OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Public Health Goals

FIRST PUBLIC REVIEW DRAFT

1,4-Dioxane in Drinking Water

September 2025

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Proposed Public Health Goal for 1,4-Dioxane in Drinking Water

September 2025

Prepared by

Pesticide and Environmental Toxicology Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

This page intentionally left blank.

PREFACE

Public Health Goal (PHG) technical support documents provide information on health effects from contaminants in California drinking water. PHGs are developed for chemical contaminants based on the best available data in the scientific literature and using the most current principles, practices, and methods used by public health professionals. These documents and the analyses contained herein provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

Under the California Safe Drinking Water Act of 1996 (Health and Safety Code section 116365), the Office of Environmental Health Hazard Assessment (OEHHA) develops PHGs for drinking water contaminants in California based exclusively on public health considerations. OEHHA periodically reviews PHGs and revises them as necessary based on the occurrence of the respective contaminant in California drinking water supplies and the availability of new scientific data. This document presents the proposed PHG for 1,4-dioxane.

PHGs published by OEHHA are used by the State Water Resources Control Board (SWRCB) in establishing primary drinking water standards (California Maximum Contaminant Levels, or CA MCLs). Whereas PHGs are based solely on scientific and public health considerations, MCLs adopted by SWRCB consider economic factors and technological feasibility. State law requires that MCLs be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory and represent goals that SWRCB and California's public water systems strive to achieve if it is feasible to do so. Under federal law, CA MCLs established by SWRCB must be at least as stringent as the corresponding federal MCL if one exists.

CONTENTS

SUMMARY

This draft document presents the proposed Public Health Goal (PHG) for 1,4-dioxane, based on the most sensitive health effects and includes consideration of sensitive populations, such as infants and children. The proposed PHG is 0.04 µg/L (micrograms per liter), equivalent to 0.04 ppb (parts per billion), based on hepatic tumors in female mice (Kano et al. 2009) and multiple tumor types in male mice (Kasai et al. 2009). This document also identifies a noncancer health-protective concentration (HPC) of 33 ppb for 1,4-dioxane, based on degeneration and necrosis in the liver and kidneys of male rats.

PHGs are not regulatory requirements and are based solely on protection of public health without regard to cost impacts or other factors. PHGs form the basis of California's Maximum Contaminant Levels (MCLs) for drinking water, which are established by the State Water Resources Control Board (SWRCB). Each MCL must be set as close to the corresponding PHG as is economically and technologically feasible. California MCLs may be set at the same or a more stringent level than the federal MCLs established by the United States Environmental Protection Agency (US EPA).

INTRODUCTION

Purpose

The purpose of this document is to derive health-protective concentrations (HPCs) and develop a PHG for 1,4-dioxane in drinking water. A PHG is the concentration of a contaminant in drinking water that is estimated to pose no significant health risk to individuals consuming the water on a daily basis over a lifetime. If a chemical has been identified as a human or animal carcinogen, HPCs are determined for both cancer and noncancer effects and the lower of the two values is selected as the PHG. The proposed HPCs are based on a comprehensive analysis of the toxicology and pharmacokinetics of 1,4-dioxane. HPCs for carcinogens are set at a de minimis risk level of one in a million (10^{-6}) for exposures over a lifetime. When estimating lifetime cancer risks, OEHHA accounts for the greater sensitivity of infants and children to carcinogens.

1,4-Dioxane is used as a solvent in multiple industrial applications, e.g., as a wetting and dispersing agent in textile processing, degreasing agent, polymerization catalyst and as a component in various paints, varnishes and related products (NTP, 2021). In 1998, the Drinking Water Program at the California Department of Public Health[1] established a notification level (NL) of 3 ppb for 1,4-dioxane. NLs are non-regulatory health-based advisory levels for chemicals in drinking water that lack MCLs (SWRCB, 2021). In 2010, US EPA concluded a one-in-one-million cancer risk would correspond to a 1,4-dioxane drinking water concentration of 0.35 ppb and as a result, the notification level was revised to 1 ppb. The notification level was revised to 1 ppb instead of 0.35 ppb due to limitations in accurately quantifying 1,4-dioxane at levels below 1 ppb. In 2019, SWRCB requested that OEHHA establish a PHG for 1,4-dioxane,

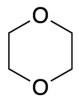
[1] The program was transferred to SWRCB in 2014 and became the Division of Drinking Water.

which will be used to establish an enforceable drinking water MCL. In deriving the PHG, OEHHA performed a systematic literature search of human and animal toxicity and pharmacokinetics studies published from 2009 onward, just prior to the US EPA (2010) assessment. OEHHA also reviewed health assessments by US EPA (2013a) and the Agency for Toxic Substances and Disease Registry (ATSDR (2012), where studies published before 2009 were identified.

Physical and Chemical Properties

1,4-Dioxane exists at room temperature as a colorless liquid. It is miscible in water, oils, and most solvents. Physical and chemical properties of 1,4-dioxane are presented in Table 1. Synonyms of 1,4-dioxane include dioxane, dioxan, 1,4-diethylene dioxide, diethylene oxide, diethylene ether, di(ethylene oxide), 1,4-dioxacyclohexane, p-dioxane, p-dioxin, para-dioxane, dioxane-1,4, tetrahydro-; tetrahydro-1,4-dioxin; tetrahydro-para-dioxin; tetrahydro-*p*-dioxin and glycol ethylene ether (ATSDR, 2012).

Table 1. Physical and Chemical Properties

Properties	**1,4-dioxane**
Formula	$C_4H_8O_2$
Chemical Structure	
CAS No.	123-91-1
Molecular weight (g/mol)	88.11
Physical state at ambient temperature	Colorless liquid
Melting point (ºC)	11.75 (HSDB, 2020)
Boiling point (ºC)	101.2 (HSDB, 2020)
Density (g/cm^3)	1.0337 (20º C) (HSDB, 2020)
Solubility in water (g/L)	>800 (25º C) (HSDB, 2020)
Vapor pressure (mm Hg)	38.1 (25º C) (HSDB, 2020)
Henry's Law constant at 25°C (atm-cm^3/mol)	4.8 ×10^{-6} (HSDB, 2020)
Log K_{ow} (unitless)	-0.27 (HSDB, 2020)

METHODOLOGY

Systematic Literature Search and Toxicological Evaluation

The toxicological evaluation of a chemical starts with a review of the available literature. A systematic literature search is conducted, using a comprehensive search string and multiple scientific databases (Pubmed, Embase, Scopus, etc.). Details of the literature search are provided in Appendix A. Briefly, a PECO (populations, exposures, comparators, outcomes) statement that outlines inclusion/exclusion criteria is developed for initial reference screening. The title and abstract of each reference are screened by a minimum of two reviewers, and each reference is included or excluded based on criteria outlined in the PECO statement.

Subsequently, a full-text review of included references is conducted to select the studies that are relevant for PHG development.

Study Evaluation

Animal Studies

The findings from studies that meet the PECO criteria are critically evaluated, and the quality of each study is assessed. OEHHA's criteria for study quality evaluation include the following:

- appropriate number of animals per dose group
- an untreated control group plus a minimum of two dose groups
- appropriate exposure duration
- relevant route of exposure
- adequate test species
- adequate statistical analysis
- biological significance of endpoints
- adequate reporting.

The search of recent literature revealed 1,306 human, animal, and in vitro studies. Eighteen studies met the PECO criteria, and seven studies were retained following the full-text screen. A total of 79 studies were tagged as supplemental material, e.g., mechanistic studies, exposure characteristics, and toxicokinetics evaluations.

Human Studies

Epidemiology studies were evaluated based on modified Bradford-Hill criteria (Hill, 1965), including study design, factors related to bias, exposure and outcome methods, and confounding.

PHG Derivation

After a review of the toxicity studies of suitable quality and identification of relevant hazards, the most sensitive endpoints from studies determined to be relevant to human health are selected as candidate critical studies, and analyses of the dose-response relationships are performed. The adverse effect or a physiological change that leads to an adverse effect that occurs at the lowest dose is selected as the critical effect from which a PHG is derived.

If a chemical has been identified as a human or animal carcinogen, health-protective water concentrations are determined for both cancer and noncancer effects and the lower value is selected as the PHG.

Deriving Health-Protective Concentrations for Noncancer Effects

Calculation of an HPC for noncancer effects involves a four-step approach: determination of the point of departure (POD), estimation of an acceptable daily dose (ADD), determination of the relative source contribution (RSC) and calculation of an HPC.

Point of Departure (POD)

The POD is the dose of a chemical (in units of milligrams per kilogram of body weight per day, mg/kg-day) from a study in animals or humans that is used as a starting point for calculation of the ADD. The POD is typically determined by fitting a mathematical model to the dose-response data. OEHHA generally uses the publicly available Benchmark Dose Software (BMDS version 3.2) developed and maintained by the US EPA (https://www.epa.gov/bmds). BMDS fits mathematical models to the data and determines the dose (benchmark dose or BMD) that corresponds to a pre-determined level of response (benchmark response or BMR). The BMR is typically set at a response level of 5% extra risk for dichotomous data (OEHHA, 2008). For continuous data, a BMR of one standard deviation from the control mean is typically used when there are no data to indicate what level of response is biologically significant (US EPA, 2012). To account for the uncertainty in a BMD estimate, the 95% lower confidence limit of the BMD, called the BMDL (L stands for the lower confidence limit) is calculated. For PHG development, OEHHA uses the BMDL as the POD for the calculation of an HPC when the data are amenable to BMD modeling. When data are not amenable to BMD modeling (e.g., when the models fit the data poorly), OEHHA uses the no-observed-adverse-effect level or concentration (NOAEL or NOAEC), or lowest-observed-adverse-effect level or concentration (LOAEL or LOAEC) approach in identifying the POD.

Model selection criteria when comparing outputs of different models for the same endpoint/dataset are:

- the lowest Akaike's information criterion (AIC);
- goodness of fit p-value ≥0.05;
- scaled residual at the dose close to the BMD estimate ≤ the absolute value of 2;
- consideration of the BMD/BMDL ratio; and
- visual inspection of the dose-response curve.

A model is classified as questionable if any of the following is true: the goodness of fit p-value is <0.05, the absolute value of the scaled residual is >2, the BMD/BMDL ratio is >20, the BMD or BMDL is 10-fold lower than lowest non-zero dose, or degrees of freedom = 0 (US EPA, 2022).

Application of BMD modeling for noncancer effects mitigates some of the limitations of the NOAEL/LOAEL approach, including:

- dependence on dose spacing and sample size;
- inability to account for uncertainty and variability in the experimental results due to the characteristics of the study design;
- the need to use an additional uncertainty factor when a NOAEL cannot be determined in a study;
- inability to account for the shape of the dose-response curve; and
- difficulty in quantitatively comparing studies with distinct dosing designs.

Acceptable Daily Dose (ADD)

The ADD is the estimated maximum average daily dose of a chemical (in mg/kg-day) that can be consumed by a human for a lifetime without adverse effects. This is similar to the term "reference dose" used by US EPA. To determine the ADD, the POD is adjusted by factors that account for uncertainties and variabilities in the risk assessment, such as differences between animals and humans, and differences among humans in response to a chemical exposure. These factors form a composite uncertainty factor (UF).

Uncertainty and Variability Factors (UF)

When developing HPCs for noncancer effects based on animal toxicity studies, OEHHA generally applies a composite UF of 300 (OEHHA, 2008).

These UFs are:

- 10 for interspecies extrapolation, accounting for possible differences in the way laboratory animals and humans respond to the chemical, consisting of
    - √10 for pharmacodynamics
    - √10 for pharmacokinetics
- 30 for intraspecies variability, which accounts for some human subpopulations, such as children and the elderly, possibly being more sensitive to the chemical than the general population, consisting of
    - √10 for pharmacodynamics
    - 10 for pharmacokinetics.

These default factors are applied unless data support an alternative value. A table of default UFs for ADD derivation is presented in Appendix B. Additional adjustments may be included depending on the limitations of available data. For 1,4-dioxane, there was only one reproductive/developmental animal study identified. In addition, it had a number of limitations for use in deriving a PHG, and therefore OEHHA included an additional √10 for database deficiencies[2].

The ADD is calculated using the following equation:

$$\text{ADD} = \text{POD} \div \text{combined UF}.$$

Daily Water Intake Equivalent (DWI)

To calculate a PHG for a chemical, the ADD is converted to a concentration in drinking water that accounts for the total exposure to the chemical that people receive from using tap water. It includes intake from ingestion as well as inhalation and dermal contact with the chemical in tap water from household uses (e.g., drinking, cooking, bathing, and showering). Inhalation

[2] The Giavini et al, (1985) was the only reproductive study identified. It was not a multi-generational study and was not informative for dose-response modeling.

exposure can take place when the chemical volatilizes out of the water during cooking or showering. Dermal absorption of the chemical can occur during bathing and other household uses of tap water.

The daily water intake (DWI) equivalent is expressed in units of liters or liter equivalents per kilogram of body weight per day (L/kg-day or L_{eq}/kg-day, respectively). Liter equivalents represent the equivalent of the amount of tap water one would have to drink to account for the exposure to a chemical in tap water through oral, inhalation, and dermal routes, taking into consideration route-specific toxicokinetic mechanisms.

For oral intake rates, the PHG program uses age-specific water ingestion estimates (OEHHA, 2012) derived from a nationwide survey of food and beverage intake from approximately 20,000 people (US Department of Agriculture's Continuing Survey of Food Intake of Individuals 1994 – 1996, 1998 dataset). These age-specific intake rates are normalized to body weight and expressed as L/kg-day. Updated water ingestion rates indicate that drinking water ingestion per unit body weight is higher in infants than in adults. These refined estimates replace previous ingestion rates of 2 L/day for adults and 1 L/day for a 10 kg child used in older PHGs. For noncancer endpoints, the time-weighted average daily water ingestion rate for a 70-year lifetime is typically used for the general population. However, if there is a particularly sensitive age group or other subgroup that comprises a meaningful portion of the general population, the high end estimates of the age-specific water ingestion rate for this subgroup will be used in the PHG calculations in place of a life-time average value (OEHHA, 2012). In PHG development, OEHHA is mandated by Health and Safety Code section 116365.2 to give special consideration to sensitive subgroups, such as children and infants, who may have greater susceptibility to contaminants and be at greater risk of adverse health effects due to their disproportionately high exposure to contaminants in comparison to adults.

As noted above, exposure to a chemical in tap water can occur via oral, inhalation and dermal pathways. For example, volatile organic compounds (VOCs) are released from tap water in the shower and can be inhaled by the person showering. In older PHG documents, OEHHA assumed that inhalation and dermal exposures to volatile contaminants in tap water were equivalent to drinking 2 L/day of water, effectively doubling the total exposure, since ingestion was also assumed to be 2 L/day. However, McKone (1987) demonstrated that exposures to volatile chemicals from routes other than oral ingestion may be larger than exposure from ingestion alone. Thus, to estimate inhalation and dermal exposures to chemicals in tap water, OEHHA uses equations extracted from the CalTOX 4.0[3] multimedia total exposure model developed for the California Department of Toxic Substances Control by Lawrence Berkeley National Laboratory. The equations, parameters, and inputs used in calculating multiroute exposures are described in Appendix C. The resulting DWI is expressed in L_{eq}/kg-day, and accounts for all exposures from tap water.

Relative Source Contribution

The relative source contribution (RSC) is the proportion of exposures to a chemical attributed to tap water, as part of total exposure from all sources (including food and air). The RSC values typically range from 20% to 80% (expressed as 0.20 to 0.80) and are determined based on

[3] Available at: https://dtsc.ca.gov/caltox-download-instructions/

available exposure data. For certain PHGs, the RSC can be as high as 1.0 (tap water is the only source of the chemical) when it is deemed appropriate. OEHHA uses this approach to ensure that the PHG identifies a concentration of a drinking water contaminant that would pose no significant health risk after taking into account exposures to all other sources.

Derivation of the Health-Protective Concentration

Following the determination of the ADD, the HPC (in milligrams per liter, mg/L, or in micrograms per liter, µg/L) in drinking water can be derived by incorporating the DWI and RSC of the chemical:

$$HPC = (ADD \times RSC) \div DWI.$$

Deriving Health-Protective Concentrations for Cancer Effects

Calculation of an HPC for cancer effects involves a three-step approach: determination of a POD from which a cancer potency can be determined, estimation of an average daily dose, and calculation of the HPC.

Cancer Dose-Response Analyses and Cancer Potency Derivation

Standard methods for estimation of lifetime theoretical cancer risks are employed in the development of cancer potencies based on animal studies (OEHHA, 2009; US EPA, 2005a, 2012). The estimated carcinogenic potency, also referred to as the cancer slope factor (CSF), is a measure of the carcinogenic potential of a compound. It is often reported in units of 1/(mg/kg-day) or $(mg/kg\text{-}day)^{-1}$ and is derived by fitting US EPA's BMDS Multistage-Cancer model (US EPA, 2012) to the tumor incidence data from an animal carcinogenicity bioassay.. When data are not amenable to BMD modeling or there is early mortality in experimental animals, other models may be used.

Method for Calculating Cancer Potency

Development of cancer potency estimates from animal bioassays includes consideration of:

- the quality, suitability, and sensitivity of the available animal bioassay studies; for example, the thoroughness of experimental protocol, the temporal exposure pattern, the degree to which dosing resembles the expected manner of human exposure, the duration of the study, the purity of test material, the number and size of exposed groups, and the extent of tumor occurrence
- the cancer sites and types from the selected experiments most appropriate for characterizing the cancer potency; where there are multiple sites with significant tumor findings in a selected experiment, a multisite analysis is performed to describe the overall carcinogenic potential
- whether a dose-response model that assumes the absence of a carcinogenic threshold dose should be used or whether there are compelling mechanistic data to support an alternative approach
- interspecies scaling of animal cancer potency to human cancer potency
- physiologic, toxicokinetic, and metabolic information for possible use in extrapolating from test animals to humans, from high to low dose, and from one exposure route to another.

Calculating Average Daily Dose

A mathematical model is fit to dose-response data from animal studies. For studies that do not involve daily administration of a fixed mg/kg body weight amount, an average daily dose (in units of mg/kg-day) is calculated. This is done by adjusting the administered or nominal dose, accounting for days of dosing during the week and total dosing weeks during the experimental period. For studies using variable doses, the weighted mean dose is calculated considering the dosing frequency and duration of the various administered doses.

Dose-Response Model

Information on the mode of action (MOA) involved in the carcinogenesis of a chemical is evaluated to determine whether human cancer risk should be estimated using the default assumption of low dose linearity or otherwise. Unless there is sufficiently compelling evidence, OEHHA uses a non-threshold approach and a linearized multistage (LMS) cancer model to calculate the chemical's cancer potency, expressed as the CSF. This is accomplished by using the BMDS Multistage-Cancer model developed by US EPA (BMDS version 3.2). The model calculates the lifetime probability of developing a tumor (p) induced by an average daily dose (d) using the following equation:

$$p(d) = \beta + (1- \beta) \times \exp[-(q_1 d + q_2 d^2 + ... + q_i d^i)].$$

The q_i are parameters of the model, which are taken to be constants and are estimated from the animal cancer bioassay data. As recommended by US EPA (2012), $q_i \geq 0$ for all i. For example, with four dose groups, the Multistage-Cancer model can have a maximum of four parameters, β, q_1, q_2, and q_3. When dose is expressed in units of mg/kg-day, q_1 is given in units of $(mg/kg\text{-}day)^{-1}$. The q_1 parameter is, for small doses, the ratio of excess lifetime cancer risk to the average daily dose received. The parameter β provides the basis for estimating the background lifetime probability of the tumor (i.e., when dose d is zero, the probability of cancer, p, is equal to β).

The $BMDL_{05}$ (the 95% lower confidence limit of the BMD that corresponds to a 5% increase in tumor incidence above background) is used to estimate the cancer potency in animals, also called the "animal cancer slope factor" or CSF_{animal}. As shown below, the CSF_{animal} is calculated by dividing the BMR of 5%, or 0.05, by the $BMDL_{05}$. The result is typically a value close to the upper 95% confidence bound on the parameter q_1.

$$CSF_{animal} = BMR \div BMDL_{05} = 0.05 \div BMDL_{05}.$$

The Multistage-Cancer model defines the probability of developing a tumor at a single site. For carcinogens that induce tumors at multiple sites and/or in different cell types at the same site in a particular species and sex, US EPA's BMDS can be used to derive maximum likelihood estimates (MLEs) for the parameters of the multisite carcinogenicity model by summing the MLEs for the individual multistage models from the different sites and/or cell types. This multisite model provides a basis for estimating the cancer potency of a chemical that causes tumors at multiple sites (OEHHA, 2009).

Adjusting for Early Mortality in Experimental Animals

When data are available, OEHHA uses the effective number approach to analyze tumor incidence. The incidence is presented as the number of animals with the tumor divided by the number of animals alive at the first occurrence the tumor. For instances where treatment related mortality is observed early (before 85 weeks) in the duration of a cancer bioassay, time to tumor approaches such as a Poly-3 or multistage-in-dose Weibull-in-time (multistage Weibull, MSW) model can be used. Poly-3 adjustment (Bailer and Portier, 1988) is used to adjust the denominator (N) of tumor incidence (see Appendix F for details). Poly-3 adjusted incidences can be assessed with US EPA's BMDS. When a large fraction of animals dies before the end of the study, the MSW model can be used to estimate the cancer potency. The model can fit two types of tumor-related responses, fatal and non-fatal (incidental) tumors. Fatal tumors are tumors that are directly responsible for the death of the animal, whereas incidental tumors are tumors that are observed during necropsy and are incidental to another cause of death.

Adjusting for Experimental Duration

When the total experimental duration is shorter than the natural lifespan of the animals (104 weeks for rats and mice), an adjustment is applied to account for the expected increased incidence of cancer with time. For experiments of duration T_e, rather than the natural lifespan of the animals (T), it is assumed that the lifetime incidence of cancer increases with the third power of age:

$$CSF_{animal,adj} = CSF_{animal} \times (T \div T_e)^3.$$

Adjusting for Human-Animal Differences

In the absence of reliable toxicokinetic information, the human cancer slope factor (CSF_{human}) is estimated by assuming the chemical dose per body weight scaled to the three-quarters power produces the same degree of effect in different species. Under this assumption, the CSF_{animal} is multiplied by the ratio of human to animal body weights raised to the one-fourth power when animal cancer potency is expressed in units of $(mg/kg\text{-}day)^{-1}$:

$$CSF_{(human)} = CSF_{(animal)} \times (\text{body weight}_{(human)} \div \text{body weight}_{(animal)})^{1/4}.$$

When data are available, separate oral and inhalation cancer potencies may be calculated and they are applied to each specific exposure route. Since it is unusual to have a cancer bioassay through dermal exposure, OEHHA generally uses the oral cancer potency for estimating cancer risk through the dermal route. Similarly, when an inhalation cancer potency is not available, the oral cancer potency is used to estimate cancer risk through the inhalation route. If only an inhalation cancer potency is available, then it will be applied to all routes when determining the HPC.

Accounting for Increased Susceptibility During Early-in-Life Exposures

When determining cancer risk, OEHHA applies age sensitivity factors (ASFs, unitless) to account for the increased susceptibility of infants and children to carcinogens (OEHHA, 2009). A weighting factor of 10 is applied for exposures that occur from the 3rd trimester to <2 years of age, and a factor of 3 is applied for exposures that occur from 2 to 16 years of age (Table 1).

These default factors are applied regardless of the mechanism of action, unless chemical-specific data exist to better guide the risk assessment.

Table 1. Duration and age sensitivity factors of different life stages

Life Stage	Fractional Duration[a] (d)	Age Sensitivity Factor (ASF)[b]
3rd Trimester	0.25/70	10
Infant (0-2 yr)	2/70	10
Child (2-16 yr)	14/70	3
Adult (16-70 yr)	54/70	1

[a]An average lifetime of 70 years is assumed for the general population
[b]Age sensitivity factors for different life stages adopted by OEHHA (2009)

ASFs for each life stage are multiplied by the fractional duration (d) of each life stage and the daily water intake (DWI, in L/kg-day or L_{eq}/kg-day if accounting for inhalation and dermal exposures). This generates the ASF-adjusted exposure at each life stage, as shown in Appendix G. The sum of the ASF-adjusted exposures across all life stages is the lifetime exposure value for the chemical.

Derivation of the Health-Protective Concentration

The HPC for carcinogenic effects that addresses the oral, inhalation, and dermal routes of exposure can be calculated using the following equation, which combines the separate calculations for each exposure period (shown in Appendix G) into a single bracket:

$$HPC = \frac{R}{CSF_{oral} \times (\sum_j [ASF_j \times d_j \times DWI^{oral}_j]) + CSF_{inh} \times (\sum_j [ASF_j \times d_j \times DWI^{inh}_j]) + CSF_{derm} \times (\sum_j [ASF_j \times d_j \times DWI^{derm}_j])}$$

where:

R	=	default risk level of one in one million, or 10^{-6}
CSF_{oral}	=	oral cancer slope factor, in $(mg/kg\text{-}day)^{-1}$
CSF_{inh}	=	inhalation cancer slope factor, in $(mg/kg\text{-}day)^{-1}$
CSF_{derm}	=	dermal cancer slope factor, in $(mg/kg\text{-}day)^{-1}$
$\sum_j$	=	sum of contributions at each age range
ASF_j	=	age sensitivity factors for the 3rd trimester + infants, children, and adults
d_j	=	duration of exposure for the 3rd trimester + infant, child, and adult life stages
$DWI^{inh/oral/derm}_j$	=	equivalent water exposure values for each age range.

Water consumption rates and multiroute exposure calculations are described in the noncancer methodology section, and the underlying principles do not change when examining cancer endpoints.

PRODUCTION, USE, AND ENVIRONMENTAL OCCURRENCE

Production and Use

1,4-Dioxane is commonly used as a solvent in several industrial applications, including the manufacturing of adhesives, resins and oils (US EPA, 2013a). Between 10 – 50 million pounds per year of 1,4-dioxane were produced between 1986 and 1990 (US EPA, 2013a). According to US EPA's Chemical Data Reporting database, the total aggregate production volume of 1,4-dioxane was between 0.5 to 1 million pounds total per year in between 2012 and 2015 (US EPA, 2020). Historically, it was primarily used as a stabilizer for 1,1,1-trichloroethane but since 1995, 1,1,1-trichloroethane has been phased out of production in the US. Currently it is used as a solvent for resins, oils, varnishes, adhesives, waxes, lacquers, and greases, and in the manufacturing of insecticides, herbicides, rubber, and plastics (US EPA, 2020).

Environmental Occurrence and Human Exposure

1,4-Dioxane is found in the environment from industrial and commercial production. According to the US EPA's Toxics Release Inventory, in 2023, 397,204 pounds were released into the environment, down from a total of over 1.7 million pounds in 2014 (IRTC, 2021). Of the 2023 total, 9% of 1,4-dioxane was released to surface water, with the majority released to landfills.

Air

Industrial processes that produce 1,4-dioxane cause release of the chemical into the air. Once in the air, it is quickly degraded by photo-oxidation with a reaction half-life of 6.7 hours (US EPA, 2013a). The California Air Resources Board reported almost 19,000 pounds of total 1,4-dioxane emissions in 2021, compared to an estimated 210,000 pounds in 1997 (ATSDR, 2012; CARB, 2023).

Hart et al. (2018) used estimates of 1,4-dioxane concentration in air from US EPA's National Air Toxics Assessment (NATA) models to investigate the impacts of hazardous air pollutants on the risk of invasive breast cancer in the US. The estimated 1,4-dioxane exposure in the study population (which consisted of female nurses from California, Connecticut, Indiana, Iowa, Kentucky, Massachusetts, Michigan, Missouri, New York, North Carolina, Ohio, Pennsylvania, South Carolina and Texas) ranged from 0 to 0.9 microgram per meter cubed ($\mu g/m^3$) with a mean of 2.46×10^{-4} $\mu g/m^3$. A study by Kalkbrenner et al. (2018) also used NATA models to investigate associations of perinatal exposure to air toxicants with autism spectrum disorder. The study estimated a mean exposure concentration of 1.63×10^{-4} $\mu g/m^3$ 1,4-dioxane in the air.

Soil

1,4-Dioxane is released into soil through industrial manufacturing processes or through its uses. Due to its low organic carbon-water partition coefficient (K_{oc}), 1,4-dioxane is not expected to sorb to soil, but readily migrates to ground and surface water (ATSDR, 2012). Levels of 1,4-dioxane in soil have not been measured.

Water

In 2015, 35,402 lbs of 1,4-dioxane were released to surface water from industrial sources in the US (US EPA, 2020). In the last three years, detected levels of 1,4-dioxane in California public

water supply wells ranged from 0.02 to 42 ppb.[4] Studies in California have reported elevated levels of 1,4-dioxane in groundwater in the vicinity of industrial sites. In 1998, levels ≥250,000 ppb were detected in groundwater near a solvent recycling facility in San Jose, California (Mohr, 2004). The groundwater near the Stanford Linear Accelerator Center in Menlo Park, California, had as much as 7,300 ppb 1,4-dioxane (Mohr, 2004). Sampling of groundwater from well fields adjacent to contaminated sites or from known contaminant plumes in California detected 1,4-dioxane in 21% of the samples (13 out of 62), with concentrations ranging from 1.1 to 18 ppb (Draper et al., 2000).

Consumer Products

1,4-Dioxane residue in consumer products is a significant source of human exposure. It can be found as a residual contaminant in paints, coatings, lacquers, coolants, spray foam, household detergents, cosmetic products and textile dyes. The Environmental Working Group (EWG) reported that 22% of 15,000 cosmetic and personal care products tested contained 1,4-dioxane (EWG, 2007). Since the 1980s, 1,4-dioxane concentrations in personal care and cleaning products have been reported in the literature. Doherty et al. (2023) reported detections of 1,4-dioxane in 66% of all products tested, with the highest mean concentrations in shampoo, conditioner and laundry detergent. Lower levels were measured in dish soap, body wash, hand soap, household cleaner and bubble bath while even lower levels were measured in lotion, cream, and body scrub (Doherty et al., 2023). It is also present in adhesives and anti-freeze (NTP, 2021).

Food

Studies measuring 1,4-dixoane in food are limited. However, it has been detected in some food sources such as volatiles from fried chicken, other meats, tomato products, cooked shrimp, adipose tissue in lambs, food additives, and food flavorings (ATSDR, 2012).

PHARMCOKINETICS

Absorption

A study by Young et al. (1977) investigated the kinetics of 1,4-dioxane in humans by exposing four healthy male volunteers to 50 ppm (parts per million) of 1,4-dioxane vapor for 6 hours. Blood and urine analyses were conducted to determine the concentration of 1,4-dioxane and its metabolite, β-hydroxyethoxyacetic acid (HEAA). 1,4-Dioxane was rapidly absorbed and could be detected in the plasma within one hour of exposure. The peak 1,4-dioxane concentration in the plasma occurred at the 6th hour of exposure. After the 6-hour exposure period, plasma concentration decreased linearly with a half-life of 59 ± 7 min. Based on these data, the authors suggested that the pharmacokinetics of 1,4-dioxane can be described with a one-compartment

[4] Data accessed with GeoTracker GAMA on May 30, 2025: https://gamagroundwater.waterboards.ca.gov/gama/gamamap/public/. The data for public water supply wells accessed with GeoTracker GAMA do not indicate whether the source is raw (untreated) water or treated water; therefore, the results in the dataset may not be representative of the water delivered to customers.

model and a first-order rate of elimination. The authors also noted that the exposure concentration of 50 ppm in vapor presumably did not saturate the metabolism of 1,4-dioxane.

In another inhalation study in humans by Göen et al. (2016), three groups of six volunteers were exposed to 20 ppm of 1,4-dioxane in vapor for 8 hours. One exposure group was exposed to 1,4-dioxane at rest, the second and third groups were told to exercise for 10 minutes every hour at different amounts of physical exercise (50 Watts and 75 Watts). At 4 hours in the resting group, 0.98 mg/L of 1,4-dioxane was measured in blood and at 8 hours, 1.1 mg/L was measured. The exercised groups had higher levels at 4 and 8 hours (1.07 and 1.48 mg/L at 4 hours and 1.24 and 1.47 mg/L at 8 hours for the second and third groups, respectively).

There are no studies available investigating the absorption of 1,4-dioxane through oral exposure in humans. Male rats receiving a single dose of 10 – 1,000 mg/kg-day of radiolabeled [^{14}C]-1,4-dioxane by oral gavage absorbed most of the chemical through the gastrointestinal tract, as 75 – 98% of the total radioactivity was detected in the urine at 8 – hour intervals for 24 – 72 hours (Young et al., 1978). Takano et al. (2010) showed that 1,4-dioxane was rapidly absorbed and cleared within 24 hours of oral treatment in rats. Another study in male rats showed that an hour after a single oral dose of 65 mg/kg 1,4-dioxane, maximum blood concentration was reached and it was no longer detectable in the blood after 6 hours (Take et al., 2012).

There are limited studies of dermal absorption of 1,4-dioxane in humans and animals. An in vitro study using excised human skin showed rapid evaporation and low absorption (Bronaugh, 1982). Monkeys exposed to 1,4-dioxane in methanol or in skin lotion for 24 hours had 2.3% and 3.4%, respectively, of the applied dose in the urine and the peak urinary concentration was measured between 0 to 4 hours (Marzulli et al., 1981).

Distribution

Data on the distribution of 1,4-dioxane following inhalation, oral or dermal exposure in humans or animals are limited.

Young et al. (1977) developed a pharmacokinetic model from four male volunteers exposed to 50 ppm of 1,4-dioxane vapor. They calculated the volume of distribution to be 104 ± 30 ml/kg. Woo et al. (1977b) found that distribution of 1,4-dioxane was uniform in liver, kidney, spleen, lung, colon and skeletal tissue in Sprague Dawley rats exposed via intraperitoneal (i.p.) injection of radiolabeled [^{3}H]1,4-dioxane. Organs were collected 1, 2, 6, and 12 hours post-exposure and the specific activity (expressed in nmoles/g wet weight) was similar among the tissues. In a similar study looking at the distribution of [^{14}C]1,4-dioxane in the blood, kidney and brain of rats between 5 minutes to 6 hours after i.p. injection, Mikheev et al. (1990) found that the time to reach maximum concentration of radiolabeled 1,4-dioxane was shorter in the liver and kidney compared to the blood and testes. Concentrations in the tissues were not reported. Distribution of 1,4-dioxane in human tissues has not been studied.

Woo et al. (1977b) also measured the extent of covalent binding of 1,4-dioxane to tissues and subcellular fractions in rats and found greater covalent binding of 1,4-dioxane in liver, colon and spleen compared to skeletal muscle and blood. At the subcellular level, 1,4-dioxane was found to be highest in the cytosol of the liver, followed by whole homogenate, microsomal, mitochondrial and nuclear fractions in rats exposed via i.p injection or drinking water. Pretreatment of rats with inducers of cytochromes P-450 (CYPs) to increase in vivo metabolism

of 1,4-dioxane did not change the amount of covalent binding in the hepatic subcellular fractions (Woo et al., 1977b). Take et al. (2012) found that 1,4-dioxane was distributed to the lungs, liver, brain and abdominal fat after oral, inhalation and combined oral and inhalation administration in rats.

Metabolism

1,4-Dioxane is metabolized to a single predominant metabolite, HEAA, in rats and humans (Young et al., 1976; Young et al., 1977; Göen et al., 2016). Another metabolite, p-dioxane-2-one, has been measured in rats (Woo et al., 1977b; Woo et al., 1977c). However, in a study by the US Army Public Health Command, in blood and urine samples of male and female Sprague Dawley rats exposed to 0, 10, or 1,000 mg/kg of 1,4-dioxane by oral gavage, there was no p-dioxane-2-one present up to 8 hours post-exposure (Eck, 2010). A hypothesized metabolic pathway of 1,4-dioxane is shown in the figure below (Godri Pollitt et al., 2019). 1,4-Dioxane is oxidized by CYPs to diethylene glycol or 1,4-dioxane-2-one, or to 1,4-dioxane-2-ol and β-hydroxyethoxy acetaldehyde to produce HEAA.

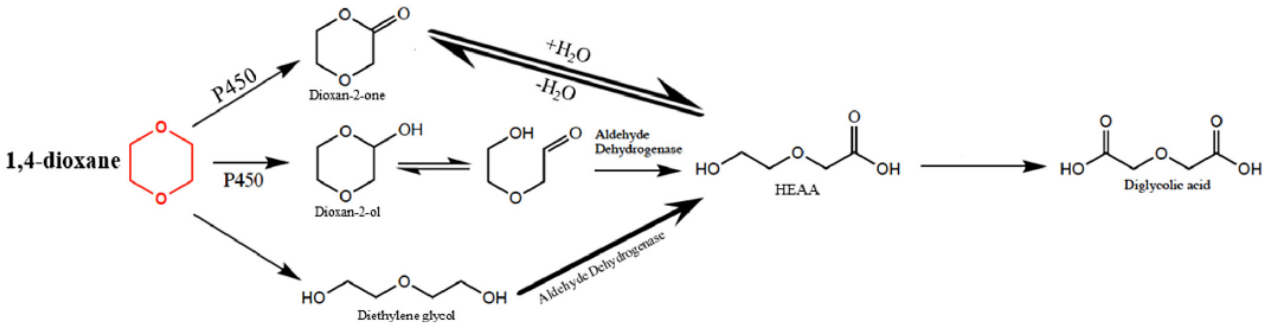


Figure 1. Proposed metabolic pathway of 1,4-dioxane by Woo et al. (1977a) adapted by Godri Pollitt et al. (2019).

Workers had higher urine levels of HEAA compared to levels of 1,4-dioxane when exposed to higher levels of 1,4-dioxane vapor (Young et al., 1976). The peak plasma concentration of HEAA in healthy male volunteers exposed to 1,4-dioxane vapor for 6 hours occurred at 7 hours (1 hour after cessation), after which concentrations decreased linearly but remained higher than concentrations of 1,4-dioxane (Young et al., 1977). In a study by Göen et al. (2016), peak concentrations of HEAA in the urine of humans were detected nearly 10 hours after an 8-hour period of exposure to 1,4-dioxane.

