) and NTP (1999) rodent studies were chosen for the dose-response analysis. In these studies, significantly increased tumors were found at multiple sites in male and female mice and in male rats. Increased tumor incidence was observed in one site in female rats. The NTP (1995) stop-exposure study in rats and mice was not used to estimate the IUR due to its short exposure period (6 months) and less-than-lifetime observation period of one year.

Dose-Response Model

Based upon the toxicological information presented in the preceding sections, OEHHA determined that isoprene's likely mode of carcinogenic action is via genotoxicity. For carcinogenic substances that appear to act via genotoxicity and/or mutagenicity, OEHHA's 2009 cancer risk assessment guidelines recommend using the multistage cancer model, as implemented in US EPA's BMDS. Thus, OEHHA used the multistage cancer model and adopted the linear low-dose hypothesis[11].

Dose Metric for Quantitative Analysis

OEHHA chose to use the applied dose based on the inhaled isoprene concentration as the metric for dose-response modeling. Two other dose metrics— (1) the internal blood or tissue concentration of one or more of isoprene's epoxides (or the diepoxide), and (2) the rate of the first oxidative step of isoprene's metabolism ("the metabolized dose")—

[11] The linear low-dose hypothesis asserts that the incremental risk of exposure to a carcinogen increases in direct (linear) proportion to the long-term average daily dose of the substance. Thus, any amount of exposure greater than zero produces some amount of extra cancer risk.

were also considered. However, these alternatives were not used because of insufficient toxicokinetic information, including gaps in the available physiologically-based pharmacokinetic or toxicokinetic (PBPK) models. The following section briefly describes three PBPK models for isoprene that OEHHA identified in the literature. Reasons for not using the models to define dose metrics for the risk assessment are also provided.

Toxicokinetic Models

Three publicly available PBPK models for isoprene were identified by OEHHA: NTP (1999), Bogaards et al. (2001), and Csanády and Filser (2001). Each model was evaluated to determine whether it was complete, with methods and results of sufficient quality for use in a dose-response analysis. The adequacy of the models was based upon criteria relating to model applicability, biological relevance (e.g., correct mathematics for the biological mechanisms being modeled), and performance/reliability.

The NTP (1999) model was developed for inhalation exposure and intraperitoneal injection in rats. It included compartments for the lungs, liver, kidneys, gastrointestinal tract, fat, slowly-perfused tissues, venous and arterial blood, peritoneal space, viscera, and urine. The model was designed to simulate concentrations of isoprene and its mono-epoxides in these tissues and to predict concentrations of vinyl lactic acid, isoprene diols, and other metabolic products in urine. CYP450-mediated oxidative metabolism of isoprene to its mono-epoxides was assumed to occur in the liver, kidneys, and lungs, with metabolic activity at 88%, 7%, and 5%, respectively. Oxidation of the mono-epoxides to the diepoxide was assumed to occur only in the liver. Enzymatic hydrolysis and glutathione conjugation of isoprene mono-epoxides were assumed to occur in the liver and lungs. Despite the model's relevance to developing internal dose metrics in rats, its lack of components for humans and mice precluded its use for the dose-response analysis.

The Bogaards et al. (2001) model was formulated for inhalation exposure in rats, mice, and humans. It included formation, hydrolysis, and conjugation of the mono-epoxides and isoprene diepoxide, assuming oxidative metabolism in the liver and lungs (approximately 87% metabolism in the liver and 13% in the lungs). The model was capable of estimating concentrations of isoprene in lungs, liver, fat, kidneys, and rapidly- and slowly-perfused tissue compartments. For the mono-epoxides and isoprene diepoxide, the lungs and liver were modeled separately, and the rest of the body was lumped into one compartment. This model was more complete than the NTP (1999) model and defined internal dose metrics, allowing simulation of exposures in rats, mice, and humans and estimation of the mutagenic isoprene diepoxide tissue concentrations. However, the authors noted that the model was preliminary and designed mainly "to

explain differences in isoprene toxicity between mouse and rat based on *in vitro* metabolism data." Model validation was restricted to isoprene concentrations in the mouse. Due to the lack of relevant published data in humans and rodents, no additional validation was attempted to gauge the model's accuracy in predicting any epoxide or diepoxide metabolites. As such, the model was judged by OEHHA to be of questionable reliability for use in the dose-response evaluation.

The Csanády and Filser (2001) model simulated CYP450-mediated isoprene clearance in rats, mice, and humans, including five tissue compartments (lung, liver, richly-perfused tissue, fat, and muscle). Isoprene metabolism was assumed in the model to occur in the liver (90%) and richly-perfused tissue (10%). Although this model is relatively simple and adequately reproduced limited measured data on isoprene in rats, mice, and humans, it lacks components for simulating isoprene epoxide concentrations in blood or other organs. Further, OEHHA could not replicate the results of the published model simulations in rats, mice, and humans based on information on model structure, model equations, and parameter values retrieved from the peer-reviewed literature.

