

**Presentation to the Scientific Review Panel on Toxic Air Contaminants**

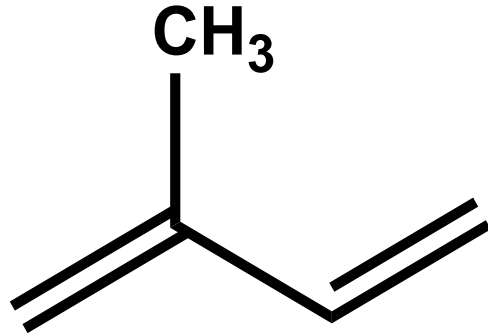
**Isoprene Cancer Inhalation Unit Risk (IUR)  
Factor – Technical Support Document for  
Cancer Potency Factors**

**Office of Environmental Health Hazard Assessment**

**August 16, 2024**



# Isoprene Structure



- A diene containing two carbon double bonds with a methyl group on the second carbon
- Also known as 2-methyl-1,3-butadiene



# Chemical and Physical Properties

- Colorless liquid with mild petroleum-like odor
- Soluble in organic solvents; solubility in water: 642 mg/L @ 25°C
- Boiling point: 34°C (93.2 °F) at 760 mm Hg
- Vapor pressure: 550 mm Hg (torr) @ 25°C
- Unit conversion: 1 part per billion (ppb) = 2.79 micrograms per cubic meter ( $\mu\text{g}/\text{m}^3$ )



# Uses and Emission Sources

- Naturally emitted by plants and trees
- Produced endogenously by humans and some mammals
- Occurs as a by-product of the thermal cracking of naphtha
- Is used to make synthetic rubber for vehicle tires
- Other sources include biomass combustion, wood pulping, tobacco smoking, exhaust from turbines and automobiles, and wildfires
- Emissions in California
  - 186 tons per year, primarily from mobile sources (2017)
  - 12 Facilities (CEIDARS)

# Airborne Concentrations

- Urban air levels of isoprene correlate with other chemicals (e.g., benzene) found in vehicle emissions
- Air concentrations reported in California (average and maximum):
  - South Coast Air Basin: 0.5 and 1.8  $\mu\text{g}/\text{m}^3$ , respectively
  - San Joaquin Valley: 0.1 and 0.8  $\mu\text{g}/\text{m}^3$ , respectively
- In exhaled breath, steady-state concentrations of 195 and 371  $\mu\text{g}/\text{m}^3$  in end-tidal breath for the 25<sup>th</sup>–75<sup>th</sup> quantile range for adults at rest



# Cancer Classification

- Listed as a carcinogen under California's Proposition 65 Program since 1996
- Possibly carcinogenic to humans (Group 2B) - International Agency for Research on Cancer (IARC)
- Reasonably anticipated to be a human carcinogen - the United States National Toxicology Program (NTP) Report on Carcinogens

# Toxicokinetics

- Metabolism of inhaled isoprene in humans and rodents:
  - Primarily occurs through oxidative metabolism via P450 enzymes to epoxide intermediates
  - Also occurs via hydrolysis, conjugation with glutathione and further oxidation of diols formed via hydrolysis.
- Main urinary metabolites in rodents are 2-methyl-3-butene-1,2-diol and its glucuronide, and vinyl lactic acid (2-hydroxy-2-methyl-3-butenic acid)
- Carcinogenicity thought to be related to formation of oxidized reactive metabolites including mono-epoxides, a diepoxide, and diol-epoxides

# Three Sets of Rodent Carcinogenicity Bioassays

1. NTP (1995): One-year stop-exposure studies in male rats and male mice
  - 6-month exposure (6 hours/day, 5 days/week) plus 6 months clean air
  - 30 rodents/species/group
2. Placke et al. (1996): Two-year studies in male and female mice
  - 80-Week exposure (8 hours/day, 5 days/week) with sacrifice at 105 weeks for two lowest exposure groups, and 96 weeks for the other three exposure groups
  - 50 mice/group/sex
3. NTP (1999): Two-year studies in male and female rats
  - 104-Week exposure (6 hours/day, 5 days/week)
  - 50 rats/group/sex