In male Sprague Dawley rats exposed to a single oral dose of 1,4-dioxane, metabolism was saturated at the high dose of 1,000 mg/kg, but was rapid at the low dose of 10 mg/kg (Young et al., 1978). Following intravenous (i.v.) administration in rats, 1,4-dioxane was eliminated linearly at low doses (3 and 10 mg/kg) but slowed down with increasing doses (30 – 5,600 mg/kg) due to metabolic saturation. However, during inhalation exposure, saturation of metabolism does not appear to be evident. In male and female rats exposed to 400 – 3,200 ppm of 1,4-dioxane for 6 hours/day, 5 days/week for 13 weeks, there was a dose-dependent linear increase in plasma concentrations of 1,4-dioxane across all concentrations tested (Kasai et al., 2008).

Although the exact metabolic pathway of 1,4-dioxane is not certain, CYPs are likely involved. In a study by Woo et al. (1977c), male Sprague Dawley rats pretreated with 50 mg/kg of

phenobarbital via i.p. injection four days before i.p. administration of 3,000 mg/kg of 1,4-dioxane had increased metabolite concentrations in the urine compared to control rats receiving 1,4-dioxane and no phenobarbital. The increase in metabolites with CYP inducers such as phenobarbital illustrates the involvement of P450s in 1,4-dioxane metabolism.

Increased metabolism of 1,4-dioxane does not appear to increase toxicity as shown in a study by Nannelli et al. (2005). Pretreatment of male Sprague Dawley rats with CYP2E1 and CYP2B1/2 inducers (fasting or phenobarbital) followed by administration of 2 mg/kg 1,4-dioxane via i.p. injection did not change hepatic glutathione and plasma alanine aminotransferase activity in the liver compared to controls. The authors posited that there was a lack of or poor formation of toxic metabolites of 1,4 dioxane by pretreatment with P450 inducers.

Excretion

1,4-Dioxane is eliminated primarily via the urine as HEAA in humans and in rats. Young et al. (1977) determined the pharmacokinetics of 1,4-dioxane in male volunteers exposed to 50 ppm 1,4-dioxane vapor for six hours. The urinary half-life of elimination of unmetabolized 1,4-dioxane was 48 ± 17 minutes based on the regression of the urinary concentration data after the cessation of exposure. Almost all of the absorbed dose of 1,4-dioxane was eliminated in the urine in the form of HEAA within 24 hours. Within the 6-hour exposure period, 47% of the total HEAA was excreted, while the remaining 53% was excreted between the 6-hour (cessation of exposure) and 24-hour timepoints. The urinary elimination half-life of HEAA was calculated to be 2.7 ± 0.5 hours. The HEAA renal clearance was 121 ml/min, similar to that of creatinine (124 ml/min), leading the authors to suggest that HEAA was cleared by glomerular filtration. In contrast, the renal clearance of unmetabolized 1,4-dioxane was 0.34 ml/min, suggesting poor renal elimination. In rats exposed to a single dose of 1,4-dioxane orally (10, 100 or 1,000 mg/kg), the primary route of excretion was through the urine (Young et al., 1978). Almost all of the total urinary excretion of inhaled 1,4-dioxane at 50 ppm in rats was measured as urinary HEAA. At the end of exposure, the plasma concentration of 1,4-dioxane decreased log-linearly with a half-life of 1.01 ± 0.15 hours (Young et al., 1978).

At high doses where metabolism is saturated, a greater percentage of administered dose is eliminated via expiration. Young et al. (1978) showed that in rats orally exposed to a single dose of 1,4-dioxane (10, 100 or 1,000 mg/kg), as the dose is increased, there was a decrease in the percentage of administered 1,4-dioxane in the urine and an increase in the percentage in expired air. Rats exposed to 30 – 5,600 mg/kg of 1,4-dioxane intravenously displayed a slower rate of elimination than rats exposed to 3 and 10 mg/kg (Young et al. 1978). The metabolic clearance was calculated from the difference between the plasma clearance and the total renal and pulmonary clearance. The metabolic clearance decreased from 2.82 ml/min at the 10 mg/kg dose to 0.17 ml/min at the 1,000 mg/kg dose, indicative of metabolic saturation. Excretion of 1,4-dioxane in expired air and in urine follows a first-order process.

Physiologically-Based Pharmacokinetic Models

Several physiologically-based pharmacokinetic (PBPK) models have been published for 1,4-dioxane and a description of each model is provided below.

Leung and Paustenbach (1990)

This model simulates the pharmacokinetics of 1,4-dioxane in four compartments, consisting of liver, fat, slowly perfused and richly perfused tissues in rats and in humans. The authors used the pharmacokinetic model for styrene by Ramsey and Andersen (1984) as a framework for the 1,4-dioxane model. The model also simulates 1,4-dioxane concentrations in arterial and venous blood and the concentration of the metabolite HEAA in the urine. It simulates inputs from inhalation and oral ingestion via drinking water. For inhalation, 1,4-dioxane input was modeled to equal cardiac output. For oral ingestion, the input was simulated as a zero-order process from the GI to the liver. Partition coefficients of the model were determined by the vial equilibrium technique. Human partition coefficient values were assumed to be equal to rat coefficient values. Human ventilation rate and cardiac output values were calculated using body weight scaling from rat values. Calibration of the model was conducted using data from Young et al. (1977, 1978). The model was not validated with independent data.

Reitz et al. (1990)

This model simulates the pharmacokinetics of 1,4-dioxane in six compartments consisting of liver, lung, fat, venous blood, slowly perfused and richly perfused tissues in rats, mice and humans. The authors also used the model for styrene by Ramsey and Andersen (1984) as a framework for the model. The model simulates concentrations of 1,4-dioxane in tissues, arterial blood, and venous blood, and the formation of the metabolite HEAA. The model was optimized using rat and human data from Young et al. (1977, 1978). While mouse parameters, partition coefficients, flow and cardiac output were assumed to be similar to rat parameters, metabolic rate constants were allometrically scaled from human and rat data. The model simulates inputs from inhalation, i.v. and oral exposure. Partition coefficients for rat and human blood were determined experimentally via vial equilibrium by the authors. Organ volumes used for rats were similar to volumes used in the model by Andersen et al. (1987). The human blood/air partition coefficients were increased by a factor of two to improve the fit with the Young et al. (1977) data. The model was not validated with independent data.

Sweeney et al. (2008)

To fill in data gaps from the previously developed 1,4-dioxane PBPK models (Leung and Paustenbach, 1990; Reitz et al., 1990), Sweeney et al. (2008) conducted studies to determine tissue/ blood partition coefficients (for mice, rats and humans), mouse blood concentrations over time, and metabolic rate constants. The PBPK model structure is similar to the model by Leung and Paustenbach (1990) and Reitz et al. (1990). The model simulates the pharmacokinetics of 1,4-dioxane in four compartments consisting of liver, fat, slowly perfused tissues, and richly perfused tissues in rats, mice and humans, as well as 1,4-dioxane concentrations in venous blood and the formation, and elimination of the metabolite HEAA. Parameters not determined experimentally were taken from kinetic studies by Young et al. (1978), Young et al. (1976) and Young et al. (1977). Tissue volumes and fractional blood flow rate values from Brown et al. (1997) were used. Oral absorption rate in mice was determined experimentally by the authors

and the rat value was assumed to be similar to that of mice. The human oral absorption rate was not determined in humans. The body weight adjusted maximum rate of metabolism (VmaxC) and Michaelis constant (Km) for rats were determined from data by Young et al. (1978). For mice, the Km was assumed to be the same as that of rats while VmaxC values were determined experimentally by the authors. Human VmaxC and Km values were derived from scaling human in vitro hepatocyte data determined by the authors with the ratio of rat optimized VmaxC and scaled VmaxC from rat hepatocytes. In the rat model, simulation of exhaled levels of 1,4-dioxane matched experimental data for 1,000 mg/kg i.v. and gavage experiments but at 10 mg/kg, the model over predicted exhaled levels by a factor of three for i.v. administration and by a factor of five for gavage administration, indicating that the model was better at predicting at high doses than at low doses. The authors validated the models with additional data. Using the rat model, for simulations of inhalation exposure to 50 ppm of 1,4-dioxane for 6 hours, the predicted HEAA urinary concentrations were threefold lower than experimental values while predicted blood 1,4-dioxane concentrations were similar to reported values. In the mouse model, predicted HEAA levels in the urine after an exposure of 20 mg/kg by oral gavage were lower than experimental data. The mouse model also greatly underpredicted HEAA levels in blood up to 1 hour after exposure, and overpredicted blood HEAA levels at later time points (up to 6 hours). Blood levels of 1,4-dioxane were predicted to be indistinguishable from background levels, which is consistent with measured data. In the human model, simulation of inhalation exposure predicted 1,4-dioxane levels in the blood and HEAA levels in the urine were sixfold lower than observed data from human volunteers in the study by Young et al. (1977). The authors also validated their model using occupational worker data in the study by Young et al. (1976) and found that predicted body burden was in agreement with observed values following 1,4-dioxane inhalation exposure. The authors provided no explanation for the different outcomes.

Takano et al. (2010)

This model, which was constructed for rats and humans, has three compartments: liver as the metabolizing compartment, gut as the absorption compartment and general circulation as the central distribution compartment. Human PBPK parameters were estimated from repeated oral exposure of 1,4-dioxane at 500 mg/kg-day for 14 days, a single i.p. injection of 500 mg/kg, and from in vitro studies in rat and human liver microsomes conducted by Takano et al. (2010). Partition coefficients were estimated by in silico analysis. Values of plasma unbound fraction and octanol-water partition coefficient were obtained by in silico estimation using SimCYP and ChemDrawBioUltra software. The model was not validated with independent data.

Use of PBPK models in OEHHA Assessment

The major limitation of the available models for 1,4-dioxane (Leung and Paustenbach, 1990; Reitz et al., 1990; Takano et al., 2010; Sweeney et al., 2008) is that no validation with human oral studies was conducted. While the Sweeney et al. (2008) rat model was validated with external data, the model was not very accurate in simulating exhalation data at lower doses following oral exposure, which suggests that the model does not perform well at dose ranges that would be relevant for assessing risk from chronic exposure in humans. The use of only a single relatively high oral dose in the development of the model by Takano et al. (2010) makes this model unsuitable for low dose prediction and extrapolation required in POD determinations. Additionally, the Sweeney et al. (2008) human model poorly simulated inhalation data from

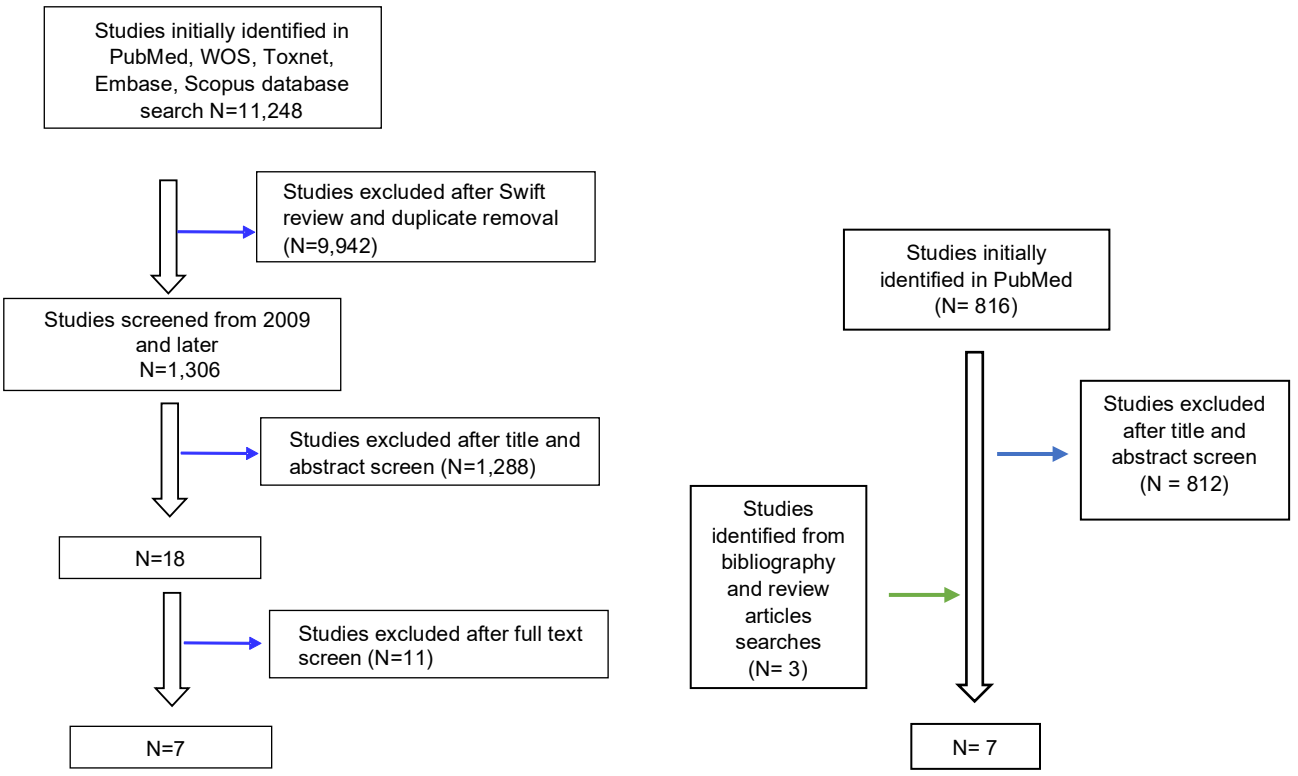
human volunteers exposed to 1,4-dioxane, which raises concerns about its suitability for risk assessment. Overall, there is considerable uncertainty associated with the available PBPK models for 1,4-dioxane.

Due to these limitations, OEHHA did not use the PBPK models for dose-response analysis.

LITERATURE SEARCH AND SCREENING RESULTS

Details of the literature search strategy and screening are briefly summarized here and described in more detail in Appendix A. Two separate literature searches were conducted. One was a general search focused on screening of recent literature for human, animal and supplemental evidence. After de-duplication and using SWIFT Review filters designed to identify human, animal and *in vitro* evidence, 1,306 studies were reviewed at the title and abstract level (Figure 2A). Eighteen studies met the PECO criteria, and seven studies were retained following the full-text screen. A total of 79 studies were tagged as supplemental material. A second search and screening effort to identify human epidemiology studies was also conducted (Figure 2B). The PubMed search generated 816 hits, but relatively few involved epidemiologic studies. The primary reasons for exclusion were that the studies were remediation or biodegradation studies, exposure studies, animal studies, in vitro studies, reviews, or were unrelated to 1,4-dioxane. Excluding case reports, seven epidemiologic studies were identified that were not identified in the general search: four from PubMed and 3 from bibliography or review article searches. Two of the studies involved breast cancer, two involved autism spectrum disorder or related conditions, and three involved other effects.

Figure 2. Literature search: recent studies of 1,4-dioxane and toxicity



A. General search of human, animal and in vitro studies

B. Search of human epidemiology studies only

HUMAN EPIDEMIOLOGY STUDIES

The US Agency for Toxic Substances and Disease Registry (ATSDR) performed a comprehensive review of the human epidemiologic research on 1,4-dioxane toxicity published prior to 2012 (ATSDR, 2012). US EPA materials from the December 2020 TSCA risk evaluation were also reviewed for pertinent citations, but the EPA review focused on studies published after the ATSDR. This research was limited to studies in occupationally exposed workers, clinical studies in mostly healthy volunteers, and case reports involving severe disease and death. No studies examining oral exposures, studies in the general population, or studies in potentially susceptible groups such as children or pregnant women were identified. In the two occupational studies reviewed by ATSDR (2012) there were no increases in deaths, cancer mortality, or biochemical markers of liver, kidney or hematologic disease in workers exposed to airborne concentrations of 1,4-dioxane up to 17 ppm (Buffler et al., 1978; Thiess et al., 1976). Importantly, firm conclusions cannot be made from these two studies given their relatively small sample sizes, limited information on exposure, and incomplete data on potential confounders and co-exposures. ATSDR (2012) also identified case reports of deaths following high inhalation and/or dermal exposures (208-650 ppm of 1,4-dioxane in Johnstone (1959)) for periods of approximately one or two weeks. Here, postmortem examinations revealed severe abnormalities involving the kidneys and liver (Barber, 1934; Johnstone, 1959).

ATSDR identified several clinical trials in which healthy volunteers were exposed to known air concentrations of 1,4-dioxane (20-2,000 ppm) for periods ranging from a few minutes to a few hours. Here, the primary outcomes reported were irritant symptoms involving the eyes and upper respiratory tract. Further details on the methods and results of these particular studies can be found in ATDSR (2012). Based on the findings of one of these clinical trials, ATSDR (2012) established a minimal risk level (MRL) of 2 ppm for acute-duration inhalation exposure (14 days or less). An MRL is an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse non-carcinogenic effects over a specified duration of exposure. This MRL was derived using the no-observed-adverse-effect level (NOAEL) of 20 ppm for eye and respiratory irritation and pulmonary function reported in the study by Ernstgård et al. (2006). In this study, six male and six female volunteers were exposed to 0 or 20 ppm of 1,4-dioxane vapor for two hours. Outcomes assessed included self-reported eye, nose or throat discomfort, breathing difficulty, solvent smell, headache, fatigue, nausea, dizziness, and the "feeling of intoxication." These symptoms were assessed before, during (3, 60, and 118 minutes), and after exposure (20 and 180 minutes). Other adverse outcomes included abnormal spirometry, nasal swelling and eye blinking, and altered serum levels of C-reactive protein (CRP) and interleukin 6. Statistically significant increases were not seen for any of the adverse outcomes included in the study. ATSDR (2012) derived the acute MRL of 2 ppm by dividing the NOAEL of 20 ppm by an uncertainty factor of 10 for human variability. ATSDR (2012) did not identify sufficient information from human studies to derive MRLs for chronic inhalation (i.e., for exposures >365 days) or oral exposure, or to estimate human cancer risks.

Human epidemiologic studies on 1,4-dioxane toxicity published in 2011 or later (i.e., those not included in the 2012 ATSDR report) are described in the following paragraphs. This research was identified by OEHHA using the literature search strategies discussed in Appendix A, as well as through bibliography searches of more recent studies, review articles, and government reports (Bilal and Iqbal, 2019; Godri Pollitt et al., 2019; MDH, 2013; NTP, 2011; SCCS, 2015;

US EPA, 2013a, 2017). All human epidemiologic studies of 1,4-dioxane and adverse human health effects published after January 1, 2011, were included in these searches. Case reports involving high accidental exposures and death, editorials and reviews, and in vitro studies of human cell lines are not reviewed here.

Only a small number of studies were identified in these searches, and most of them had weaknesses that make it difficult to interpret their results. For example, many of these studies were based on samples taken from the general population, where overall 1,4-dioxane exposures may be too low to detect real associations in most epidemiologic studies. In addition, several of these studies used exposure modeling based only on emissions data, weather patterns, and limited residential information (e.g., a single address), and it is unclear whether the exposure estimates generated by these models can be used to accurately estimate individual or long-term exposure. None of the studies using these models provided thorough validation data. Overall, none of the epidemiological studies published in 2011 or later could be used to make firm conclusions regarding 1,4-dioxane toxicity. These studies are reviewed in further detail below.

Breast cancer: Two human studies examined the association between 1,4-dioxane and breast cancer. Neither of these studies identified evidence of an association, but both involved potential weaknesses that could have limited their ability to find true effects. In a large prospective cohort study, Garcia et al. (2015) evaluated the relationship between ambient air concentrations of 24 different pollutants, including 1,4-dixoane, and the risk of incident breast cancer. The study included 112,378 female participants of the California Teachers Study who were followed for an average of 15 years (n=5,676 breast cancer cases). Exposure was assessed by linking estimated annual average ambient air concentrations of each pollutant to the census tract where the participants lived at baseline. These estimates were derived using the US EPA's Assessment System for Population Exposure Nationwide. Further details on this exposure tool can be found in Garcia et al. (2015). Information on age, race/ethnicity, family medical history, age at menarche, age at first pregnancy, breastfeeding, menopause, hormone therapy, physical activity, alcohol use, height, weight, and smoking were obtained from questionnaires given to each participant at baseline and considered in the statistical analyses. Overall, no clear increase in breast cancer risk was seen across increasing quintiles of 1,4-dioxane exposure. The breast cancer hazard ratio (HR) for the highest quintile compared to the lowest quintile was 1.02 (95% confidence interval (CI): 0.94-1.11; p-trend=0.23). The HRs in quintiles 2-4 were also all near 1.0 and not statistically significant. The corresponding HRs for estrogen or progesterone positive or negative cancers were also reportedly not statistically significant, although the actual results were not provided. Clear interactions by menopause status, body mass index (BMI), and hormone therapy use were not seen. Potential weaknesses include errors related to the exposure modeling and the generally low levels of 1,4-dioxane exposure likely experienced by participants of essentially population-based studies like this.

In the second breast cancer study, Hart et al. (2018) linked estimates of ambient air concentrations of 1,4-dioxane to the residential addresses of 109,239 participants of the Nurses' Health Study II. Addresses were available for the years 1989 to 2011. Incident cases of breast cancer (n=3,321) were ascertained using self-reports obtained through biennial questionnaires, as well as through family reports and government death records. Validation data based on pathology records confirmed 99% of the self-reported breast cancer cases. Estimates of 1,4-dioxane exposure were made using US EPA's 2002 National Air Toxics Assessment. Further details on this exposure assessment tool can be found in Hart et al. (2018). Potential confounders considered in the statistical models included age, calendar period, race, family

history of breast cancer, history of benign breast disease, age at menarche, parity, age at first birth, menopausal status, postmenopausal hormone use, oral contraception use, recent mammogram, BMI, smoking, physical activity, diet, alcohol consumption, shift work, marital status, household income, and census tract information on socioeconomic status. Overall, the researchers found no association between 1,4-dioxane and breast cancer risk (p-trend=0.818). Associations were also not seen in analyses stratified by smoking or estrogen receptor status. As in Garcia et al. (2015), errors in exposure modeling or the very narrow range of exposures may have limited the ability of this study to identify true associations.

Autism spectrum disorder (ASD): Two studies evaluated associations between 1,4-dioxane and ASD or ASD-related conditions. While one of these identified a potential association (Kalkbrenner et al., 2018), the other did not (Roberts et al., 2013). Roberts et al. (2013) used information on the children of the participants of the Nurses' Health Study II to examine associations between ASD and perinatal exposure to a large number of potentially hazardous air pollutants including 1,4-dioxane. Cases of ASD and related conditions (e.g., Asperger's syndrome) (n=325) were ascertained through self-reports obtained from the mothers in the study's 2005 questionnaire. Controls (n=22,098) were children whose mothers reported that their children did not have ASD or a related condition. Addresses at the time of birth of each child were linked to estimated ambient air concentrations of 1,4-dioxane and other air toxics using the US EPA's National Air Toxics Assessment (NATA). Further information on the exact methods used to derive these estimates can be found in Roberts et al. (2013). As with the other studies in this section that used similar exposure assessment methods, validation data were not presented. Co-variates considered in the analyses included mother's education level, family income, smoking, year of birth, maternal age, child's sex, and census tract socioeconomic information. Tests of significance were adjusted for false discovery rate using the SAS Multtest procedure. The odds ratio (OR) for comparing children in the highest tertile of 1,4-dioxane to children in the lowest tertile was 1.10 (95% CI: 0.77-1.57; p=0.60). No other results were provided for 1,4-dioxane.

In the other study of ASD, Kalkbrenner et al. (2018) used a case-control design involving participants of the Autism Genetic Resource Exchange (AGRE). One hundred fifty-five different chemical agents, including 1,4-dioxane, were assessed. The AGRE included children with ASD ("cases") and their unaffected siblings ("controls") and involved families living throughout the United States. The home addresses of each family at the time the children were born were linked to estimates of airborne 1,4-dioxane obtained from the US EPA's NATA. Information on race/ethnicity, number of siblings, maternal age, and census tract information on socioeconomic factors were also available. Mixed models with a random effect for family, as well as other methods, were used to try to account for correlated exposures among siblings. In total, 1,101 cases and 346 control children were included. Statistical models were also adjusted for birth year, family exposure levels, as well as census block population density, education level, and median rent. The OR for ASD for each logarithmic increase in 1,4-dioxane was 2.87 (95% CI: 1.43-5.76). Similar ORs were seen in both boys and girls in analyses stratified by sex. 1,4-Dioxane exposure was also found to be associated with higher Social Responsiveness Scale scores, a quantitative measure of autism traits, although this finding was not statistically significant. Elevated ORs were reported for a number of other chemicals although few were greater than 2.87. Information on the shape of the dose-response curve, including analyses based on categories of exposure, were not presented. In addition, no information was given on the presence or impact of potential outliers. Results were adjusted for multiple comparisons using the Benjamini-Hochberg approach. However, given the large number of chemicals assessed, the role of chance cannot be ruled out. Overall, given the conflicting results seen

between this study and Roberts et al. (2013), and that both studies had a number of potential weaknesses (e.g., limited address information, questions regarding the exposure modeling, lack of exposure validation data, and probable low levels of exposure), firm conclusions cannot be made regarding the relationship between 1,4-dioxane and ASD from human epidemiologic studies at this time.

Other outcomes: Niehoff et al. (2019) evaluated the associations between 1,4-dioxane and telomere length in 731 adult female participants randomly selected from the US Sister Study. Twenty-eight other toxic air pollutants were also examined. Annual average air concentrations of 1,4-dioxane were estimated using US EPA's 2005 NATA. These estimates were linked to the addresses the participants lived in at study baseline (2003 – 2009). Telomere length was evaluated in serum samples also collected at baseline. Co-variates considered in the analyses included age, race, residence location type (e.g., urban vs. rural), education, smoking, BMI, and physical activity. Each pollutant was assessed individually. In addition, Classification and Regression Tree methods were used to examine combinations of exposures (further details of this method are provided in Niehoff et al. (2019). In multiple linear regression analysis, 1,4-dioxane was associated with reduced telomere length (regression coefficient=-0.06; 95% CI: -0.13 to 0.00; p-trend=0.06). Clear interactions by BMI or physical activity were not seen. Although shorter telomere length has been linked to a number of overt adverse health conditions, the exact clinical significance of the results reported in this study is unknown. In addition, although not specifically noted by the authors, some of the potential association reported here could be due to a small number of outliers (see Niehoff et al. (2019), Figure 1). Another potential weakness is that the exposure models used in this study, like those in several of the studies discussed above, were only designed to evaluate exposures in ambient air. As such, they may not account for exposures from indoor air, occupational activities, product use, food, or drinking water. In general, if true associations did exist, the errors associated with these models would most likely have biased the results of these studies towards the null.

Finally, Ernstgård et al. (2019) presented several updated analyses using data on 1,4-dioxane from the Ernstgård et al. (2006) study discussed above. The focus of these new analyses were on the association between irritant symptoms and serum biomarkers of inflammation (e.g., CRP). Overall, the presence of irritant symptoms seemed to be associated with a decrease (i.e., "down regulation") in serum CRP levels. However, the data on 1,4-dioxane were pooled with those on several other irritant chemicals, and the specific effects of 1,4-dioxane were not reported. Similarly, Ernstgård and Bottai (2012) used data from Ernstgård et al. (2006), in this case, to investigate whether visual analogue scales (VAS) could be used as an accurate metric for more objective signs and symptoms of irritation. However, the analyses presented in this publication also involved pooling of data from several different agents, and the individual effects of 1,4-dioxane were not reported.

ANIMAL TOXICITY STUDIES

Reviews of available literature on the animal toxicity of 1,4-dioxane have been conducted by other agencies (ATSDR, 2012; IARC, 1999; US EPA, 2013a, 2020). The liver, kidney and lung are the main targets for 1,4-dioxane toxicity in rats, mice and guinea pigs (Argus et al., 1965; Argus et al., 1973; Fairley et al., 1934; Hoch-Ligeti and Argus, 1970; Hoch-Ligeti et al., 1970; Stoner et al., 1986; Stott et al., 1981). OEHHA reviewed key studies from these previous risk

assessments as well as available studies after the 2010 revision of the notification level, which was first established in 1998.

Acute and short-term toxicity studies in animals

No studies describing effects from acute or short-term exposure to 1,4-dioxane were found from 2009 and later. A list of older studies can be found in the risk assessment by US EPA (2013a). Overall, liver and kidney lesions, changes in serum enzyme activity, and nasal and central nervous system effects were observed in these studies. The oral median lethal dose (LD_{50}) of 1,4-dioxane is 5,400 – 7,120 mg/kg in rats, 5,900 mg/kg in mice, and 3,150 – 4,030 mg/kg in guinea pigs (US EPA, 2013a).

Subchronic studies in animals

Subchronic oral exposure to 1,4-dioxane affected the liver and kidney of experimental animals (Fairley et al., 1934; Gi et al., 2018; Kano et al., 2008; Lafranconi et al., 2021; Stoner et al., 1986). US EPA (2013a) previously summarized available subchronic studies. The table below lists available studies from 2009 and later to capture studies published just prior to and after the revision of the notification level in 2010.

Table 2. Summary of subchronic toxicity in animals

Sex/Species	Dose/Route/ Duration of Exposure	Endpoints	NOAEL/LOAEL	Reference
Male Sprague Dawley rats (6/dose)	0,10 or 1,000 mg/kg-day in drinking water for 11 weeks	↑ relative liver weight; ↑ DNA synthesis	NOAEL: 10 mg/kg-day	Stott et al. (1981)
Male and female F344/ DuCrj rats (10/sex/dose)	0, 640, 1,600, 4,000, 10,000 or 25,000 ppm (0, 52, 126, 274, 657, or 1,554 mg/kg-day for males and 0, 83, 185, 427, 756, 1,614 mg/kg-day for females) in drinking water for 13 weeks	Males and females: ↓ body weight; ↑ AST; ↓ plasma glucose level; nuclear enlargement in respiratory and olfactory epithelium of the nasal cavity and epithelium of the trachea; single cell necrosis and centrilobular swelling in the liver; vacuolic change in centrilobular hepatocytes; hydropic change and nuclear enlargement of the proximal tubule of the kidney; vacuolic change in the cerebrum Males only: ↑ red blood cells, hemoglobin, hematocrit, ALT Females only: nuclear enlargement of the bronchial epithelium	NOAEL: 52 mg/kg-day in males for centrilobular swelling of the liver and nuclear enlargement of the respiratory epithelium	Kano et al. (2008)

Sex/Species	Dose/Route/ Duration of Exposure	Endpoints	NOAEL/LOAEL	Reference
Male and female Crj:BDF1 mice (10/sex/dose)	0, 640, 1,600, 4,000, 10,000 or 25,000 ppm (0, 86, 231, 585, 882, 1,570 mg/kg-day for males and 0, 170, 387, 898, 1,620, 2,669 mg/kg-day for females) in drinking water for 13 weeks	Males and females: ↑ AST and ALT; ↓ plasma glucose level; nuclear enlargement of the trachea, bronchial epithelium, and olfactory epithelium of the nasal cavity; vacuolic change of the olfactory nerve; degeneration of the bronchial epithelium; single cell necrosis and centrilobular swelling of the liver Males only: ↓ body weight; ↑ red blood cells, hemoglobin, and hematocrit Females only: nuclear enlargement of the respiratory epithelium of the nasal cavity	NOAEL: 170 mg/kg-day in females for nuclear enlargement of the bronchial epithelium	Kano et al. (2008)
Female B6D2F1/Crl mice (10/dose)	0, 40, 200, 600, 2,000, or 6,000 ppm (0, 7.2, 37.3, 116, 364, or 979 mg/kg-day) in drinking water for 7, 28, or 90 days	↑ apoptosis in the liver (28 and 90 days); centrilobular hypertrophy in the liver (28 and 90 days); hepatocellular proliferation (90 days)	NOAEL: 116 mg/kg-day for ↑ relative liver weight at 28 and 90 days	Lafranconi et al. (2021)

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; NOAEL, no observed adverse effect level.

Genetic toxicity

Studies investigating the genotoxicity of 1,4-dioxane are summarized in Tables 3 and 4. OEHHA identified only a few studies published after 2009. Therefore, older studies were also included in Tables 3 and 4 to provide more information on the genotoxicity of 1,4-dioxane. In vivo genetic toxicity studies show mixed results. The majority of in vitro studies indicate that 1,4-dioxane is not genotoxic, as shown in Table 4. In the risk evaluation by US EPA (2013a), the authors concluded that 1,4-dioxane is nongenotoxic or weakly genotoxic based on results from in vitro studies. However, in a recent risk assessment, US EPA (2020) concluded that 1,4-dioxane is genotoxic in vivo at high doses based on bone marrow micronucleus assays, which are included in Table 3.

Table 3. Summary of in vivo genetic toxicity studies of 1,4-dioxane

Assay	Sex/Species	Dose/Route of Exposure/ Duration	Results	Reference
DNA alkylation in hepatocytes	Male Sprague Dawley rats (4 – 6/dose)	0 or 1,000 mg/kg by oral gavage	-	Stott et al. (1981)
DNA repair in hepatocytes	Male Sprague Dawley rats (6/dose)	0 or 1,000 mg/kg by oral gavage	-	Stott et al. (1981)
DNA repair in hepatocytes	Male F344 rats (3/dose)	0 or 1,000 mg/kg by oral gavage at 2 and 12 hours before sacrifice 0 or 1,389 mg/kg-day for 2 weeks or 2,778 mg/kg-day in drinking water for 1 week	-	Goldsworthy et al. (1991)
DNA repair in hepatocytes	Male guanine phosphoribosyl transferase (gpt) delta transgenic F344 rats (7 or 8/dose)	0, 200, 1,000, or 5,000 ppm (0, 18.7, 92.3, or 440.2 mg/kg-day) in drinking water for 16 weeks	+ (440.2 mg/kg-day)	Gi et al. (2018)
DNA repair in nasal epithelial cells	Male F344 rats (3/dose)	0 or 1,389[a] mg/kg-day in drinking water for 8 days followed by 0, 10, 100, or 1,000 mg/kg by a single oral gavage	-	Goldsworthy et al. (1991)
DNA single strand breaks	Female Sprague Dawley rats (3 – 5/dose)	0, 168, 840, 2,550, or 4,200 mg/kg by oral gavage at 4 and 21 hours before sacrifice	+ (≥2,550 mg/kg)	Kitchin and Brown (1990)
DNA adduct formation in hepatocytes	Male F344 rats (30/dose)	0, 20, 200, or 5,000 ppm (0, 2.2, 21.9, or 562.4[b] mg/kg-day) in drinking water for 16 weeks	+ (≥21.9 mg/kg-day)	Totsuka et al. (2020)
DNA double strand breaks	Female BDF1 mice (4 – 6/dose)	0, 9.6, 99, or 1,030 mg/kg-day	+ (1,030 mg/kg-day at 1 week, 927	Charkoftaki et al. (2021)

Assay	Sex/Species	Dose/Route of Exposure/ Duration	Results	Reference
and DNA repair in hepatocytes		in drinking water for 1 week 0, 9.3, 96, or 927 mg/kg-day in drinking water for 4 weeks	mg/kg-day at 4 weeks)	
DNA mutation frequency	Male guanine phosphoribosyl transferase (gpt) delta transgenic F344 rats (7 or 8/dose)	0, 200, 1,000, or 5,000 ppm (0, 18.7, 92.3, or 440.2 mg/kg-day) in drinking water for 16 weeks	+ (440.2 mg/kg-day)	Gi et al. (2018)
DNA mutation frequency	Male guanine phosphoribosyl transferase (gpt) delta transgenic F344 rats (5/dose)(	0, 0.2, 2, or 20 ppm (0, 0.02, 0.2, or 1.9 mg/kg-day) in drinking water for 16 weeks	-	Gi et al. (2018)
Micronucleus formation in hepatocytes	Male F344/DuCrlCrlj rats (4/dose)	0, 1,000, 2,000, or 3,000 mg/kg by a single oral gavage or daily oral gavage for two days	+ (≥1,000 mg/kg)	Itoh and Hattori (2019)
Micronucleus formation in bone marrow	Male F344/DuCrlCrlj rats (5/dose)	0, 1,000, 2,000, or 3,000 mg/kg by a single oral gavage	-	Itoh and Hattori (2019)
Micronucleus formation in bone marrow	Male B6C3F1 mice (5/dose)	0, 2,000, 3,000, or 4,000 mg/kg by a single or three daily i.p. injections	-	McFee et al. (1994)
Micronucleus formation in bone marrow	Male and female C57BL6 mice (10 or 4 males; 10 or 5 females/ dose)	0, 900, 1,800, or 3,600 mg/kg for males; 0 or 5,000 mg/kg for females by oral gavage at 24 or 48 hours before sacrifice	+ (≥900 mg/kg in males and 5,000 mg/kg in females)	Mirkova (1994)
Micronucleus formation in bone marrow	Male BALB/c mice (6/dose)	0 or 5,000 mg/kg by oral gavage at 24 hours before sacrifice	-	Mirkova (1994)

Assay	Sex/Species	Dose/Route of Exposure/ Duration	Results	Reference
Micronucleus formation in bone marrow	Male CBA and C57BL6 mice (4 – 8/dose)	0 or 1,800 mg/kg in CBA mice and 0 or 3,600 mg/kg in C57BL6 mice by a single oral gavage	-[c]	Tinwell and Ashby (1994)
Micronucleus formation in bone marrow	Male CD1 mice (5/dose)	0, 1,500, 2,500 or 3,500 mg/kg-day by oral gavage for 5 days	+ (≥1,500 mg/kg)	Roy et al. (2005)
Micronucleus formation in hepatocytes	Male CD1 mice (5/dose)	0, 1,000, 2,000, or 3,000 mg/kg by a single oral gavage	+ (≥2,000 mg/kg)	Morita and Hayashi (1998)
Micronucleus formation in hepatocytes	Male CD1 mice (5/dose)	0, 1,500, 2,500 or 3,500 mg/kg-day by oral gavage for 5 days	+ (≥2,500 mg/kg)	Roy et al. (2005)
Micronucleus formation in peripheral blood	Male CD1 mice (5/dose)	0, 1,000, 2,000, or 3,000 mg/kg by a single oral gavage	-	Morita and Hayashi (1998)

[a] Dose calculated by OEHHA based on default body weight value of 0.18 kg from US EPA (1988) and drinking water consumption of 0.025 L/day reported by the authors.
[b] Dose conversion in the Totsuka et al. (2020) study was extracted from Gi et al. (2018) since liver tissue for DNA adductome analysis was taken from animals from Gi et al. (2018).
[c] A positive response was observed for one 1,800 mg/kg replicate. Negative response was observed for one 1,800 mg/kg replicate and one 3,600 mg/kg replicate.

