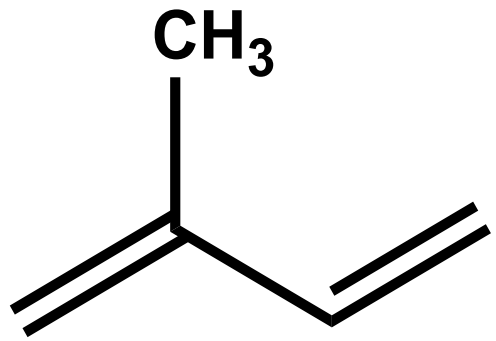
Presentation to the Scientific Review Panel on Toxic Air Contaminants

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 ($\mu g/m^3$)

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 µg/m^3, respectively
  - San Joaquin Valley: 0.1 and 0.8 µg/m^3, respectively
- In exhaled breath, steady-state concentrations of 195 and 371 µg/m^3 in end-tidal breath for the 25^{th}–75^{th} 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-butenoic 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†	11/50	12/50	15/50	24/50**	27/48**	30/50**
Liver carcinoma†	9/50	6/50	9/50	16/50	17/48*	16/50
Lung adenoma†	11/50	16/50	4/50[a]	13/50	23/50**	30/50**
Lung carcinoma†	0/50	1/50	2/50	1/50	7/50**	7/50**
Forestomach squamous cell carcinoma†	0/50	0/48	0/50	1/50	0/47	3/50
Harderian gland adenoma†	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

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

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

[a] 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†	2/49	3/49	8/49*
Spleen hemangiosarcoma	1/50	1/49	4/50
Pituitary gland adenoma†	1/49	6/46*	9/49**

† 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)†	2/50 ***2/38***	4/50 ***4/42***	8/50* ***8/40***	15/50* ***15/44****
Male mammary gland: fibroadenoma or carcinoma†	2/50 ***2/32***	5/50 ***5/33***	7/50 ***7/34***	21/50** ***21/35****
Male testes: adenoma†	33/50 ***33/48***	37/50 ***37/50***	44/50* ***44/50****	48/50** ***48/48****
Female mammary gland: fibroadenoma	19/50 ***19/49***	35/50** ***35/49****	32/50* ***32/48****	32/50* ***32/48****

† 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:

  ADD (mg/kg BW-day) = IR × C / 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: $IR\ (m^3/day) = 0.702 \times (BW)^{2/3}$ (OEHHA, 2018)

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

IUR Derivation: Average Daily Doses (ADD) in Mice

Placke et al. (1996) bioassay

Isoprene Chamber Concentration (ppm)	**0**	**10**	**70**	**280**	**700**	**2200**
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