# NTP (1995) One-Year Stop-Exposure Study

## Tumor Incidence in Male Rats

Tumor Type	0 ppm	70 ppm	220 ppm	700 ppm	2200 ppm	7000 ppm
Testes adenoma	3/30	3/30	4/30	7/30	8/29	9/30

Positive trend for tumor type ( $p = 0.021$ )



# NTP (1995) One-Year Stop-Exposure Study

## Tumor Incidence in Male Mice

Tumor Type	0 ppm	70 ppm	220 ppm	700 ppm	2200 ppm	7000 ppm
Liver adenoma or carcinoma	7/30	3/30	7/29	15/30*	18/30**	17/28**
Lung adenoma or carcinoma	2/30	2/30	1/29	5/30	10/30*	9/28*
Forestomach squamous cell papilloma or carcinoma	0/30	0/30	0/30	1/30	4/30	6/30*
Harderian gland adenoma	2/30	6/30	4/30	14/30**	13/30**	12/30**

Positive trends for all tumor types ( $p < 0.001$ )

\*  $p$ -value  $< 0.05$ , \*\*  $p$ -value  $< 0.01$



# Placke et al. (1996) 80-Week Exposure Study

## Tumor Incidence in Male Mice

Tumor Type	0 ppm	10 ppm	70 ppm	280 ppm	700 ppm	2200 ppm
Liver adenoma <sup>†</sup>	11/50	12/50	15/50	24/50**	27/48**	30/50**
Liver carcinoma <sup>†</sup>	9/50	6/50	9/50	16/50	17/48*	16/50
Lung adenoma <sup>†</sup>	11/50	16/50	4/50 <sup>a</sup>	13/50	23/50**	30/50**
Lung carcinoma <sup>†</sup>	0/50	1/50	2/50	1/50	7/50**	7/50**
Forestomach squamous cell carcinoma <sup>†</sup>	0/50	0/48	0/50	1/50	0/47	3/50
Harderian gland adenoma <sup>†</sup>	4/47	4/49	9/50	17/50**	26/49**	35/50**
Harderian gland carcinoma	0/47	0/49	0/50	1/50	3/49	2/50

<sup>†</sup> Positive trend for tumor type ( $p < 0.05$ )

\*  $p$ -value  $< 0.05$ , \*\*  $p$ -value  $< 0.01$

<sup>a</sup> Pairwise comparison of lung adenomas of the 70-ppm group was statistically significantly lower ( $p < 0.05$ ) compared to the control group.

# Placke et al. (1996) 80-Week Exposure Study

## Tumor Incidence in Female Mice

Tumor Type	0 ppm	10 ppm	70 ppm
Harderian gland adenoma <sup>†</sup>	2/49	3/49	8/49*
Spleen hemangiosarcoma	1/50	1/49	4/50
Pituitary gland adenoma <sup>†</sup>	1/49	6/46*	9/49**

<sup>†</sup> Positive trend for tumor types ( $p < 0.05$ )

\*  $p$ -value  $< 0.05$ , \*\*  $p$ -value  $< 0.01$



## Two-Year NTP (1999) Bioassays

### Overall and Effective Tumor Incidence in Male and Female Rats

Sex and Tumor Type	0 ppm	220 ppm	700 ppm	7000 ppm
Male kidney: renal tubule adenoma or carcinoma – single and step sections (combined) <sup>†</sup>	2/50 <i><b>2/38</b></i>	4/50 <i><b>4/42</b></i>	8/50* <i><b>8/40</b></i>	15/50* <i><b>15/44**</b></i>
Male mammary gland: fibroadenoma or carcinoma <sup>†</sup>	2/50 <i><b>2/32</b></i>	5/50 <i><b>5/33</b></i>	7/50 <i><b>7/34</b></i>	21/50** <i><b>21/35**</b></i>
Male testes: adenoma <sup>†</sup>	33/50 <i><b>33/48</b></i>	37/50 <i><b>37/50</b></i>	44/50* <i><b>44/50*</b></i>	48/50** <i><b>48/48**</b></i>
Female mammary gland: fibroadenoma	19/50 <i><b>19/49</b></i>	35/50** <i><b>35/49**</b></i>	32/50* <i><b>32/48**</b></i>	32/50* <i><b>32/48**</b></i>