Table 4 summarizes in vitro assays incubated with and without S9 (crude liver extract containing metabolic enzymes to simulate in vivo metabolism). Results were generally negative regardless of the presence of S9. However, in vitro systems may not be able to reliably detect the mutagenic potential of metabolites of 1,4-dioxane because they may or may not capture the complete metabolism of 1,4-dioxane. For example, Sweeney et al. (2008) did not detect HEAA when rat liver microsomes were incubated with 1,4-dioxane, but incubations with hepatocytes resulted in measurable HEAA amounts. In contrast, Takano et al. (2010) detected 1,4-dioxane metabolites in rat and human liver microsomes.

Table 4. Summary of in vitro genetic toxicity studies of 1,4-dioxane

Assay	Result Without S9	Result With S9	Concentration	Reference
Reverse mutation in *S. typhimurium* TA98, TA100, TA1535, TA1537, TA 1538	-	-	0 – 103 mg/plate	Stott et al. (1981)
Reverse mutation in *S. typhimurium* TA98, TA100, TA1535, TA1537	-	-	0 – 0.010 mg/plate	Haworth et al. (1983)
Reverse mutation in *S. typhimurium* TA100, TA1535	-	-	0 – 103 mg/plate	Nestmann et al. (1984)
Reverse mutation in *S. typhimurium* TA98, TA100, TA1530, TA1535, TA1537	-	-	Not reported	Khudoley et al. (1987)
Reverse mutation in *S. typhimurium* TA98, TA100, TA1535, TA1537	-	-	0 – 5 mg/plate	Morita and Hayashi (1998)
Reverse mutation in *E. coli* WP2 and WP2uvrA	-	-	0 – 5 mg/plate	Morita and Hayashi (1998)
DNA repair in *E. coli* K-12 uvrB/recA	-	-	0 or 1,150 mM	Hellmér and Bolcsfoldi (1992)
DNA single stand breaks in rat hepatocytes	+ (>0.3 mM)	Not determined	0, 0.03 – 30 mM	Sina et al. (1983)
DNA repair in rat primary hepatocytes F344	-	Not determined	0 – 1 mM	Goldsworthy et al. (1991)
Forward mutation assay in L5178Y mouse lymphoma cells	-	-	0, 0.05 – 1 mg/mL	McGregor et al. (1991)

Assay	Result Without S9	Result With S9	Concentration	Reference
Forward mutation assay in L5178Y mouse lymphoma cells	-	-	0 – 5mg/mL	Morita and Hayashi (1998)
Cell transformation in BALB/3T3 cells	+ (2 mg/mL)	Not determined	0, 0.25 – 2 mg/mL	Sheu et al. (1988)
Chromosomal aberration in CHO cells	-	-	0,1.05 – 10.52 mg/mL	Galloway et al. (1987)
Chromosomal aberration in CHO cells	-	-	0 – 5 mg/mL	Morita and Hayashi (1998)
Sister chromatid exchange in CHO cells	+ (10.52 mg/mL)	-	0,1.05 – 10.52 mg/mL	Galloway et al. (1987)
Sister chromatid exchange in CHO cells	-	-	0 – 5 mg/mL	Morita and Hayashi (1998)
Micronucleus formation in CHO cells	-	-	0 – 5 mg/mL	Morita and Hayashi (1998)

CHO, Chinese hamster ovary; S9, crude liver extract containing metabolic enzymes

Reproductive and developmental toxicity studies in animals

Only one reproductive/developmental toxicity study in animals was identified for 1,4-dioxane. Giavini et al. (1985) exposed female Sprague-Dawley rats to 0, 0.25, 0.5 or 1.0 ml/kg-day (0, 258.4, 516.9 or 1,033.7 mg/kg-day using a density value of 1.0337 g/ml) 1,4-dioxane by oral gavage on gestation days (GD) 6 – 15. Animals were sacrificed on GD 21. Weight gain was slightly reduced in dams at the high dose but was not statistically significant. There was a significant decrease in mean fetal weight at the high dose. There was also a significant decrease in ossification of the sternebrae at the high dose. There were no significant differences in corpora lutea, implantation numbers, live fetuses, resorptions and dead fetuses, pre- and postimplantation loss, or dams with resorptions. The authors noted that the decreased fetal weight at the high dose was an indication of fetal retardation and concluded that this was the result of maternal toxicity.

No histopathological changes were reported in the reproductive organs of male and female rats and mice exposed to 1,4-dioxane in subchronic and chronic studies (US EPA, 2015).

Chronic studies in animals

The chronic toxicity studies available for 1,4-dioxane were primarily conducted to evaluate the carcinogenic effects of this chemical. Table 5 summarizes the noncancer effects observed in the carcinogenicity studies of 1,4-dioxane. Similar to the effects seen in short-term and subchronic oral studies, key target organs of 1,4-dioxane chronic toxicity are the liver, kidney and nasal cavity. There were no chronic studies identified from 2009 onwards in the literature search, and therefore studies before the literature search cutoff date are presented.

Table 5. Summary of chronic animal studies of 1,4-dioxane

Sex/Species	Dose/ Route/ Duration of Exposure	Endpoints	NOAEL/LOAEL	Reference
Male Wistar rats (9 in control, 26 in dosed)	0, 1% v/v (0, 649[a] mg/kg-day) in drinking water for 63 weeks[b]	Glomerular nephritis; severe interstitial mononuclear infiltration in kidney arteries; epithelial proliferation in the glomeruli	LOAEL: 649 mg/kg-day	Argus et al. (1965)
Male Sprague Dawley rats (28 – 32/dose)	0, 0.75, 1, 1.4, 1.8% (0, 430, 573, 803, or 1,032 mg/kg-day)[c] in drinking water for 13 months	↑ lipid droplets in hepatocytes	NOAEL: 430 mg/kg-day	Argus et al. (1973)
Male and female Osborne-Mendel rats (35/dose)	0, 0.5, or 1% (0, 593, or 1,187 mg/kg-day for males; 0, 643, or 1,285 mg/kg-day for females) in drinking water for 42 weeks	Males and females: lung lesions	LOAEL: 593 mg/kg-day in males	King et al. (1973)
Male and female Sherman rats (60/sex/dose)	0, 0.01, 0.1 or 1% v/v (0, 9.6, 94, or 1,015 mg/kg-day for males; 0, 19, 148, or 1,599 mg/kg-day for females[d]) in drinking water for up to 716 days	Males and females: ↓ survival; ↓ body weight; ↑ absolute and relative liver weight; renal tubular epithelial degeneration and necrosis; hepatocellular degeneration and necrosis; liver hyperplastic nodule formation	NOAEL: 9.6 mg/kg-day for histopathological changes in kidney and liver in males	Kociba et al. (1974)

Sex/Species	Dose/ Route/ Duration of Exposure	Endpoints	NOAEL/LOAEL	Reference
Male and female Osborne-Mendel rats (35/sex/dose)	0, 0.5, or 1.0% v/v (0, 240, or 530 mg/kg-day for males; 0, 350, or 640 mg/kg-day for females) in drinking water for 110 – 117 weeks	Males and females: ↓ survival; renal tubular degeneration; ↓ mean body weight; nasal turbinate rhinitis (acute suppurative inflammation) Males: ↑ mean body weight (low dose only); gastric ulcer; chronic inflammation in spleen (low dose only); spleen hemosiderosis Females: hepatocellular hyperplasia	LOAEL: 240 mg/kg-day for renal tubular degeneration and acute suppurative inflammation in nasal turbinates in males	NCI (1978)
Male and female B6C3F1 mice (50/dose/sex)	0, 0.5, or 1.0% v/v (0, 720, or 830 mg/kg-day for males; 0, 380, or 860 mg/kg-day for females) in drinking water for 90 – 93 weeks	Males: ↑ mean body weight (high dose); lung inflammation Females: ↑ mean body weight (low dose only); ↓ mean body weight (high dose); lung inflammation; rhinitis (combined acute inflammation and acute suppurative inflammation in nasal turbinate); hepatocellular hyperplasia (low dose only); uterine endometrial cyst (low dose only)	LOAEL: 380 mg/kg-day for rhinitis and lung inflammation (pneumonia) in females	NCI (1978)
Male and female F344/DuCrj rats (50/dose/sex)	0, 200, 1,000, or 5,000 ppm (0, 11, 55, or 274 mg/kg-day for males; 0, 18, 83, or 429 mg/kg-day for females) in drinking water for 2 years	Males and females: ↓ survival; ↓ terminal body weight; ↑ absolute and relative liver weight; ↑ nuclear enlargement of the respiratory epithelium and olfactory epithelium; ↑ basophilic cell foci and mixed cell foci in the liver; squamous cell metaplasia of the respiratory epithelium	NOAEL: 11 mg/kg-day for increased absolute and relative liver weight in males	Kano et al. (2009); JBRC (1998)
Male and female Crj:BDF$_1$ mice (50/dose/sex)	0, 500, 2,000, or 8,000 ppm (0, 49, 191, or 677 mg/kg-day for males; 0, 66, 278, or 964 mg/kg-day for females) in drinking water for 2 years	Males and females: ↓ terminal body weight; ↑ nuclear enlargement of the respiratory epithelium and olfactory epithelium Females: ↓ survival; ↓ food and water consumption	NOAEL: 49 mg/kg-day for decreased terminal body weight in males	Kano et al. (2009); JBRC (1998)

Sex/Species	Dose/ Route/ Duration of Exposure	Endpoints	NOAEL/LOAEL	Reference
Male F344/DuCrj rats (50/dose)	0, 50, 250, or 1,250 ppm (v/v) vapor (0, 30.11, 150.55, or 752.76 mg/kg-day) for 6 hours/day, 5 days/week for 2 years (whole body exposure)	↓ terminal body weight; ↓ hemoglobin, MCV, MCH, and urinary pH; ↑ AST, ALT, ALP, and ɣ-GTP	NOAEL: 150 mg/kg-day for changes in hematology, blood chemistry and urinalysis	Kasai et al. (2009)
Male guanine phosphor-ribosyl transferase (gpt) delta transgenic F344 rats (7 or 8/dose)	0, 200, 1,000, or 5,000 ppm (0, 18.7, 92.3, or 440.2 mg/kg-day) in drinking water for 16 weeks	↓ terminal body weight	NOAEL: 92.3 mg/kg-day	Gi et al. (2018)
Male wild-type F344 rats (30/dose)	0, 2, 20, 200, 2,000, or 5,000 ppm (0, 0.2, 2.2, 21.9, 222.2, or 562.4 mg/kg-day) in drinking water for 16 weeks	↓ body weight	NOAEL: 222.2 mg/kg-day	Gi et al. (2018)

[a] Estimated by OEHHA using the male Wistar rat reference chronic body weight of 0.462 kg from (US EPA, 1988) and drinking water intake rate of 0.03 L/day reported by the authors.
[b] Authors indicated that the study duration was 63 weeks; however hepatic tumors were reported for animals sacrificed at 64 weeks (448 days) and 65 weeks (455 days).
[c] Estimated by OEHHA based on water consumption reported by the authors and default values for body weight (US EPA, 1988).
[d] Daily dose values were calculated from measured daily water consumption rates on days 114 to 198.

Argus et al. (1965) exposed male Wistar rats to 0 or 1% v/v (0 or 649 mg/kg-day) 1,4-dioxane in drinking water for approximately 63 weeks (the exact timing was unclear in the report). Rats treated with 1,4-dioxane showed effects in the kidneys, mainly glomerular nephritis, severe interstitial mononuclear infiltration in the arteries, and epithelial proliferation in the glomeruli.

Argus et al. (1973) exposed male Sprague Dawley rats to 0, 0.75, 1, 1.4, or 1.8% v/v (0, 430, 573, 803, or 1,032 mg/kg-day) 1,4-dioxane in drinking water for 13 months. Progression of liver tumors was studied, and effects observed in treated rats included increased lipid droplets, decreased glycogen, and increased smooth endoplasmic reticulum.

King et al. (1973) reported lung lesions in male and female Osborne-Mendel rats exposed to 0, 0.5 or 1% 1,4-dioxane in drinking water for 42 weeks. Specific descriptions of the lesions were not included in the report. It is unclear whether the lesions were related to the reported increased incidences of chronic bronchopneumonia in treated animals compared to controls. Lung lesions were not evaluated in treated mice.

Kociba et al. (1974) exposed male and female 6–8-week-old Sherman rats to 0, 0.01, 0.1 or 1% v/v (0, 9.6, 94, or 1,015 mg/kg-day for males and 0, 19, 148, or 1,599 mg/kg-day for females) 1,4-dioxane in drinking water for up to 716 days. Male and female rats at the high dose had significantly increased mortality in the first four months of the study. Rats in the high dose also had significantly decreased body weight and increased absolute and relative liver weight compared to controls. Measurements of hematological endpoints (packed cell volumes, total erythrocyte count, hemoglobin, and total and differential white blood cell counts) were not presented, although the authors noted they were not significantly different between control and treated groups. Upon histopathological examination, the authors noted renal tubular degeneration and necrosis, and hepatocellular degeneration, necrosis, and liver hyperplastic nodule formation in the mid- and high-dose groups.

The National Cancer Institute (NCI, 1978) exposed male and female Osborne-Mendel rats to 0, 0.5 or 1% v/v (0, 240, or 530 mg/kg-day for males and 0, 350, or 640 mg/kg-day for females) 1,4-dioxane in drinking water for 110 – 117 weeks. Mean body weights of the high dose males and females were lower than the control group, while the males in the low dose group had higher mean body weights than controls. The authors found a significant dose-dependent trend in mortality for either sex. Results showed that exposure to 1,4-dioxane caused the following: tubular degeneration in the kidney, decreases in mean body weight, and acute suppurative inflammation in the nasal turbinate of males and females; stomach ulcers, chronic inflammation of the spleen, and spleen hemosiderosis in males; and hepatocellular hyperplasia in females.

NCI (1978) also exposed male and female B6C3F1 mice to 0, 0.5 or 1% v/v (0, 720, or 830 mg/kg-day for males and 0, 380, or 860 mg/kg-day for females) 1,4-dioxane in drinking water for 90 – 93 weeks. Mean body weights in high dose males and low dose females were higher than controls, whereas high dose females had lower mean body weights than control animals. A significant trend in mortality was observed in females at all doses. The authors noted that there was a significant increase in lung inflammation in both males and females at all doses. In females, there was significant combined acute inflammation and acute suppurative inflammation in the nasal turbinate, and rhinitis. Additionally, hepatocellular hyperplasia and uterine endometrial cysts were observed only in the low dose group females.

Kano et al. (2009) and JBRC (1998) exposed male and female F344/DuCrj rats to 0, 200, 1,000 or 5,000 ppm (0, 11, 55, or 274 mg/kg-day for males and 0, 18, 83, or 429 mg/kg-day for females) 1,4-dioxane in drinking water for 2 years. There was significant mortality in the high dose male and female groups attributed to tumors. The terminal body weight was significantly decreased in both male and female high-dose groups. Absolute and relative liver weight were significantly increased in males in the mid- and high-dose groups. Absolute and relative liver weight were increased in females in the high-dose group. Animals in the high dose group also displayed increased incidences of nuclear enlargement and squamous cell metaplasia of the respiratory epithelium, and nuclear enlargement of the olfactory epithelium, compared to controls. Glutathione S-transferase placental form (GST-P) positive hepatocellular foci were

observed at the high dose for both sexes. An increase is GST-P positive hepatocellular foci has been shown to be predictive of hepatocarcinogenicity (Ito et al., 2000).

Kano et al. (2009) and JBRC (1998) also exposed male and female Crj:BDF_1 mice to 0, 500, 2,000 or 8,000 ppm (0, 49, 191, or 677 mg/kg-day for males and 0, 66, 278, or 964 mg/kg-day for females) 1,4-dioxane in drinking water for 2 years. There was significant mortality in the mid- and high-dose female groups that was attributed to hepatic tumors. Terminal body weight was significantly decreased in the mid- and high-dose groups.

Kasai et al. (2009) exposed male F344/CuCrj (SPF) rats to 0 (clean air), 50, 250, or 1,250 ppm (v/v) 1,4-dioxane vapor for 6 hours/day, 5 days/week (equivalent to 0, 30.11, 150.55, or 752.76 mg/kg-day) for 2 years. Significant differences in effects such as decreased body weight, and changes in hematology and blood biochemistry markers were observed only in high-dose animals.

Cancer studies in animals

A summary of cancer studies in animals reviewed by OEHHA is listed in Table 6. There were no cancer studies identified from 2009 onwards in the literature search. Therefore, studies before the literature search cutoff date are presented. 1,4-Dioxane causes tumors in multiple organs including liver, nasal cavity, mammary gland, subcutis and peritoneum (Argus et al., 1965; Argus et al., 1973; Kano et al., 2009; Kociba et al., 1974; NCI, 1978; JBRC, 1998; Kasai et al., 2009).

Several agencies have identified 1,4-dioxane as a carcinogen or potential carcinogen to humans. In 1988, 1,4-dioxane was listed as a carcinogen under California's Proposition 65 by the state's Carcinogen Identification Committee[5]. The International Agency for Research on Cancer (IARC) classified 1,4-dioxane as "possibly carcinogenic to humans (Group 2B)" based on inadequate evidence in humans and sufficient evidence in animals for carcinogenicity (IARC (1999). US EPA (2013a) classified 1,4-dioxane as "likely to be carcinogenic to humans" based on evidence in animals (including hepatic tumors in multiple species and strains, as well as peritoneal mesotheliomas, mammary gland, and nasal tumors) and inadequate evidence in humans. The National Toxicology Program (NTP) classified 1,4-dioxane as "reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals" (NTP, 2021).

Table 6. Summary of carcinogenicity studies of 1,4-dioxane

Sex/ Species	Dose/ Route/ Duration of Exposure	Tumor Incidence	Reference
Male Wistar rats (9 in control, 26 in dosed)	0 or 1% v/v (0 or 649 mg/rat-day) in drinking water for 63[a] weeks	Liver tumors:[b] 1/9, 7/26	Argus et al. (1965)

[5] OEHHA Proposition 65 List: https://oehha.ca.gov/proposition-65/proposition-65-list

Sex/ Species	Dose/ Route/ Duration of Exposure	Tumor Incidence	Reference
Male Sprague Dawley rats (30/dose)	0, 0.75, 1, 1.4, or 1.8% (0, 430, 573, 803, or 1,032 mg/kg-day)[c] in drinking water for 13 months	Nasal tumors: 0/30, 1/30, 1/30, 2/30, 2/30	Hoch-Ligeti et al. (1970)
Male Sprague-Dawley rats (28-32/dose)	0, 0.75, 1, 1.4, or 1.8% (0, 430, 573, 803, or 1,032 mg/kg-day)[c] in drinking water for 13 months	Hepatocellular adenomas (incipient tumors, starting at the lowest dose): 4/31, ₸ 9/32, 13/30, 11/28[d,*] Hepatocellular carcinomas: 0/31,₸ 0/32, 3/30, 12/28 [d**] Combined hepatocellular adenomas (incipient tumors) and carcinomas (starting at the lowest dose): 4/31,₸ 9/32, 16/30,** 23/28[d**]	Argus et al. (1973)
Male and female Swiss-Webster mice (30/sex/dose)	Initiated with 50 µg of DMBA; 0 or 0.2 ml[e] on shaved skin on the backs of mice 3 times a week for 78 weeks	<u>Skin tumors</u> Males: Papillomas: 2/4 Suspected carcinomas: 3/4 Subcutaneous tumors: 2/4 Females: Papillomas: 2/5 Suspected carcinomas: 3/5 Spleen tumors: 3/15 Kidney tumors: 3/15 Lung tumors: 9/15	King et al. (1973)
Male and female Sherman rats (60/sex/dose)	0, 0.01, 0.1 or 1% v/v (0, 9.6, 94, or 1,015 mg/kg-day for males; 0, 19, 148, or 1,599 mg/kg-day for females) in drinking water for 2 years	Males and females: All hepatic tumors: 2/106,₸ 0/110, 1/106, 12/66* Hepatocellular carcinomas: 1/106,₸ 0/110, 1/106, 10/66* Nasal carcinomas: 0/106,₸ 0/110, 0/106, 3/66	Kociba et al. (1974)

Sex/ Species	Dose/ Route/ Duration of Exposure	Tumor Incidence	Reference
Male and female Osborne-Mendel rats (35/sex/dose)	0, 0.5, or 1.0% v/v (0, 240, or 530 mg/kg-day for males; 0, 350, or 640 mg/kg-day for females) in drinking water for 110 – 117 weeks	Males: Nasal cavity squamous cell carcinoma: 0/34,[Ŧ] 12/26, 16/33* Nasal cavity adenocarcinoma: 0/31,[Ŧ] 0/12, 3/18* Nasal cavity rhabdomyoma: 0/31,[Ŧ] 1/16, 0/22 Nasal cavity squamous cell carcinoma, adenocarcinoma, or rhabdomyoma: 0/34,[Ŧ] 13/26,* 19/33* Hepatocellular adenoma: 1/16, 1/32, 1/31 Hepatocellular carcinoma: 0/16, 1/32, 0/31 Hepatocellular adenoma or carcinoma: 1/16, 2/32,1/31 Mesothelioma of the tunica vagilis: 2/31, 4/15, 5/22 Females: Nasal cavity squamous cell carcinoma: 0/34,[Ŧ] 10/28,* 8/23* Nasal cavity adenocarcinoma: 0/33, 1/26, 1/22 Nasal cavity squamous cell carcinoma or adenocarcinoma: 0/34,[Ŧ] 11/28,* 9/23* Hepatocellular adenoma: 0/33,[Ŧ] 10/24,* 11/22* Hepatocellular carcinoma: 0/28,[Ŧ] 0/3, 1/2 Hepatocellular adenoma or carcinoma: 0/33,[Ŧ] 10/24,* 12/22*	NCI (1978)
Male and female B6C3F1 mice (50/sex/dose)	0, 0.5, or 1.0% v/v (0, 720, or 830 mg/kg-day for males; 0, 380, or 860 mg/kg-day for females) in drinking water for 90 – 93 weeks	Males: Hepatocellular adenoma: 4/48, 1/47, 4/45 Hepatocellular carcinoma: 4/29,[Ŧ] 18/49,* 24/50* Hepatocellular adenoma or carcinoma: 8/29,[Ŧ] 19/49,* 28/50* Females: Hepatocellular adenoma: 0/49,[Ŧ] 9/47,* 6/42* Hepatocellular carcinoma: 0/48,[Ŧ] 12/47,* 29/41* Hepatocellular adenoma or carcinoma: 0/49,[Ŧ] 21/47,* 35/42*	NCI (1978)

Sex/ Species	Dose/ Route/ Duration of Exposure	Tumor Incidence	Reference
Male and female F344/DuCrj rats (50/dose/sex)	0, 200, 1,000, or 5,000 ppm (0, 11, 55, or 274 mg/kg-day for males; 0, 18, 83, or 429 mg/kg-day for females) in drinking water for 2 years	Males: Nasal cavity squamous cell carcinoma: 0/44,[Ŧ] 0/45, 0/39, 3/33* Nasal cavity rhabdomyosarcoma: 0/40,[Ŧ] 0/45, 0/35, 1/22 Nasal cavity sarcoma NOS: 0/44,[Ŧ] 0/45, 0/38, 2/32 Nasal cavity esthesioneuroepithelioma: 0/48,[Ŧ] 0/47, 0/44, 1/42 Nasal cavity squamous cell carcinoma, rhabdomyosarcoma, sarcoma NOS or esthesioneuroepithelioma: 0/48,[Ŧ] 0/47, 0/44, 7/42* Hepatocellular adenoma: 0/48,[Ŧ] 2/47, 4/40,* 24/39* Hepatocellular carcinoma: 0/49,[Ŧ] 0/48, 0/43, 14/44* Hepatocellular adenoma or carcinoma: 0/49,[Ŧ] 2/48, 4/43, 33/44* Mammary gland fibroadenoma: 1/40,[Ŧ] 1/45, 0/35, 4/22* Mammary gland adenoma: 0/40,[Ŧ] 1/45, 2/35, 2/23 Mammary gland fibroadenoma or adenoma: 1/40,[Ŧ] 2/45, 2/35, 6/23* Subcutis fibroma: 6/47,[Ŧ] 3/45, 6/39, 12/36* Peritoneal mesothelioma: 2/49,[Ŧ] 2/48, 5/44, 28/47* Females: Nasal cavity squamous cell carcinoma: 0/49,[Ŧ] 0/49, 0/45, 7/47* Nasal cavity esthesioneuroepithelioma: 0/38,[Ŧ] 0/37, 0/38, 1/24 Nasal cavity squamous cell carcinoma or esthesioneuroepithelioma: 0/49,[Ŧ] 0/49, 0/45, 8/47* Hepatocellular adenoma: 0/49,[Ŧ] 0/50, 0/49, 7/50 Hepatocellular carcinoma: 0/38,[Ŧ] 0/37, 0/38, 1/24 Hepatocellular adenoma or carcinoma: 0/49,[Ŧ] 0/50, 0/49, 8/50* Mammary gland fibroadenoma: 3/49, 2/49, 1/45, 3/45 Mammary gland adenoma: 6/49,[Ŧ] 7/50, 10/49, 16/49* Mammary gland adenocarcinoma: 0/49, 1/50, 1/46, 0/47 Mammary gland fibroadenoma, adenoma or adenocarcinoma: 8/49,[Ŧ] 9/50, 12/49, 18/49*	Kano et al. (2009), JBRC (1998)

Sex/ Species	Dose/ Route/ Duration of Exposure	Tumor Incidence	Reference
Male and female Crj:BDF_1 mice (50/dose/sex)	0, 500, 2,000, or 8,000 ppm (0, 49, 191, or 677 mg/kg-day for males; 0, 66, 278, or 964 mg/kg-day for females) in drinking water for 2 years	Males: Hepatocellular adenoma: 7/48, 16/45,* 22/49,* 8/46 Hepatocellular carcinoma: 15/50,Ŧ 20/46, 23/50, 36/48* Hepatocellular adenoma or carcinoma: 21/50,Ŧ 31/46,* 37/50,* 39/48* Hemangioendothelioma of the heart: 0/43,Ŧ 0/40, 1/41, 3/39 Females: Hepatocellular adenoma: 4/49, 30/50,* 20/47,* 2/47 Hepatocellular carcinoma: 0/49,Ŧ 6/50, 30/48,* 45/48* Hepatocellular adenoma or carcinoma: 4/49,Ŧ 34/50,* 41/48,* 46/48*	Kano et al. (2009), JBRC (1998)
Male F344/DuCrj rats (50/dose)	0, 50, 250, or 1,250 ppm (v/v) vapor (0, 30.11, 150.55, or 752.76 mg/kg-day) for 6 hours/day, 5 days/week for 2 years (whole body exposure)	Nasal squamous cell carcinoma: 0/50,Ŧ 0/50, 1/50, 6/50* Hepatocellular adenoma or carcinoma:[f] 1/50,Ŧ 2/50, 4/50, 22/50** Peritoneal mesothelioma: 2/50,Ŧ 4/50, 14/50,** 41/50** Mammary gland fibroadenoma:[g] 1/50,Ŧ 2/50, 3/50, 5/50 Zymbal gland adenoma: 0/50,Ŧ 0/50, 0/50, 4/50 Renal cell carcinoma: 0/50,Ŧ 0/50, 0/50, 4/50 Subcutis fibroma: 1/50, 4/50, 9/50,** 5/50	Kasai et al. (2009)

DMBA, dimethylbenzanthracene; DEN, diethylnitroasmine; NOS, not otherwise specified.

[a] Authors indicated that the study duration was 63 weeks; however hepatic tumors were reported for animals sacrificed at 64 weeks (448 days) and 65 weeks (455 days).

[b] Hepatic tumors included multifocal hepatocellular carcinomas, but the study did not report tumor counts by specific tumor type.

[c] Dose calculated by OEHHA based on default body weight value of 0.523 kg from US EPA (1988) and drinking water consumption of 0.030 L/day reported by the authors.

[d] Tumor incidences were not reported for control animals. Additionally, tumor incidence for dosed groups were reported graphically as a percentage. Denominators were calculated by OEHHA using GetData graph digitizer and information provided by the authors within the description of the study results.

[e] Authors did not state concentration.

[f] US EPA (2013): "Incidence data was provided via email from Dr. Tatsuya Kasai (JBRC) to Dr. Reeder Sams (U.S.EPA) on 12/23/2008. Statistics were not reported for these data by study authors, so statistical analyses were conducted by EPA."

[g] Mammary gland adenomas were not included to prevent double counting because it is unclear if mammary gland fibroadenomas counts were included mammary gland adenomas.

*,** significantly different from control at p ≤ 0.05 or 0.01 by Fisher's exact test, respectively, calculated by OEHHA
Ŧ significant trend

Nasal and liver tumors are the most common tumors reported in cancer studies of 1,4-dioxane (Argus et al., 1965) (Argus et al., 1973; Hoch-Ligeti et al., 1970; JBRC, 1998; Kano et al., 2009; Kociba et al., 1974; NCI, 1978; Kasai et al., 2009). Only one study (King et al., 1973) reported skin tumors via dermal exposure; however, the study was not complete at the time of publication and omission of key details (e.g. vehicle concentration, number of animals evaluated) precludes further consideration of this study as a candidate critical study. Of the studies listed in Table 6, OEHHA selected the studies by NCI (1978), Kano et al. (2009) and JBRC (1998), and Kasai et al. (2009) as candidate critical studies because they were the best quality studies. These studies had large numbers of animals per dose group, multiple dose groups, adequate reporting of results including pathology, and were the most sensitive.

NCI (1978) exposed male and female Osborne-Mendel rats to 0, 0.5 or 1% v/v 1,4-dioxane in drinking water (0, 240, or 530 mg/kg-day for males and 0, 350, or 640 mg/kg-day for females) for 110-117 weeks. Tumors were observed in the nasal cavity and the liver. Statistically significant increases in combined nasal cavity squamous cell carcinomas, adenocarcinomas or rhabdomyomas with statistically significant positive trend were observed in male rats. Statistically significant increases in combined nasal cavity squamous cell carcinomas or adenocarcinomas and combined hepatocellular adenomas or carcinomas with statistically significant positive trends were observed in female rats. Incidences of mesotheliomas in the vaginal tunics of the testis in male rats were found to be treatment-related by the authors. This type of tumor is rare in this strain of rats and may pose biological significance in the understanding of the cancer effects of 1,4-dioxane. Upon inspection of the individual animal data obtained from NTP, OEHHA did not find statistically significant differences between control and dosed groups for this tumor type. As a result, OEHHA did not include mesotheliomas in the dose-response analysis.

NCI (1978) also exposed male and female B6C3F1 mice to 0, 0.5 or 1% v/v 1,4-dioxane in drinking water (0, 720, or 830 mg/kg-day for males and 0, 380, or 860 mg/kg-day for females) for 90-93 weeks. Statistically significant increases in combined hepatocellular adenomas or carcinomas with a statistically significant positive trend were observed in male and female mice. There was a significant increase in combined incidences of hemangioma or hemangiosarcoma from all sites only in low dose male mice. There was no significant dose-related trend in the incidence of these tumors. The authors concluded that hemangiomas or hemangiosarcomas were not related to 1,4-dioxane exposure.

Kano et al. (2009) and JBRC (1998) exposed male and female F344/DuCrj rats to 0, 200, 1,000 or 5,000 ppm 1,4-dioxane in drinking water (0, 11, 55, or 274 mg/kg-day for males and 0, 18, 83, or 429 mg/kg-day for females) for 2 years. Statistically significant increases in combined nasal cavity squamous cell carcinomas, rhabdomysarcomas, sarcomas (NOS, not otherwise specified) or esthesioneuroepitheliomas, combined hepatocellular adenomas or carcinomas, combined mammary gland fibroadenomas or adenomas, subcutis fibromas, and mesothelioma of the peritoneum with statistically significant positive trend were observed in male rats. Statistically significant increases in combined nasal cavity squamous cell carcinomas or ethesioneuroepitheliomas, combined hepatocellular adenomas or carcinomas, and combined

mammary gland fibroadenomas, adenomas or adenocarcinomas with statistically significant positive trend were observed in female rats. Although single occurrences of nasal esthesioneuroepitheliomas, rhabdomyosarcomas and sarcomas NOS were observed only in the high dose male rats and a single occurrence of nasal esthesioneuroepithelioma was observed in high dose female rats, OEHHA included these counts in the combined tumors of the nasal cavity because the authors indicated that these tumors have not been observed in the historical control data of the Japan Bioassay Research Center (JBRC) and thus were determined to be treatment-related.

Kano et al. (2009) and JBRC (1998) also exposed male and female Crj:BDF_1 mice to 0, 500, 2,000 or 8,000 ppm 1,4-dioxane in drinking water (0, 49, 191, or 677 mg/kg-day for males and 0, 66, 278, or 964 mg/kg-day for females) for 2 years. Statistically significant increases in combined hepatocellular adenomas or carcinomas with a statistically significant positive trend were observed in male and female mice. There was a significant positive trend for hemangioendotheliomas of the heart in male mice.

Kasai et al. (2009) exposed male F344/CuCrj (SPF) rats to 0 (clean air), 50, 250, or 1,250 ppm (v/v) 1,4-dioxane vapor for 6 hours/day, 5 days/week (equivalent to 0, 30.11, 150.55, or 752.76 mg/kg-day) for 2 years. There was a significant decrease in survival at the high dose primarily due to peritoneal mesotheliomas, although nasal tumors also contributed to the cause of death. Statistically significant increases in nasal cell carcinomas and hepatocellular adenomas or carcinomas were reported at the high dose. There were also statistically significant increases in mesotheliomas of the peritoneum at the mid and high dose, and subcutis fibroma at the mid dose. There were significant positive trends of mammary gland fibroadenomas, Zymbal gland adenomas, and renal cell carcinomas.

Mode of action and mechanistic considerations

1,4-Dioxane causes tumors in multiple sites in rodents (liver, nasal cavity, mammary gland, subcutis and peritoneum). The 1,4-dioxane carcinogenic mode of action (MOA) is not well understood; however, mechanistic studies have been conducted to elucidate the MOA for liver tumor formation. In this section, mechanistic data related to 1,4-dioxane carcinogenicity is presented, organized by the key characteristics (KCs) of carcinogens (described by Smith et al. (2016)). These data support 1,4-dioxane is genotoxic (KC 2), induces chronic inflammation (KC 6), and alters cell proliferation (KC 10) but does modulate receptor-mediated effects (KC 8). Other proposed MOAs and mechanisms not related to the KCs (such as metabolic saturation, cytotoxicity and activation of transcription profiling) are also be discussed.

Key Characteristics of Carcinogens

KC2: Is genotoxic

Genotoxic agents cause damage to DNA or induce changes to the DNA sequence (Smith et al., 2016). Examples of DNA damage include the formation of adducts, strand breaks, or crosslinks and DNA alkylation. Examples of DNA sequence changes include gene or point mutations (such as base substitutions, frameshifts and small deletions or insertions) and chromosomal mutations (such as chromosomal aberrations, micronuclei and aneuploidy). Genotoxicity studies of 1,4-

dioxane are summarized in the Genetic Toxicity section. 1,4-Dioxane is not mutagenic in the majority of in vitro systems tested; however, it is possible that metabolites of 1,4-dioxane are mutagenic. The in vitro studies are limited, as they do not adequately capture the complete metabolism of 1,4-dioxane and in vivo studies show mixed results. While some studies of animals orally exposed to 1,4-dioxane showed negative results (Goldsworthy et al., 1991; 1994; Stott et al., 1981; Tinwell and Ashby, 1994), several studies showed positive results from single and repeated oral doses of 21.9 mg/kg and higher (Gi et al., 2018; Goldsworthy et al., 1991; Kitchin and Brown, 1990; Mirkova, 1994; Miyagawa et al., 1999; Roy et al., 2005; Stott et al., 1981; Uno et al., 1994).

Some studies found evidence of mutagenicity in rodents. Gi et al. (2018) investigated the in vivo mutagenicity of 1,4-dioxane in the liver of guanine phosphoribosyl transferase (gpt) delta transgenic F344 rats, an animal model for in vivo genotoxicity. Male gpt delta transgenic F344 rats were exposed to 0, 18.7, 92.3, or 440.2 mg/kg-day 1,4-dioxane in drinking water for 16 weeks. 1,4-Dioxane caused an increase in DNA mutation frequency at 92.3 and 440.2 mg/kg-day and induction of DNA repair enzymes at 440.2 mg/kg-day in the transgenic rats; wild-type (WT) rats were not evaluated for mutagenicity.

Several studies reported DNA damage in rodents. In a study by Totsuka et al. (2020) there was a dose dependent increase in DNA adduct formation in rats exposed to 0, 2.2, 21.9 and 562.4 mg/kg-day 1,4-dioxane in drinking water for 16 weeks. Control and 2.2 mg/kg-day dosed rats exhibited different types of DNA adducts compared to rats given 21.9 and 562.4 mg/kg-day, suggesting that adduct formation at the two higher doses was likely treatment related. One of the adducts formed contained 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage. Oxidative DNA damage in the liver was also seen in mice exposed to 651 mg/kg-day 1,4 dioxane for 3 months (Chen et al., 2022).