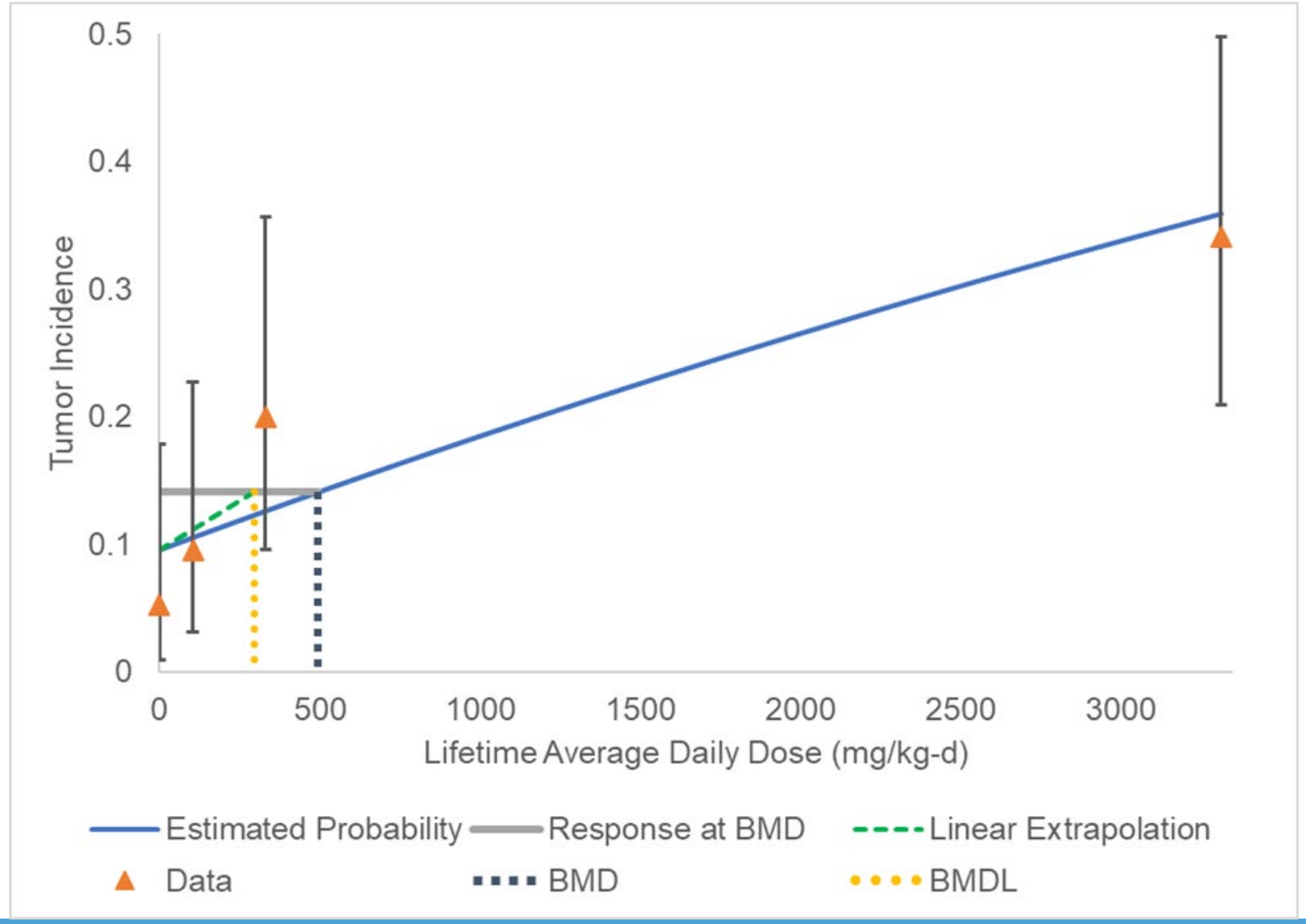
IUR Derivation: Determination of Cancer Slope Factor