None of the available PBPK models were considered by OEHHA to be fully adequate for simulating the alternative dose metrics relevant to risk assessment. Moreover, the appropriate dose metric for cancer risk assessment has not been definitively identified for isoprene [i.e., parent compound, metabolites (primary, secondary, or tertiary), or a combination thereof]. Thus, OEHHA used the applied dose (based on the inhaled concentration of isoprene) as the metric for estimating the cancer potency of inhaled isoprene.

Dose Calculations for Mice and Rats

For mice in the Placke et al. (1996) studies, the isoprene chamber concentrations of 0, 10, 70, 280, 700, and 2200 ppm were time-adjusted and converted to mg/m^3 (8.12 hours ÷ 24 hours × 5 days ÷ 7 days × weeks on study ÷ 104 weeks (or time to necropsy) × 2.79 mg/m^3 ÷ 1 ppm). Time adjustment is carried out to convert the intermittent chamber exposure conditions to continuous exposure over the life span of the animals (i.e., to simulate an annualized average air concentration). There were 96 weeks on study (time to necropsy) for the 280-, 700-, and 2200-ppm male mice and 104 weeks for the other groups, with 80 weeks of isoprene exposure (weeks on study) for all groups. The time-adjusted concentrations based on time to necropsy were 0, 5.19, 36.31, 157.33, 393.31, and 1236.13 mg/m^3, respectively.

For rats in the NTP (1999) studies, the isoprene chamber concentrations (0, 220, 700, and 7000 ppm) were also time-adjusted and converted to mg/m^3 (6.2 hours ÷ 24 hours

× 5 days ÷ 7 days × 104 weeks on study ÷ 104 weeks × 2.79 mg/m^3 ÷ 1 ppm). The time-adjusted concentrations were 0, 113.26, 360.38, and 3603.75 mg/m^3, respectively.

The lifetime average daily dose, in mg/kg-d, is used for calculating the cancer potencies (Tables 5a and 5b). The time-weighted average body weight throughout the study is used to determine the inhalation rate (IR) to calculate the daily dose. Body weight data were not provided for mice in the Placke et al. (1996) studies. Thus, standard body weight values of 0.03 kg and 0.025 kg were used in the present assessment for male and female B6C3F$_1$ mice, respectively (Gold and Zeiger, 1997). In the NTP rat studies, the weighted average lifetime body weights for the control group in both sexes were calculated based on the regular reporting of group mean body weights during the two-year exposure (NTP, 1999). The time-weighted average body weights were 0.446 and 0.274 kg for the control male and female rats, respectively.

The formulas to calculate the IR based on rodent body weight reflect proportional differences of body weight (BW$^{2/3}$) on the respiratory rate within a species. The IR for mice was determined using Equation 6.1a by Anderson et al. (1983).

$$\text{Mice: IR (m}^3\text{/day)} = 0.0345\ \text{m}^3\text{/day} \times (\text{BW} \div 0.025\ \text{kg})^{2/3} \quad \text{Equation 6.1a}$$

Where: IR = Inhalation rate (m^3/day)
BW = Time-weighted average body weight (kg)

The IR was determined for rats using Equation 6.1b by OEHHA (2018).

$$\text{Rats: IR (m}^3\text{/day)} = 0.702\ \text{m}^3\text{/day-kg} \times (\text{BW})^{2/3} \quad \text{Equation 6.1b}$$

The calculated daily IRs for mice were 0.039 and 0.0345 m^3/day for males and females, respectively. The calculated daily IRs for rats were 0.410 and 0.296 for males and females, respectively. The lifetime average daily doses for male and female mice and rats (shown in Tables 5a and 5b) were calculated using the following equation.

$$\text{Dose (mg/kg BW-day)} = \text{IR} \times \text{C} \div \text{BW}$$

Where C = time-adjusted isoprene concentration (mg/m^3).

Table 5a. Calculated average daily dose of isoprene in male and female mice (Placke et al., 1996).

Parameter	Sex	Isoprene Chamber Concentration					
		0 ppm, 0 mg/m^3	10 ppm, 28 mg/m^3	70 ppm, 195 mg/m^3	280 ppm, 781 mg/m^3	700 ppm, 1953 mg/m^3	2200 ppm, 6138 mg/m^3
Average daily dose (mg/kg-d)	Males	0	6.74	47.20	204.52	511.31	1606.96
	Females	0	7.16	50.10	ND	ND	ND

Abbreviations: mg/kg-d – milligrams per kilogram of body weight per day; mg/m^3 – milligrams per cubic meter; ppm – parts per million; ND – no data (no exposure group at this concentration).

Table 5b. Calculated average daily dose of isoprene in male and female rats (NTP, 1999).

Parameter	Sex	Isoprene Chamber Concentration			
		0 ppm, 0 mg/m^3	220 ppm, 614 mg/m^3	700 ppm, 1953 mg/m^3	7000 ppm, 19,530 mg/m^3
Average daily dose (mg/kg-d)	Males	0	104.12	331.29	3312.86
	Females	0	122.35	389.31	3893.10

Abbreviations: mg/kg-d – milligrams per kilogram of body weight per day; mg/m^3 – milligrams per cubic meter; ppm – parts per million.