<sup>†</sup> Positive trend for all tumor type ( $p < 0.001$ )

\*  $p$ -value  $< 0.05$ , \*\*  $p$ -value  $< 0.01$

Effective tumor incidence in italics and bold below overall “uncorrected” tumor incidence



# Genotoxicity Summary

- DNA damage assay – positive via comet assay in human cells with both isoprene and epoxide metabolites
- Bacterial reverse mutation assays – negative with isoprene, positive with metabolite (2-methyl-1,2,3,4-diepoxybutane)
- Chromosomal damage – positive *in vivo* (but not *in vitro*) – for micronuclei formation and sister chromatid exchange



# PBPK Models

- OEHHA evaluated the three available models based on biological relevance and applicability, completeness, and performance/reliability
- Issues identified in each model:
  - NTP (1999): Only included rats (incomplete)
  - Bogaards et al. (2001): Validation limited to isoprene concentrations in the mouse
  - Csanady and Filser (2001): Lacks components to simulate epoxide concentrations; also OEHHA could not replicate model output from peer-reviewed literature
- OEHHA found none of these models adequate for quantitative dose-response assessment

# Cancer Hazard Evaluation

- No epidemiology studies for carcinogenicity
- Three rodent long-term inhalation bioassays: 1) carcinogenic in multiple species, and 2) induced tumors at one or more sites in rats and mice
- Positive genotoxicity studies: primarily in *in vitro* DNA damage assays and *in vivo* chromosomal damage assays
- The structurally-related compound 1,3-butadiene is a known human carcinogen



# IUR Derivation: Calculation of Average Daily Dose

- Convert the air exposure concentration to average daily dose (ADD), in mg/kg BW-day:

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

Where:

C = time-adjusted concentration to annual average  
(6 or 8 hrs / 24 hrs x 5 days / 7 days)

BW = body weight

IR = inhalation rate – equation based on BW of animal

- IR calculation:

Rats:  $\text{IR (m}^3\text{/day)} = 0.702 \times (\text{BW})^{2/3}$  (OEHHA, 2018)

Mice:  $\text{IR (m}^3\text{/day)} = 0.0345 \text{ m}^3\text{/day} \times (\text{BW} / 0.025 \text{ kg})^{2/3}$  (Anderson, 1983)



# IUR Derivation: Average Daily Doses (ADD) in Mice

Placke et al. (1996) bioassay

Isoprene Chamber Concentration (ppm)	<b>0</b>	<b>10</b>	<b>70</b>	<b>280</b>	<b>700</b>	<b>2200</b>
Male mice ADD (mg/kg BW-day)	0	6.74	47.20	204.52	511.31	1606.96
Female mice ADD (mg/kg BW-day)	0	7.16	50.10	ND	ND	ND

ND: No Data



# IUR Derivation: Average Daily Doses (ADD) in Rats

NTP (1999) bioassay

Isoprene Chamber Concentration (ppm)	0	220	700	7000
Male rat ADD (mg/kg BW-day)	0	104.12	331.29	3312.86
Female rat ADD (mg/kg BW-day)	0	122.35	389.31	3893.10