In female rats, a single gavage exposure to ≥2,550 mg/kg 1,4-dioxane for 4 or 21 hours showed DNA single strand breaks in the absence of cytotoxicity (Kitchin and Brown, 1990). In the study by Charkoftaki et al. (2021), mice exposed to 5,000 mg/L 1,4-dioxane in drinking water for one week (equivalent to 1,030 mg/kg-day) or four weeks (equivalent to 927 mg/kg-day) caused an increase in expression of $_{\gamma}$H2AX, a marker for DNA double-stranded breaks, without inducing gross pathological changes in the liver. The study also found increased cytokeratin-7 (CK-7) positive cells following 4 weeks of exposure to 5,000 mg/L (927 mg/kg-day) 1,4-dioxane, which shows proliferation of precholangiocytes in the liver. Cell proliferation was observed at the same doses in which DNA damage occurred suggesting that DNA damage was occurring simultaneously with DNA repair.

Additionally, several studies observed increases in chromosomal effects after exposure to 1,4-dioxane. Mice exposed to doses of 1,4-dioxane between 900 and 3,500 mg/kg by oral gavage had micronucleus formation in bone marrow at 24 or 48 hours after a single exposure (Mirkova, 1994) and in bone marrow and hepatocytes after 5 days of exposure (Itoh and Hattori, 2019; Mirkova, 1994; Roy et al., 2005). Rats dosed with 1,000-3,000 mg/kg-day 1,4-dioxane by oral gavage had micronucleus formation in hepatocytes (single or two daily doses), but not in bone marrow (single dose) (Itoh and Hattori, 2019).

KC5: Induces oxidative stress

Carcinogens can induce oxidative stress by altering the redox balance within tissues, which leads to reactive oxygen species (ROS) production. Oxidative stress can cause oxidative damage to DNA, which includes base modification and DNA-protein crosslinks (Smith et al., 2016). In recent mechanistic studies, there are inconsistencies in whether 1,4-dioxane causes oxidative stress in wildtype and transgenic models. Transcriptome analysis showed that mice exposed to 927 mg/kg-day 1,4-dioxane for 4 weeks exhibited oxidative stress via changes in gene expression of glutathione (GSH)-mediated detoxification and nuclear factor erythroid 2-related factor 2 (NRF2)-mediated oxidative stress response genes (Charkoftaki et al., 2021). In contrast, histopathological analysis of the liver showed no difference in expression of 4-hydroxynonenal, a biomarker for oxidative damage, between control and treated mice. In a follow-up study by Chen et al. (2022), GSH-deficient glutamate-cysteine ligase modifier subunit (Gclm)-null mice (knockout, KO), a model for oxidative stress, and WT mice were exposed to 1,000 mg/kg-day 1,4-dioxane by oral gavage for one week or 5,000 mg/L 1,4-dioxane in drinking water (651 mg/kg-day in WT mice and 647 mg/kg-day in KO mice) for three months to investigate the effect of 1,4-dioxane on redox homeostasis. Consistent with the study by Charkoftaki et al. (2021), the NRF2 mediated oxidative stress response was upregulated for KO mice exposed to 1,000 mg/kg-day of 1,4-dioxane for one week and 647 mg/kg-day of 1,4-dioxane for three months . A progressive induction of CYP2E1 protein expression and activity, oxidative stress in the form of increased lipid peroxidation, and oxidation of the GSH pool were observed. Increased expression of $H2AX_{\gamma}$ was observed in 1,4-dioxane-exposed KO and WT mice. Additionally, levels of 8-OHdGincreased in the liver of 1,4-dioxane exposed mice (Chen et al., 2022). In contrast, Gi et al. (2018) measured formation of 8-OHdG in the liver DNA of gpt delta transgenic F344 rats, and found that there was no significant difference between controls and rats treated with 18.7- 440.2 mg/kg-day 1,4-dioxane in drinking water for 16 weeks. The authors concluded that oxidative DNA damage is not involved in 1,4-dioxane carcinogenesis. Totsuka et al. (2020) showed DNA adduct formation in F344 rats exposed to 0, 2.2, 21.9 and 562.4 mg/kg-day 1,4-dioxane in drinking water for 16 weeks and determined the structures of the adducts using high-resolution accurate-mass (HRAM) mass spectroscopy. One of three adducts that were found to be unique to 1,4-dioxane exposure was 8-oxo-dG, which is produced during oxidative stress. The authors, however, noted that the formation of this adduct may not be the main cause for genotoxicity observed in the liver because the difference in 8-oxo-dG formation in the two highest doses was moderate.

KC6: Induces chronic inflammation

Carcinogens can cause chronic inflammation by persistent infection or irritation that cause cell death (Smith et al., 2016). This can cause deregulated compensatory cell proliferation and aberrant repair (Smith et al., 2020). In a study by Zhou et al. (2020), male C57BL/6 mice (n=10/dose) exposed to 0, 0.5, 5, 50, and 500 ppm 1,4-dioxane in drinking water for 12 weeks had hepatic inflammation confirmed by histopathological examination. Levels of lipopolysaccharide (LPS, also referred to as endotoxin), are inflammation triggering molecules normally produced by gut bacteria, were increased in the serum and in the liver of mice treated with 50 and 500 ppm 1,4-dioxane. At the highest dose, a decrease in intestinal goblet cells was observed, indicating intestinal injury. The authors concluded that the intestinal changes and increase in LPS levels due to 1,4-dioxane exposure induced the hepatic inflammation seen in the mice. In C57BL/6J mice exposed to 5,000 mg/L (651 mg/kg-day) 1,4-dioxane in drinking

water for three months, hepatic inflammation via histopathological examination of the liver was significantly increased (Chen et al., 2022).

KC8: Modulates receptor-mediated effects

Only one study investigating receptor mediated effects of 1,4-dioxane was identified. Gi et al. (2018) measured the mRNA expression of CYPs induced by constitutive androstane receptor (CAR), pregnane X receptor (PXR), peroxisome proliferator-activated receptor α (PPARα) and aryl hydrocarbon receptor (AhR) in the liver of gpt delta transgenic F344 rats exposed to 1,4-dioxane in drinking water (0, 18.7, 93.3, 440.2 mg/kg-day) for 16 weeks and found that there were no changes in expression. The authors concluded that the carcinogenic mechanism of 1,4-dioxane is not likely to be mediated via CAR, PXR, PPARα, or AhR.

KC10: Alters cell proliferation, cell death or nutrient supply

Carcinogens can increase cell proliferation, decrease apoptosis, and cause changes in cellular energetics, growth factors, angiogenesis and cellular signaling (Smith et al., 2016). Table 7 shows a summary of animal studies of 1,4-dioxane that measured effects on replicative DNA synthesis, an indicator of cell proliferation. The studies below show that 1,4-dioxane causes increased DNA synthesis in the liver of rats.

Table 7. Cell proliferation assays on 1,4-dioxane

Assay	Sex/Species	Dose/Route of Exposure/ Duration	Results	Reference
Replicative DNA synthesis (cell proliferation) in hepatocytes	Male F344 rats (3–5/dose)	0 or 1,000 mg/kg by oral gavage for 2 and 12 hours 0, 1,500 mg/kg-day for 2 weeks or 3,000 mg/kg-day in drinking water for 1 week	+ (1,500 mg/kg-day for 2 weeks)	Goldsworthy et al. (1991)
Replicative DNA synthesis (cell proliferation) in hepatocytes	Male F344 rats (4/dose)	0*, 1,000, 1,500, 2,000 or 4,000 mg/kg by a single oral gavage for 24 hours	+ (1,000 – 2,000 mg/kg)	Miyagawa et al. (1999)
Replicative DNA synthesis (cell proliferation) in hepatocytes	Male F344 rats (4/dose)	0, 1,000, or 2,000 mg/kg by a single oral gavage	+ (2,000 mg/kg 24 hours post administration)	Uno et al. (1994)
Replicative DNA synthesis (cell proliferation) in nasal epithelial cells	Male F344 rats (3/dose)	0 or 1,500 mg/kg-day in drinking water for 2 weeks	-	Goldsworthy et al. (1991)

Assay	Sex/Species	Dose/Route of Exposure/ Duration	Results	Reference
Cell proliferation in hepatocytes	Male F344 rats (10/dose)	0, 2, 20, 200, 1,000, or 5,000 ppm (0, 0.2, 2.2, 21.9, 222.2, or 562.4 mg/kg-day) in drinking water for 16 weeks	+ (562.4 mg/kg-day)	Gi et al. (2018)

* Study authors stated that control data were obtained from a previous study.

Other mechanistic considerations

Proposed mechanism via metabolic saturation, cytotoxicity, and proliferation

It has been suggested that a possible MOA for the carcinogenicity of 1,4-dioxane is metabolic saturation, followed by accumulation in the blood which leads to cytotoxicity, regenerative proliferation, and the development of liver tumors (Dourson et al., 2014; Dourson et al., 2017). Toxicokinetic studies show that metabolism of 1,4-dioxane is saturated at high doses in single oral gavage and i.v. studies in rats (Young et al., 1978). Doses that resulted in plasma levels greater than 100 µg/ml were metabolized and eliminated more slowly. However, in a 13-week inhalation study in rats, levels of 1,4-dioxane in the blood increased linearly to 730 µg/ml in male rats and 1,054 µg/ml in female rats (Kasai et al, 2008). The authors concluded that the linear increase in plasma levels of 1,4-dioxane indicates that the metabolic capacity was not saturated. However, the data presented in the study shows that the ratio of plasma 1,4-dioxane levels over concentration of inhaled 1,4-dioxane increased with increasing concentration of inhaled 1,4-dioxane, indicating that metabolism or elimination of 1,4-dioxane has slowed down, which may be suggestive of saturation. Nonetheless, the authors suggested that the reason for the lack of metabolic saturation is the result of enhanced metabolism through the induction of P450 enzymes after repeated exposure to 1,4-dioxane. Sweeney et al. (2008) suggest that metabolic saturation in mice occurs at a dose in the range of 245 to 2,230 mg/kg-day. In the Kano et al. (2009) and JBRC (1998) study, liver tumors were observed in male and female mice at doses well below this range, which suggests that metabolic saturation is not essential for tumorigenesis.

Dourson et al. (2014), hypothesized that once metabolic saturation is reached, cytotoxicity occurs in the form of hypertrophy and necrosis, which then leads to regenerative hyperplasia in rodents. In the study by Stott et al. (1981), cytotoxicity in the liver was observed in rats administered 1,000 mg/kg-day of 1,4-dioxane in drinking water for 11 weeks, as well as increased liver-to-body weight ratio and DNA synthesis, a marker of cell proliferation. At lower doses, these effects were not observed (Stott et al., 1981). In contrast, in the study by Kitchin and Brown (1990), female Sprague-Dawley rats were given 1,4-dioxane orally for 4 and 21 hours at 0, 168, 840, 2,550, or 4,200 mg/kg. DNA damage was observed at 2,550 or 4,200 mg/kg of 1,4-dioxane while no liver cytotoxicity was observed. In long term studies, there are also inconsistent data on whether cytotoxicity is necessary prior to cell proliferation (Kano et al., 2009; Kociba et al., 1974). A summary of the presence of cytotoxicity, cell proliferation, and liver

tumor counts is presented in Table 8. Kociba et al. (1974) reported hepatocellular degeneration and necrosis, as well as hepatic regeneration at doses at and below those that resulted in statistically significant tumor formation. In the studies by Kano et al. (2009) and JBRC (1998), hyperplasia was observed at doses at and below levels resulting in tumor formation in rats, but no incidence of cytotoxicity was reported, suggesting that cell proliferation can occur in the absence of cytotoxicity. Liver tumors were present in rats and mice administered 1,4-dioxane in drinking water in the studies by NCI (1978), but cytotoxicity was not reported. In female mice, liver tumors were observed in high- and low-dose animals. However, hyperplasia was only observed in low-dose animals. Additionally, Gi et al. (2018) did not observe necrosis in the liver of gpt delta transgenic F344 rats treated with 1,4-dioxane in drinking water for 16 weeks at doses shown to cause tumors in other studies (92.3 – 440.2 mg/kg-day). The authors concluded that cytotoxicity is not involved in 1,4-dioxane carcinogenesis. There was, however, an increase in mutation frequency in these animals

Table 8. 1,4-Dioxane-induced liver toxicity in chronic studies (adapted from US EPA (2013a))

Reference	Sex/Species/ Dose/Route of Exposure/ Duration	Dose (mg/kg-day)	Cytotoxicity	Proliferation/ hyperplasia	Incidence of Liver Tumors[a]
Kociba et al. (1974)	Male and female Sherman rats, drinking water, 2 years	0	-	-	2/106
		9.6/19[b]	-	-	0/110
		94/148 [b]	+	+	1/106
		1,015/1,599[b]	+	+	12/66*
NCI (1978)	Male Osborne-Mendel rats, drinking water, 110-117 weeks	0	-	+	1/16
		240	NR	+	2/32
		530	NR	+	1/31
NCI (1978)	Female Osborne-Mendel rats, drinking water, 110-117 weeks	0	NR	+	0/33
		350	NR	+	10/24*
		640	NR	+	12/22*
NCI (1978)	Male B6C3F1 mice, drinking water, 90-93 weeks	0	NR	-	8/29
		720	NR	+	19/49*
		830	NR	+	28/50*
NCI (1978)	Female B6C3F1 mice, drinking water, 90-93 weeks	0	NR	-	0/49
		380	NR	+	21/47*
		860	NR	-	35/42*
Kano et al. (2009), JBRC (1998)	Male F344/DuCrj rats, drinking water, 2 years	0	NR	-	0/49
		11	NR	-	2/48
		55	NR	-	4/43
		274	NR	+	33/44*
Kano et al. (2009),	Female F344/DuCrj	0	NR	-	1/49
		18	NR	-	0/50

Reference	Sex/Species/ Dose/Route of Exposure/ Duration	Dose (mg/kg-day)	Cytotoxicity	Proliferation/ hyperplasia	Incidence of Liver Tumors[a]
JBRC (1998)	rats, drinking water, 2 years	83	NR	-	5/49
		429	NR	+	40/50*
Kano et al. (2009), JBRC (1998)	Male Crj:BDF1 mice, drinking water, 2 years	0	NR	-	21/50
		49	NR	-	31/46*
		191	NR	-	37/50*
		677	NR	-	39/48*
Kano et al. (2009), JBRC (1998)	Female Crj:BDF1 mice, drinking water, 2 years	0	NR	-	4/49
		66	NR	-	34/50*
		278	NR	-	41/48*
		964	NR	-	46/48*
Kasai et al. (2009)	Male F344/DuCrj rats, vapor, 2 years	0	+	NR	1/50
		30.11	+	NR	2/50
		150.55	+	NR	4/50
		752.76	+	NR	22/50*

-, negative result; NR, not reported

[a] numerator denotes incidence of tumors and denominator denotes effective number of animals

[b] first value is administered dose in males and second value is administered dose in females

*significantly different from control in Fisher's exact test ($p<0.05$) calculated by OEHHA

Gene Expression Profiling

A study by Furihata et al. (2018) used gene expression profiling to compare 1,4-dioxane with genotoxic and nongenotoxic hepatocarcinogens. Mice were exposed to 1,4-dioxane in drinking water for 4 weeks while other groups of mice were exposed to typical genotoxic hepatocarcinogens, N-nitrosodiethylamine (DEN) and 3,3'-dimethylbenzidine-2HCl (DMB) and a typical nongenotoxic hepatocarcinogen, di (2-ethylhexyl)phthalate (DEHP), in drinking water or food. Selected gene expression patterns in the liver were analyzed by principal component analysis. When compared to untreated controls, the gene expression profile of animals exposed to 1,4-dioxane differed significantly from animals exposed to genotoxic and non-genotoxic hepatocarcinogens. Gene expression profiles with1,4-dioxane treatment differed significantly from those with DEN and DMB, as well as DEHP. The authors concluded that 1,4-dioxane has an intermediate gene expression profile between genotoxic and nongenotoxic hepatocarcinogens.

Tumor promotion

Tumor promotion may be a possible MOA of 1,4-dioxane, as shown in studies in mouse skin and rat liver (King et al., 1973; Lundberg et al., 1987). 1,4-Dioxane demonstrated tumor-promoting activity in a study of Swiss-Webster mice. Administration of 0.2 mL of 1,4-dioxane (1% in acetone) three times a week on the shaved backs of male and female mice for 78 weeks following initiation with 50 µg of dimethylbenzanthracene (DMBA) induced increased incidences of lung, spleen, skin, and kidney tumors compared to DMBA alone (King et al., 1973). When male Sprague-Dawley rats were given 1,000 mg/kg-day 1,4-dioxane in drinking water 5 days/week for 7 weeks following initiation with diethylnitrosoamine (DEN), there was an increase in the number of gamma-glutamyl transpeptidase-positive foci and total foci volume

compared to animals administered DEN alone (Lundberg et al., 1987) indicating foci development that may progress to carcinogenesis (Bannasch et al., 1982). 1,4-Dioxane elicited a concentration dependent increase in upregulated tumor promoter marker genes in an in vitro study using gene expression analysis to identify chemicals for tumor promoting potential (Maeshima et al., 2010).

Summary

Overall, the available evidence for a singular predominant mechanism for 1,4-dioxane carcinogenesis is not conclusive. As shown above, there are studies that support cytotoxicity as a key event in tumor formation, but there are also studies that show tumor formation in the absence of cytotoxicity. Similarly, oxidative stress, which can cause DNA damage, has also been implicated as a plausible mechanism; however, there are multiple studies reporting conflicting results. Furthermore, the in vivo genotoxicity studies showing DNA mutations, DNA strand breaks and micronucleus and adduct formation indicate that genotoxicity remains a plausible mechanism. Multiple mechanisms appear involved based on evidence for genotoxicity in in vivo studies, increased oxidative stress, induction of chronic inflammation, and increased cell proliferation; therefore, OEHHA's default linear low-dose extrapolation is appropriate for cancer dose-response analysis.

DOSE-RESPONSE ASSESSMENT

Noncancer Dose-Response Analyses and Acceptable Daily Dose Calculation

The liver and kidney are common targets for 1,4-dioxane toxicity as evidenced in the studies by Kano et al. (2009), Kociba et al. (1974) and NCI (1978). These studies were chronic in duration, exposed animals via drinking water, had large sample sizes, and were the most sensitive for effects in the liver and kidney. Other studies, including the only study measuring reproductive and developmental effects, were significantly less sensitive (Argus et al., 1965; Argus et al., 1973; Giavini et al., 1985). The study by Kociba et al. (1974) was the most sensitive study with a NOAEL of 9.6 mg/kg-day for histopathological changes in the liver and kidney. Renal tubular epithelial and hepatocellular degeneration and necrosis were observed at doses of 94 mg/kg-day and higher. Quantitative data were not reported for all the endpoints in this study. Therefore, BMD modeling was not performed on this dataset (Table 9). The study by Kano et al. (2009) had a NOAEL of 11 mg/kg-day for increased absolute and relative liver weight and nuclear enlargement of the olfactory epithelium in male rats. For female rats, the NOAEL was 83 mg/kg-day for increased relative liver weight. Quantitative data and BMD modeling results are presented in Table 9. Because it is unclear whether 1,4-dioxane or its metabolites are responsible for noncancer and cancer effects observed, dose-response modeling was based on administered doses.

Table 9. Dose-response modeling results for 1,4-dioxane candidate critical noncancer studies

Study Sex/Species (N) Duration	Dose (mg/kg-day)	Critical Effect	Mean ± SD	NOAEL or LOAEL (mg/kg-day)	BMD/BMDL (mg/kg-day) p-value Model Type
Kociba et al. (1974) Male and female rats (60) 2 years	0 9.6/19[a] 94/148[a] 1,015/1,599[a]	Renal tubular epithelial degeneration and necrosis; hepatocellular degeneration and necrosis in males and females	N/A	NOAEL: 9.6 (males)	Quantitative data not reported
Kano et al. (2009), JBRC (1998) Male rats (50) 2 years	0 11 55 274	↑ Rel. liver weight	2.92 ± 0.31 3.03 ± 0.6 3.33 ± 0.52** 5.01 ± 1.1**	NOAEL: 11	Questionable model fit[b]
Kano et al. (2009), JBRC (1998) Female rats (50) 2 years	0 18 83 429	↑ Rel. liver weight	2.72 ± 0.69 2.63 ± 0.59 2.73 ± 0.41 7.35 ± 2.28**	NOAEL: 83	Questionable model fit
Kano et al. (2009), JBRC (1998) Male mice (50) 2 years	0 49 191 677	↓ terminal body weight	48.7 ± 6.1 47.3 ± 6.8 44.1 ± 7.6* 27 ± 3.0**	NOAEL: 49	316.32/212.81[c] p = 0.85 Frequentist polynomial degree 2
Kano et al. (2009), JBRC (1998) Female mice (50) 2 years	0 66 278 964	↓ terminal body weight	35.3 ± 5.1 33.8 ± 6.4 29.7 ± 4.7** 19.3 ± 2.8**	NOAEL: 66	261.85/191.79[c] p = 1.00 Exponential 3

NOAEL, no-observed-adverse-effect level, SD, standard deviation
*p<0.05 ** p<0.01
[a] Values indicate dose for male/female
[b] Refer to Methodology section for definition
[c] Values are BMD_{1SD} and $BMDL_{1SD}$, BMD and BMDL resulted in a benchmark response of one standard deviation from the control mean

BMD modeling of the Kano et al. (2009) and JBRC (1998) liver weight data in rats produced questionable model fits. Modeling of the male mouse terminal body weight data obtained a BMD_{1SD} (benchmark dose resulting from a benchmark response of one standard deviation from

the control mean) of 316.32 mg/kg-day and a $BMDL_{1SD}$ of 212.81 mg/kg-day. Modeling of the female mouse terminal body weight data obtained a BMD_{1SD} of 261.85 mg/kg-day and a $BMDL_{1SD}$ of 191.79 mg/kg-day. Details of the BMD modeling are shown in Appendix D.

The BMDLs obtained from the Kano et al. (2009) and JBRC (1998) datasets are higher than the NOAELs for the candidate critical studies listed in Table 9. As a result, the Kociba et al. (1974) study was selected as the critical study because the histopathological changes in the liver and kidney were the most sensitive endpoint. The resulting POD is 9.6 mg/kg-day. A composite UF of 1,000 is applied for ADD calculation, consisting of 10 for interspecies extrapolation, 30 for human variability (√10 for pharmacodynamics and 10 for pharmacokinetics variability, including infants and children with no kinetic data (OEHHA, 2008)), and √10 for database deficiencies for the limited database of reproductive and developmental toxicity studies. For details on uncertainty factors, refer to Appendix B. The intraspecies uncertainty factor also takes into account variability in CYP enzyme expression, which is involved in the metabolism of 1,4-dioxane and may contribute to varying susceptibilities among the human population. The composite UF of 1,000 is applied to the POD to derive the ADD using the following equation:

$$\text{ADD} = 9.6 \text{ mg/kg-day} \div 1{,}000 = 0.0096 \text{ mg/kg-day}.$$

Cancer Dose-Response Analyses and Cancer Potency Derivation

Oral Cancer Slope Factor

The most sensitive and consistent endpoints among the cancer bioassays listed in Table 6 are liver and nasal cavity tumors. Among the available cancer bioassays, the rat and mouse studies by NCI (1978), and Kano et al. (2009) and JBRC (1998) were selected as candidate critical studies to derive the oral cancer slope factor. These studies used multiple doses, exposed animals via drinking water for 2 years, had large sample sizes, and were the most sensitive for liver and nasal cavity tumors. Other tumors observed in the candidate critical studies were mammary gland tumors in male and female rats, as well as subcutis fibroma and mesothelioma of the peritoneum in male rats in the Kano et al. (2009) and JBRC (1998) studies. In male mice, there was a significant trend in hemangiothelioma of the heart (Kano et al., 2009; JBRC, 1998). These are also included in the dose-response analysis.

Incidences for the different tumor types were modeled using the BMDS linear multistage (LMS) model (BMDS version 3.2, US EPA) and multistage-in-dose Weibull-in-time (MSW) model (US EPA, 2010). Results of BMDS analyses are shown in Table 10. The lower 95% confidence limit of the doses associated with a 5% increase in risk of developing a tumor ($BMDL_{05}$) was estimated and used to derive the cancer slope factors (CSFs) shown in Table 11. In instances where tumors were found at multiple sites and/or in different cell types at the same site, the BMDS multitumor model (MS_Combo) was used. Additional details on the methodology for deriving CSFs are provided in Appendix F.

Table 10. Dose-response modeling results for 1,4-dioxane oral candidate critical cancer studies

Reference Sex/Species (N) Duration	Dose (mg/kg-day)	Tumor Type	Incidences	Model	BMD/BMDL (mg/kg-day) p-value[a]
NCI (1978) Male Osborne-Mendel rats (35) 110-117 weeks	0 240 530	Nasal cavity squamous cell carcinoma, adenocarcinoma, or rhabdomyoma	0/35[b] 13/35*[b] 19/35*[b]	MSW	14.49/9.29 N/A
NCI (1978) Female Osborne-Mendel rats (35) 110-117 weeks	0 350 640	Nasal cavity squamous cell carcinoma or adenocarcinoma	0/35[b] 11/35*[b] 9/35*[b]	MSW	48.17/29.17 N/A
NCI (1978) Female Osborne-Mendel rats (35) 110-117 weeks	0 350 640	Hepatocellular adenoma or carcinoma	0/35[b] 10/5*[b] 12/35*[b]	MSW	11.20/5.30 N/A
NCI (1978) Female Osborne-Mendel rats (35) 110-117 weeks	0 350 640	Multisite: Nasal cavity squamous cell carcinoma or adenocarcinoma and hepatocellular adenoma or carcinoma	N/A	Multisite time-to-tumor	Not reported[c]
NCI (1978) Male B6C3F1 mice (50) 90-93 weeks	0 720 830	Hepatocellular adenoma or carcinoma	8/49 19/49* 28/50*	LMS 2nd degree polynomial	254.08/62.70 p-value = 0.31
NCI (1978) Female B6C3F1 mice (50) 90-93 weeks	0 380 860	Hepatocellular adenoma or carcinoma	0/49.16[d] 21/47.07*[d] 35/45.16*[d]	LMS 1st degree polynomial	30.97/24.71 p-value = 0.70

Reference Sex/Species (N) Duration	Dose (mg/kg-day)	Tumor Type	Incidences	Model	BMD/BMDL (mg/kg-day) p-value[a]
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats (50) 2 years	0 11 55 274	Nasal cavity squamous cell carcinoma, rhabdomyosarcoma, sarcoma NOS or esthesioneuroepithe-lioma	0/48 0/47 0/44 7/42*	LMS 3rd degree polynomial	180.10/96.98 p-value = 1.00
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats (50) 2 years	0 11 55 274	Hepatocellular adenoma or carcinoma	0/49 2/48 4/43 33/44*	LMS 2nd degree polynomial	24.48/12.79 p-value = 0.49
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats (50) 2 years	0 11 55 274	Mammary gland fibroadenoma or adenoma	1/40 2/45 2/35 6/23*	LMS 1st degree polynomial	54.83/29.46 p-value = 0.87
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats (50) 2 years	0 11 55 274	Subcutis fibroma	5/47 3/45 5/39 12/36*	LMS 1st degree polynomial	45.81/26.83 p-value = 0.68
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats (50) 2 years	0 11 55 274	Peritoneal mesothelioma	2/49 2/48 5/44 28/47*	LMS 1st degree polynomial	17.95/13.35 p-value = 0.40

Reference Sex/Species (N) Duration	Dose (mg/kg-day)	Tumor Type	Incidences	Model	BMD/BMDL (mg/kg-day) p-value[a]
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats (50) 2 years	0 11 55 274	Multisite: Nasal cavity squamous cell carcinoma or adenocarcinoma, rhabdomyosarcoma, sarcoma NOS or esthesioneuroepithe-lioma, hepatocellular adenoma or carcinoma, mammary gland fibroadenoma or adenoma, subcutis fibroma, and peritoneal mesothelioma	N/A	MS_Combo	7.53/5.54 N/A
Kano et al. (2009), JBRC (1998) Female F344/DuCrj rats (50) 2 years	0 18 83 429	Nasal cavity squamous cell carcinoma or esthesioneuroepithe-lioma	0/49 0/49 0/45 8/47*	LMS 3rd degree polynomial	279.66/151.30 p-value = 1.00
Kano et al. (2009), JBRC (1998) Female F344/DuCrj rats (50) 2 years	0 18 83 429	Hepatocellular adenoma or carcinoma	1/49 0/50 5/49 40/50*	LMS 2nd degree polynomial	68.31/31.22 p-value = 0.22
Kano et al. (2009), JBRC (1998) Female F344/DuCrj rats (50) 2 years	0 18 83 429	Mammary gland fibroadenoma, adenoma or adenocarcinoma	8/49 9/50 12/49 18/49*	LMS 1st degree polynomial	79.15/43.82 p-value = 0.87

Reference Sex/Species (N) Duration	Dose (mg/kg-day)	Tumor Type	Incidences	Model	BMD/BMDL (mg/kg-day) p-value[a]
Kano et al. (2009), JBRC (1998) Female F344/DuCrj rats (50) 2 years	0 18 83 429	Multisite: Nasal cavity squamous cell carcinoma or ethesioneuroepithe-lioma, hepatocellular adenoma or carcinoma, and mammary gland fibroadenoma, adenoma or adenocarcinoma	N/A	MS Combo	43.10/22.88 N/A
Kano et al. (2009), JBRC (1998) Male Crj:BDF1 mice (50) 2 years	0 49 191 677	Hepatocellular adenoma or carcinoma	21/50 31/46* 37/50* 39/48*	LMS 1st degree polynomial	Questionable model fit with all doses 11.82/7.23 p-value = 0.10 with high dose dropped
Kano et al. (2009), JBRC (1998) Male Crj:BDF1 mice (50) 2 years	0 49 191 677	Hemangioendothe-lioma of the heart	0/43 0/40 1/41 3/39	LMS 1st degree polynomial	449.71/218.06 p-value = 0.97
Kano et al. (2009), JBRC (1998) Male Crj:BDF1 mice (50) 2 years	0 49 191 677	Multisite: Hepatocellular adenoma or carcinoma and hemangioendothe-lioma of the heart	N/A	MS Combo	11.52/7.11 N/A
Kano et al. (2009), JBRC (1998) Female Crj:BDF1 mice (50) 2 years	0 66 278 964	Hepatocellular adenoma or carcinoma	4/50[b] 34/50*[b] 41/50*[b] 46/50*[b]	MSW	2.64/1.85 N/A

LMS, linear multistage; MSW, multistage Weibull; MS_Combo, multitumor model in BMDS; NOS, not otherwise specified

*significantly different from control in Fisher's exact test ($p<0.05$)

[a] p-value is not calculated by MSW or multisite (MS_COMBO) models

[b] Denominators differ from denominators reported in Table 6 because the MSW program takes into account different tumor contexts for all animals in the study. Denominators in Table 6 denote effective number of animals
[c] BMD and BMDL not reported, only CSF (shown in Table 11)
[d] poly-3 adjusted incidences

There was no significant treatment-related early mortality in male mice in the NCI (1978) studies. Therefore, the number of animals alive at the first occurrence of tumors (also known as the effective number) was used as the denominator for reporting tumor incidence in Table 10. The tumors in male mice were modeled using the LMS model. There were significant differences in survival between control and dosed groups for the male and female rats and female mice in the NCI (1978) study. The significant differences in survival occurred early in the study, therefore the dose-response relationship was analyzed with time-to-tumor adjustments. In female mice, the observed early mortality was significant but not severe. Therefore, a poly-3 adjustment (Bailer and Portier, 1988) was used (see Appendix F for details). Mortality in male and female rats was significant and severe, so the dose-response analysis was conducted using the multistage Weibull (MSW) model (US EPA, 2010). The MSW model requires inputs for the dose and tumor detection time for individual animals. To account for tumors at multiple sites in female rats, dose-response analyses were conducted using a multisite approach (details in Appendix F). Tumor incidences were modeled using incidental risk since the cause of death was not indicated.

In the Kano et al. (2009) and JBRC (1998) studies, the authors indicated survival differences in male and female rats and female mice. Upon examination of the individual animal data, the differences in survival in male and female rats occurred later (after 85 weeks) in the study, therefore the dose-response relationship was modeled using the effective animal number and the LMS model. Although the authors reported survival differences for male mice, OEHHA determined there was no significant treatment related early mortality and the data were modeled similarly to male and female rats. In female mice, significant differences in mortality were observed early in the study (before 85 weeks). Therefore the dose-response relationship was modeled using incidental risk in a time-to-tumor MSW model since both incidental and fatal tumors were present, and the model estimated the “appearance of detectable tumors” rather than “death from tumor” (US EPA, 2010).

Table 11. Oral cancer slope factor calculations for 1,4-dioxane

Reference Species Study Duration	Tumor Type	Animal CSF $(mg/kg\text{-}day)^{-1}$	Time-Weighted Average Animal Body Weight (kg)[a]	Human CSF_b $(mg/kg\text{-}day)^{-1}$
NCI (1978) Male Osborne-Mendel rats 110-117 weeks	Nasal cavity squamous cell carcinoma, adenocarcinoma, or rhabdomyoma	0.0054	0.5369	0.018
NCI (1978) Female Osborne-Mendel rats 110-117 weeks	Nasal cavity squamous cell carcinoma or adenocarcinoma	0.0071	0.34489	0.0065

Reference Species Study Duration	Tumor Type	Animal CSF $(mg/kg\text{-}day)^{-1}$	Time-Weighted Average Animal Body Weight (kg)[a]	Human CSF_b $(mg/kg\text{-}day)_{-1}$
NCI (1978) Female Osborne-Mendel rats 110-117 weeks	Hepatocellular adenoma or carcinoma	0.0094	0.34489	0.036
NCI (1978) Female Osborne-Mendel rats 110-117 weeks	Multisite: Nasal cavity squamous cell carcinoma or adenocarcinoma and hepatocellular adenoma or carcinoma	0.0098	0.34489	0.037
NCI (1978) Male B6C3F1 mice 90-93 weeks	Hepatocellular adenoma or carcinoma	0.0008[c]	0.03352	0.0083[c]
NCI (1978) Female B6C3F1 mice 90-93 weeks	Hepatocellular adenoma or carcinoma	0.0020[c]	0.0314	0.021[c]
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats 2 years	Nasal cavity squamous cell carcinoma, rhabdomyosarcoma, sarcoma NOS or esthesioneuroepithelioma	0.0005	0.4256	0.0018
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats 2 years	Hepatocellular adenoma or carcinoma	0.0039	0.4256	0.014
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats 2 years	Mammary gland fibroadenoma or adenoma	0.0017	0.4256	0.0061
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats 2 years	Subcutis fibroma	0.0019	0.4256	0.0067

Reference Species Study Duration	Tumor Type	Animal CSF $(mg/kg\text{-}day)^{-1}$	Time-Weighted Average Animal Body Weight (kg)[a]	Human CSF_b $(mg/kg\text{-}day)_{-1}$
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats 2 years	Peritoneal mesothelioma	0.0037	0.4256	0.013
Kano et al. (2009), JBRC (1998) Male F344/DuCrj rats 2 years	Multisite: Nasal cavity squamous cell carcinoma or adenocarcinoma, hepatocellular adenoma or carcinoma, mammary gland fibroadenoma or adenoma, subcutis fibroma, and peritoneal mesothelioma	0.0090	0.4256	0.032
Kano et al. (2009), JBRC (1998) Female F344/DuCrj rats 2 years	Nasal cavity squamous cell carcinoma or esthesioneuroepithelioma	0.0003	0.2628	0.0013
Kano et al. (2009), JBRC (1998) Female F344/DuCrj rats 2 years	Hepatocellular adenoma or carcinoma	0.0015	0.2628	0.0061
Kano et al. (2009), JBRC (1998) Female F344/DuCrj rats 2 years	Mammary gland fibroadenoma, adenoma or adenocarcinoma	0.0011	0.2628	0.0046
Kano et al. (2009), JBRC (1998) Female F344/DuCrj rats 2 years	Multisite: Nasal cavity squamous cell carcinoma or ethesioneuroepithelioma, hepatocellular adenoma or carcinoma, and mammary gland fibroadenoma, adenoma or adenocarcinoma	0.0022	0.2628	0.0088

Reference Species Study Duration	Tumor Type	Animal CSF $(mg/kg\text{-}day)^{-1}$	Time-Weighted Average Animal Body Weight (kg)[a]	Human CSF_b $(mg/kg\text{-}day)^{-1}$
Kano et al. (2009), JBRC (1998) Male Crj:BDF_1 mice 2 years	Hepatocellular adenoma or carcinoma	0.0069	0.0474	0.043
Kano et al. (2009), JBRC (1998) Male Crj:BDF_1 mice 2 years	Hemangioendothelioma of the heart	0.0002	0.0474	0.0014
Kano et al. (2009), JBRC (1998) Male Crj:BDF_1 mice 2 years	Multisite: Hepatocellular adenoma or carcinoma and hemangioendothelioma of the heart	0.0070	0.0474	0.044
Kano et al. (2009), JBRC (1998) Female Crj:BDF_1 mice 2 years	Hepatocellular adenoma or carcinoma	0.02708	0.0353	**0.18**

[a] The animal weights are the time-weighted average body weight in the control group. In NCI (1978), animal body weights were reported graphically; therefore, values were extracted using GetData graph digitizer.
[b] The equation used for the calculation of the human CSF from the animal CSF is shown in the text following this table. The default value of 70 kg was used for human body weight.
[c] Adjusted for duration to 104 weeks

The female mouse study by Kano et al. (2009) and JBRC (1998) was the most sensitive among the available cancer studies, producing the highest CSF with the MSW model (Table 11). The dose associated with a 5% extra risk of developing liver tumors (BMD_{05}) and the 95% lower confidence limit on that dose ($BMDL_{05}$) are 2.64 and 1.85 mg/kg-day, respectively, for the 1st-degree polynomial model (Table 10). The model output is shown in Appendix E. The animal CSF was calculated as follows:

$$CSF_{animal} = BMR \div BMDL_{05} = 0.05 \div 1.85 \text{ mg/kg-day} = 0.027 \text{ (mg/kg-day)}^{-1}.$$

The human CSF was calculated based on allometric conversion using the default human body weight of 70 kg and the time-weighted average female mouse body weight in the control group (0.0353 kg) from the Kano et al. (2009) study:

$$CSF_{human} = CSF_{(animal)} \times (\text{body weight}_{(human)} \div \text{body weight}_{(animal)})^{1/4}$$

$$= 0.027 \text{ (mg/kg-day)}^{-1} \times (70 \text{ kg} \div 0.0353 \text{ kg})^{1/4} = 0.18 \text{ (mg/kg-day)}^{-1}.$$

This value is utilized, along with the inhalation CSF, for derivation of the HPC for 1,4-dioxane based on cancer.