Effective Tumor Incidences

When available, individual animal survival data in carcinogenicity studies are used to determine the effective tumor incidence. The effective tumor incidence is the number of tumor-bearing animals (numerator) over the number of animals alive at the time of the first occurrence of the tumor (denominator). This method of tallying tumor incidence

removes animals from the assessment that died before they are considered at risk for tumor development. Animals with missing tissue or tissues (e.g., due to autolysis) at the tumor site were also removed from the assessment. Individual survival data were not presented for mice in the Placke et al. (1996) studies, so the effective tumor incidence could not be determined. In these circumstances, the overall incidence data in Tables 2a and 2b were used for cancer risk assessment in the mice. The effective tumor incidences in rats (Table 6) were determined from individual rat survival data from the NTP (1999) studies. Statistical analysis of the effective tumor incidence data was performed by OEHHA using the exact conditional Cochran-Armitage test for linear trend (i.e., exact trend test) and the one-tailed Fisher's exact test for pairwise comparisons as recommended for carcinogen risk assessment (US EPA, 2005).

Table 6. Effective tumor incidence in male and female rats exposed to isoprene by inhalation for two years (NTP, 1999)[a,b].

Sex and Species	Tumor Type	Incidence by concentration				Statistical *p*-values for trend test or pairwise comparison with controls			
		0 ppm, 0 mg/m^3	220 ppm, 614 mg/m^3	700 ppm, 1953 mg/m^3	7000 ppm, 19,530 mg/m^3	Trend[c]	220 ppm, 614 mg/m^3	700 ppm, 1953 mg/m^3	7000 ppm, 19,530 mg/m^3
Male Rats	Kidney: Renal Tubule Adenoma or Carcinoma – Single + step sections (combined)[d]	2/38	4/42	8/40	15/44**	0.0004	0.387	0.052	0.001
	Mammary Gland: Fibroadenoma	2/32	4/33	6/34	21/35**	<0.0001	0.351	0.149	<0.001
	Mammary Gland: Carcinoma	0/21	1/15	1/18	2/18	0.1087	0.417	0.461	0.206
	Mammary Gland: Fibroadenoma or Carcinoma	2/32	5/33	7/34	21/35**	<0.0001	0.226	0.089	<0.001
	Testis: Interstitial Cell Adenoma	33/48	37/50	44/50*	48/48**	<0.0001	0.657	0.027	<0.001
Female Rats	Mammary Gland: Fibroadenoma	19/49	35/49**	32/48**	32/48**	0.1273	0.002	0.008	0.008

(a) Incidence ratio after adjusting for intercurrent mortality using the effective number adjustment method (i.e., number alive on the day of the first tumor). Effective tumor incidences were determined from data provided by NTP (1999) in Table A2.

(b) * = $p < 0.05$, ** = $p < 0.01$; *p*-value indicators are from pairwise comparisons with controls using one-tailed Fisher's exact tests performed by OEHHA.

(c) *p*-values in the trend column are for the exact trend test performed by OEHHA

(d) A single kidney renal tubule carcinoma was found during single sectioning in a 700-ppm (1953-mg/m^3) male rat that also had an adenoma. No further carcinomas were found following step sectioning.

Benchmark Dose Calculations

The US EPA's BMD methodology and BMDS (version 3.3) were used to perform the multistage cancer model calculations (US EPA, 2022a). In the multistage model, cancer potency is estimated based on the following expression relating the lifetime probability of a tumor at a specific site (*p*) to dose (d):

$$p(d) = \beta_0 + (1 - \beta_0)\,(1 - \exp\,[-\,(\beta_1 d + \beta_2 d^2 + \ldots + \beta_j d^j)])$$

In the above equation, “d” represents the average daily dose resulting from a uniform, continuous exposure over the nominal lifetime of the animal (two years for both rats and mice). When using a study in which the exposures vary in time, the exposures are averaged over the study period and modeled as uniform and continuous. The coefficients (β_0, β_1, etc.) are parameters estimated by fitting the data using maximum likelihood methods.

BMD analyses were run for the mouse and rat tumor data that were identified as treatment-related and showed a statistically significant increase above control values and/or a statistically significant positive trend. Tumors of the same histological cell type or tissue type were combined for dose-response assessment (McConnell et al., 1986; Brix et al., 2010).

For large datasets such as those by NTP, a Benchmark Response (BMR) of 5% is recommended by OEHHA (2008) for the BMD and the 95% lower confidence bound (i.e., BMDL). First-, 2nd-, and 3rd-degree multistage models were run for all suitable tumor data sets, and the most appropriate model fit was chosen based on BMD technical guidance (US EPA, 2022).

Since isoprene induced significant increases in tumors at multiple sites in male mice, male rats, and female mice, the combined cancer potency was estimated using the multisite tumor module provided in BMDS. The BMDS procedure for summing risks over several tumor sites is based on the profile likelihood method. In this method, the maximum likelihood estimates (MLEs) for the multistage model parameters (β_i) for each tumor type are added together (i.e., $\sum\beta_0$, $\sum\beta_1$, $\sum\beta_2$, etc.), and the resulting model is used to determine a combined BMD. Then, a confidence interval for the combined BMD is calculated by computing the desired percentile of the chi-squared distribution associated with a likelihood ratio test having one degree of freedom.