# IUR Derivation: Determination of Cancer Slope Factor

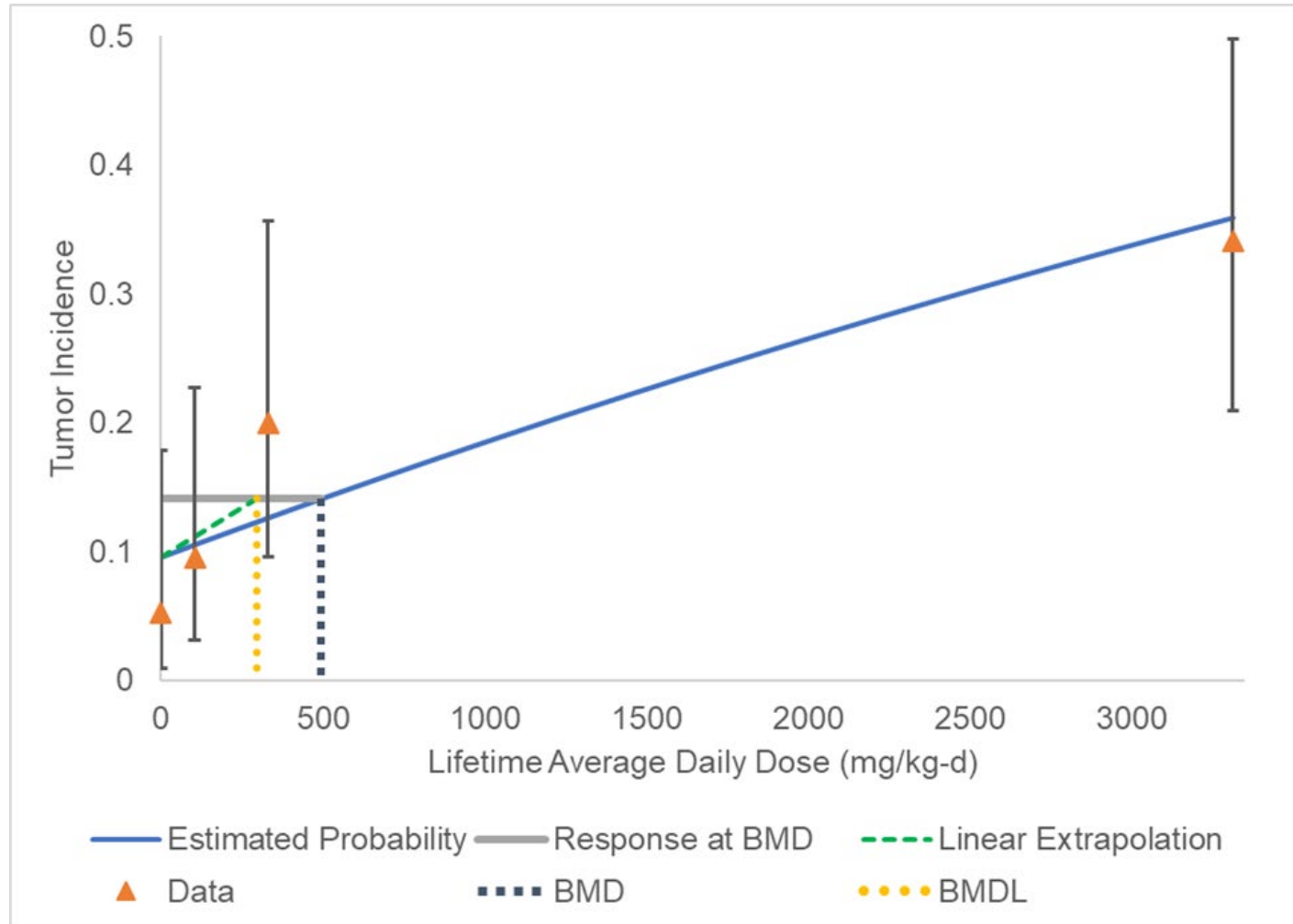
- Determined the Cancer Slope Factor (CSF) using the U.S. EPA Multistage Cancer Model in the Benchmark Dose Software (U.S. EPA, 2023)
  - Used a Benchmark Response (BMR) of 5% tumor incidence above control to determine the Benchmark Dose (BMD)
  - The 95% lower confidence bound of the dose producing 5% tumor response ( $BMDL_{05}$ ) is used to calculate cancer potency
  - $CSF = 0.05 / BMDL_{05}$
- Combined tumor potency was determined for animals with tumors occurring at multiple sites using the U.S. EPA multi-site model