- 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

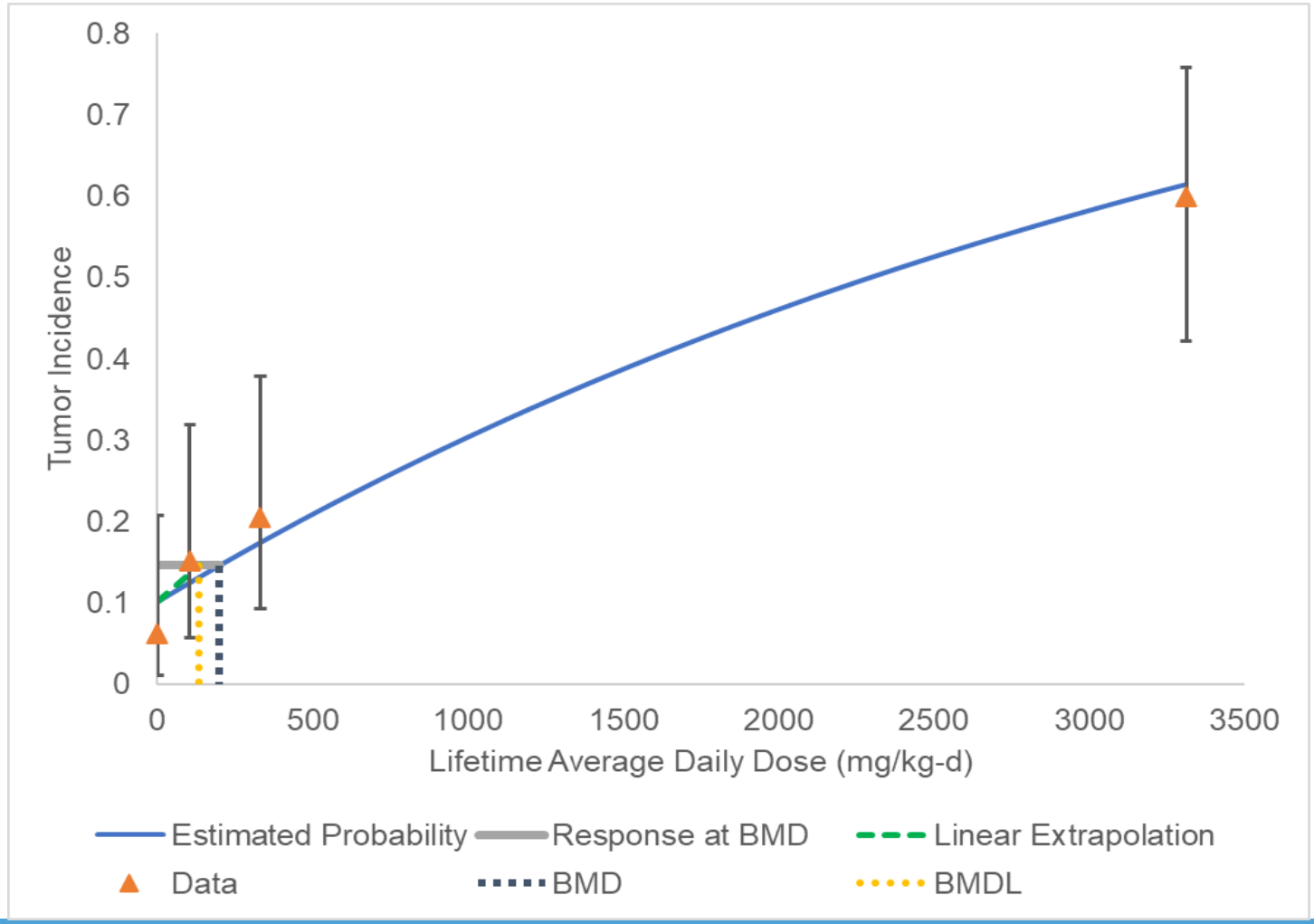
IUR Derivation: Determination of Cancer Slope Factor (CSF)