Benchmark Dose Results

The BMDS results, including the BMD and BMDL values and adequacy measures related to the model fit, are presented in Tables 7 and 8. CSFs for mice and rats in

units of $(mg/kg\text{-}d)^{-1}$ were calculated as 0.05 ÷ BMDL, where 0.05 represents the 5% tumor response. Equivalent human CSFs (i.e., CSF_h values) were calculated from animal CSFs (CSF_a values) by multiplying the CSF_a by the ratio of human-to-animal body weights ($BW_h \div BW_a$) raised to the one-fourth power when animal potency is expressed in units of $(mg/kg\text{-}d)^{-1}$:

$$CSF_h = CSF_a \times (BW_h \div BW_a)^{1/4}$$

The body weights for mice and rats applied in the equation were the same values described above for the average daily dose calculation. The default body weight for humans is 70 kg (OEHHA, 2009). This interspecies scaling approach is used to account for differences between test animals and humans in pharmacokinetics (e.g., breathing rate, metabolism), and pharmacodynamics (e.g., tissue responses to chemical exposure) (U.S. EPA, 2005).

BMD modeling results of mouse data from Placke et al. (1996) are presented in Table 7. Combined adenoma/carcinoma data in individual mice were not reported. Thus, OEHHA chose to model the data for adenomas since, for each of the sites modeled (liver, lung, and Harderian gland), the increase of adenomas was larger than that of carcinomas. BMD modeling of the male mouse alveolar/bronchiolar lung adenoma data did not provide a model with adequate goodness of fit (p = 0.02).

Following US EPA (2012) Benchmark Dose Modeling Guidance, the highest dose group was removed, and modeling was repeated, with no success. Repetition of this exercise by sequentially removing two additional dose groups did not yield a model with acceptable goodness of fit. Overall, the male mouse lung adenoma data from Placke et al. (1996) were not amenable to BMD modeling and CSF derivation, likely due to a single treatment group (70-ppm; 195-mg/m^3) with significantly lower incidence than both the controls and the 10-ppm (27.9-mg/m^3) dose group (Table 2a). Subsequently, for the purpose of multisite analysis, an adequate model fit was obtained by omitting the 70-ppm (195-mg/m^3) dose group while modeling the male mouse lung adenoma dataset (p = 0.41; Table 7). However, as shown in Table 7, including the 70-ppm dose group resulted in a similar CSF_h value (shown in brackets).

While the incidence of forestomach carcinomas in male mice was statistically significant by trend, the number of tumors observed at that site was relatively low compared to the other treatment-related tumor sites (Table 2a). Since the contribution to the overall potency would have been trivial, the male mouse forestomach carcinoma data were not included in the multisite CSF calculation.

Table 7. BMDS modeling results for 80-week isoprene inhalation exposure study in male and female mice (Placke et al., 1996).

Mouse Sex	Tumor Site	BMD (mg/kg-d)	BMDL (mg/kg-d)	Goodness-of-Fit *p*-value	Animal CSF $(mg/kg\text{-}d)^{-1}$	Human CSF $(mg/kg\text{-}d)^{-1}$
Male	Liver	103.8414	70.7637	0.06	7.07×10^{-4}	4.91×10^{-3}
	Lung[a]	126.1022 [110.0349]	84.9722 [78.0350]	0.41 [0.02]	5.88×10^{-4} [6.41×10^{-4}]	4.09×10^{-3} [4.46×10^{-3}]
	Harderian gland	58.2709	45.3000	0.14	1.10×10^{-3}	7.65×10^{-3}
	Multisite[b]	28.8007 [27.8712]	23.6918 [23.0883]	NA	2.11×10^{-3} [2.17×10^{-3}]	1.47×10^{-2} [1.51×10^{-2}]
Female	Harderian gland	18.8411	9.6078	0.96	5.20×10^{-3}	3.78×10^{-2}
	Pituitary	14.6151	7.5741	0.08	6.60×10^{-3}	4.80×10^{-2}
	Multisite	8.2306	4.9923	NA	1.00×10^{-2}	7.27×10^{-2}

Abbreviations: BMD – Benchmark Dose; BMDL – Benchmark Dose (Lower confidence level); CSF – cancer slope factor; mg/kg-d – milligrams per kilogram of body weight per day; NA – not applicable (value not available for modeling procedure; $(mg/kg\text{-}d)^{-1}$ – per milligram per kilogram of body weight per day.

(a) BMD modeling of the entire data set yielded a goodness-of-fit *p*-value < 0.05 indicating poor model fit [values given in square brackets], likely due to a single treatment group (70-ppm; 195-mg/m^3) with significantly lower incidence than both the controls and the 10-ppm (27.9-mg/m^3) dose group. Subsequently, for the purpose of multisite analysis, an adequate fit to this dataset was obtained by omitting the 70-ppm (195-mg/m^3) dose group. However, it is notable that inclusion of the 70-ppm dose group resulted in a similar CSF_h value.