Inhalation Cancer Slope Factor

The Kasai et al. (2009) inhalation study was selected for slope factor derivation because it included multiple exposure concentrations, had a study duration of 2 years, and had large sample sizes. Similar to the oral cancer bioassays, liver and nasal cavity tumors were observed. Incidences for the different tumor types in the Kasai et al. (2009) study were modeled using the LMS model (BMDS version 3.3.2, US EPA) and the resulting $BMDL_{05}$ values (Table 12) were used to derive the CSFs shown in Table 13. In animals where tumors were observed at multiple sites, the BMDS multitumor model (MS_Combo) was used.

Table 12. Dose-response modeling results for tumors in male F344 rats exposed to 1,4-dioxane vapor for two years (Kasai et al., 2009)

Tumor	Concentration (ppm): 0, 50, 250, 1,250 Dose[a] (mg/kg-day): 0, 30.11, 150.55, 752.76 Tumor Incidence	Model	BMD/BMDL (mg/kg-day) p-value
Nasal squamous cell carcinoma	0/50, 0/50, 1/50, 6/50*	LMS 1st degree polynomial	324.56/184.67 p-value = 0.96
Hepatocellular adenoma or carcinoma[c]	1/50, 2/50, 4/50, 22/50**	LMS 1st degree polynomial	74.11/53.43 p-value = 0.69
Peritoneal mesothelioma	2/50, 4/50, 14/50,** 41/50**	LMS 1st degree polynomial	24.10/18.87 p-value = 0.85
Mammary gland fibroadenoma[d]	1/50, 2/50, 3/50, 5/50	LMS 1st degree polynomial	479.48/206.12 p-value = 0.79
Zymbal gland adenoma	0/50, 0/50, 0/50, 4/50	LMS 1st degree polynomial	578.96/280.76 p-value = 0.80
Renal cell carcinoma	0/50, 0/50, 0/50, 4/50	LMS 1st degree polynomial	578.96/280.76 p-value = 0.80
Subcutis fibroma	1/50, 4/50, 9/50,** 5/50[e]	LMS 1st degree polynomial	41.56/24.01 p-value = 0.52
Multisite: all tumors in this table	N/A	MS_Combo	11.41/9.17 p-value = N/A[b]

LMS, linear multistage; MS-Combo, multitumor model in BMDS; N/A, not available

[a] To convert ppm to mg/kg-day, the equation shown below was used.[6] The animal weights are the time-weighted average body weight of the control group. Animal body weights were reported graphically; therefore, values were extracted using GetData graph digitizer.
[b] p-value not calculated by the multisite (MS_Combo) model
[c] US EPA (2013): "Incidence data was provided via email from Dr. Tatsuya Kasai (JBRC) to Dr. Reeder Sams (US EPA) on 12/23/2008. Statistics were not reported for these data by study authors, so statistical analyses were conducted by EPA."
[d] Mammary gland adenomas were not pooled with mammary gland fibroadenomas to prevent double counting.
[e] The high dose was dropped to allow for model fit. Authors noted high mortality at the high dose; thus, the lack of statistical significance and decreased incidence rate at the high dose may be attributed to increased mortality.
*,** significantly different from control at $p \leq 0.05$ or 0.01 by Fisher's exact test, respectively

Table 13. Inhalation cancer slope factor calculations based on tumors in male F344 rats exposed to 1,4-dioxane vapor for two years (Kasai et al., 2009)

Tumor Type	Animal CSF $(mg/kg\text{-}day)^{-1}$	Time-Weighted Average Animal Body Weight (kg)[a]	Human CSF[b] $(mg/kg\text{-}day)^{-1}$
Nasal squamous cell carcinoma	0.000271	0.427	0.0010
Hepatocellular adenoma or carcinoma [b]	0.000936	0.427	0.0033
Peritoneal mesothelioma	0.00265	0.427	0.0095
Mammary gland fibroadenoma	0.000243	0.427	0.00087
Zymbal gland adenoma	0.000178	0.427	0.00064
Renal cell carcinoma	0.000178	0.427	0.00064
Subcutis fibroma	0.00208	0.427	0.0074
Multisite: all tumors in this table	0.00545	0.427	**0.020**

[a] The animal weights are the time-weighted average body weight of the control group. Animal body weights were reported graphically; therefore, values were extracted using GetData graph digitizer.
[b] The equation used for the calculation of the human CSF from the animal CSF is shown in the text following this table. The default value of 70 kg was used for human body weight.

[6] mg/kg-day = ppm × UC × BR/BW x ETA × EFA
UC = unit conversion factor, (Y mg/m^3 = (X ppm)(molecular weight)/24.45) = (Y mg/m^3 = (X ppm)(88.1)/24.45): 1 ppm = 3.6 mg/m^3
ETA = exposure time adjustment, 6 hr/24 hr (unitless)
EFA = exposure frequency factor, 5 days/7 days (unitless)
BR = breathing rate of rat, (0.702 x $BW^{2/3}$ (OEHHA 2018)), 0.40 m^3/day
BW = body weight of male Fischer 344 rat, 0.427 kg (time-weighted average body weight of the control group). Animal body weights were reported graphically; therefore, values were extracted using GetData graph digitizer.

The male rat cancer study by Kasai et al. (2009) was the only inhalation study appropriate for dose-response modeling for deriving the inhalation CSF. As treatment-related tumors were induced at multiple sites in this experiment, the multisite approach provides a basis for estimating the combined risk. The BMD_{05} and the $BMDL_{05}$ were 11.41 and 9.17 mg/kg-day, respectively (Table 12). The model output is shown in Appendix E. The animal CSF was calculated as follows:

$$CSF_{animal} = BMR \div BMDL_{05} = 0.05 \div 9.17 mg/kg\text{-}day = 0.00545\ (mg/kg\text{-}day)^{-1}.$$

The human CSF was calculated based on allometric conversion using the average human body weight of 70 kg and the time-weighted average animal weight in the male rat control group (0.427 kg):

$$CSF_{human} = CSF_{(animal)} \times (body\ weight_{(human)} \div body\ weight_{(animal)})^{1/4}$$

$$= 0.00545\ (mg/kg\text{-}day)^{-1} \times (70\ kg \div 0.427\ kg)^{1/4} = 0.020\ (mg/kg\text{-}day)^{-1}.$$

This value is utilized, along with the oral CSF, for derivation of the HPC for 1,4-dioxane based on cancer.

HEALTH-PROTECTIVE DRINKING WATER CONCENTRATIONS

Noncancer Health-Protective Drinking Water Concentration

To calculate an HPC for a chemical, the ADD is converted to a concentration in drinking water that accounts for the total exposure to the chemical from using tap water. This includes intake from multiple routes of exposure (oral, inhalation, and dermal) to contaminants in tap water from household uses (e.g., drinking, cooking, bathing, and showering). To estimate inhalation and dermal exposures, the CalTOX 4.0 multimedia total exposure model developed for the California Department of Toxic Substances Control by Lawrence Berkeley National Laboratory was used. The estimated relative contributions from each route at different life stages are shown in Table 14. Detailed parameters and equations used are presented in Appendix C.

Table 14. Estimated relative contributions of multiple routes of exposure to 1,4-dioxane in tap water at different life stages

Life Stage	Oral Ingestion (%)	Inhalation (%)	Dermal (%)
Fetus (pregnancy)	89	11	0
Infant	100	0[a]	0
Child	83	17	0
Adult	91	9	0

[a] It is anticipated that infants are not exposed to chemicals in tap water via inhalation because they typically do not shower or flush toilets. These are the predominant inhalation exposure scenarios; therefore, the inhalation pathway is excluded for infants.

From studies dioxane in human volunteers, absorption via inhalation and ingestion routes is assumed to be 100% (Young et al., 1976; ATSDR, 2012). There are limited studies investigating dermal absorption in humans and animals but from the data available, dermal absorption is minimal due to the volatility of 1,4-dioxane (Bronaugh, 1982; Marzulli et al., 1981). Liter equivalent (L_{eq}) values for inhalation and dermal exposures (Table 15) are calculated using life

stage-specific oral ingestion rates (OEHHA, 2012) and the relative contributions of the routes of exposure listed in Table 14.

Table 15. Liter equivalent values for multiroute exposure to 1,4-dioxane in tap water

Life Stage	Age range (years)	Fractional duration (years)	Tap Water Exposure Level (L_{eq}/kg-day)			
			Oral	Inhalation[a,b]	Dermal	Total
Pregnancy	NA	0.75/70	0.047	0.006	0	0.053
Infant	0-2	2/70	0.196	0	0	0.196
Child	2-16	14/70	0.061	0.012	0	0.073
Adult	16-70	54/70	0.045	0.004	0	0.049
Time-weighted average over lifetime (L_{eq}/kg-day)						**0.059[c]**

[a] Inhalation estimate is calculated using life stage-specific oral ingestion rates (OEHHA, 2012) and relative contributions of this route of exposure.
[b] L_{eq} for inhalation assumes100% absorption in the lung (Young et al., 1976).
[c] Multiroute lifetime tap water exposure = [(0.75 × 0.053) + (2 × 0.196) + (14 × 0.073) + (54 × 0.049)]÷70 = 0.059 L_{eq}/kg-day.

The relative source contribution (RSC) is the proportion of exposures to a chemical attributed to tap water (including inhalation and dermal exposures, e.g., during showering), as part of total exposure from all sources (including food and air pollution). The RSC of 0.2 is selected for 1,4-dioxane as the general population can be exposed to 1,4-dioxane through other sources such as air, soil, food, and consumer and industrial products, in addition to drinking water. However, data on the exposure patterns of these other sources are not available, thus the default value of 0.2 is applied. An RSC of 0.2 is consistent with other states such as Minnesota and New Jersey in their derivation of a noncancer health-based value for 1,4-dioxane (MDH, 2013; NJDWQI, 2020).

The noncancer health-protective drinking water concentration of 1,4-dioxane is calculated as:

HPC = (0.0096 mg/kg-day × 0.2) ÷ 0.059 L_{eq}/kg-day = 0.033 mg/L = 33 ppb or µg/L

The health-protective drinking water concentration for noncancer effects is 33 ppb.

Cancer Health-Protective Drinking Water Concentration

When determining cancer risk, OEHHA applies ASFs to account for the increased susceptibility of infants and children to carcinogens (OEHHA, 2009). A weighting factor of 10 is applied for exposures that occur from the 3rd trimester to <2 years of age, and a factor of 3 is applied for exposures that occur from 2 through 16 years of age. These factors are applied regardless of the mechanism of action, unless chemical-specific data exist to better guide the risk assessment. ASFs for each life stage are multiplied by the fractional duration (d) of each life stage and the daily water intake (DWI, in L/kg-day or L_{eq}/kg-day if accounting for inhalation and dermal exposures). The sum of the ASF-adjusted exposures across all life stages is the lifetime exposure value for the chemical (Tables 16 and 17; detailed equations in Appendix G).

Table 16. ASF-adjusted oral exposures from tap water use

Stage	Age Sensitivity Factor (ASF)[a]	Fractional Duration[b] (d)	Daily Water Intake (DWI, L/kg-day)	ASF × d × DWI (L/kg-day)
3rd trimester (Pregnancy)	10	0.25/70	0.047	0.0017
Infant (0-2 yr)	10	2/70	0.196	0.0560
Child (2-16 yr)	3	14/70	0.061	0.0366
Adult (16-70 yr)	1	54/70	0.045	0.0347
			Total Lifetime Exposure	**0.1290**

[a] Age sensitivity factors for different life stages adopted by OEHHA (2009)
[b] An average lifetime of 70 years is assumed for the general population

Table 17. ASF-adjusted inhalation exposures from tap water use

Stage	Age Sensitivity Factor (ASF)[a]	Fractional Duration[b] (d)	Daily Water Intake (DWI, L_{eq}/kg-day)	ASF × d × DWI (L_{eq}/kg-day)
3rd trimester (Pregnancy)	10	0.25/70	0.006	0.0002
Infant (0-2 yr)	10	2/70	0[c]	0.0000
Child (2-16 yr)	3	14/70	0.012	0.0072
Adult (16-70 yr)	1	54/70	0.004	0.0031
			Total Lifetime Exposure	**0.0105**

[a] Age sensitivity factors for different life stages adopted by OEHHA (2009)
[b] An average lifetime of 70 years is assumed for the general population
[c] It is anticipated that infants are not exposed to chemicals in tap water via inhalation because they typically do not shower or flush toilets. These are the predominant inhalation exposure scenarios; therefore the inhalation pathway is excluded for infants.

The study by Kano et al. (2009) and JBRC (1998) via the oral route and the study by Kasai et al. (2009) via the inhalation route provide the most appropriate dose-response data for cancer effects and were selected as the critical studies. The resulting human oral CSF of 0.18 (mg/kg-day)$^{-1}$ and the human inhalation CSF of 0.02 (mg/kg-day)$^{-1}$ were used to calculate the risk-specific concentration for a one in one million lifetime cancer risk.

$$HPC = \frac{R}{[CSF_{oral} \times (\sum_j [ASF_j \times d_j \times DWI^{oral}_j]) + CSF_{inh} \times (\sum_j [ASF_j \times d_j \times DWI^{inh}_j])]}$$

where:

R	=	default risk level of one in one million, or 10^{-6}
CSF_{oral}	=	oral cancer slope factor, in (mg/kg-day)$^{-1}$
CSF_{inh}	=	inhalation cancer slope factor, in (mg/kg-day)$^{-1}$
$\sum_j$	=	sum of contributions at each age range
ASF_i	=	age sensitivity factors for the 3rd trimester + infants, children, and adults
d_j	=	duration of exposure for the 3rd trimester + infant, child, and adult life stages
$DWI^{inh/oral}_j$	=	equivalent water exposure values for each age range.

$$HPC = \frac{10^{-6}}{[(0.18\ (mg/kg\text{-}day)^{-1} \times 0.1290\ Leq/kg\text{-}day) + (0.02\ (mg/kg\text{-}day)^{-1} \times 0.0105\ Leq/kg\text{-}day)]}$$

$$= 0.000043\ mg/L = 0.04\ \mu g/L \text{ or ppb (rounded)}$$

The estimated health-protective drinking water concentration for cancer effects is 0.04 ppb.

Public Health Goal

The HPC of 0.04 ppb for cancer is proposed as the PHG. Since this value is lower than the HPC of 33 ppb derived for noncancer effects, the PHG should protect against both cancer and noncancer effects of 1,4-dioxane in drinking water.

RISK CHARACTERIZATION

The proposed PHG of 0.04 ppb for 1,4-dioxane is based on liver cancer observed in female mice by Kano et al. (2009) and JBRC (1998) following chronic exposure to 1,4-dioxane in drinking water and tumors at multiple sites observed in male rats by Kasai et al. (2009) following chronic exposure to 1,4-dioxane vapor. There are no epidemiological studies that link 1,4-dioxane exposure to cancer in humans. IARC (1999) classified 1,4-dioxane as “possibly carcinogenic to humans (Group 2B)” based on inadequate evidence in humans and sufficient evidence in animals for carcinogenicity. US EPA (2013a) classified 1,4-dioxane as “likely to be carcinogenic to humans” based on evidence in animals and inadequate evidence in humans. NTP classified 1,4-dioxane as “reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals.” Additionally, 1,4-dioxane is currently listed as a carcinogen under California’s Proposition 65.

Mechanistic evidence, some key uncertainties, and other issues considered in the development of the proposed PHG and HPCs for 1,4-dioxane are summarized below:

- **Genotoxicity.** Evidence of genotoxicity for 1,4-dioxane was mixed. In vitro genetic toxicity tests were negative while in vivo genetic toxicity tests showed mixed results in mice and rats. Additionally,1,4-dioxane caused cancer in both sexes of two species of laboratory animals, and at multiple sites. Taken together, a genotoxic MOA for 1,4-dioxane cannot be ruled out.
- **Linear model of cancer dose-response.** As illustrated in the mode of action and mechanistic considerations section, there are multiple plausible MOAs for 1,4-dioxane carcinogenicity. An alternative MOA of cytotoxicity leading to cell proliferation has been proposed in the literature. However, several animal studies show evidence that cytotoxicity did not occur prior to tumor formation. Oxidative stress is another plausible MOA; however, there are several studies with conflicting results. Therefore, because the exact MOA remains unknown, OEHHA employed the default linear extrapolation for CSF derivation. This is consistent with US EPA’s calculation of cancer risk using a linear model (US EPA, 2020).

- **Interspecies extrapolation for cancer.** To estimate the risk of any human cancer based on animal data, OEHHA uses the interspecies scaling factor of body weight to the ¾ power to account for toxicokinetic and toxicodynamic differences between rodents and humans that might result in carcinogenic response to 1,4-dioxane exposure. This adjustment was approximately seven-fold for mouse-based values.
- **Dose metric.** It remains unknown whether 1,4-dioxane or its metabolite is responsible for the cancer and noncancer effects observed in test animals. Administered doses were used as the dose-metric in dose-response analysis.
- **Sensitive subgroups.** OEHHA is mandated to consider sensitive subgroups, such as children and infants, who may be at greater risk of adverse health effects than the general population due to exposure to drinking water contaminants. Improvements in water ingestion estimates and adjustments for age sensitivity are crucial to the assessment of risk to these sensitive subgroups. Additionally, human variability in CYP enzyme expression may contribute to the variation in human susceptibility since CYP enzymes are involved in 1,4-dioxane metabolism. This potential in human variability is taken into account in the intraspecies uncertainty factor (UF_H, see appendix B). In addition, when determining cancer risk, OEHHA has adopted the use of ASFs to account for elevated risk in infants and children exposed to carcinogens (OEHHA, 2009). A weighting factor of 10 is applied for exposures that occur from the 3rd trimester to <2 years of age, and a factor of 3 is applied for exposures that occur from 2 through 16 years of age. These factors are applied regardless of the mechanism of action, unless chemical-specific data exist to better guide the risk assessment. To account for increased exposure during these sensitive periods, OEHHA uses age group-specific 95th percentile drinking water consumption rates.
- **Uncertainty factors.** OEHHA generally applies a default composite UF of 300, consisting of 10 for interspecies extrapolation (√10 for toxicokinetics and √10 for toxicodynamics) and 30 for intraspecies variability (10 for toxicokinetics and √10 for toxicodynamics). The intraspecies uncertainty factor also includes variability in CYP enzyme expression. An additional √10 for database deficiencies was included for the limited database of reproductive and developmental toxicity studies. A composite uncertainty factor of 1000 was used to calculate the noncancer health-protective concentration.

Other regulatory standards

Regulatory standards for 1,4-dioxane from federal, state and international agencies are shown below.

Table 18. Summary of state, federal and international drinking water health advisory levels and regulations for 1,4-dioxane

State/ Organization	Advisory or Regulatory Value	Reference
US EPA	Drinking Water Unit Risk: 0.35 ppb at 10^{-6} cancer risk	US EPA (2013a); (US EPA, 2013b)
Maine	Maximum Exposure Guideline: 4 ppb	MEDEP (2015)
Massachusetts	Drinking Water Guideline: 0.3 ppb	Mass DEP (2020)
Health Canada	Health Based Value: 50 ppb	Health Canada (2021)
Connecticut	Action Level: 3 ppb	Connecticut Department of Public Health (2022)
New Jersey	MCL: 0.33 ppb	New Jersey Drinking Water Quality Institute (2021)
Groundwater		
Alaska	Groundwater Human Health Cleanup Level: 4.6 ppb	AL DEC (2021)
New Jersey	Groundwater Quality Standard: 0.4 ppb	NJDEP (2015)
Colorado	Groundwater Organic Chemical Standards: 0.35 ppb	CDPHE (2012)
Delaware	Groundwater screening level: 0.46 ppb	Delaware Department of Natural Resources and Environmental Control (2022)
Florida	Health Advisory Level: 0.35 ppb	Florida Department of Health Environmental Health (2016)
Illinois	Groundwater standard: 7.7 ppb	Illinois Pollution Control Board (2022)

State/ Organization	Advisory or Regulatory Value	Reference
Indiana	Groundwater standard: 7.7 ppb	Indiana Department of Environmental Management (2022)
Iowa	Protected Groundwater Source Standard: 200 ppb Unprotected Groundwater Source Standard: 1000 ppb	Iowa Department of Natural Resources (2022)
Minnesota	Health Based Value: 1 ppb	MDH (2013)
Mississippi	Target Remediation Goal in Groundwater: 6.09 ppb	Mississippi Department of Environmental Quality (2002)
New Hampshire	Ambient Groundwater Quality Standard: 0.32 ppb	New Hampshire Department of Environmental Services (2007)
North Carolina	Groundwater Standard: 3 ppb	North Carolina Department of Environmental Quality (2013)
Pennsylvania	Medium-Specific Concentration in Groundwater: 6.4 ppb	Pennsylvania Department of Environmental Protection Land Recycling Program (2016)
Texas	Protective Concentration Level for Groundwater at a Residential setting: 9.1 ppb	Texas Commission on Environmental Quality (2018)
Vermont	Groundwater Enforcement Standard and Preventative Action level: 0.3 ppb	Vermont Agency of Natural Resources Department of Environmental Conservation (2019)
Washington	Water Quality Criteria for Groundwater: 7 ppb	Washington Department of Ecology (2003)
West Virginia	Groundwater Standard: 0.46 ppb	West Virginia Department of Environmental Protection (2018)

REFERENCES

AL DEC (2021). Environmental Conservation. https://dec.alaska.gov/media/533hr3p0/18-aac-75.pdf. date accessed:2022.

Andersen ME, Clewell HJ, Gargas ML, Smith FA, Reitz RH (1987). Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol 87(2): 185-205.

Argus MF, Arcos JC, Hochligeti C (1965). Studies on the carcinogenic activity of protein-denaturing agents: hepatocarcinogenicity of dioxane. J Natl Cancer Inst 35(6): 949-958.

Argus MF, Sohal RS, Bryant GM, Hoch-Ligeti C, Arcos JC (1973). Dose-response and ultrastructural alterations in dioxane carcinogenesis.: Influence of methylcholanthrene on acute toxicity. Eur J Cancer 9(4): 237-243.

ATSDR (2012). Toxigological Profile for 1,4-dioxane. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Bailer AJ, Portier CJ (1988). Effects of Treatment-Induced Mortality and Tumor-Induced Mortality on Tests for Carcinogenicity in Small Samples. Biometrics 44(2): 417-431.

Bannasch P, Moore MA, Klimek F, Zerban H (1982). Biological markers of preneoplastic foci and neoplastic nodules in rodent liver. Toxicol Pathol 10(2): 19-34.

Barber H (1934). Haemorrhagic nephritis and necrosis of the liver from dioxan poisoning. Guy's Hospital Reports 84: 267-280.

Bilal M, Iqbal HMN (2019). An insight into toxicity and human-health-related adverse consequences of cosmeceuticals - A review. Sci Total Environ 670: 555-568.

Bronaugh RL (1982). Percutaneous absorption of cosmetic ingredients. Principles of cosmetics for the dermatologist. PH Frost, S.N. Minneapolis, MN: University of Minnesota Press.

Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP (1997). Physiological Parameter Values for Physiologically Based Pharmacokinetic Models. Toxicol Ind Health 13(4): 407-484.

Buffler PA, Wood SM, Suarez L, Kilian DJ (1978). Mortality Follow-up of Workers Exposed to 1,4-Dioxane. J Occup Environ Med 20(4).

CARB (2023). California Air Resources Board (CARB): CARB Emission Inventory Data for 2021 (extracted August 2, 2023).accessed,date.

CDPHE (2012). Regulation No. 41: The Basic Standards for Ground Water (5 CCR 1002-41). https://www.sos.state.co.us/CCR/GenerateRulePdf.do?ruleVersionId=5034&fileName=5%20CCR%201002-41 [accessed July 29, 2021 Access 2012].

Charkoftaki G, Golla JP, Santos-Neto A, et al. (2021). Identification of Dose-Dependent DNA Damage and Repair Responses From Subchronic Exposure to 1,4-Dioxane in Mice Using a Systems Analysis Approach. Toxicol Sci 183(2): 338-351.

Chen Y, Wang Y, Charkoftaki G, et al. (2022). Oxidative stress and genotoxicity in 1,4-dioxane liver toxicity as evidenced in a mouse model of glutathione deficiency. Sci Total Environ 806(Pt 2): 150703.

Connecticut Department of Public Health (2022). Action Level List for Drinking Water. https://portal.ct.gov/DPH/Environmental-Health/Environmental-and-Occupational-Health-Assessment/Action-List-for-Drinking-Water. date accessed:2022.

Delaware Department of Natural Resources and Environmental Control (2022). Hazardous Substance Cleanup Act Screening Level Table Guidance. https://documents.dnrec.delaware.gov/dwhs/remediation/HSCA-Screening-Level-Table-Guidance.pdf. date accessed:2022.

Doherty A-C, Lee C-S, Meng Q, et al. (2023). Contribution of household and personal care products to 1,4-dioxane contamination of drinking water. Curr Opin Environ Sci Hlth 31: 100414.

Dourson M, Reichard J, Nance P, et al. (2014). Mode of action analysis for liver tumors from oral 1,4-dioxane exposures and evidence-based dose response assessment. Regul Toxicol Pharmacol 68(3): 387-401.

Dourson ML, Higginbotham J, Crum J, et al. (2017). Update: Mode of action (MOA) for liver tumors induced by oral exposure to 1,4-dioxane. Regul Toxicol Pharmacol 88: 45-55.

Draper WM, Dhoot JS, Remoy JW, Perera SK (2000). Trace-level determination of 1,4-dioxane in water by isotopic dilution GC and GC-MS. Analyst 125(8): 1403-1408.

Eck WS (2010). Toxicology Report No. 87-XE-08WR-09 Studies on Metabolism of 1,4-Dioxane Aberdeen Proving Ground, MD 21010-5403.

Ernstgård L, Bottai M (2012). Visual analogue scales: How can we interpret them in experimental studies of irritation in the eyes, nose, throat and airways? J Appl Toxicol 32(10): 777-782.

Ernstgård L, Bottai M, Johanson G, Sjögren B (2019). Down-regulation of the inflammatory response after short-term exposure to low levels of chemical vapours. Occup Environ Med 76(7): 482-487.

Ernstgård L, Iregren A, Sjögren B, Johanson G (2006). Acute effects of exposure to vapours of dioxane in humans. Hum Exp Toxicol 25(12): 723-729.

EWG (2007). EWG Research Shows 22 Percent of All Cosmetics May Be Contaminated With Cancer-Causing Impurity. http://www.ewg.org/node/21286. date accessed:08/01/2022.

Fairley A, Linton EC, Ford-Moore AH (1934). The Toxicity to Animals of 1:4 Dioxan. J Hyg 34(4): 486-501.

Florida Department of Health Environmental Health (2016). Maximum Contaminant Levels and Health Advisory Levels. https://www.floridahealth.gov/environmental-health/drinking-water/_documents/hal-list.pdf. date accessed:2022.

Furihata C, Toyoda T, Ogawa K, Suzuki T (2018). Using RNA-Seq with 11 marker genes to evaluate 1,4-dioxane compared with typical genotoxic and non-genotoxic rat hepatocarcinogens. Mutat Res Genet Toxicol Environ Mutagen 834: 51-55.

Galloway SM, Armstrong MJ, Reuben C, et al. (1987). Chromosome aberrations and sister chromatid exchanges in chinese hamster ovary cells: Evaluations of 108 chemicals. Environ Mol Mutagen 10(S10): 1-35.

Garcia E, Hurley S, Nelson DO, Hertz A, Reynolds P (2015). Hazardous air pollutants and breast cancer risk in California teachers: a cohort study. Environ Health 14: 14.

Gi M, Fujioka M, Kakehashi A, et al. (2018). In vivo positive mutagenicity of 1,4-dioxane and quantitative analysis of its mutagenicity and carcinogenicity in rats. Arch Toxicol 92(10): 3207-3221.

Giavini E, Vismara C, Broccia ML (1985). Teratogenesis study of dioxane in rats. Toxicol Lett 26(1): 85-88.

Godri Pollitt KJ, Kim JH, Peccia J, et al. (2019). 1,4-Dioxane as an emerging water contaminant: State of the science and evaluation of research needs. Sci Total Environ 690: 853-866.

Göen T, von Helden F, Eckert E, et al. (2016). Metabolism and toxicokinetics of 1,4-dioxane in humans after inhalational exposure at rest and under physical stress. Arch Toxicol 90(6): 1315-1324.

Goldsworthy TL, Monticello TM, Morgan KT, et al. (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes. Arch Toxicol 65(1): 1-9.

Hart JE, Bertrand KA, DuPre N, et al. (2018). Exposure to hazardous air pollutants and risk of incident breast cancer in the nurses' health study II. Environ Health 17(1): 28.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. Environ Mutagen 5(S1): 3-49.

Health Canada (2021). 1,4 dioxane in Drinking Water. Ottawa, ON https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-guideline-1-4-dioxane.html Access 2021].

Hellmér L, Bolcsfoldi G (1992). An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds. Mutat Res 272(2): 145-160.

Hill A (1965). The environment and disease: Association or causation? Proc R Soc Med 58(5): 295-300.

Hoch-Ligeti C, Argus MF, Eds. (1970). Effect of carcinogens on the lung of guinea pigs. Oak Ridge, TN, United States Atomic Energy Comission, Division of Technical Information.

Hoch-Ligeti C, Argus MF, Arcos JC (1970). Induction of carcinomas in the nasal cavity of rats by dioxane. Br J Cancer 24(1): 164-167.

HSDB (2020). 1,4-Dioxane. Bethesda, MD National Library of Medicine, National Institutes of Health https://pubchem.ncbi.nlm.nih.gov/compound/Dioxane Access 2020].

IARC (1999). 1,4-Dioxane. IARC Monographs on the Evaluation of Carciongenic Risks to Humans. Lyon, France. 71 part 2**:** 589-602.

Illinois Pollution Control Board (2022). Section 620.410 Groundwater Quality Standards for Class I: Potable Resource Groundwater. http://www.ilga.gov/commission/jcar/admincode/035/035006200D04100R.html. date accessed:2022.

Indiana Department of Environmental Management (2022). Section 620.410 Groundwater Quality Standards for Class I: Potable Resource Groundwater. https://www.in.gov/idem/cleanups/2392.htm. date accessed:2022.

Iowa Department of Natural Resources (2022). Cumulative risk calculator https://programs.iowadnr.gov/riskcalc/Home/statewidestandards. date accessed:2022.

IRTC (2021). Regulatory framework 1,4-dioxane. https://14d-1.itrcweb.org/wp-content/uploads/2021/02/14d_regulatory_framework_021621.pdf Access 2021].

Ito N, Imaida K, Asamoto M, Shirai T (2000). Early detection of carcinogenic substances and modifiers in rats. Mutation Research/Reviews in Mutation Research 462(2): 209-217.

Itoh S, Hattori C (2019). In vivo genotoxicity of 1,4-dioxane evaluated by liver and bone marrow micronucleus tests and Pig-a assay in rats. Mutat Res 837: 8-14.

JBRC (1998). Two-year studies of 1,4-dioxane in F344 rats and BDF1 mice (drinking water). Kanagawa, Japan.

Johnstone RT (1959). Death due to dioxane? AMA Arch Ind Health 20: 445-447.

Kalkbrenner AE, Windham GC, Zheng C, et al. (2018). Air Toxics in Relation to Autism Diagnosis, Phenotype, and Severity in a U.S. Family-Based Study. Environ Health Perspect 126(3): 037004.

Kano H, Umeda Y, Kasai T, et al. (2009). Carcinogenicity studies of 1,4-dioxane administered in drinking-water to rats and mice for 2 years. Food Chem Toxicol 47(11): 2776-2784.

Kano H, Umeda Y, Saito M, et al. (2008). Thirteen-week oral toxicity of 1,4-dioxane in rats and mice. J Toxicol Sci 33(2): 141-153.

Kasai T, Kano H, Umeda Y, et al. (2009). Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats. Inhal Toxicol 21(11): 889-897.

Kasai T, Saito M, Senoh H, et al. (2008). Thirteen-week inhalation toxicity of 1,4-dioxane in rats. Inhal Toxicol 20(10): 961-971.

Khudoley VV, Mizgireuv I, Pliss GB (1987). The study of mutagenic activity of carcinogens and other chemical agents with Salmonella typhimurium assays: testing of 126 compounds. Arch. Geschwulstforsch 57(6): 453-462.

King ME, Shefner AM, Bates RR (1973). Carcinogenesis bioassay of chlorinated dibenzodioxins and related chemicals. Environ Health Perspect 5: 163-170.

Kitchin KT, Brown JL (1990). Is 1,4-dioxane a genotoxic carcinogen? Cancer Lett 53(1): 67-71.

Kociba RJ, McCollister SB, Park C, Torkelson TR, Gehring PJ (1974). 1,4-Dioxane. I. Results of a 2-year ingestion study in rats. Toxicol Appl Pharmacol 30(2): 275-286.

Lafranconi M, Budinsky R, Corey L, et al. (2021). A 90-day drinking water study in mice to characterize early events in the cancer mode of action of 1,4-dioxane. Regul Toxicol Pharmacol 119: 104819.

Leung HW, Paustenbach DJ (1990). Cancer risk assessment for dioxane based upon a physiologically-based pharmacokinetic approach. Toxicol Lett 51(2): 147-162.

Lundberg I, Högberg J, Kronevi T, Holmberg B (1987). Three industrial solvents investigated for tumor promoting activity in the rat liver. Cancer Lett 36(1): 29-33.

Maeshima H, Ohno K, Nakano S, Yamada T (2010). Validation of an in vitro screening test for predicting the tumor promoting potential of chemicals based on gene expression. Toxicol In Vitro 24(3): 995-1001.

Marzulli FN, Anjo DM, Maibach HI (1981). In vivo skin penetration studies of 2,4-toluenediamine, 2,4-diaminoanisole, 2-nitro-p-phenylenediamine, p-dioxane and N-nitrosodiethanolamine in cosmetics. Food Cosmet Toxicol 19(6): 743-747.

Mass DEP (2020). Standards and Guidelines or Contaminants in Massachusetts Drinking Waters. https://www.mass.gov/doc/2020-standards-and-guidelines-for-contaminants-in-massachusetts-drinking-waters/download. date.

McFee AF, Abbott MG, Gulati DK, Shelby MD (1994). Results of mouse bone marrow micronucleus studies on 1,4-dioxane. Mutat Res 322(2): 145-148.

McGregor DB, Brown AG, Howgate S, et al. (1991). Responses of the L5178Y mouse lymphoma cell forward mutation assay. V: 27 coded chemicals. Environ Mol Mutagen 17(3): 196-219.

McKone TE (1987). Human exposure to volatile organic compounds in household tap water: the indoor inhalation pathway. Environ Sci Technol 21(12): 1194-1201.

MDH (2013). Toxicological Summary for 1,4-Dioxane. https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/14dioxane.pdf Access 2013].

MEDEP (2016). Maine Remedial Action Guidelines (RAGs) for Sites Contaminated with Hazardous Substances. https://www.maine.gov/dhhs/mecdc/environmental-health/eohp/wells/documents/megtable2016.pdf [accessed July 29, 2021].

Mikheev MI, Gorlinskaya Ye P, Solovyova TV (1990). The body distribution and biological action of xenobiotics. J Hyg Epidemiol Microbiol Immunol 34(4): 329-336.

Mirkova ET (1994). Activity of the rodent carcinogen 1,4-dioxane in the mouse bone marrow micronucleus assay. Mutat Res 322(2): 142-144.

Mississippi Department of Environmental Quality (2002). Risk evaluation procedures for voluntary cleanup and redevelopment of brownfield sites. https://www.mdeq.ms.gov/wp-content/uploads/2017/05/Proced.pdf. date accessed:2022.

Miyagawa M, Shirotori T, Tsuchitani M, Yoshikawa K (1999). Repeat-assessment of 1,4-dioxane in a rat-hepatocyte replicative DNA synthesis (RDS) test: evidence for stimulus of hepatocyte proliferation. Exp Toxicol Pathol 51(6): 555-558.

Mohr T (2004). GRA's 1,4-Dioxane Conference Profiles National Challenge of Emerging and Unregulated Contaminants. Hydro Visions 13 No. 1.

Morita T, Hayashi M (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay. Environ Mol Mutagen 32(3): 269-280.

Nannelli A, De Rubertis A, Longo V, Gervasi PG (2005). Effects of dioxane on cytochrome P450 enzymes in liver, kidney, lung and nasal mucosa of rat. Arch Toxicol 79(2): 74-82.

NCI (1978). Bioassay of 1,4-dioxane for possible carcinogenicity. National Cancer Institute Carcinogenesis Technical Report Series No. 80. Bethesda, MD. 80**:** 1-123.

Nestmann ER, Otson R, Kowbel DJ, Bothwell PD, Harrington TR (1984). Mutagenicity in a modified Salmonella assay of fabric-protecting products containing 1,1,1-trichloroethane. Environ Mol Mutagen 6(1): 71-80.

New Hampshire Department of Environmental Services (2007). New Hampshire code of administrative rules, . https://www.des.nh.gov/sites/g/files/ehbemt341/files/documents/2020-01/Env-Or%20600.pdf. date accessed:2022.

New Jersey Drinking Water Quality Institute (2021). Maximum Contaminant Level Recommendation for 1,4-Dioxane in Drinking Water, New Jersey Drinking Water Quality Institute.

Niehoff NM, Gammon MD, Keil AP, et al. (2019). Hazardous air pollutants and telomere length in the Sister Study. Environ Epidemiol 3(4).

NJDEP (2015). Interim Ground Water Quality Standards. New Jersey Department of Environmental Protection Water Monitoring and Standards Bureau of Environmental Assessment Restoration and Standards
https://www.nj.gov/dep/wms/bears/docs/1,4%20dioxane%20final%20draft%20for%20posting2.pdf Access 2015].