# **IUR Derivation:**

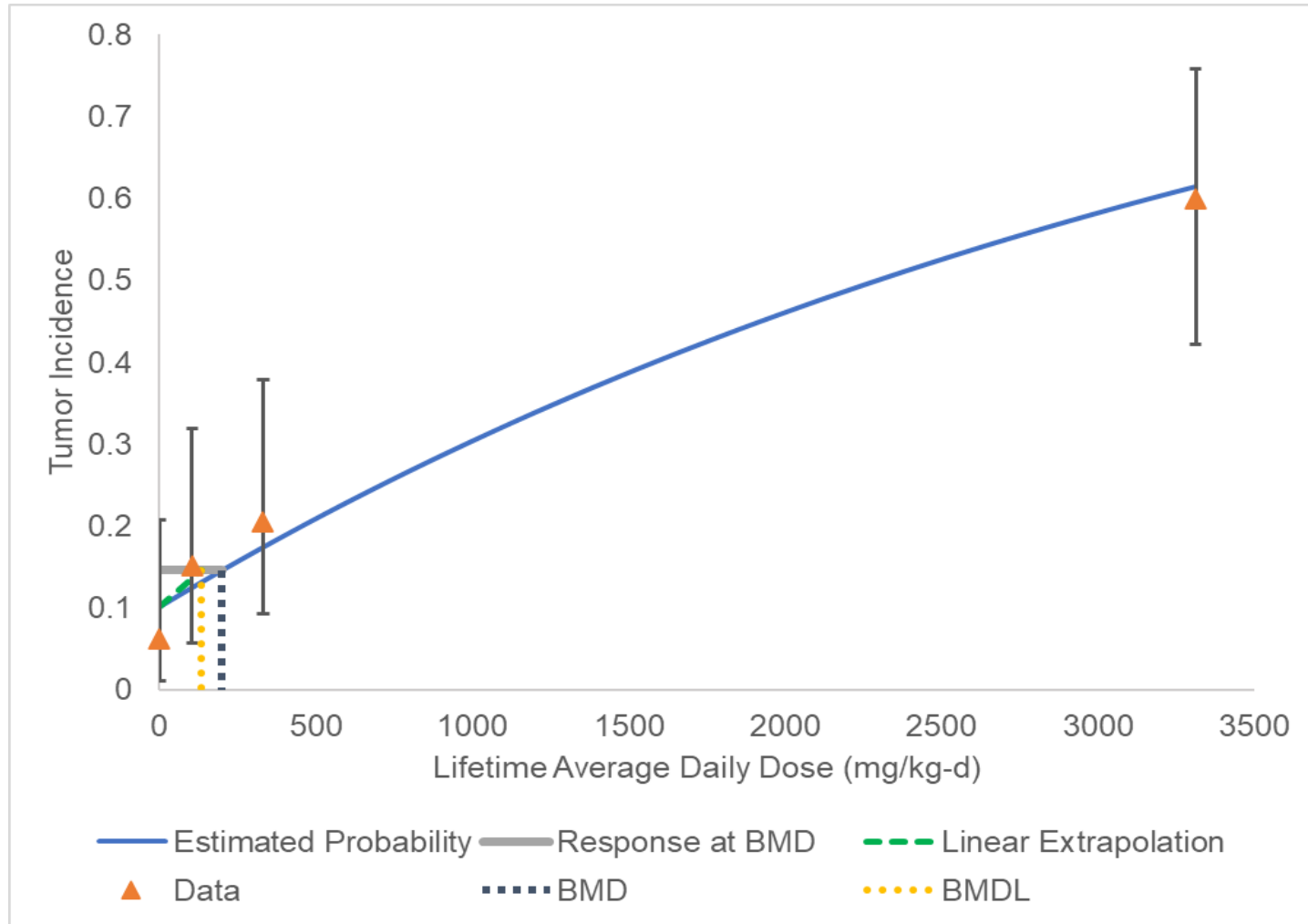
## **Determination of Cancer Slope Factor (CSF)**