(b) Multisite analysis includes liver, lung [sans 70-ppm (195-mg/m^3) dose group], and Harderian gland adenomas [see footnote (a)].

The male mouse multisite tumor analysis for the three organs provided a multisite CSF_h of 1.47×10^{-2} $(mg/kg\text{-}d)^{-1}$, while the multisite tumor analysis for female mice provided a CSF_h of 7.27×10^{-2} $(mg/kg\text{-}d)^{-1}$. Because both benign and malignant tumors were significantly increased in the male mouse, whereas only benign tumors were modeled in the female mouse, OEHHA considered the male mouse to provide the more representative estimate of the CSF_h in the Placke et al. studies.

The multisite tumor analysis of male rat data in the NTP (1999) study yielded a CSF_h of 1.88×10^{-2} $(mg/kg\text{-}d)^{-1}$ (Table 8). BMD modeling of the female rat mammary gland fibroadenoma incidence data resulted in a poor goodness-of-fit (p-value = 0.005). The highest dose groups were sequentially dropped until an acceptable goodness-of-fit value was achieved. For mammary gland tumor incidence, the model fit was poor (p = 0.017) with the control and two lowest isoprene dose groups. Therefore, the CSF_a was determined using only the control and low-dose (220-ppm, 614-mg/m^3) groups. This finding is supported by NTP's conclusion that the dose response for this tumor type would be better characterized at concentrations below the lowest isoprene dose that NTP (1999) used. Additionally, the female rat tumors were benign in nature (fibroadenoma), whereas both malignant and benign tumors were observed in male rats. Therefore, OEHHA considered the male rat to provide the more representative estimate of the CSF_h in the NTP (1999) studies.

Table 8. BMDS modeling results for the two-year isoprene inhalation exposure study in male and female rats (NTP, 1999).

Rat Sex	Tumor Site	BMD (mg/kg-d)	BMDL (mg/kg-d)	Goodness-of-Fit *p*-value	Animal CSF $(mg/kg\text{-}d)^{-1}$	Human CSF $(mg/kg\text{-}d)^{-1}$
Male	Kidney	493.9275	294.8393	0.28	1.70×10^{-4}	6.02×10^{-4}
	Mammary gland	200.7235	135.0588	0.60	3.70×10^{-4}	1.31×10^{-3}
	Testes	18.0411	10.1144	0.98	4.94×10^{-3}	1.75×10^{-2}
	Multisite	16.0165	9.4390	NA	5.30×10^{-3}	1.88×10^{-2}
Female	Mammary gland	8.2344	5.1825	NA	9.65×10^{-3}	3.86×10^{-2}

Abbreviations: BMD – Benchmark Dose; BMDL – Benchmark Dose (Lower confidence level); mg/kg-d – milligrams per kilogram of body weight per day; NA – not available (value not available for modeling procedure); NTP – National Toxicology Program; $(mg/kg\text{-}d)^{-1}$ – per milligram per kilogram of body weight per day.

The calculated CSF_h values in Tables 7 and 8 give a range of values across tumor sites and species. The four data sets analyzed are from sensitive studies of sufficient quality.

The CSF_h from the Placke et al. (1996) study in male mice was based on benign tumor incidence data for the treatment-related sites modeled (liver, lung, Harderian gland). Both benign and malignant tumors were significantly elevated but, as discussed previously, the combined adenoma/carcinoma data in individual mice were not reported in the study. The CSF_h based on the NTP (1999) male rat study was derived by modeling tumor incidence data for each of the three treatment-related tumors (renal tubule adenoma and carcinoma combined, mammary gland fibroadenoma and carcinoma combined, testicular interstitial cell adenoma). In contrast to the Placke et al. study, the tumors modeled in the NTP study included both benign and malignant tumors.

Based on the modeled results, the multisite analysis in the NTP (1999) male rats was chosen by OEHHA as the critical data set, with a CSF_h value of $1.9 \times 10^{-2}\ (mg/kg\text{-}d)^{-1}$, rounded to two significant figures in the final assessment. This value is similar to the other robust CSF_h estimate, $1.5 \times 10^{-2}\ (mg/kg\text{-}d)^{-1}$, from the

Placke et al. study in male mice. Graphical presentations of the BMD model results for male rat kidney adenomas or carcinomas combined, mammary gland fibroadenomas or carcinomas combined, and testicular interstitial cell adenomas are shown in Appendix B.

Inhalation Unit Risk Factor

The IUR describes the excess cancer risk associated with inhalation exposure to a concentration of 1 μg/m^3 and is derived from the CSF_h as shown below.

$$IUR = (CSF_h \times BR_h) \div (BW_h \times CF)$$

Where:

BR_h = mean human breathing rate (20 m^3/day)
BW_h = mean human body weight (70 kg)
CF = mg-to-μg conversion factor of 1000

Use of the equation above with the isoprene CSF_h of 1.9 × 10^{-2} (mg/kg-d)$^{-1}$ results in a calculated IUR of 5.4 × 10^{-6} (μg/m^3)$^{-1}$ [1.5 × 10^{-5} (ppb)$^{-1}$]. Thus, the extra cancer risk associated with continuous "adult" lifetime exposure to 1 μg/m^3 isoprene is 5.4 in a million.