NJDWQI (2020). Health-Based Maximum Contaminant Level Support Document 1,4-Dioxane. https://www.state.nj.us/dep/watersupply/pdf/14-dioxane-pub-rev-health-sub.pdf. date accessed:July 29, 2021.

North Carolina Department of Environmental Quality (2013). Groundwater Standards Table. https://files.nc.gov/ncdeq/documents/files/02L%20Groundwater%20Standards%20Table%205-21%202013_0.pdf. date accessed:2022.

NTP (2011). 1,4-dioxane. In Report on carcinogens, fourteenth edition, U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program.

NTP (2021). 1,4-dioxane. In Report on carcinogens, fifteenth edition, U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program.

OEHHA (2008). Air toxics hot spots risk assessment guidelines: Technical support document for the derivation of noncancer reference exposure levels. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

OEHHA (2009). Technical support document for cancer potency factors: methodologies for derivation, listing of available values, and adjustments to allow for early life stage exposures. Appendix J. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

OEHHA (2012). Air toxics hot spots program risk assessment guidelines: technical support document for exposure assessment and stochastic analysis. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

Pennsylvania Department of Environmental Protection Land Recycling Program (2016). Table 1-medium-specific concentrations (mscs) for organic regulated substances in groundwater. https://files.dep.state.pa.us/EnvironmentalCleanupBrownfields/LandRecyclingProgram/LandRecyclingProgramPortalFiles/SWHTables-2016/Table%201.pdf. date accessed:2022.

Ramsey JC, Andersen ME (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol Appl Pharmacol 73(1): 159-175.

Reitz RH, McCroskey PS, Park CN, Andersen ME, Gargas ML (1990). Development of a physiologically based pharmacokinetic model for risk assessment with 1,4-dioxane. Toxicol Appl Pharmacol 105(1): 37-54.

Roberts AL, Lyall K, Hart JE, et al. (2013). Perinatal air pollutant exposures and autism spectrum disorder in the children of Nurses' Health Study II participants. Environ Health Perspect 121(8): 978-984.

Roy SK, Thilagar AK, Eastmond DA (2005). Chromosome breakage is primarily responsible for the micronuclei induced by 1,4-dioxane in the bone marrow and liver of young CD-1 mice. Mutat Res 586(1): 28-37.

SCCS (2015). The Report of the ICCR Working Group: Considerations on Acceptable Trace Level of 1,4-Dioxane in Cosmetic Products.

Sheu CW, Moreland FM, Lee JK, Dunkel VC (1988). In vitro BALB/3T3 cell transformation assay of nonoxynol-9 and 1,4-dioxane. Environ Mol Mutagen 11(1): 41-48.

Sina JF, Bean CL, Dysart GR, Taylor VI, Bradley MO (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat Res 113(5): 357-391.

Smith MT, Guyton KZ, Gibbons CF, et al. (2016). Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis. Environ Health Perspect 124(6): 713-721.

Smith MT, Guyton KZ, Kleinstreuer N, et al. (2020). The Key Characteristics of Carcinogens: Relationship to the Hallmarks of Cancer, Relevant Biomarkers, and Assays to Measure Them. Cancer Epidemiol Biomarkers Prev 29(10): 1887-1903.

Stoner GD, Conran PB, Greisiger EA, et al. (1986). Comparison of two routes of chemical administration on the lung adenoma response in strain AJ mice. Toxicol Appl Pharmacol 82(1): 19-31.

Stott WT, Quast JF, Watanabe PG (1981). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. Toxicol Appl Pharmacol 60(2): 287-300.

Sweeney LM, Thrall KD, Poet TS, et al. (2008). Physiologically Based Pharmacokinetic Modeling of 1,4-Dioxane in Rats, Mice, and Humans. Toxicol Sci 101(1): 32-50.

SWRCB (2021). Drinking Water Notification Levels. https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/NotificationLevels.html. date.

Takano R, Murayama N, Horiuchi K, et al. (2010). Blood concentrations of 1,4-dioxane in humans after oral administration extrapolated from in vivo rat pharmacokinetics, in vitro human metabolism, and physiologically based pharmacokinetic modeling. J Health Sci 56(5): 557-565.

Take M, Ohnishi M, Yamamoto S, et al. (2012). Distribution of 1,4-dioxane by combined inhalation plus oral exposure routes in rats. Int. J. Environ. Anal. Chem 92(15): 1715-1728.

Texas Commission on Environmental Quality (2018). PCL Tables. https://www.tceq.texas.gov/downloads/remediation/trrp/2018-pcl-tables.xlsx. date accessed:2022.

Thiess AM, Tress E, Fleig I (1976). Industrial-medical investigation results in the case of workers exposed to dioxane. Arb.med. Soz.med. Präv.med. 11: 35-46.

Tinwell H, Ashby J (1994). Activity of 1,4-dioxane in mouse bone marrow micronucleus assays. Mutat Res 322(2): 148-150.

Totsuka Y, Maesako Y, Ono H, et al. (2020). Comprehensive analysis of DNA adducts (DNA adductome analysis) in the liver of rats treated with 1,4-dioxane. Proc Jpn Acad Ser B Phys Biol Sci 96(5): 180-187.

Uno Y, Takasawa H, Miyagawa M, et al. (1994). An in vivo-in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 noncarcinogens. Mutat Res 320(3): 189-205.

US EPA (1988). Recommendations for and Documentation of Biological Values for Use in Risk Assessment. EPA-600/6-87/008. NTIS PB88-179874/AS. Cincinnati, Ohio.:Environmental Criteria and Assessment Office, Office of Research and Development, US Environmental Protection Agency https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=34855 Access 1988].

US EPA (2005a). Guidelines for carcinogen risk assessment. EPA/630/P-03/001F. Washington, DC:Risk Assessment Forum, US Environmental Protection Agency https://www.epa.gov/sites/default/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf Access 2005a].

US EPA (2010). User Manual for "MSW" Multistage Weibull Time-to-Tumor Model. Washington, DC:Quantitative Risk Management Group National Center for Environmental Assessment (Washington Division) Office of Research and Development, US Environmental Protection Agency https://cfpub.epa.gov/ncea/bmds/recordisplay.cfm?deid=217055 Access 2010].

US EPA (2012). Benchmark Dose Technical Guidance. EPA/100/R-12/001. EPA/100/R-12/001. Washington, DC:Risk Assessment Forum, US Environmental Protection Agency https://www.epa.gov/sites/default/files/2015-01/documents/benchmark_dose_guidance.pdf Access 2012].

US EPA (2013a). Toxicological review of 1,4-Dioxane (with inhalation update) (CAS No. 123-91-1) in support of summary information on the Integrated Risk Information System (IRIS). EPA-635/R-11/003-F. Washington, DC:US Environmental Protection Agency https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0326tr.pdf Access 2013a].

US EPA (2013b). 1,4-dioxane IRIS Summary (with inhalation update). Washington, DC:US Environmental Protection Agency https://cfpub.epa.gov/ncea/iris2/chemicallanding.cfm?substance_nmbr=326 Access 2013b].

US EPA (2015). TSCA Work Plan Chemical Problem Formulation and Initial Assessment 1,4-Dioxane CASRN: 123-91-1. 740-R1-5003. Washington, DC:Office of Chemical Safety and Pollution Prevention, US Environmental Protection Agency https://www.epa.gov/sites/default/files/2017-06/documents/14_dioxane_problem_formulation_and_intial_assessment.pdf Access 2015].

US EPA (2017). Technical Fact Sheet – 1,4-Dioxane. Washington, DC:Office of Land and Emergency Management, U.S. Environmental Protection Agency https://nepis.epa.gov/Exe/ZyNET.exe/P100THWG.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2016+Thru+2020&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C16thru20%5CTxt%5C00000004%5CP100THWG.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL Access 2017].

US EPA (2020). Final Risk Evaluation for 1,4-Dioxane. EPA-740-R1-8007. Washington, DC:Office of Chemical Safety and Pollution Prevention, US Environmental Protection Agency https://www.epa.gov/sites/default/files/2020-12/documents/1._risk_evaluation_for_14-dioxane_casrn_123-91-1.pdf Access 2020].

US EPA (2022). Benchmark Dose Software Version 3.3 User Guide. Washington, DC:US Environmental Protection Agency https://assessments.epa.gov/bmds/document/&deid%3D353980#downloads Access 2022].

Vermont Agency of Natural Resources Department of Environmental Conservation (2019). Chapter 12 of the Environmental Protection Rules: Groundwater Protection Rule and Strategy. https://dec.vermont.gov/sites/dec/files/dwgwp/DW/2019.07.06%20-%20GWPRS.pdf. date accessed:2022.

Washington Department of Ecology (2003). WAC 173-200-040 Criteria. https://app.leg.wa.gov/WAC/default.aspx?cite=173-200-040&pdf=true. date accessed:2022.

West Virginia Department of Environmental Protection (2018). 60CSR3—voluntary remediation and redevelopment rule. https://apps.sos.wv.gov/adlaw/csr/readfile.aspx?DocId=50235&Format=PDF. date accessed:2022.

Woo Y, Argus MF, Arcos JC (1977b). Tissue and subcellular distribution of 3H-dioxane in the rat and apparent lack of microsome-catalyzed covalent binding in the target tissue. Life Sci 21(10): 1447-1456.

Woo YT, Arcos JC, Argus MF, Griffin GW, Nishiyama K (1977a). Structural identification of p-dioxane-2-one as the major urinary metabolite of p-dioxane. Naunyn Schmiedebergs Arch Pharmacol 299(3): 283-287.

Woo YT, Argus MF, Arcos JC (1977c). Metabolism in vivo of dioxane: Effect of inducers and inhibitors of hepatic mixed-function oxidases. Biochem Pharmacol 26(16): 1539-1542.

Young JD, Braun WH, Gehring PJ (1978). The dose-dependent fate of 1,4-dioxane in rats. J Environ Pathol Toxicol 2(2): 263-282.

Young JD, Braun WH, Gehring PJ, Horvath BS, Daniel RL (1976). 1,4-Dioxane and beta-hydroxyethoxyacetic acid excretion in urine of humans exposed to dioxane vapors. Toxicol Appl Pharmacol 38(3): 643-646.

Young JD, Braun WH, Rampy LW, Chenoweth MB, Blau GE (1977). Pharmacokinetics of 1,4-dioxane in humans. J Toxicol Environ Health 3(3): 507-520.

Zhou Q, Jiang L, Qiu J, et al. (2020). Oral Exposure to 1,4-Dioxane Induces Hepatic Inflammation in Mice: The Potential Promoting Effect of the Gut Microbiome. Environ Sci Technol 54(16): 10149-10158.

APPENDIX A. LITERATURE SEARCH STRATEGY

Literature search and screening methods for human epidemiology studies

Studies published prior to 2011 were identified from the 2012 ATSDR review. Studies published in 2011 or later were identified through a variety of sources including PubMed, Google Scholar, Embase, review articles, government reports, and the bibliographies of all relevant articles identified through these other sources. Search terms included 1,4-dioxane and common synonyms such as p-dioxane, diethylene-dioxide, diethylene-oxide, diethylene-ether, and glycol-ethylene-ether. In order to keep the searches as broad as possible (e.g., increase sensitivity), terms for specific health effects or terms commonly used to identify epidemiologic studies were not included. All epidemiologic studies in humans involving 1,4-dioxane exposure and any adverse health effect were initially included. The PubMed search generated 816 hits, but relatively few involved epidemiologic studies. The primary reasons for exclusion were that the studies were remediation or biodegradation studies, exposure studies, animal studies, in vitro studies, reviews, or were unrelated to 1,4-dioxane. Excluding case reports, seven epidemiologic studies were identified: four from PubMed and 3 from bibliography or review article searches. Two of the studies involved breast cancer, two involved autism spectrum disorder or related conditions, and three involved other effects.

Literature search and screening methods for animal studies

OEHHA conducted a search of the literature on the toxicity of 1,4-dioxane. The goal was to identify peer-reviewed open source and proprietary journal articles, print and digital books, reports, and gray literature that potentially reported relevant information on the toxicity of this chemical. Searches were limited to literature published from January 2009 (to overlap the publication of the notification level in 2010) up to the time the searches were executed in April 2019. An updated literature search was conducted in August 2021 and August 2023.

Search process

PubChem was used to identify the Chemical Abstracts Service (CAS) Registry Number and synonyms for 1,4-dioxane. The PubMed MeSH browser was used to identify subject headings and other index terms and additional synonyms for the chemical.

The following is the search strategy devised and executed in PubMed. The search was limited to years January 2009 to April 15, 2019 when the search was conducted.

((dioxane[tiab] OR 1,4-dioxan[tiab] OR 1,4-dioxane[tiab] OR 1,4-dioxane[Supplementary Concept] OR 1,4-dioxano[tiab] OR 123-91-1[rn] OR Dioxano[tiab] OR p-dioxane[tiab] OR dioxanes[mh:noexp] OR 1,4-diethylene-dioxide[tiab] OR 1,4-dioxanne[tiab] OR Dioxyethylene-ether[tiab] OR p-dioxan[tiab] OR 1,4-dioxacyclohexane[tiab] OR diethylene-dioxide[tiab] OR diethylene-ether[tiab] OR dioxanne[tiab]) AND ("2009"[PDAT] : "3000"[PDAT]))

This strategy was then tailored for use in additional databases: Embase, Scopus, Web of Science TOXLINE (National Library of Medicine): Toxicology Literature Online (https://toxnet.nlm.nih.gov/newtoxnet/toxline.htm).

Data sources and results

The following is a list of the databases searched and the number of references retrieved from each.

Table A1 Database search results

Source	Results
PubMed (National Library of Medicine)	2,232
Web of Science	4,705
ToxNet[a]	509
Embase	633
Scopus	3,169

[a] In 2019, when the literture search was initiated, TOXNET was an available database; however, since then, the database has been moved to other databases within the National Library of Medicine (NLM).

Literature screening

Relavant literature was identified through a multi-step screening process outlined in Figure A1. Because the number of studies retrieved was large even after duplicate removal, studies were imported into SWIFT Review software (https://www.ncbi.nlm.nih.gov/ pubmed/27216467) to identify human, animal and in vitro studies. In brief, SWIFT Review has pre-set literature search filters developed by information scientists that that can be applied to reduce the number of studies that undergo additional screening. SWIFT Review filters for “human” and “animal’ and “in vitro” were applied. Application of these filters reduced the number of studies for further consideration to 1,306.

These studies were imported into a web-based systematic review software, DistillerSR, for title/abstract and full-text screening. Both title/abstract and full-text screening were conducted by two independent reviewers. Studies were screened for inclusion or exlcusion based on the Populations, Exposures, Comparators, and Outcomes (PECO) criteria outline in Table A2. Studies that met PECO criteria during title and abstract screening were considered for full-text screening. At both the title/abstract and full-text review levels, screening conflicts were resolved by discussion.

Table A2. Populations, exposures, comparators, and outcomes (PECO) criteria

PECO element	Evidence
Populations	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages).
Exposures	Relevant forms: 1,4-dioxane (CAS number 123-91-1). Metabolites of interest, including β-hydroxyethoxy acetic acid (HEAA). Measures of metabolites used to estimate exposures to 1,4-dioxane. Studies of the effects of exposure to the metabolites themselves. **Human:** Any exposure to 1,4-dioxane via oral and inhalation routes. Other exposure routes, including dermal or unknown/multiple routes, will be tracked during title and abstract screening and tagged as "potentially relevant supplemental information." **Animal:** Any exposure to 1,4-dioxane via oral route or inhalation routes. Studies involving exposures to mixtures will be included only if they include exposure to 1,4-dioxane alone. Other exposure routes, including dermal or injection, will be tagged during title and abstract screening as "potentially relevant supplemental information."
Comparators	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits) of 1,4-dioxane, or exposure to 1,4-dioxane for shorter periods of time. Case reports and case series will be tagged as "potentially relevant supplemental information." **Animal:** A concurrent control group exposed to vehicle-only treatment or untreated control.
Outcomes	All health outcomes (both cancer and noncancer). Exclude studies where 1,4-dioxane is only used as a model cytotoxic agent.
PBPK models	Studies describing physiologically-based pharmacokinetic (PBPK) models for 1,4-dioxane will be included.

During title/abstract or full-text level screening in DistillerSR, studies that did not meet the PECO criteria, but which could provide supporting information were categorized as supplemental material. Studies that were categorized as supplemental material were not necessarily excluded from consideration in the assessment and may be used as supporting evidence. Studies were tagged as potentially relevant supplemental material if they were:

- Mechanistic: cancer and noncancer (including in vitro studies)
- Non-mammalian model
- Non-oral routes of administration

A total of 79 studies were tagged as supplemental material.

APPENDIX B. DEFAULT UNCERTAINTY FACTORS

This appendix describes the default uncertainty factors OEHHA generally uses to calculate the Acceptable Daily Dose when deriving PHGs. When scientific evidence is compelling, these defaults are supplanted by alternative factors or modeled results. Table B1 below is adapted from OEHHA's "Technical Support Document for the Development of Noncancer Reference Exposure Levels" (OEHHA, 2008).

Table B1. Default uncertainty factors for PHG derivation, adapted from OEHHA (2008)

Uncertainty Factor	Value
Interspecies uncertainty factor (UF_A)	
Combined interspecies uncertainty factor (UF_A):	1 human observation √10 animal observation in nonhuman primates 10 where no data are available on toxicokinetic or toxicodynamic differences between humans and a non-primate test species
Toxicokinetic component (UF_{A-k}) of UF_A:	1 where animal and human PBPK models are used to describe interspecies differences √10 nonprimate studies with no chemical or species specific kinetic data
Toxicodynamic component (UF_{A-d}) of UF_A:	1 where animal and human mechanistic data fully describe interspecies differences (*This is unlikely to be the case.)* 2 for residual susceptibility differences where there are some toxicodynamic data √10 nonprimate studies with no data on toxicodynamic interspecies differences
Intraspecies uncertainty factor (UF_H)	
Toxicokinetic component (UF_{H-k}) of UF_H:	1 human study including sensitive subpopulations (e.g., infants and children), or where a PBPK model is used and accounts for measured interindividual variability √10 for residual susceptibility differences where there are some toxicokinetic data (e.g., PBPK models for adults only) 10 to allow for diversity, including infants and children, with no human kinetic data
Toxicodynamic component (UF_{H-d}) of UF_H:	1 human study including sensitive subpopulations (e.g., infants and children) √10 human studies with normal adult subjects only, but no reason to suspect additional susceptibility of children 10 suspect additional susceptibility of children (e.g., exacerbation of asthma, neurotoxicity)

Uncertainty Factor	**Value**
LOAEL uncertainty factor (UF_L)	
Values used:	10 LOAEL, any effect 1 NOAEL or BMDL used
Subchronic uncertainty factor (UF_S)[1]	
Values used:	1 study duration >12% of estimated lifetime √10 study duration 8-12% of estimated lifetime 10 study duration <8% of estimated lifetime
Database deficiency factor (UF_D)	
Values used:	1 no substantial data gaps √10 substantial data gaps including, but not limited to, developmental toxicity

[1] Exposure durations of 13 weeks or less are subchronic regardless of species (OEHHA, 2008)

Reference

OEHHA (2008). Air Toxics Hot Spots Risk Assessment Guidelines: Technical Support Document for the Derivation of Noncancer Reference Exposure Levels. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

APPENDIX C. DETERMINATION OF MULTIROUTE EXPOSURES

Human exposure to chemical contaminants in tap water can occur via oral ingestion, as well as inhalation or dermal contact while performing common household activities, such as bathing, showering, and flushing toilets. This appendix describes the multiroute exposure assessment of chemicals in drinking water using equations extracted from CalTOX.[7] CalTOX is a multimedia total exposure model with built-in physicochemical property values for over 200 chemicals and mathematical equations to calculate total human exposure to contaminants in the environment (air, soil, and water).

For PHG development, exposures to chemicals in tap water over a lifetime (70 years) are considered. Exposure estimates differ across life stages (fetus, infant, child, and adult) due to physiological and activity pattern changes. CalTOX equations are used to calculate how much each route (oral, inhalation, and dermal) contributes to the total daily exposure to a contaminant in tap water. The relative contributions of the different routes are then used to estimate a daily drinking water intake equivalent (DWI, in L_{eq}/kg-day) of multiroute exposure to tap water for each life stage. The lifetime daily multiroute intake rate of tap water in L_{eq}/kg-day is the time-weighted average of these life-stage specific tap water intake rates.[8] The liter equivalent (L_{eq}/kg-day) value represents the equivalent of how much water a person would have to drink to account for exposures via ingestion, inhalation and dermal uptake. Table C1 shows the descriptions and values of parameters applied in the exposure equations. Tables C2 and C3 show life-stage specific exposure parameter values.

[7] A multimedia total exposure model developed for the Department of Toxic Substances Control, California Environmental Protection Agency (Cal/EPA), by the Lawrence Berkeley National Laboratory (2002, Version 4.0 Beta).

[8] A 0.75-yr exposure duration for the fetus is used to derive the time-weighted average for the lifetime daily tap water exposure rate (e.g., 0.75/70*0.047+2/70*0.196+14/70*0.061+54/70*0.045=0.053 L/kg-day for exposure via oral ingestion) in calculating the noncancer HPC (OEHHA, 2012). A 0.25-yr duration (3rd trimester) is applied to the life-stage-specific exposure of the fetus in calculating the age sensitivity factor (ASF)-adjusted life-stage-specific exposures to tap water.

Table C1. Descriptions and values of model defaults, chemical-specific and exposure-specific parameters

Symbol	Parameter	Value	Unit	Source
Inputs and Calculated Outputs				
$Intake_{oral}$	chemical intake via oral ingestion of tap water	-	mg/kg-day	calculated
$Intake_{inh}$	chemical intake via inhalation	-	mg/kg-day	calculated
$Uptake_{dermal}$	chemical uptake via dermal contacts	-	mg/kg-day	calculated
C_{tap_water}	chemical concentration in tap water	100[a]	mg/L	input
C_{air}	chemical concentration in indoor air	-	mg/m^3	calculated
C_{bath_air}	chemical concentration in bathroom air	-	mg/m^3	calculated
Exposure Parameters				
Ifl	fluid (water) intake, normalized to body weight	0.045 to 0.196[b]	L/kg-day	OEHHA, 2012
BR_a	active breathing rate, normalized to body weight	0.012 to 0.045[b]	m^3/kg-hr	OEHHA, 2012
BR_r	resting breathing rate, normalized to body weight	0.012 to 0.045[b]	m^3/kg-hr	OEHHA, 2012
SA_b	surface area, normalized to body weight	0.029 to 0.059[b]	m^2/kg	OEHHA, 2012
ET_{ai}	exposure time, active indoors	5.71 to 8[c]	hr/day	model default
ET_{ri}	exposure time, resting indoors	8 to 11[c]	hr/day	model default
ET_{sb}	exposure time, in shower or bath	0.27[c]	hr	model default
δ_{skin}	skin thickness	0.0025	cm	model default
f_s	fraction of skin in contact of water during showering or bathing	0.80	unitless	model default
CF	conversion factor for dermal uptake calculation	10	L/cm-m^2	calculated
Physicochemical and Other Parameters				
W_{house}	Water use in the house	40	L/hr	model default
VR_{house}	Room ventilation rate, house	750	m^3/hr	model default
W_{shower}	Water use in the shower	8	L/min	model default
VR_{bath}	Room ventilation rate, bathroom	1	m^3/min	model default
D_{water}	Diffusion coefficient in pure water	chemical specific	m^2/day	literature
D_{air}	Diffusion coefficient in pure air	chemical specific	m^2/day	literature
Z_{water}	fugacity capacity of pure water	volatiles=1/H semivolatiles=1 (H: Henry's Law constant)	mole/Pa-m^3	literature
R_{gas}	gas constant	8.31	Pa-m^3/mol-K	literature
t_{lag}	diffusion lag time in skin	chemical specific	hr	calculated
K_m	skin-water partition coefficient	chemical specific	unitless	literature
K_p^w	steady-state skin permeability coefficient	chemical specific	cm/hr	literature
MW	molecular weight	chemical specific	g/mole	literature
K_{ow}	octanol/water partition coefficient	chemical specific	unitless	literature

[a] As long as the chemical concentration in tap water is low (well below the saturation concentration in water), the input value of C_{tap_water} does not affect the calculation of relative contributions from the multiroute exposures and 100 ppm is an arbitrarily assigned low value.
[b] See Table C2 for life-stage specific values.
[c] See Table C3 for life-stage specific values.

Table C2. OEHHA calculated exposure parameters (OEHHA, 2012[9])

Life Stage	Water Intake Rate[a] (L/kg-day)	Breathing Rate[b] (m^3/kg-hr)	Surface Area[c] (m^2/kg)
Infant (0<2 yrs)	0.196	0.045	0.059
Child (2<16 yrs)	0.061	0.031	0.045
Adult (16-70 yrs)	0.045	0.012	0.029
Fetus[d]	0.047	0.015	0.029

[a] 95th percentile water intake rates (L/kg-day) are obtained from Table 8.11 of OEHHA (2012) risk assessment guidelines.
[b] 95th percentile breathing rates (L/kg-day) are obtained from Table 3.26 of OEHHA (2012) risk assessment guidelines and converted to m^3/kg-hr. The same life stage-specific breathing rate is used for BR_a and BR_r.
[c] 95th percentile values for total body surface area over body weight (m^2/kg) are obtained from Table 6.3 of OEHHA (2012) risk assessment guidelines.
[d] In utero exposure dose of the fetus is assumed to be the same as that of the pregnant mothers. Therefore, the breathing rate and water intake rate for pregnant women are applied in the exposure estimates for fetuses (OEHHA, 2012). Pregnant women are assumed to have the same total body surface area over body weight as adults. Therefore, the total body surface area per body weight for adults is applied in the fetal dermal exposure estimation.

Table C3. CalTOX model default exposure durations

Life Stage	CalTOX Exposure Factors Set[a]	Exposure Time, Active Indoors (hr/day)	Exposure Time, Resting Indoors (hr/day)	Exposure Time, Shower or Bath (hr/day)
Infant (0<2 yrs)	Female 0-1	5.71	11.01	0.27
Child (2<16 yrs)	Female 7-9	5.71	11.01	0.27
Adult (16-70 yrs)	Female 19+	8.00	8.00	0.27
Fetus	Female 19+	8.00	8.00	0.27

[a] These Exposure Factors Sets provide the best estimates of the multiroute exposure for the corresponding life stages. Between the age groups within a particular life stage, the differences in relative contribution of a particular route are negligible, predominantly well below 1%. Within the same age group, the male and female inputs provide almost the same model outputs. Therefore, for internal consistency, use of the female Exposure Factor Sets is recommended for all life stages.

A. Oral Intake: Ingestion of Tap Water

Oral intake through ingestion of tap water can be calculated as follows:[10]

$$\mathbf{Intake_{oral}} = \mathbf{C_{tap\_water}} \times \mathbf{Ifl}$$

[9] OEHHA (2012). Air Toxics Hot Spots Program Risk Assessment Guidelines: Technical Support Document for Exposure Assessment and Stochastic Analysis. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, California.
[10] Abbreviations and symbols used in equations are defined in Table C1.

B. Inhalation Intake: Inhalation of Indoor Air in Active State, Resting State, and Shower/Bath

Chemicals in tap water can be transferred to indoor air during domestic activities such as showering, bathing, and toilet flushing. The total inhalation intake ($Intake_{inh}$) for a chemical in indoor air is obtained by summing the inhalation intakes in the active state, resting state, and in the shower/bath for each life-stage, as shown in the following equation:

$$\mathbf{Intake_{inh}} = \mathbf{C_{air}} \times (\mathbf{BR_a} \times \mathbf{ET_{ai}} + \mathbf{BR_r} \times \mathbf{ET_{ri}} - \mathbf{BR_a} \times \mathbf{ET_{sb}}) + \mathbf{C_{bath\_air}} \times \mathbf{BR_a} \times \mathbf{ET_{sb}}$$

The chemical concentration in indoor air and bathroom air are derived from the two equations below:

$$\mathbf{C_{air}} = \frac{\mathbf{3 \times 10^6 \times 0.7 \times \left(\frac{W_{house}}{VR_{house}}\right) \times C_{tap\_water}}}{\frac{\mathbf{2.5}}{(\mathbf{D_{water}}/\mathbf{86400})^{2/3}} + \frac{\mathbf{R_{gas} \times 298 \times Z_{water}}}{(\mathbf{D_{air}}/\mathbf{86400})^{2/3}}}$$

and

$$\mathbf{C_{bath\_air}} = \frac{\mathbf{3 \times 10^6 \times 0.6 \times \left(\frac{W_{shower}}{VR_{bath}}\right) \times C_{tap\_water}}}{\frac{\mathbf{2.5}}{(\mathbf{D_{water}}/\mathbf{86400})^{2/3}} + \frac{\mathbf{R_{gas} \times 298 \times Z_{water}}}{(\mathbf{D_{air}}/\mathbf{86400})^{2/3}}}$$

C. Dermal Uptake: Dermal Exposure to Tap Water during Shower/Bath

Dermal uptake of a chemical is dependent on exposure time and chemical-specific parameters, including diffusion through the skin. As a result, the dermal uptake of chemicals in tap water while showering or bathing are derived from one of the following equations:

1. When exposure time < diffusion lag time in skin[11] (t_{lag}):

    a. Exposure time << diffusion lag time, i.e. $\frac{t_{lag} \times 2}{ET_{sb}} > 3$:

$$\mathbf{Uptake_{dermal}} = \mathbf{C_{tap\_water}} \times \left(\frac{\delta_{skin} \times \mathbf{K_m}}{\mathbf{2}}\right) \times \mathbf{f_s} \times \mathbf{CF} \times \mathbf{SA_b} \times \frac{\mathbf{ET_{sb}}}{\mathbf{2 \times tlag}} \times \frac{\mathbf{1\ event}}{\mathbf{day}}$$

[11] Diffusion lag time in the skin is the amount of time it takes a chemical to permeate through the skin until it reaches a steady state of diffusion.

b. For $1 \le \frac{t_{lag} \times 2}{ET_{sb}} \le 3$:

$$\mathbf{Uptake_{dermal}} = \mathbf{C_{tap\_water}} \times \left(\frac{\delta_{\mathbf{skin}} \times \mathbf{K_m}}{\mathbf{2}}\right) \times \mathbf{f_s} \times \mathbf{CF} \times \mathbf{SA_b} \times \frac{\mathbf{1\ event}}{\mathbf{day}}$$

2. When exposure time > diffusion lag time, i.e. $\frac{t_{lag} \times 2}{ET_{sb}} < 1$:

$$\mathbf{Uptake_{dermal}} = \mathbf{C_{tap\_water}} \times \left[\frac{\delta_{\mathbf{skin}} \times \mathbf{K_m}}{\mathbf{2}} + \left(\frac{\mathbf{ET_{sb}}}{\mathbf{2}} - \mathbf{t_{lag}}\right) \times \mathbf{K_p^w}\right] \times \mathbf{f_s} \times \mathbf{CF} \times \mathbf{SA_b} \times \frac{\mathbf{1\ event}}{\mathbf{day}}$$

where the chemical-specific t_{lag} is obtained from:

$$\mathbf{t_{lag}} = \frac{\delta_{\mathbf{skin}} \times \mathbf{K_m}}{\mathbf{6} \times \mathbf{K_p^w}}$$

For chemicals with no steady-state skin permeability coefficient (K_p^w) and skin/water partition coefficient (K_m) available in the literature, these values are derived from the following equations, using chemical molecular weight (MW) and octanol/water partition coefficient (K_{ow}):

1. K_p^w is calculated using one of the equations below:

   a. Chemicals with MW < 280 g/mole:

   $$\mathbf{K_p^w} = \frac{\mathbf{1}}{\mathbf{(MW)^{0.6}}} \times \frac{\mathbf{2.4 \times 10^{-6} + 3 \times 10^{-5} \times (K_{ow})^{0.8}}}{\delta_{\mathbf{skin}}}$$

   b. Chemicals with MW ≥ 280 g/mole:

   $$\mathbf{K_p^w = 0.0019 \times (K_{ow})^{0.71} \times 10^{(-0.0061 \times MW)}}$$

   c. Chemicals with calculated K_p^w > 1:

   $$\mathbf{K_p^w = 1}$$

2. K_m is calculated using this equation:

$$\mathbf{K_m = 0.64 + 0.25 \times (K_{ow})^{0.8}}$$

D. Relative Contributions from Each Route of Exposure

Finally, the relative contributions of chemical exposure to tap water via multiple routes are derived from the $Intake_{oral}$, $Intake_{inh}$, and $Uptake_{dermal}$ as follows:

Relative Contribution from Oral Ingestion (%)

$$= \frac{\mathbf{Intake_{oral}}}{\mathbf{Intake_{oral} + Intake_{inh} + Uptake_{dermal}}} \times 100\%$$

Relative Contribution from Inhalation[12] (%)

$$= \frac{\mathbf{Intake_{inh}}}{\mathbf{Intake_{oral} + Intake_{inh} + Uptake_{dermal}}} \times 100\%$$

Relative Contribution from Dermal Uptake (%)

$$= \frac{\mathbf{Uptake_{dermal}}}{\mathbf{Intake_{oral} + Intake_{inh} + Uptake_{dermal}}} \times \mathbf{100}\%$$

E. Calculation of Liter Equivalent Values for Dermal and Inhalation Exposures

Determine dermal and inhalation tap water intake equivalents (L_{eq}) using OEHHA calculated life-stage-specific water intake rate (Table C2) and corresponding relative contributions from ingestion, dermal, and inhalation exposures (Table 14) using the following equations:

Dermal Tap Water Intake Equivalent (Leq/kg-day)

$$= \frac{\text{Dermal (\%)} \times \text{Water Intake Rate (L/kg} - \text{day)}}{\text{Ingestion (\%)}}$$

Inhalation Tap Water Intake Equivalent (Leq/kg-day)

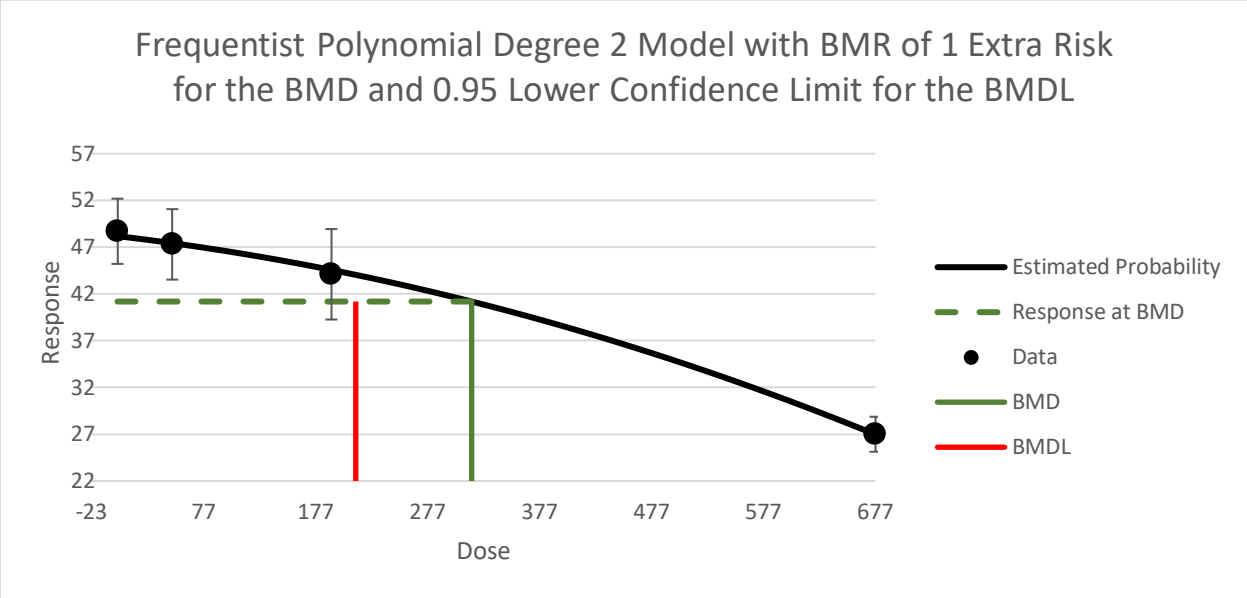
$$= \frac{\text{Inhalation (\%)} \times \text{Water Intake Rate (L/kg} - \text{day)}}{\text{Ingestion (\%)}}.$$

[12] Infant exposure to chemicals in tap water via inhalation are anticipated to be negligible, compared to the other exposure pathways, because they typically do not shower or flush toilets. Thus, the relative contribution from inhalation is zero for infants.

APPENDIX D. BENCHMARK DOSE RESPONSE ANALYSIS RESULTS FOR NONCANCER ENDPOINTS

This appendix provides the BMD modeling outputs for noncancer endpoints of 1,4-dioxane where data were amenable to dose-response modeling. All models were run with default parameters and a benchmark response of either 5% extra risk or 1 standard deviation from the control mean. Model selection criteria when comparing outputs of different models for the same endpoint/dataset were: the lowest Akaike's information criterion (AIC), goodness of fit p-value ≥0.05, scaled residual at the dose close to the BMD estimate ≤ the absolute value of 2, and visual inspection of the dose-response curve. When using BMD modeling, the BMDL, which is the lower limit of the 95% confidence interval of the BMD resulting in the benchmark response, is selected as the POD. The model for each study used to derive a POD is presented below.