- CSFs from Placke et al. (1996) were calculated for:
  - Liver, lung and Harderian gland - adenomas or carcinomas, separately and combined, in male mice
  - Pituitary and Harderian gland adenomas (benign) in female mice – not used for final CSF determination
- CSFs from NTP (1999) were calculated for:
  - Kidney, mammary gland and testes – adenoma, fibroadenoma or carcinoma, separately and combined, in male rats
  - Mammary gland fibroadenoma (benign) in female rats – not used for final CSF determination

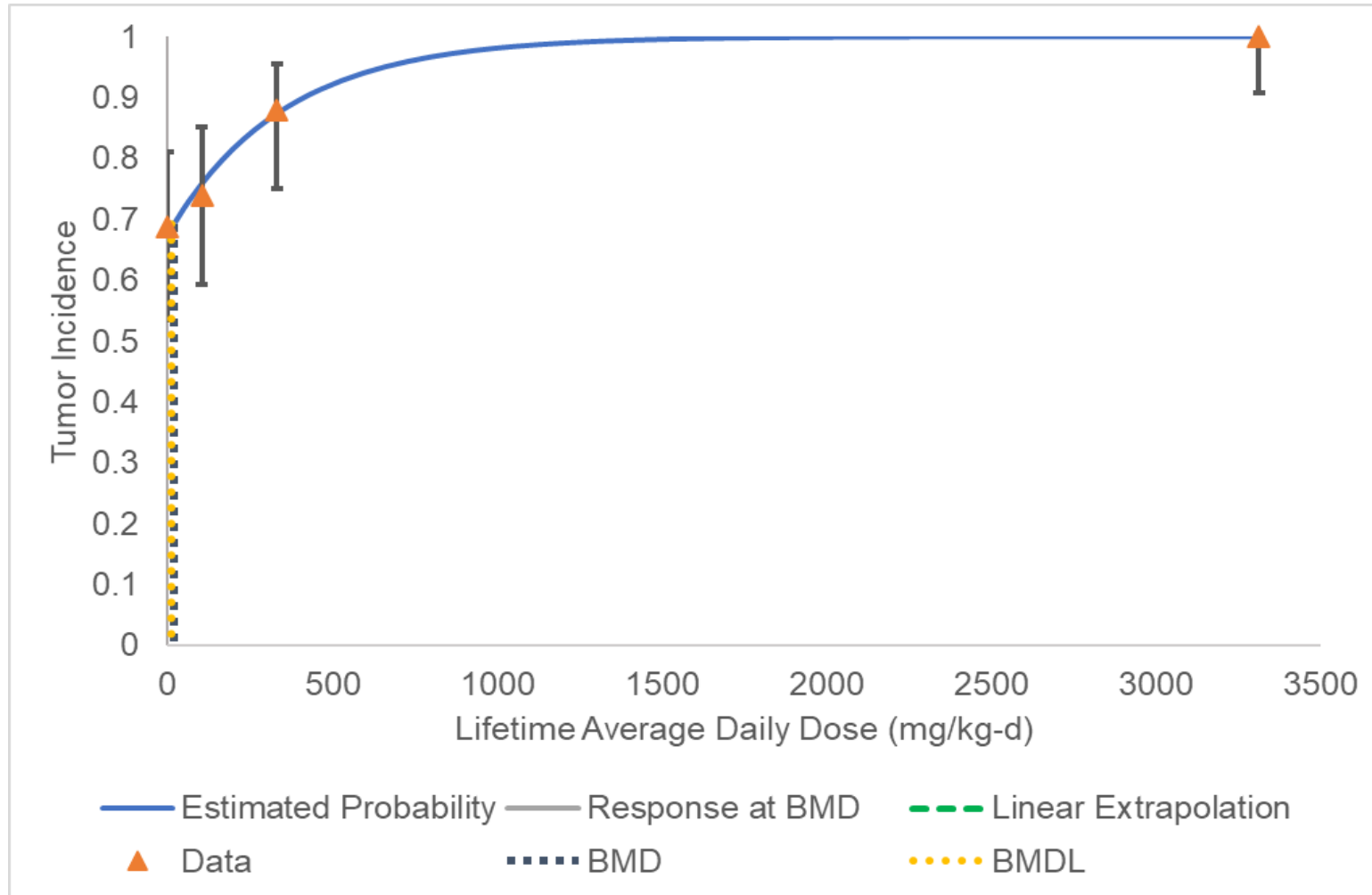
# Benchmark Dose results for renal tubule adenoma or carcinoma in male rats (NTP, 1999)