The US Environmental Protection Agency does not have an inhalation unit risk value for isoprene. The Texas Commission on Environmental Quality (TCEQ) developed a cancer unit risk factor (URF) for isoprene in 2015 (Haney et al.). TCEQ's URF of 2.2 × 10^{-8} (μg/m^3)$^{-1}$ [6.2 × 10^{-8} (ppb)$^{-1}$] was based on a single tumor type (liver carcinomas) in male mice, as reported by Placke et al. (1996). This URF included a 20-fold adjustment for cross-species differences in pharmacokinetics. As noted above, OEHHA did not consider that there was an adequate basis for choosing dose metrics different from administered concentrations in conducting the risk assessment.

Isoprene is the 2-methyl analog of 1,3-butadiene. The OEHHA Hot Spots IUR for 1,3-butadiene is 1.7 × 10^{-4} (μg/m^3)$^{-1}$, approximately 30 times more potent a carcinogen than isoprene (OEHHA, 2009). This difference aligns with genotoxicity and structure-activity data, in which comparison studies of the two chemicals show that 1,3-butadiene is the more potent carcinogen (Watson et al., 2001; Soeteman-Hernandez et al., 2016; Golding et al., 2022). For comparison purposes, the IUR values for isoprene other air toxics commonly found in urban ambient air of California are summarized below.

Hexavalent chromium	1.5×10^{-1} $(\mu g/m^3)^{-1}$
Arsenic	3.3×10^{-3} $(\mu g/m^3)^{-1}$
Diesel exhaust	3.0×10^{-4} $(\mu g/m^3)^{-1}$
Nickel	2.6×10^{-4} $(\mu g/m^3)^{-1}$
Carbon tetrachloride	4.2×10^{-5} $(\mu g/m^3)^{-1}$
Benzene	2.9×10^{-5} $(\mu g/m^3)^{-1}$
Formaldehyde	6.0×10^{-6} $(\mu g/m^3)^{-1}$
Isoprene	**5.4×10^{-6} $(\mu g/m^3)^{-1}$**
Acetaldehyde	2.7×10^{-6} $(\mu g/m^3)^{-1}$
Ethyl benzene	2.5×10^{-6} $(\mu g/m^3)^{-1}$

The complete list of IURs for Toxic Air Contaminants developed under the Hot Spots Program can be found at https://oehha.ca.gov/media/downloads/crnr/appendixa.pdf.

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APPENDIX A: LITERATURE SEARCH

The literature summarized and referenced in the present document covers the relevant publicly available reports and original peer-reviewed research articles on isoprene through July 2024. Searches were executed in PubMed, Embase, Scopus and SciFinder. Synonyms for isoprene were identified using the United States Environmental Protection Agency's (US EPA's) CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/), and PubMed's MeSH database (https://www.ncbi.nlm.nih.gov/mesh/). The search was run initially in PubMed, then the search terms and syntax were adapted to suit the other databases used. In addition to the formal database searches, the reference lists of included papers and citations in later publications were reviewed and supplemental periodic keyword searches were done in internet search engines, such as Google Scholar.

APPENDIX B: BENCHMARK DOSE MODELING OF TUMORS IN MALE RATS (NTP, 1999)