Figure D1. BMD modeling of decreased terminal weight in male Crj:BDF$_1$ mice from Kano et al. (2009)



Model run output for Figure D1: Linear model for increased relative liver weight in female Crj:BDF$_1$ mice from Kano et al. (2009)

Benchmark Dose

BMD	316.3263665
BMDL	212.8126963
BMDU	416.1398253
AIC	732.8925458
P-value	0.845949867
D.O.F.	1

Model Parameters
# of Parameters 5

Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	48.17915033	1.020264347	46.1794689	50.1788317
beta 1	-0.014004198	1.18E-02	-0.0371272	0.0091188
beta 2	-2.55791E-05	1.60E-05	-5.696E-05	5.7992E-06
rho	2.888603445	6.09E-01	1.69546471	4.08174219
alpha	0.000672616	1.03E-06	0.0006706	0.00067463

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	31	48.17915033	48.7	48.7	6.9894017	6.1	6.1	0.414909365
49	33	47.43152924	47.3	47.3	6.83329688	6.8	6.8	-0.110572974
191	25	44.57119763	44.1	44.1	6.24619621	7.6	7.6	-0.377187662
677	26	26.9746677	27	27	3.02423835	3	3	0.042711538

Likelihoods of Interest

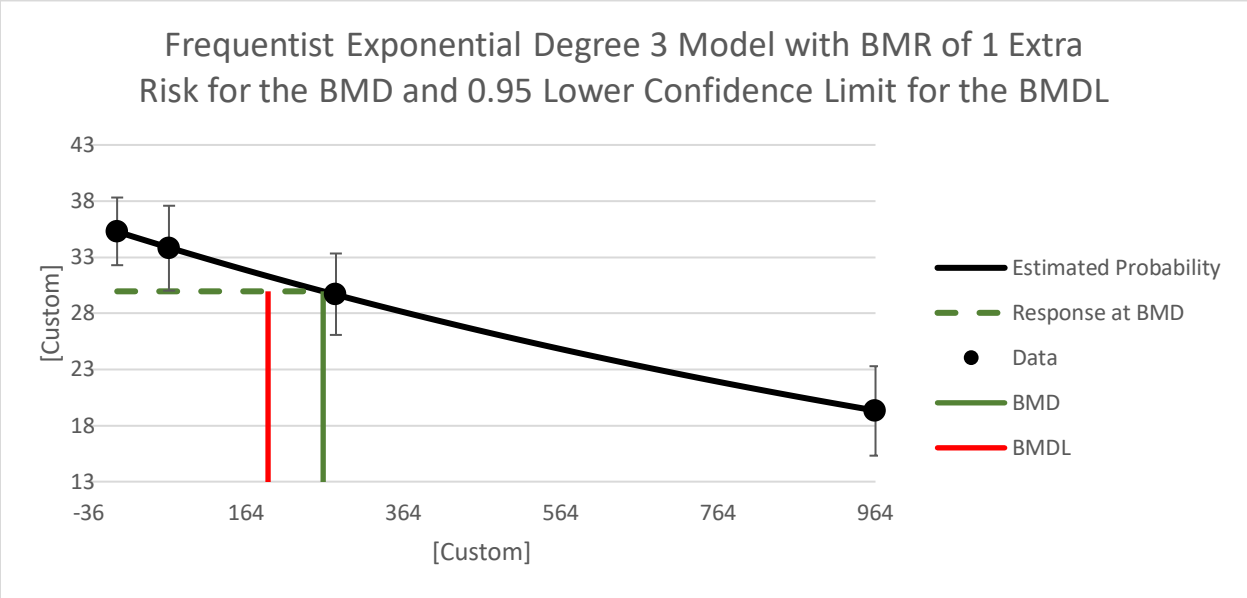
Model	Log Likelihood*	# of Parameters	AIC
A1	-370.1563831	5	750.312766
A2	-359.7248363	8	735.449673
A3	-361.4273988	6	734.854798
fitted	-361.4462729	5	732.892546
R	-432.6238524	2	869.247705

* Includes additive constant of -105.67793. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	145.7980323	6	<0.0001
2	20.86309354	3	0.0001124
3	3.405125117	2	0.18221599
4	0.037748082	1	0.84594987

Figure D2. BMD modeling of decreased terminal weight in female Crj:BDF$_1$ mice from Kano et al. (2009)



Model run output for Figure D2: Linear model for decreased terminal weight in female Crj:BDF$_1$ mice from Kano et al. (2009)

Benchmark Dose

BMD	261.8481637
BMDL	191.7858124
BMDU	440.1289857
AIC	500.320856
Test 4 P-value	0.99768765
D.O.F.	2

Model Parameters

# of Parameters 4

Variable	Estimate	Std Error	Lower Conf	Upper Conf
a	35.27160078	0.739546152	33.8221169	36.7210846
b	0.000623775	1.12E-04	0.00040497	0.00084258
d	Bounded	NA	NA	NA
log-alpha	3.341133623	1.58E-01	3.03123622	3.65103103

Goodness of Fit

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
0	29	35.27160078	35.3	35.3	5.31517965	5.1	5.1	0.028773152
66	29	33.84898303	33.8	33.8	5.31517965	6.4	6.4	-0.049627991
278	17	29.65612194	29.7	29.7	5.31517965	4.7	4.7	0.034037211

Dose	Size	Estimated Median	Calc'd Median	Observed Mean	Estimated SD	Calc'd SD	Observed SD	Scaled Residual
964	5	19.33194048	19.3	19.3	5.31517965	2.8	2.8	-0.013437193

Likelihoods of Interest

Model	Log Likelihood*	# of Parameters	AIC
A1	-247.158113	5	504.316226
A2	-243.9615433	8	503.923087
A3	-247.158113	5	504.316226
fitted	-247.160428	3	500.320856
R	-265.1144822	2	534.228964

* Includes additive constant of -73.51508. This constant was not included in the LL derivation prior to BMDS 3.0.

Tests of Interest

Test	-2*Log(Likelihood Ratio)	Test df	p-value
1	42.30587776	6	<0.0001
2	6.393139328	3	0.09397344
3	6.393139328	3	0.09397344
4	0.004630056	2	0.99768765

APPENDIX E. BENCHMARK DOSE RESPONSE ANALYSIS RESULTS FOR CANCER ENDPOINTS

This appendix provides the BMD and Multistage Weibull (MSW) modeling outputs for 1,4-dioxane. The Multistage-Cancer model within BMDS, which is optimized for cancer data, was run with default parameters and a benchmark response of 5% extra risk. Outputs were checked for goodness of fit p-value ≥ 0.05, scaled residual at the dose close to the BMD estimate ≤ the absolute value of 2, and visual inspection of the dose-response curve prior to selection for use in the PHG calculation. For analysis using MSW, a benchmark response of 5% extra risk was used. Tumor incidences were modeled using incidental risk since the cause of death was not indicated.

Model run output for MSW model for nasal cavity squamous cell carcinoma, adenocarcinoma, or rhabdomyomas in male Osborne-Mendel rats from NCI (1978)

```
=================================================================
        Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
        Solutions are obtained using donlp2-intv, (c) by P. Spellucci
        Input Data File: EXAMPLE2.(d)
        Thu Apr 28 13:42:41 2022
 =================================================================

 EXAMPLE 2, To estimated, BMD for Risk Type = Incidental

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:
P[response] = 1-EXP{-(t - t_0)^c *
             (beta_0+beta_1*dose^1)}

  The parameter betas are restricted to be positive

Dependent variable = class
Independent variables = dose, time

Total number of observations = 105
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 1
Degree of polynomial = 1

User specifies the following parameters:
        t_0    =         0

 Maximum number of iterations = 64
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
c      =      1.73684
t_0    =            0   Specified
beta_0 =            0
beta_1 =  3.3073e-008
```

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -t_0 -beta_0
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	c	beta_1
c	1	-1
beta_1	-1	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
c	1.6279	0.890633	-0.117712	3.37351
beta_0	0	NA		
beta_1	6.58586e-008	3.7099e-007	-6.61269e-007	7.92986e-007

NA - Indicates that this parameter has hit a
bound implied by some inequality constraint
and thus has no standard error.

Model	Log(likelihood)	# Param's	AIC
Fitted Model	-42.3473	3	90.6946

Data Summary

dose	class				Total
	C	F	I	U	
0	35	0	0	0	35
2.4e+002	22	0	13	0	35
5.3e+002	16	0	19	0	35

Benchmark Dose Computation
Risk Response = Incidental
Risk Type = Extra
Specified effect = 0.05
Confidence level = 0.95

Time = 805

BMD = 14.4914
BMDL = 9.28875
BMDU = 23.0882

Model run output for MSW model for nasal cavity squamous cell carcinoma or adenocarcinoma in female Osborne-Mendel rats from NCI (1978)

```
=======================================================================
Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
Solutions are obtained using donlp2-intv, (c) by P. Spellucci
Input Data File: EXAMPLE2.(d)
Wed Mar 30 14:48:20 2022
=======================================================================
EXAMPLE 2, To estimated, BMD for Risk Type = Incidental
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:
P[response] = 1-EXP{-(t - t_0)^c *
(beta_0+beta_1*dose^1)}

The parameter betas are restricted to be positive

Dependent variable = class
Independent variables = dose, time

Total number of observations = 105
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 1
Degree of polynomial = 1

User specifies the following parameters:
t_0    =         0

Maximum number of iterations = 64
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
c  = 1.1
t_0   = 0   Specified
beta_0 = 0
beta_1 = 5.48111e-006

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s)  -t_0       -beta_0
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

               c        beta_1
 c             1        -1
 beta_1        -1       1

Parameter Estimates
 Variable              Estimate          Std. Err.         95.0% Wald Confidence Interval
                                                           Lower Conf. Limit     Upper Conf. Limit
 c                     1.05623           0.983993          -0.872364             2.98482
 beta_0                0                 NA
 beta_1                6.66213e-006      2.92587e-005      -5.06839e-005         6.40082e-005
```

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Model	Log(likelihood)	# Param's	AIC
Fitted Model	-41.8595	3	89.7189

Data Summary

dose	class				Total
	C	F	I	U	
0	35	0	0	0	35
3.5e+002	24	0	11	0	35
6.4e+002	26	0	9	0	35

Benchmark Dose Computation
Risk Response = Incidental
Risk Type = Extra
Specified effect = 0.05
Confidence level = 0.95

Time = 122

BMD = 48.17
BMDL = 29.1662
BMDU = 78.9864

Model run output for MSW model for hepatocellular adenoma or carcinoma in female Osborne-Mendel rats from NCI (1978)

```
================================================================================
Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
Solutions are obtained using donlp2-intv, (c) by P. Spellucci
Input Data File: EXAMPLE2.(d)
Wed Mar 30 10:04:09 2022
================================================================================
 EXAMPLE 2, To estimated, BMD for Risk Type = Incidental

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:
P[response] = 1-EXP{-(t - t_0)^c *
(beta_0+beta_1*dose^1)}

The parameter betas are restricted to be positive

Dependent variable = class
Independent variables = dose, time

Total number of observations = 105
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 1
Degree of polynomial = 1

User specifies the following parameters:
t_0    =         0

Maximum number of iterations = 64
Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
c      =      5.07692
t_0    =            0   Specified
beta_0 = 5.93007e-035
beta_1 =  1.0427e-013

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s)  -t_0       -beta_0
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

                c        beta_1
 c              1        -1
 beta_1        -1        1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
c	5.62177	2.10871	1.48877	9.75477
beta_0	0	NA		
beta_1	8.5528e-015	8.29238e-014	-1.53975e-013	1.7108e-013

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Model	Log(likelihood)	# Param's	AIC
Fitted Model	-29.9379	3	65.8758

Data Summary

dose	class				Total
	C	F	I	U	
0	35	0	0	0	35
3.5e+002	25	0	10	0	35
6.4e+002	23	0	12	0	35

Benchmark Dose Computation
Risk Response = Incidental
Risk Type = Extra
Specified effect = 0.05
Confidence level = 0.95

Time = 122

BMD = 11.1922
BMDL = 5.30002
BMDU = 24.0594

Model run output for multisite MSW model for nasal cavity squamous cell carcinoma or adenocarcinoma and hepatocellular adenoma or carcinoma in female Osborne-Mendel rats from NCI (1978)

Individual sites (yellow highlighted line is the upper 95% confidence bound on β_1 estimated at the assumed standard lifetime which is the CSF_{animal})

Hepatocellular adenoma or carcinoma		Nasal cavity squamous cell carcinoma or adenocarcinoma	
Probability	**q1**	**Probability**	**q1**
0.000%	0.00016	0.00%	0
0.000%	0.00031	0.01%	0.000156
0.000%	0.00047	0.10%	0.000313
0.000%	0.00063	0.94%	0.000469
0.001%	0.00078	6.01%	0.000625
0.004%	0.00094	18.09%	0.000781
0.017%	0.00109	35.30%	0.000938
0.054%	0.00125	53.28%	0.001094
0.142%	0.00141	68.68%	0.00125
0.318%	0.00156	80.20%	0.001406
0.630%	0.00172	88.05%	0.001563
1.131%	0.00188	88.66%	0.001578
1.873%	0.00203	89.25%	0.001594
2.902%	0.00219	89.80%	0.001609
4.254%	0.00234	90.34%	0.001625
5.950%	0.00250	90.84%	0.001641
7.996%	0.002656	91.33%	0.001656
10.386%	0.00281	91.79%	0.001672
13.097%	0.00297	92.23%	0.001688
16.097%	0.00313	92.64%	0.001703
19.346%	0.00328	93.04%	0.001719
22.799%	0.00344	93.42%	0.001734
26.408%	0.00359	93.78%	0.00175
30.124%	0.00375	94.12%	0.001766
33.901%	0.00391	94.44%	0.001781
37.696%	0.00406	94.75%	0.001797
41.470%	0.00422	94.81%	0.0018
45.188%	0.00438	94.87%	0.001803
48.821%	0.00453	94.92%	0.001806
52.344%	0.00469	94.98%	0.001809
55.738%	0.00484	95.01%	0.001811
58.987%	0.00500	95.02%	0.001812

Hepatocellular adenoma or carcinoma		Nasal cavity squamous cell carcinoma or adenocarcinoma	
Probability	q1	Probability	q1
62.081%	0.00516	95.04%	0.001813
65.013%	0.00531	95.31%	0.001828
67.778%	0.00547	95.58%	0.001844
70.376%	0.00563	95.82%	0.001859
72.808%	0.00578	96.06%	0.001875
75.076%	0.00594	96.28%	0.001891
77.186%	0.00609	96.49%	0.001906
79.142%	0.00625	96.69%	0.001922
80.952%	0.00641	96.88%	0.001938
82.623%	0.00656	97.06%	0.001953
84.162%	0.00672	97.23%	0.001969
85.576%	0.00688	97.39%	0.001984
86.874%	0.00703	97.54%	0.002
88.064%	0.00719	97.68%	0.002016
89.152%	0.00734	97.82%	0.002031
90.147%	0.00750	97.94%	0.002047
91.054%	0.00766	98.06%	0.002063
91.882%	0.00781	98.18%	0.002078
92.636%	0.00797	98.28%	0.002094
93.322%	0.00813	98.39%	0.002109
93.945%	0.00828	98.48%	0.002125
94.512%	0.00844	98.57%	0.002141
94.619%	0.00847	98.66%	0.002156
94.724%	0.00850	98.74%	0.002172
94.827%	0.00853	98.81%	0.002188
94.928%	0.00856	98.88%	0.002203
94.978%	0.00858	98.95%	0.002219
95.003%	0.00859	99.01%	0.002234
95.027%	0.00859	99.07%	0.00225
95.495%	0.00875	99.13%	0.002266
95.919%	0.00891	99.18%	0.002281
96.303%	0.00906	99.23%	0.002297
96.651%	0.00922	99.28%	0.002313
96.967%	0.00937	99.32%	0.002328
97.253%	0.00953	99.36%	0.002344

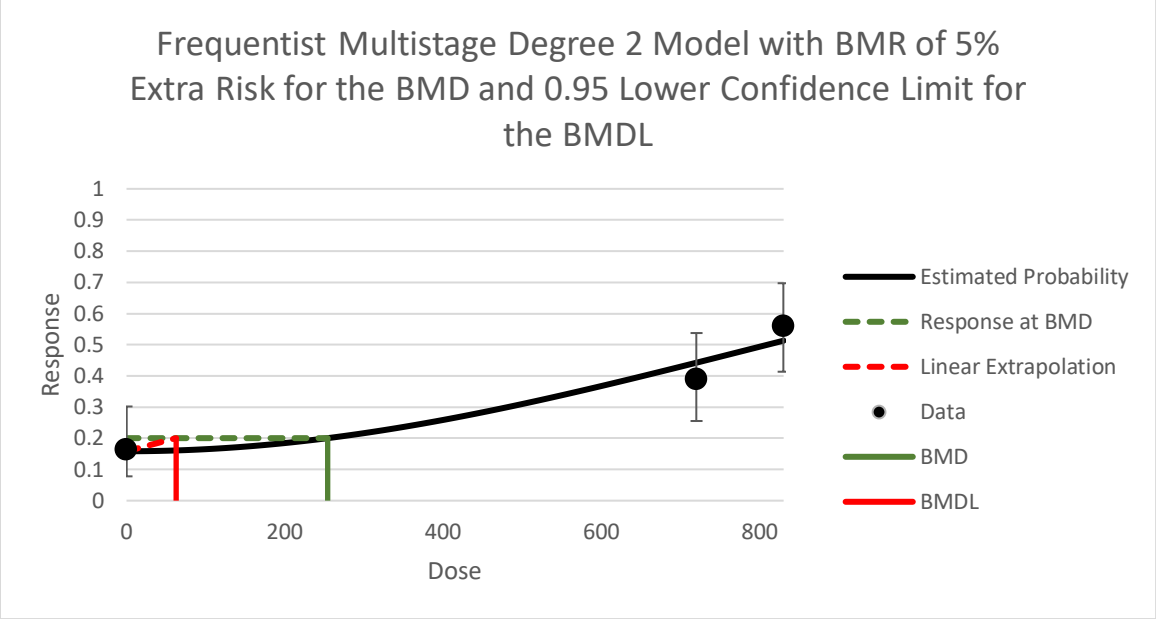
Combined sites (yellow highlighted line is the upper 95% confidence bound on the sum of β_1s which is the multisite CSF_{animal})

Probability	q1
0.001	0.002344
0.01	0.002969
0.02	0.003125
0.03	0.003422
0.04	0.003438
0.05	0.003594
0.06	0.00375
0.07	0.00375
0.08	0.003906
0.09	0.003906
0.1	0.004063
0.11	0.004063
0.12	0.004063
0.13	0.004219
0.14	0.004219
0.15	0.004219
0.16	0.004375
0.17	0.004375
0.18	0.004375
0.19	0.004531
0.2	0.004531
0.21	0.004531
0.22	0.004688
0.23	0.004688
0.24	0.004688
0.25	0.004781
0.26	0.004844
0.27	0.004844
0.28	0.004844
0.29	0.005
0.3	0.005
0.31	0.005
0.32	0.005141
0.33	0.005156
0.34	0.005156

Probability	q1
0.35	0.005156
0.36	0.005313
0.37	0.005313
0.38	0.005313
0.39	0.005313
0.4	0.005469
0.41	0.005469
0.42	0.005469
0.43	0.005578
0.44	0.005625
0.45	0.005625
0.46	0.005625
0.47	0.005781
0.48	0.005781
0.49	0.005781
0.5	0.005859
0.51	0.005938
0.52	0.005938
0.53	0.005938
0.54	0.006094
0.55	0.006094
0.56	0.006094
0.57	0.00625
0.58	0.00625
0.59	0.00625
0.6	0.006375
0.61	0.006406
0.62	0.006406
0.63	0.006547
0.64	0.006563
0.65	0.006563
0.66	0.006719
0.67	0.006719
0.68	0.006719
0.69	0.006875
0.7	0.006875
0.71	0.007016
0.72	0.007031
0.73	0.007031

Probability	q1
0.74	0.007188
0.75	0.007188
0.76	0.007344
0.77	0.007344
0.78	0.0075
0.79	0.0075
0.8	0.007656
0.81	0.007656
0.82	0.007813
0.83	0.007969
0.84	0.007969
0.85	0.008125
0.86	0.008281
0.87	0.008297
0.88	0.008438
0.89	0.008594
0.9	0.00875
0.91	0.008906
0.92	0.009172
0.93	0.009375
0.94	0.009609
0.95	0.009844
0.96	0.010313
0.97	0.010641
0.98	0.011391
0.99	0.012344
0.999	0.015938

Figure E1. BMD modeling of hepatocellular adenoma or carcinoma in male B6C3F1 mice from NCI (1978)



Model run output for Figure E1: Multistage cancer model for hepatocellular adenoma or carcinoma in male B6F3F1 mice from NCI (1978)

Benchmark Dose

BMD	254.0829462
BMDL	62.70180143
BMDU	324.1721296
AIC	182.6966984
P-value	0.306951346
D.O.F.	1
Chi^2	1.043748169
Slope Factor	0.000797425

Model Parameters

# of Parameters 3

Variable	Estimate
g	0.158412368
b1	Bounded
b2	7.94529E-07

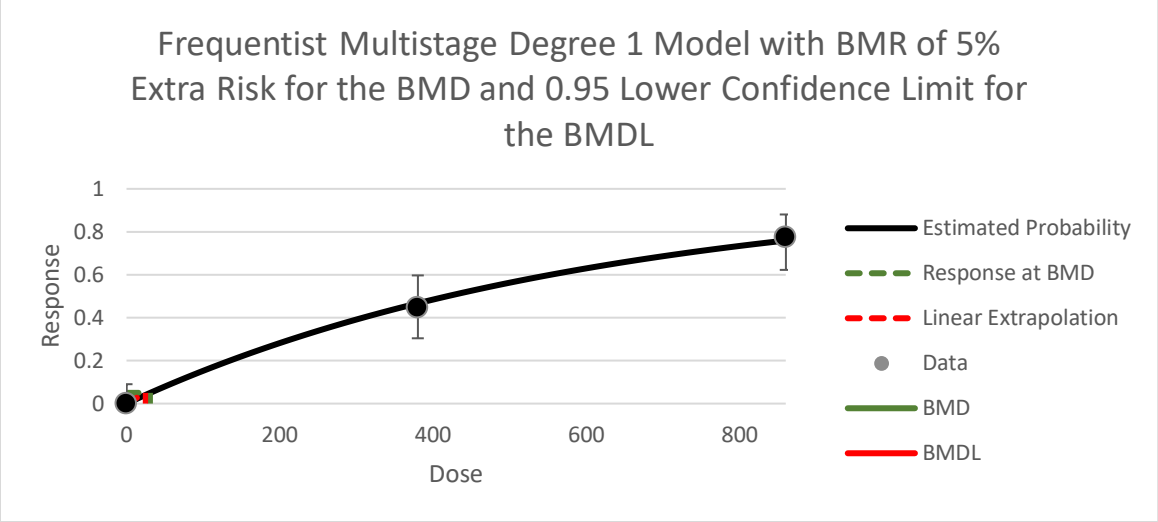
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.158412368	7.762206024	8	49	0.0930376
720	0.442531259	21.68403167	19	49	-0.771982
830	0.513158174	25.6579087	28	50	0.662673

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-88.82263011	3	-	-	NA
Fitted Model	-89.34834919	2	1.05143817	1	0.3051761
Reduced Model	-97.65233847	1	17.6594167	2	0.0001463

Figure E2. BMD modeling of hepatocellular adenoma or carcinoma in female B6C3F1 mice from NCI (1978)



Model run output for Figure E2: Multistage cancer model for hepatocellular adenoma or carcinoma in female B6F3F1 mice from NCI (1978)

Benchmark Dose

BMD	24.7062356
BMDL	39.37293856
BMDU	116.9970944
AIC	0.70442185
P-value	1
D.O.F.	0.143912681
Chi^2	0.002023781
Slope Factor	24.7062356

Model Parameters

# of Parameters 2

Variable	Estimate
g	1.60854E-08
b1	0.001656379

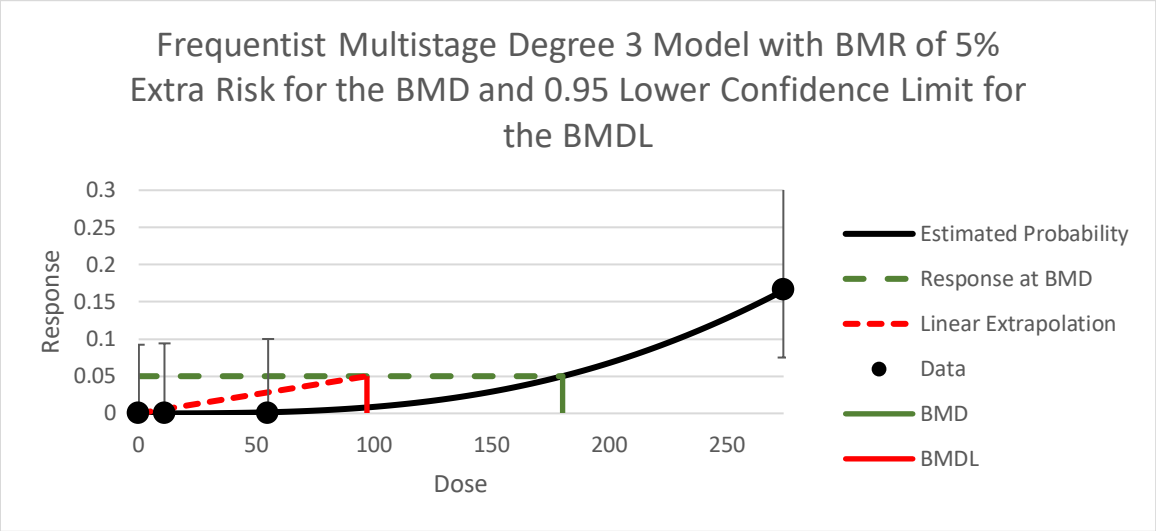
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.60854E-08	7.90692E-07	0	49.15576	-0.000889
380	0.467101365	21.98648241	21	47.07005	-0.288197
860	0.759367882	34.29137647	35	45.15779	0.246687

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-56.42603187	3	-	-	NA
Fitted Model	-56.49854719	2	0.14503064	1	0.7033302
Reduced Model	-94.9238938	1	76.9957239	2	<0.0001

Figure E3. BMD modeling of nasal cavity squamous cell carcinoma, rhabdomyosarcoma, sarcoma NOS or esthesioneuroepithelioma in male F344/DuCrj rats from Kano et al. (2009)



Model run output for Figure E3: Multistage cancer model for nasal cavity squamous cell carcinoma, rhabdomyosarcoma, sarcoma NOS or esthesioneuroepithelioma in male F344/DuCrj rats from Kano et al. (2009)

Benchmark Dose

BMD	180.097945
BMDL	96.97599048
BMDU	226.9623103
AIC	39.97740993
P-value	0.995629611
D.O.F.	3
Chi^2	0.065485455
Slope Factor	0.000515592

Model Parameters

# of Parameters 4

Variable	Estimate
g	Bounded
b1	Bounded
b2	Bounded
b3	8.7808E-09

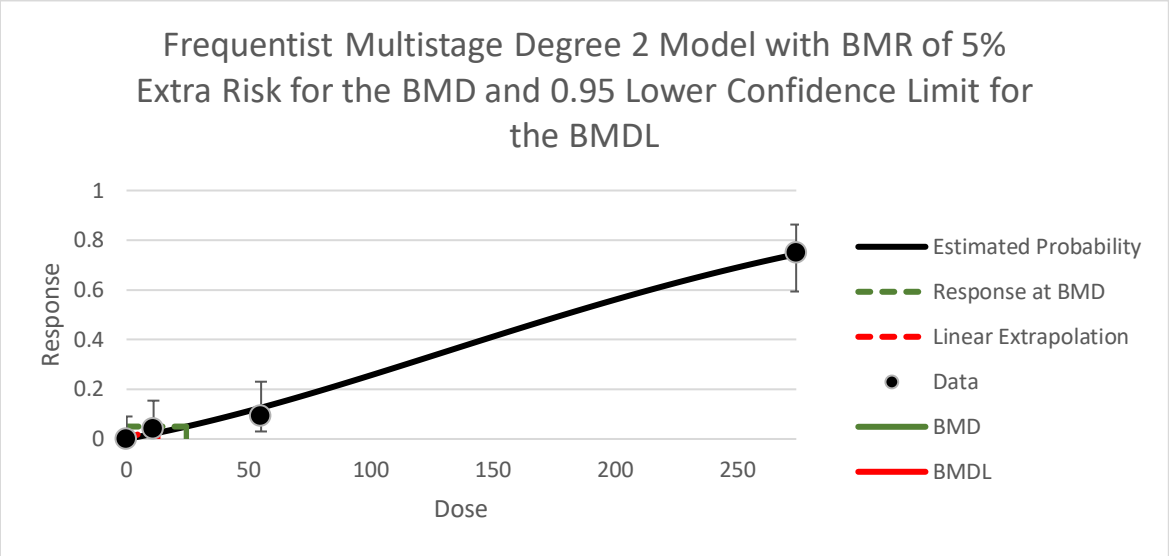
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	7.31039E-07	0	48	-0.000855
11	1.17024E-05	0.000550013	0	47	-0.023452
55	0.001459855	0.06423361	0	44	-0.253629
274	0.165254489	6.940688519	7	42	0.0246411

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-18.92357077	4	-	-	NA
Fitted Model	-18.98870496	1	0.13026838	3	0.9879727
Reduced Model	-29.63096955	1	21.4147976	3	<0.0001

Figure E4. BMD modeling of hepatocellular adenoma or carcinoma in male F344/DuCrj rats from Kano et al. (2009)



Model run output for Figure E4: Multistage cancer model for hepatocellular adenoma or carcinoma in male F344/DuCrj rats from Kano et al. (2009)

Benchmark Dose

BMD	24.48437017
BMDL	12.79464869
BMDU	57.95428689
AIC	97.96330325
P-value	0.493397491
D.O.F.	2
Chi^2	1.41288032
Slope Factor	0.003907884

Model Parameters

# of Parameters 3

Variable	Estimate
g	Bounded
b1	0.001813049
b2	1.15131E-05

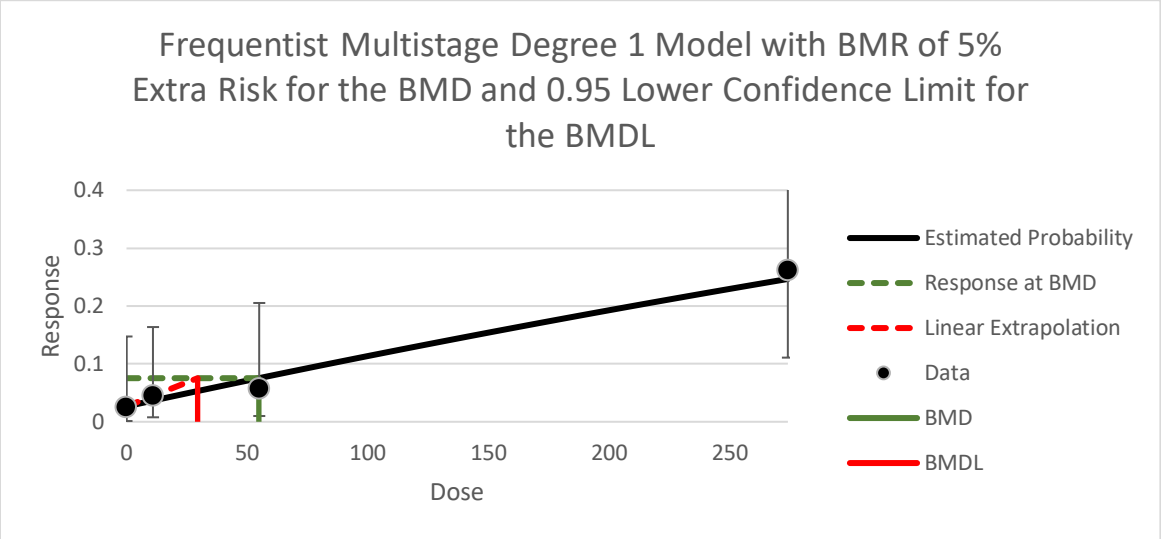
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	7.46269E-07	0	49	-0.000864
11	0.021110626	1.013310058	2	48	0.9907013
55	0.125886301	5.413110924	4	43	-0.649634
274	0.743629625	32.71970348	33	44	0.0967785

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-46.36411962	4	-	-	NA
Fitted Model	-46.98165163	2	1.23506401	2	0.5392737
Reduced Model	-95.04288255	1	97.3575259	3	<0.0001

Figure E5. BMD modeling of mammary gland fibroadenoma or adenoma in male F344/DuCrj rats from Kano et al. (2009)



Model run output for Figure E5: Multistage cancer model for mammary gland fibroadenoma or adenomas in male F344/DuCrj rats from Kano et al. (2009)

Benchmark Dose

BMD	54.82914877
BMDL	29.45901399
BMDU	140.4210694
AIC	71.73577958
P-value	0.871287921
D.O.F.	2
Chi^2	0.275565586
Slope Factor	0.001697273

Model Parameters

# of Parameters 2

Variable	Estimate
g	0.026639506
b1	0.000935512

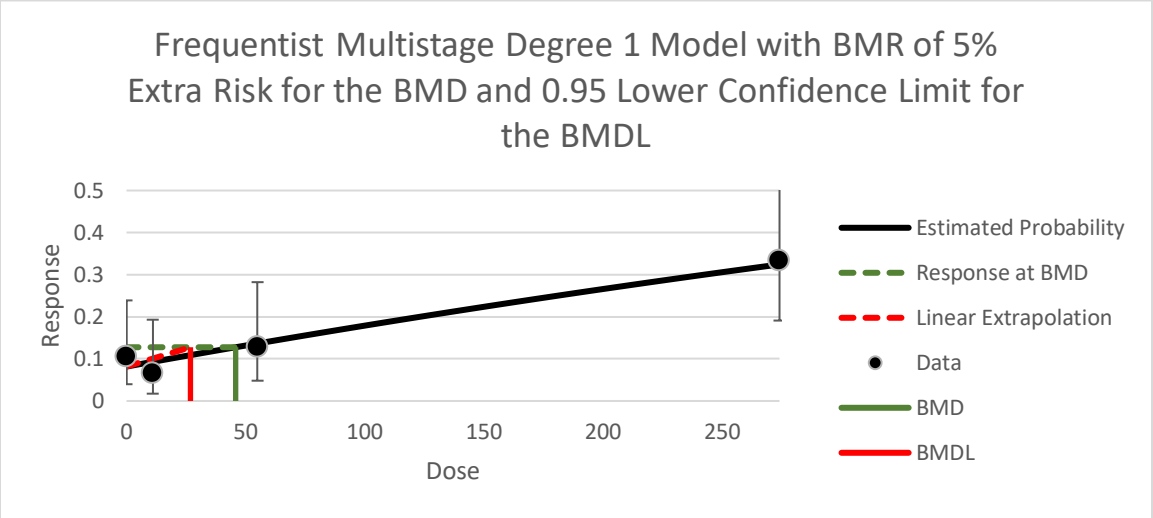
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.026639506	1.065580242	1	40	-0.064394
11	0.036604634	1.647208535	2	45	0.2800538
55	0.075455322	2.640936271	2	35	-0.410178
274	0.246729533	5.674779261	6	23	0.1572998

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-33.72550824	4	-	-	NA
Fitted Model	-33.86788979	2	0.2847631	2	0.8672903
Reduced Model	-38.78008034	1	10.1091442	3	0.0176608

Figure E6. BMD modeling of subcutis fibroma in male F344/DuCrj rats from Kano et al. (2009)



Model run output for Figure E6: Multistage cancer model for subcutis fibroma in male F344/DuCrj rats from Kano et al. (2009)

Benchmark Dose

BMD	45.80598128
BMDL	26.83092087
BMDU	103.8180031
AIC	134.3918298
P-value	0.67502759
D.O.F.	2
Chi^2	0.786003429
Slope Factor	0.001863522

Parameters

# of Parameters 2

Variable	Estimate
g	0.081766189
b1	0.001119795

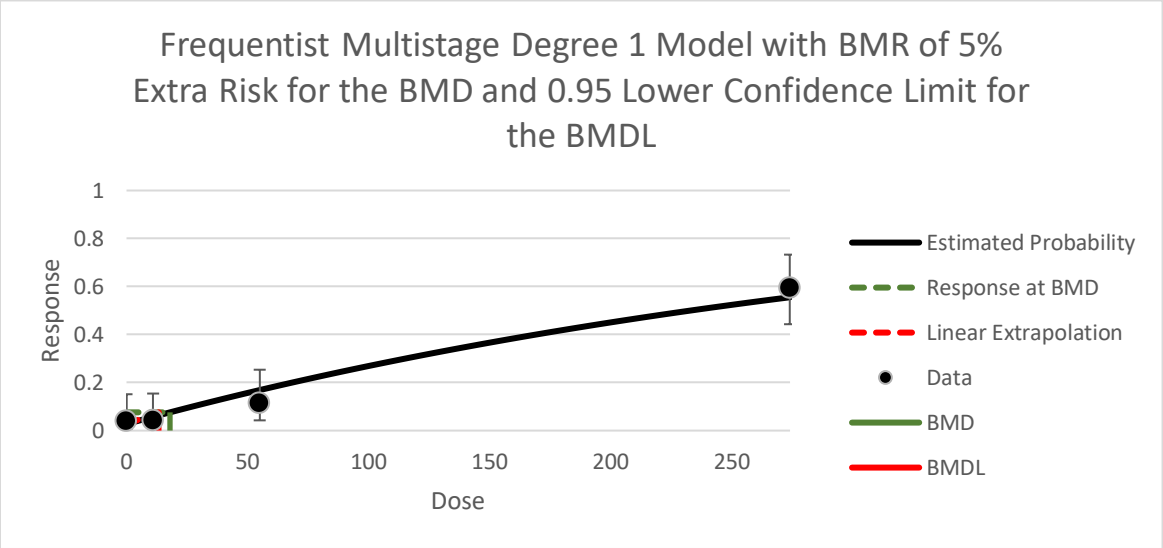
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.081766189	3.843010874	5	47	0.6159094
11	0.093007381	4.185332125	3	45	-0.608378
55	0.136612724	5.32789624	5	39	-0.152882
274	0.324381677	11.67774037	12	36	0.1147297

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-64.79944182	4	-	-	NA
Fitted Model	-65.19591492	2	0.7929462	2	0.6726884
Reduced Model	-70.50562887	1	11.4123741	3	0.0096928

Figure E7. BMD modeling of peritoneal mesothelioma in male F344/DuCrj rats from Kano et al. (2009)



Model run output for Figure E7: Multistage cancer model for peritoneal mesothelioma in male F344/DuCrj rats from Kano et al. (2009)

Benchmark Dose

BMD	17.9530893
BMDL	13.34898066
BMDU	25.17118016
AIC	133.81819
P-value	0.402807319
D.O.F.	2
Chi^2	1.818593896
Slope Factor	0.003745604

Parameters

# of Parameters 2

Variable	Estimate
g	0.027344099
b1	0.002857074

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.027344099	1.339860857	2	49	0.5782638
11	0.057437186	2.756984933	2	48	-0.469586
55	0.168782638	7.426436064	5	44	-0.97661
274	0.555392343	26.10344012	28	47	0.5567103

Analysis of Deviance

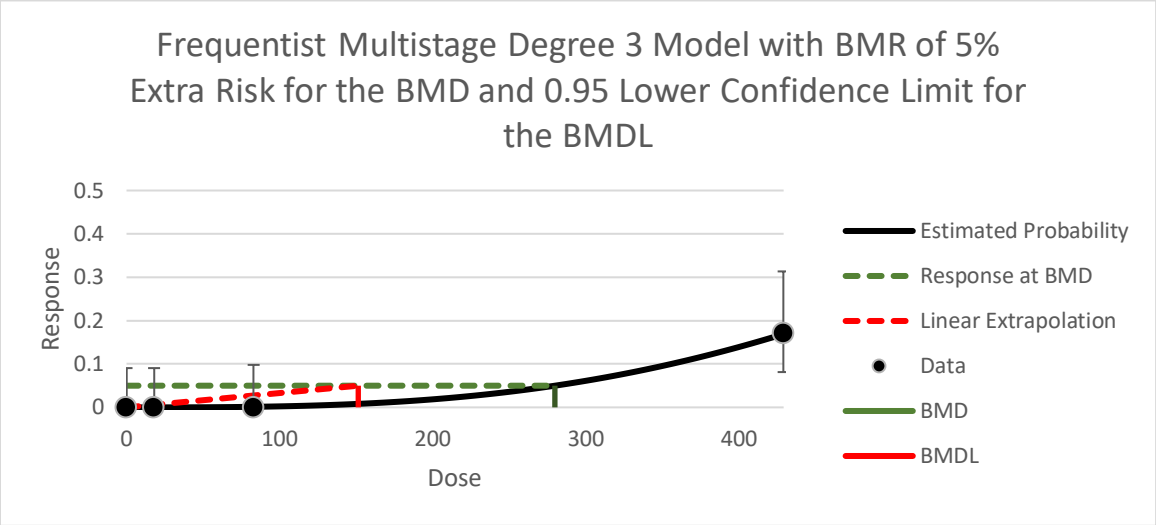
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-63.95893343	4	-	-	NA
Fitted Model	-64.90909498	2	1.9003231	2	0.3866786
Reduced Model	-93.23787089	1	58.5578749	3	<0.0001

Model run output multisite multistage model for tumors in male F344/DuCrj rats from Kano et al. (2009)

Benchmark Dose

BMD	7.52913122
BMDL	5.542679002
BMDU	11.83127762
Slope Factor	0.009020908
Combined Log-Likelihood	-229.943254
Combined Log-Likelihood Constant	202.7040302

Figure E8. BMD modeling of nasal cavity squamous cell carcinoma or esthesioneuroepithelioma in female F344/DuCrj rats from Kano et al. (2009)



Model run output for Figure E8: Multistage cancer model for nasal cavity squamous cell carcinoma or esthesioneuroepithelioma in female F344/DuCrj rats from Kano et al. (2009)

Benchmark Dose

BMD	279.6593438
BMDL	151.2949732
BMDU	346.7800116
AIC	45.00749914
P-value	0.996015341
D.O.F.	3
Chi^2	0.061524696
Slope Factor	0.00033048

Model Parameters

# of Parameters 4

Variable	Estimate
g	Bounded
b1	Bounded
b2	Bounded
b3	2.34516E-09

Goodness of Fit

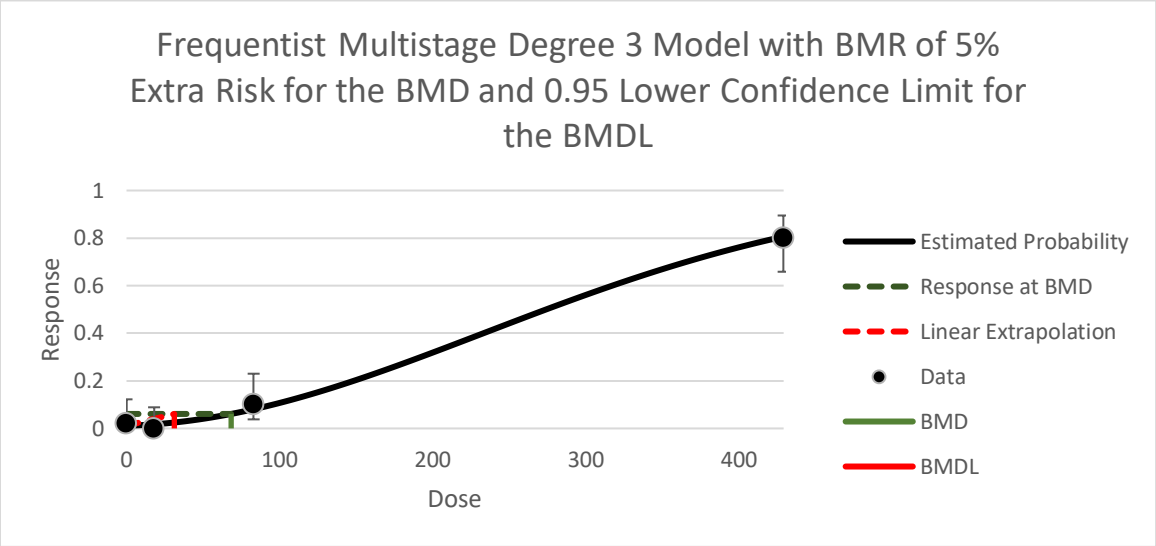
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	7.46269E-07	0	49	-0.000864
18	1.36921E-05	0.000670914	0	49	-0.025902
83	0.001340049	0.060302198	0	45	-0.24573

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
429	0.169027729	7.944303282	8	47	0.0216775

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-21.44250075	4	-	-	NA
Fitted Model	-21.50374957	1	0.12249765	3	0.9890072
Reduced Model	-33.16982433	1	23.4546472	3	<0.0001

Figure E9. BMD modeling of hepatocellular adenoma or carcinoma in female F344/DuCrj rats from Kano et al. (2009)



Model run output for Figure E9: Multistage cancer model for hepatocellular adenoma or carcinoma in female F344/DuCrj rats from Kano et al. (2009)

Benchmark Dose

BMD	68.31246731
BMDL	31.21654114
BMDU	115.6359683
AIC	100.4103232
P-value	0.216349307
D.O.F.	1
Chi^2	1.528422815
Slope Factor	0.001601715

Model Parameters

# of Parameters 4

Variable	Estimate
g	0.011558863
b1	0.000173412
b2	8.45308E-06
b3	Bounded

Goodness of Fit

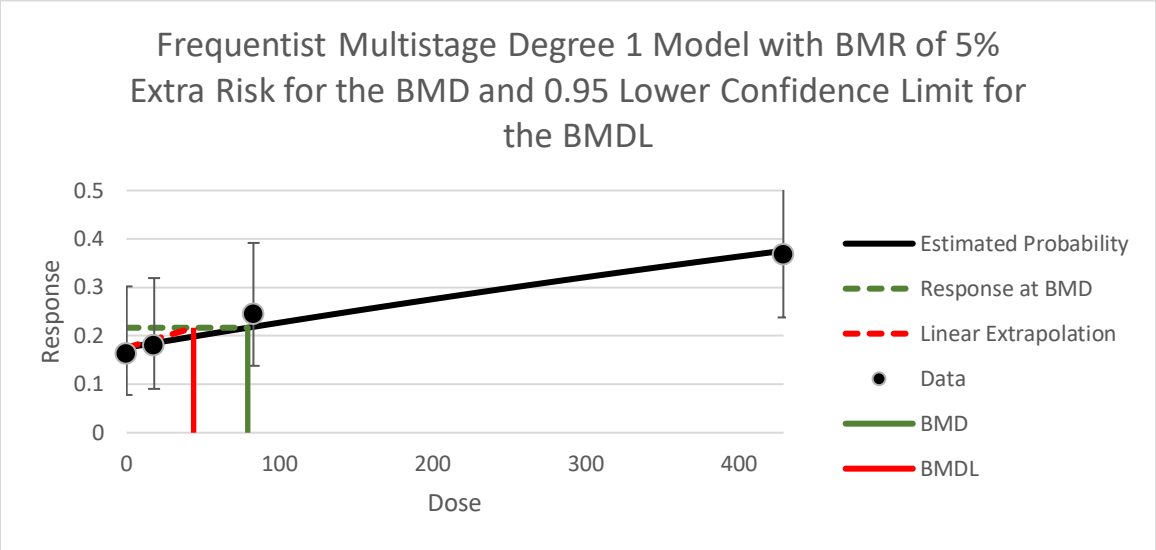
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.011558863	0.566384287	1	49	0.5795274

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
18	0.017334401	0.866720039	0	50	-0.939153
83	0.080801022	3.959250063	5	49	0.5455508
429	0.806355948	40.31779742	40	50	-0.113737

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-46.04932843	4	-	-	NA
Fitted Model	-47.20516162	3	2.31166639	1	0.1284063
Reduced Model	-107.3295287	1	122.5604	3	<0.0001

Figure E10. BMD modeling of mammary gland fibroadenoma, adenoma or adenocarcinoma in female F344/DuCrj rats from Kano et al. (2009)



Model run output for Figure E10: Multistage cancer model for mammary gland fibroadenoma, adenoma or adenocarcinoma in female F344/DuCrj rats from Kano et al. (2009)

Benchmark Dose

BMD	79.14486551
BMDL	43.8150585
BMDU	243.3140665
AIC	214.0106679
P-value	0.873424477
D.O.F.	2
Chi^2	0.270667228
Slope Factor	0.00114116

Model Parameters

# of Parameters 2

Variable	Estimate
g	0.175486418
b1	0.000648094

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.175486418	8.598834506	8	49	-0.224899
18	0.18504905	9.252452478	9	50	-0.091936
83	0.218666685	10.71466757	12	49	0.4442301

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
429	0.375618586	18.40531069	18	49	-0.119562

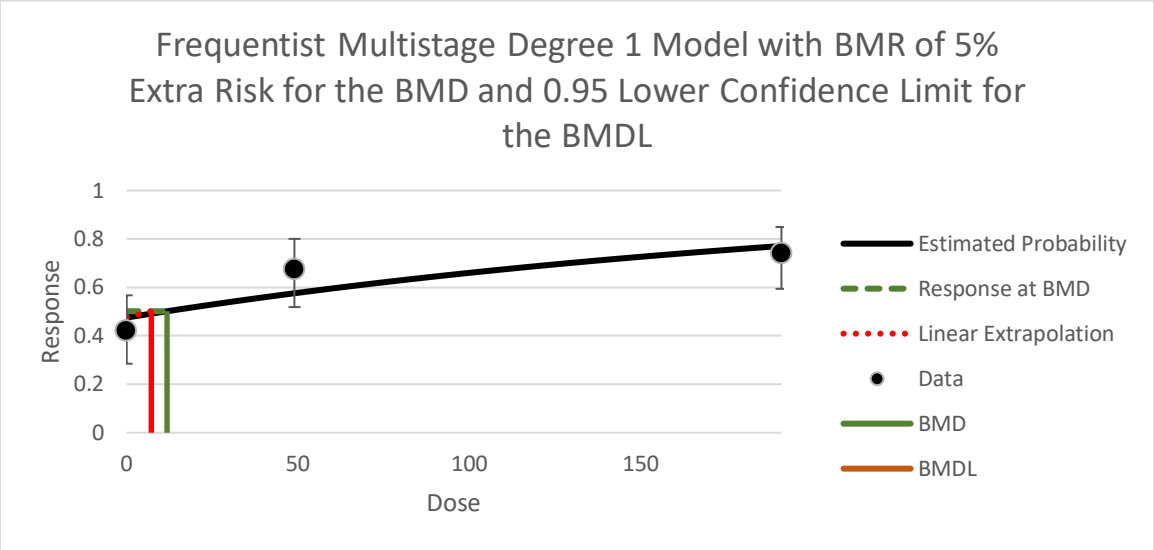
Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-104.8721335	4	-	-	NA
Fitted Model	-105.005334	2	0.2664008	2	0.8752897
Reduced Model	-108.2389032	1	6.73353923	3	0.0808936

Model run output multisite multistage model for tumors in female F344/DuCrj rats from Kano et al. (2009)
Benchmark Dose

BMD	43.10055028
BMDL	22.88185898
BMDU	65.36498865
Slope Factor	0.002185137
Combined Log-Likelihood	-173.7142425
Combined Log-Likelihood Constant	157.8665246

Figure E11. BMD modeling of hepatocellular adenoma or carcinoma with the high dose dropped in male Crj:BDF$_1$ mice from Kano et al. (2009)



Model run output for Figure E11: Multistage cancer model for hepatocellular adenoma or carcinoma with the high dose dropped in male Crj:BDF$_1$ mice from Kano et al. (2009)

Benchmark Dose

BMD	11.81900457
BMDL	7.22752028
BMDU	27.15569701
AIC	190.164584
P-value	0.10054201
D.O.F.	1
Chi^2	2.696924734
Slope Factor	0.006918002

Model Parameters

# of Parameters 2

Variable	Estimate
g	0.475358432
b1	0.0043399

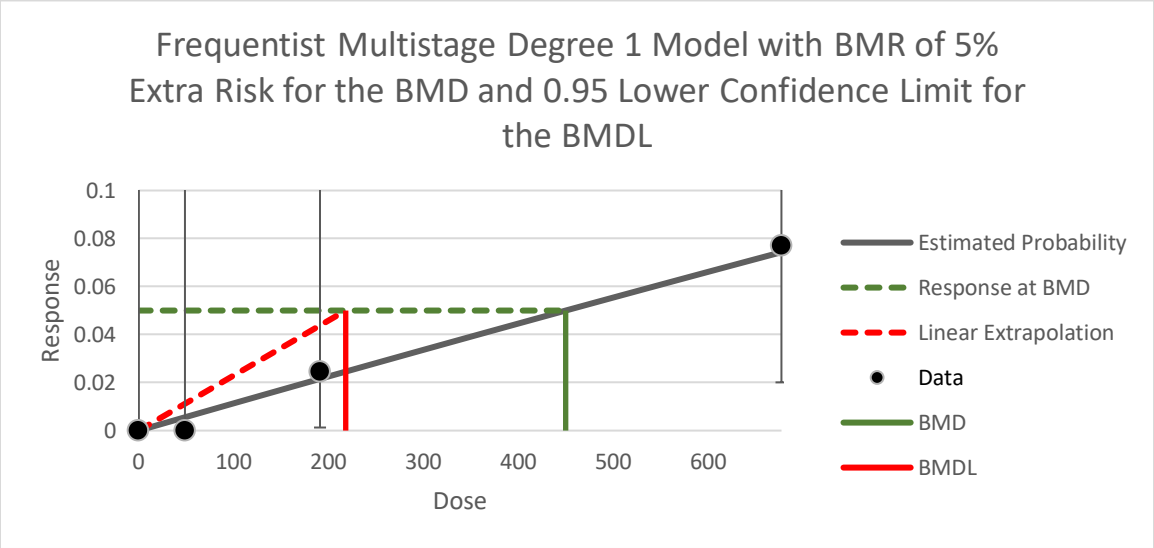
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.475358432	23.7679216	21	50	-0.783839
49	0.575861427	26.48962564	31	46	1.3456149
191	0.770983399	38.54916997	37	50	-0.521384

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-91.71059375	3	-	-	NA
Fitted Model	-93.08229201	2	2.74339651	1	0.097657
Reduced Model	-97.6640076	1	11.9068277	2	0.002597

Figure E12. BMD modeling of hemangioendothelioma of the heart in male Crj:BDF$_1$ mice from Kano et al. (2009)



Model run output for Figure E12: Multistage cancer model for hemangioendothelioma of the heart in male Crj:BDF$_1$ mice from Kano et al. (2009)

BMD	449.7088017
BMDL	218.0595584
BMDU	1261.030815
AIC	33.02131909
P-value	0.970237519
D.O.F.	3
Chi^2	0.2437379
Slope Factor	0.000229295

Model Parameters

# of Parameters 2

Variable	Estimate
g	Bounded
b1	0.000114059

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	6.54889E-07	0	43	-0.000809
49	0.005573313	0.222932515	0	40	-0.473479
191	0.021549682	0.883536948	1	41	0.1252583
677	0.074311872	2.898163019	3	39	0.0621744

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-15.27766212	4	-	-	NA
Fitted Model	-15.51065955	1	0.46599485	3	0.9262961
Reduced Model	-18.78033713	1	7.00535003	3	0.0717274

Model run output multisite multistage model for tumors in male Crj:BDF$_1$ mice from Kano et al. (2009)

Benchmark Dose

BMD	11.51639017
BMDL	7.112112395
BMDU	25.62994918
Slope Factor	0.00703026
Combined Log-Likelihood	-108.5929504
Combined Log-Likelihood Constant	98.23053814

Model run output for MSW model for hepatocellular adenoma or carcinoma female Crj:BDF$_1$ mice from Kano et al. (2009)