- 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

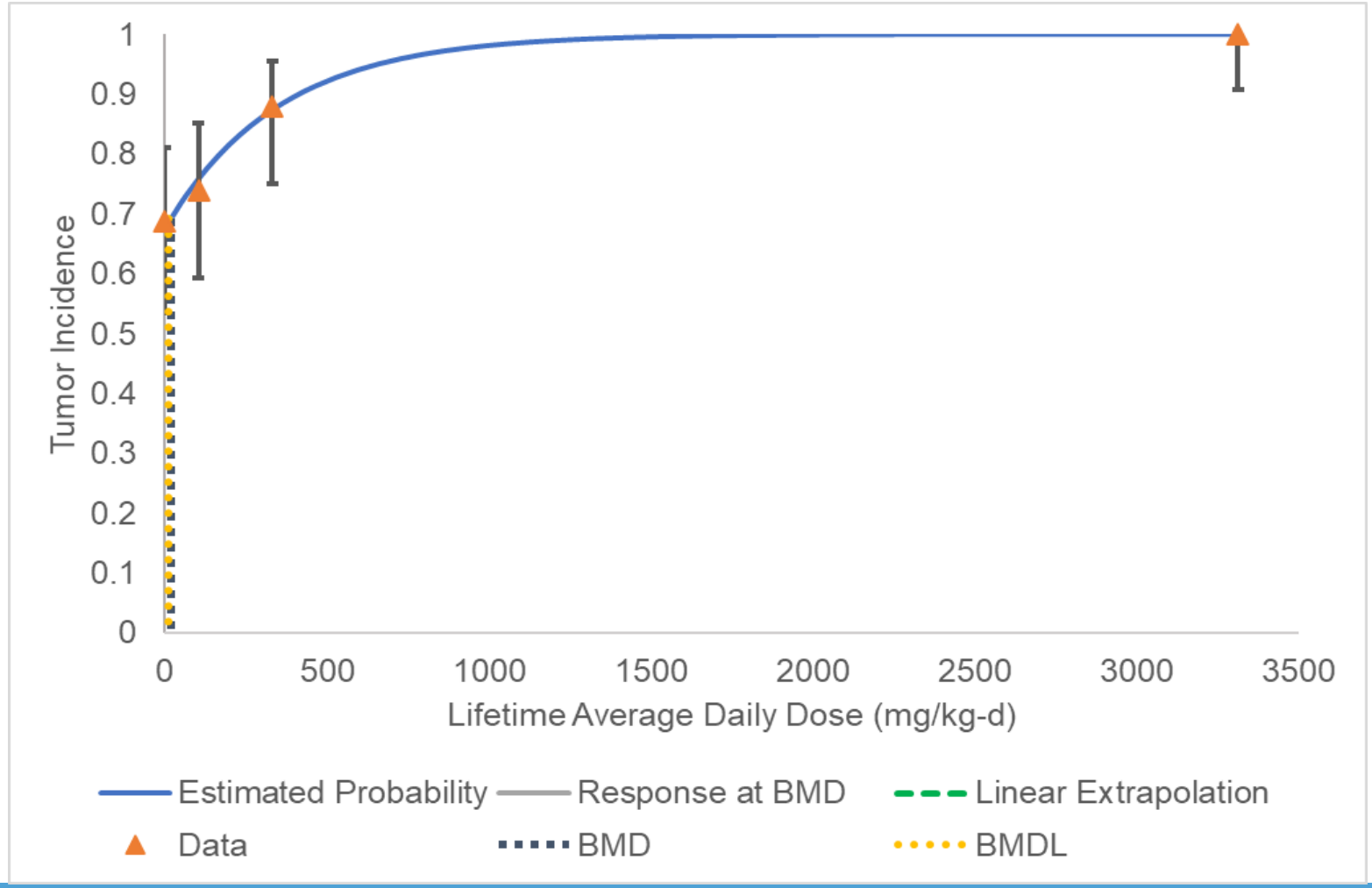
Benchmark Dose results for renal tubule adenoma or carcinoma in male rats (NTP, 1999)



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 $(mg/kg\text{-}day)^{-1}$

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

- 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_{05} (mg/kg-day)	$BMDL_{05}$ (mg/kg-day)	Animal CSF (CSF_a) $(mg/kg\text{-}day)^{-1}$	Human CSF (CSF_h) $(mg/kg\text{-}day)^{-1}$
Placke et al. (1996)	Male mice	Multi-site	28.8007	23.6918	2.11×10^{-3}	1.47×10^{-2}
NTP (1999)	Male rats	Multi-site	16.0165	9.4390	5.30×10^{-3}	**1.88×10^{-2}**

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) = $\left(\frac{CSF\ human \times Breathing\ Rate}{Body\ Weight \times Conversion\ Factor}\right)$

$$IUR = (0.019\ (mg/kg\text{-}day)^{-1} \times 20\ m^3/day) / (70\ kg \times 1000\ \mu g/mg)$$

$$= \mathbf{5.4 \times 10^{-6}\ (\mu g/m^3)^{-1}\ [1.5 \times 10^{-5}\ (ppb)^{-1}]}$$

- 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