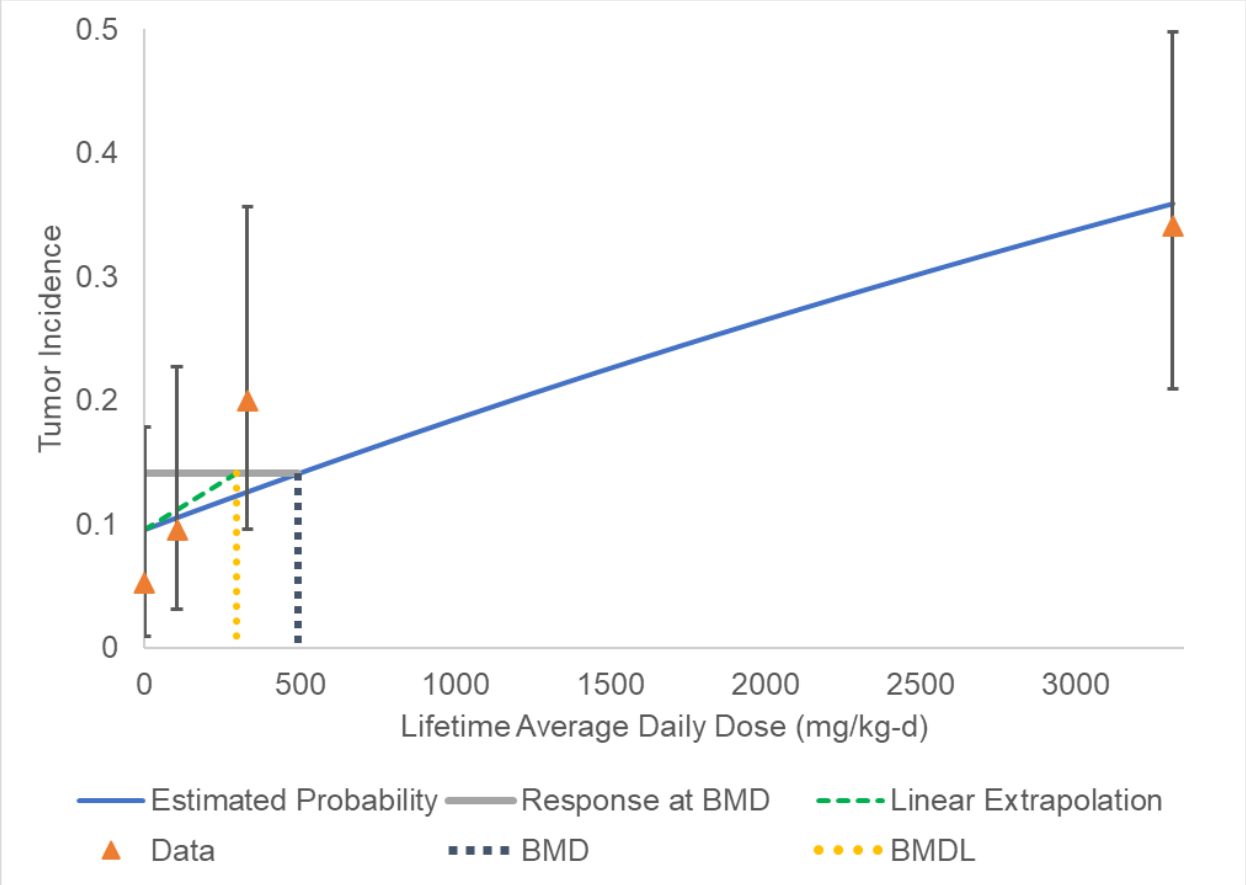

Figure B-1. Benchmark Dose results for renal tubule adenomas or carcinomas in male rats from the NTP (1999) carcinogenicity study. The line graph shows the Frequentist Multistage Degree 1 model with a benchmark response (BMR) of 5% extra risk for the benchmark dose (BMD) and 95% lower confidence limit for the benchmark dose (BMDL).