# Benchmark Dose results for mammary gland fibroadenoma or carcinoma in male rats (NTP, 1999)



# Benchmark Dose results for testis interstitial cell adenoma in male rats (NTP, 1999)





# IUR Derivation: Extrapolation to Human CSF

- Rodent CSFs ( $CSF_a$ ) were converted to human equivalents ( $CSF_h$ ) by multiplying the  $CSF_a$  by the ratio of human to animal body weights raised to the one-fourth power when animal potency is expressed in units of  $(\text{mg}/\text{kg}\cdot\text{day})^{-1}$

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

- Interspecies scaling factor accounts for
  - pharmacokinetic differences (*e.g.*, breathing rate, metabolism)
  - pharmacodynamic considerations (*i.e.*, tissue responses to chemical exposure)



# IUR Derivation: Benchmark Dose CSF Results

Study	Species Sex	Tumor Site	BMD <sub>05</sub> (mg/kg-day)	BMDL <sub>05</sub> (mg/kg-day)	Animal CSF (CSF <sub>a</sub> ) (mg/kg-day) <sup>-1</sup>	Human CSF (CSF <sub>h</sub> ) (mg/kg-day) <sup>-1</sup>
Placke et al. (1996)	Male mice	Multi-site	28.8007	23.6918	$2.11 \times 10^{-3}$	$1.47 \times 10^{-2}$
NTP (1999)	Male rats	Multi-site	16.0165	9.4390	$5.30 \times 10^{-3}$	<b><math>1.88 \times 10^{-2}</math></b>



# Limitations of Placke et. al. (1996) Mouse Study

- Limitations of male mice data set for CSF determination:
  - Combined adenoma and carcinoma incidence was not reported for liver, lung and Harderian gland tumor sites, therefore, modeling was performed with adenoma incidence data
  - No data on individual survival or appearance of first tumor – cannot determine effective tumor incidence, thus overall incidence rate was used (50 or 49 animals per group)



# IUR Calculation: Final Step

- Isoprene IUR based on male rat data from NTP (1999)

- Inhalation unit risk (IUR) =  $\left( \frac{CSF\ human \times Breathing\ Rate}{Body\ Weight \times Conversion\ Factor} \right)$

$$\begin{aligned} IUR &= (0.019\ (mg/kg\text{-}day)^{-1} \times 20\ m^3/day) / (70\ kg \times 1000\ \mu g/mg) \\ &= 5.4 \times 10^{-6}\ (\mu g/m^3)^{-1} [1.5 \times 10^{-5}\ (ppb)^{-1}] \end{aligned}$$

- Lifetime “adult” exposure to 1  $\mu g/m^3$  isoprene results in an extra cancer risk of 5.4 cases in a million.



# Public Comments

- **OEHHA did not receive any public comments on the draft Isoprene IUR**
- Public comment period: February 16, 2024 - April 2, 2024
- Public workshops were held on March 8, 2024 in Southern California and on March 15, 2024 in Northern California