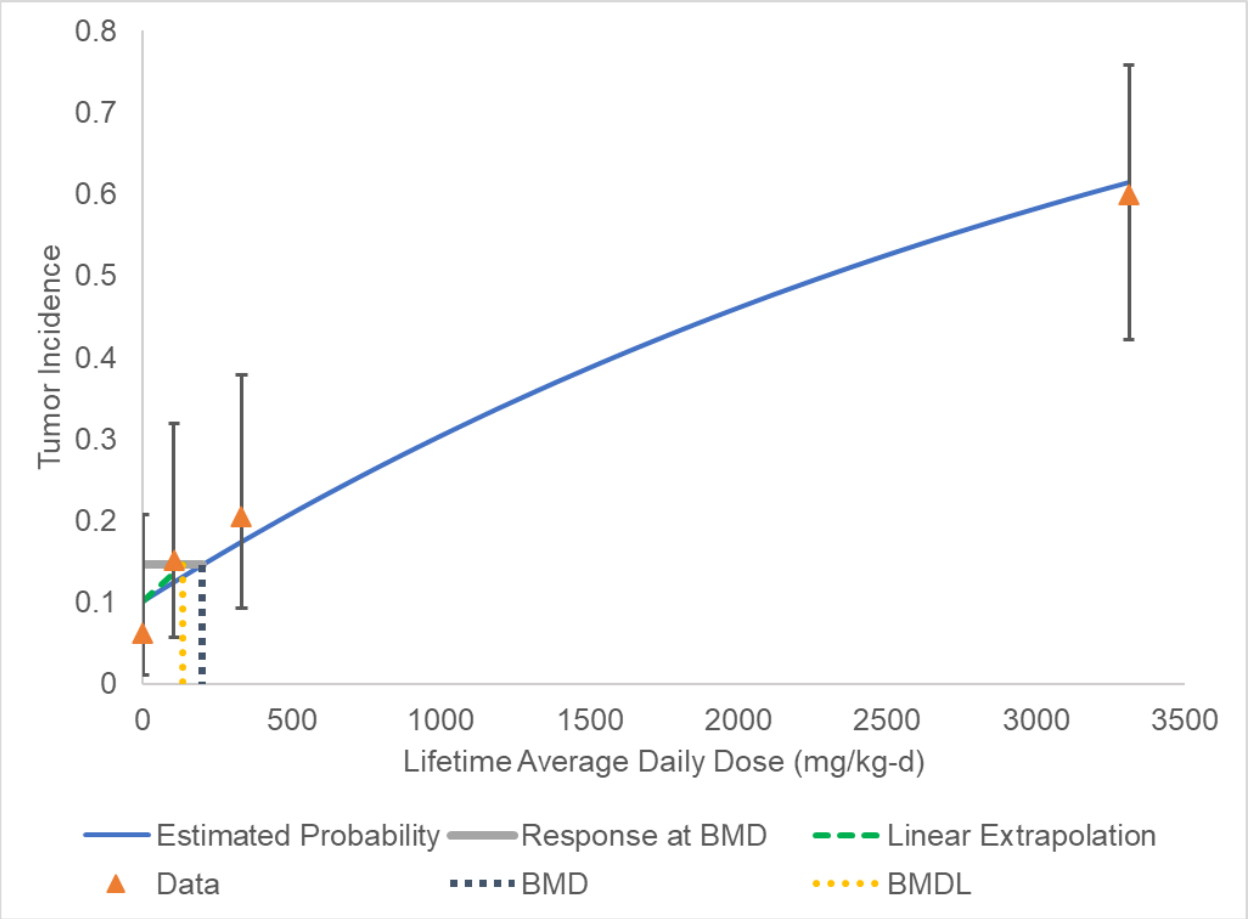

Figure B-2. Benchmark Dose results for mammary gland fibroadenomas and carcinomas (combined) in male rats from the NTP (1999) carcinogenicity study. The line graph shows the Frequentist Multistage Degree 1 model with a benchmark response (BMR) of 5% extra risk for the benchmark dose (BMD) and 95% lower confidence limit for the benchmark dose (BMDL).

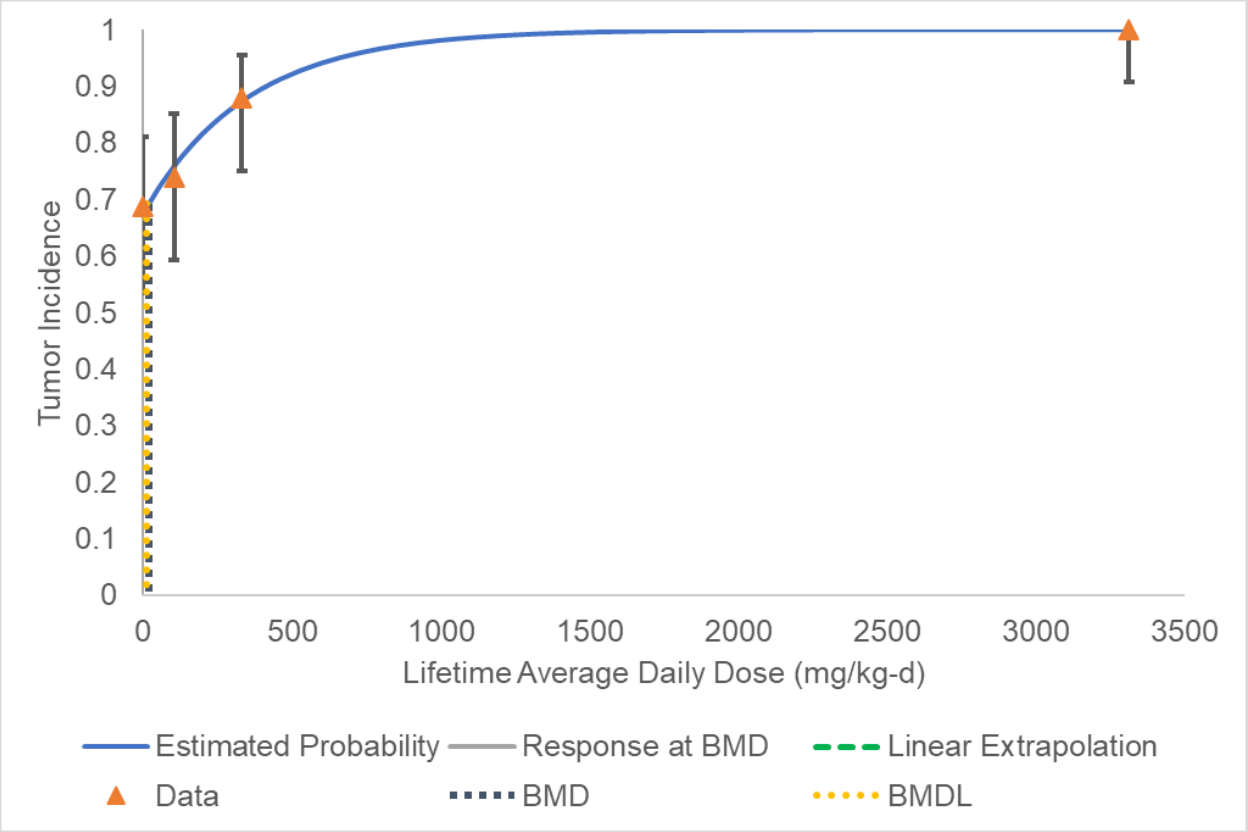

Figure B-3. Benchmark Dose results for testis adenomas in male rats from the NTP (1999) carcinogenicity study. The line graph shows the Frequentist Multistage Degree 1 model with a benchmark response (BMR) of 5% extra risk for the benchmark dose (BMD) and 95% lower confidence limit for the benchmark dose (BMDL).