```
================================================================================
Multistage Weibull Model. (Version: 1.6.1;  Date: 11/24/2009)
Solutions are obtained using donlp2-intv, (c) by P. Spellucci
Input Data File: EXAMPLE2.(d)
Thu Apr 28 13:33:39 2022
================================================================================

EXAMPLE 2, To estimated, BMD for Risk Type = Incidental

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:
P[response] = 1-EXP{-(t - t_0)^c *
 (beta_0+beta_1*dose^1)}

The parameter betas are restricted to be positive

Dependent variable = class
Independent variables = dose, time

Total number of observations = 200
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 1
Degree of polynomial = 1

User specifies the following parameters:
t_0    =         0

Maximum number of iterations = 64
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
c      =         5.5
t_0    =           0   Specified
beta_0 = 9.10766e-013
beta_1 = 1.49265e-013

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s)  -t_0
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

              c          beta_0     beta_1
 c            1          -0.99      -1
 beta_0       -0.99      1          0.99
 beta_1       -1         0.99       1

Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
c	5.46245	1.09553	3.31525	7.60965
beta_0	1.08569e-012	5.61713e-012	-9.92367e-012	1.20951e-011
beta_1	1.76752e-013	8.72105e-013	-1.53254e-012	1.88605e-012

Model	Log(likelihood)	# Param's	AIC
Fitted Model	-60.4204	3	126.841

Data Summary

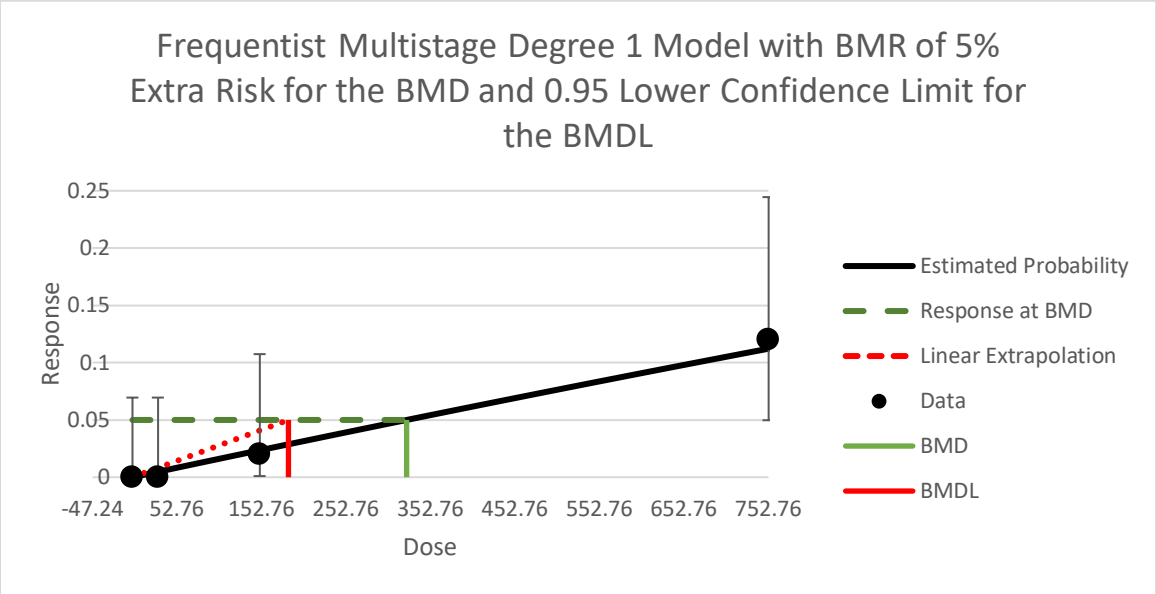
dose	class				Total
	C	F	I	U	
0	46	0	4	0	50
66	16	0	34	0	50
2.8e+002	9	0	41	0	50
9.6e+002	4	0	46	0	50

Benchmark Dose Computation
Risk Response = Incidental
Risk Type = Extra
Specified effect = 0.05
Confidence level = 0.95

Time = 105

BMD = 2.64274
BMDL = 1.84632
BMDU = 3.87024

Figure E13. BMD modeling of nasal squamous cell carcinomas from Kasai et al. (2009)



Model run output for Figure E13: Multistage cancer model for nasal squamous cell carcinomas from Kasai et al. (2009)

Benchmark Dose

BMD	324.5574688
BMDL	184.6736043
BMDU	649.3726672
AIC	49.03078553
P-value	0.960740868
D.O.F.	3
Chi^2	0.296203731
Slope Factor	0.000270748

Model Parameters

# of Parameters 2

Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	Bounded	NA	NA	NA
b1	0.000158041	4.50E-02	-0.088016	0.08833207

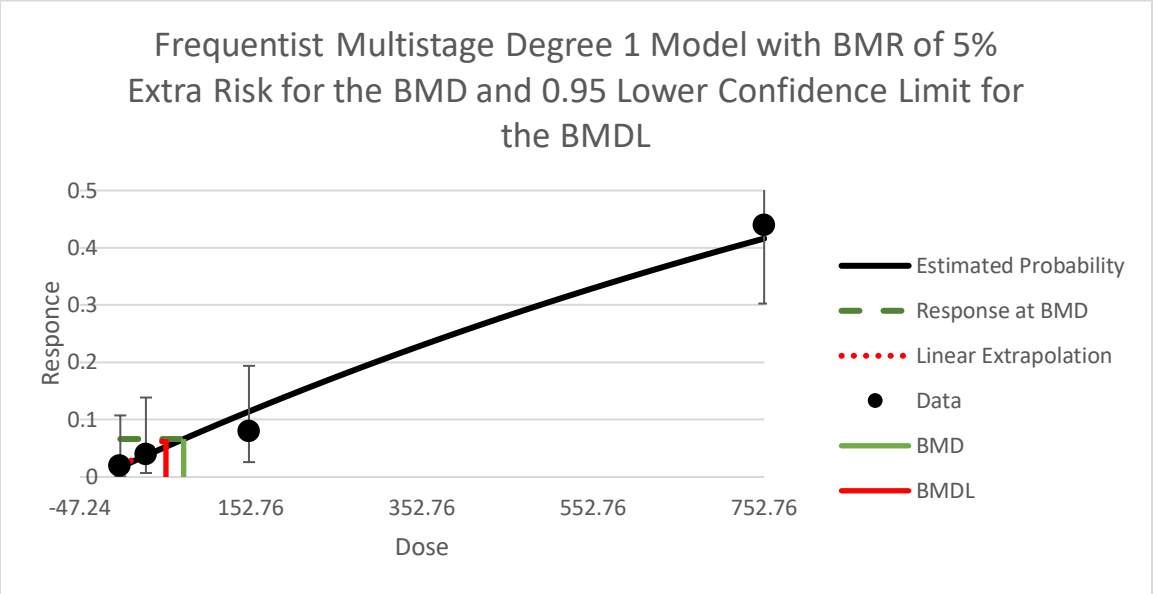
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	7.61499E-07	0	50	-0.000873
30.11	0.004747316	0.237365812	0	50	-0.488363
150.55	0.023512219	1.175610947	1	50	-0.163903
752.76	0.11216266	5.608132989	6	50	0.1756155

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-23.24820523	4	-	-	NA
Fitted Model	-23.51539276	1	0.53437507	3	0.911277
Reduced Model	-30.34289581	1	14.1893812	3	0.0026584

Figure E14. BMD modeling of hepatocellular adenomas or carcinomas from Kasai et al. (2009)



Model run output for Figure E14: Multistage cancer model for hepatocellular adenomas or carcinomas from Kasai et al. (2009)

Benchmark Dose

BMD	74.1145651
BMDL	53.43140332
BMDU	108.9063791
AIC	127.8603124
P-value	0.692819003
D.O.F.	2
Chi^2	0.733972985
Slope Factor	0.000935779

Model Parameters

# of Parameters 2

Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	0.017067943	5.33E-02	-0.0874522	0.12158808
b1	0.000692081	0.11108493	-0.2170304	0.21841454

Goodness of Fit

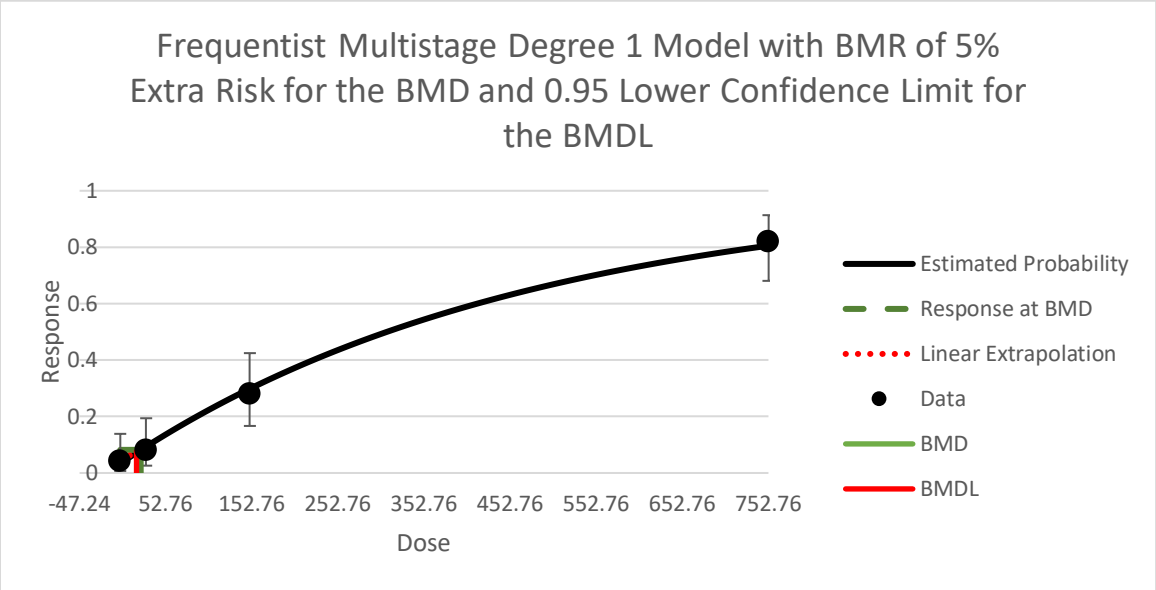
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.017067943	0.853397152	1	50	0.1600682
30.11	0.037338892	1.866944589	2	50	0.0992499
150.55	0.114327549	5.716377469	4	50	-0.762809

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
752.76	0.416193835	20.80969174	22	50	0.3415012

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-61.53412165	4	-	-	NA
Fitted Model	-61.93015622	2	0.79206914	2	0.6729834
Reduced Model	-82.78742608	1	42.5066089	3	<0.0001

Figure E15. BMD modeling of peritoneal mesotheliomas from Kasai et al. (2009)



Model run output for Figure E15: Multistage cancer model for peritoneal mesotheliomas from Kasai et al. (2009)

Benchmark Dose

BMD	24.1006963
BMDL	18.8742695
BMDU	31.51467935
AIC	155.4328986
P-value	0.85094324
D.O.F.	2
Chi^2	0.322819702
Slope Factor	0.002649109

Model Parameters

# of Parameters 2

Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	0.033631251	4.06E-02	-0.0458793	0.11314176
b1	0.002128291	0.248034731	-0.4840109	0.48826743

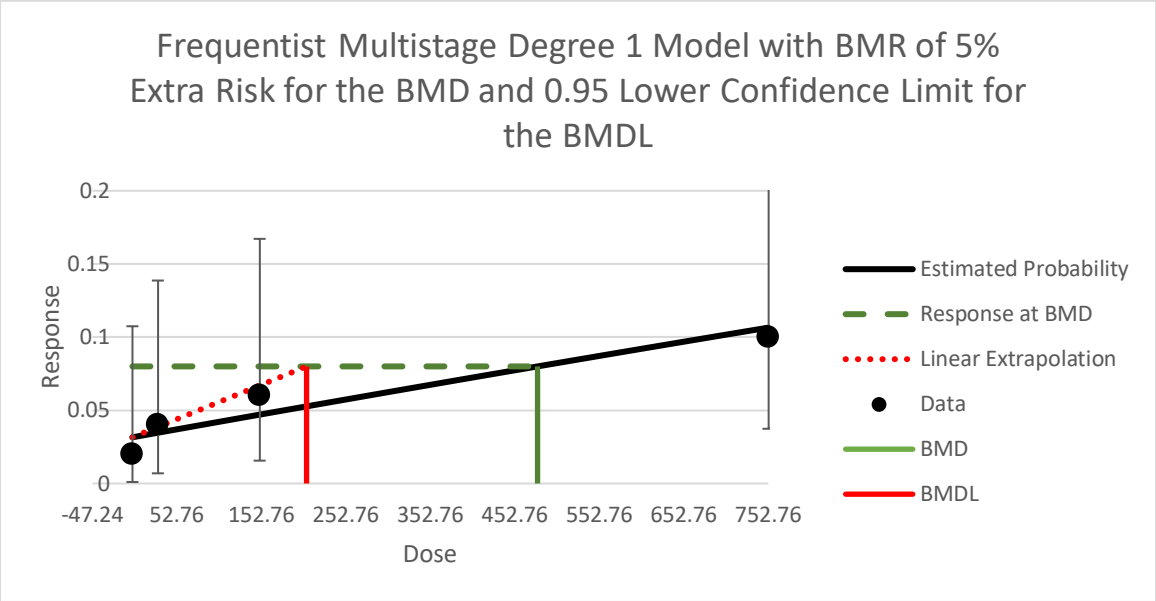
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.033631251	1.681562539	2	50	0.2498022
30.11	0.09361636	4.680817999	4	50	-0.330532
150.55	0.298562821	14.92814104	14	50	-0.286825
752.76	0.805301253	40.26506265	41	50	0.262485

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-75.55301619	4	-	-	NA
Fitted Model	-75.71644929	2	0.32686621	2	0.8492233
Reduced Model	-123.0082909	1	94.9105494	3	<0.0001

Figure E16. BMD modeling of mammary gland fibroadenomas from Kasai et al. (2009)



Model run output for Figure E16: Multistage cancer model for mammary gland fibroadenomas from Kasai et al. (2009)

Benchmark Dose

BMD	479.4801855
BMDL	206.1164676
BMDU	Infinity
AIC	86.29004471
P-value	0.790403009
D.O.F.	2
Chi^2	0.470424652
Slope Factor	0.000242581

Model Parameters

# of Parameters 2

Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	0.031583735	3.54E-02	-0.037874	0.10104147
b1	0.000106977	5.49E-02	-0.1073978	0.10761178

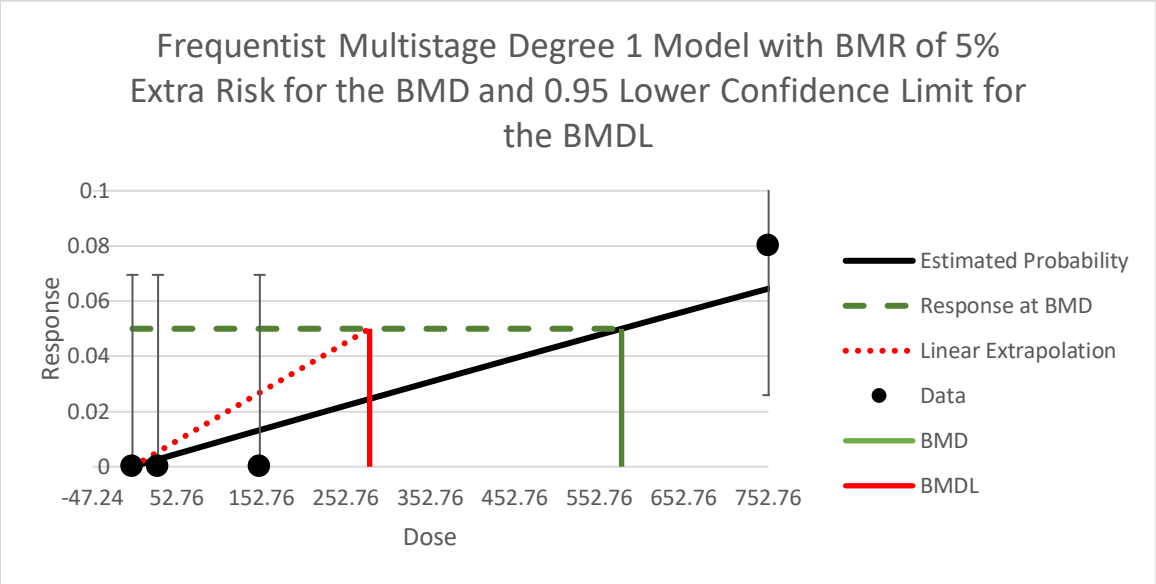
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.031583735	1.579186733	1	50	-0.46835
30.11	0.034698057	1.734902853	2	50	0.20485
150.55	0.047055515	2.352775749	3	50	0.4322457
752.76	0.106510939	5.325546952	5	50	-0.149241

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-40.90168785	4	-	-	NA
Fitted Model	-41.14502236	2	0.48666902	2	0.7840092
Reduced Model	-42.59643946	1	3.38950323	3	0.3353785

Figure E17. BMD modeling of Zymbal gland adenomas from Kasai et al. (2009)



Model run output for Figure E17: Multistage cancer model for Zymbal gland adenomas from Kasai et al. (2009)

Benchmark Dose

BMD	578.957191
BMDL	280.7555535
BMDU	1503.142382
AIC	31.66290257
P-value	0.800392567
D.O.F.	3
Chi^2	1.003551632
Slope Factor	0.000178091

Model Parameters

# of Parameters 2

Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	Bounded	NA	NA	NA
b1	8.8596E-05	3.33E-02	-0.0652694	0.06544661

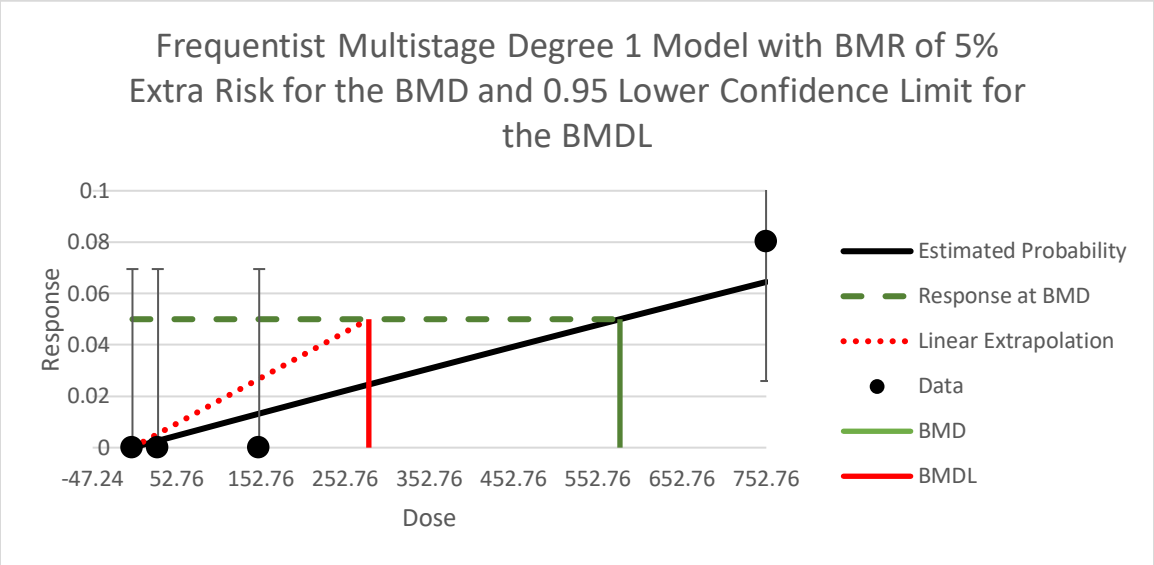
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	7.61499E-07	0	50	-0.000873
30.11	0.002664086	0.13320431	0	50	-0.365459
150.55	0.013249586	0.662479309	0	50	-0.819375
752.76	0.064516294	3.225814676	4	50	0.4456635

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-13.93846859	4	-	-	NA
Fitted Model	-14.83145129	1	1.7859654	3	0.6179938
Reduced Model	-19.60782266	1	11.3387081	3	0.0100285

Figure E18. BMD modeling of renal cell carcinomas from Kasai et al. (2009)



Model run output for Figure E18: Multistage cancer model for renal cell carcinomas from Kasai et al. (2009)

Benchmark Dose

BMD	578.957191
BMDL	280.7555535
BMDU	1503.142382
AIC	31.66290257
P-value	0.800392567
D.O.F.	3
Chi^2	1.003551632
Slope Factor	0.000178091

Model Parameters

# of Parameters 2

Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	Bounded	NA	NA	NA
b1	8.8596E-05	3.33E-02	-0.0652694	0.06544661

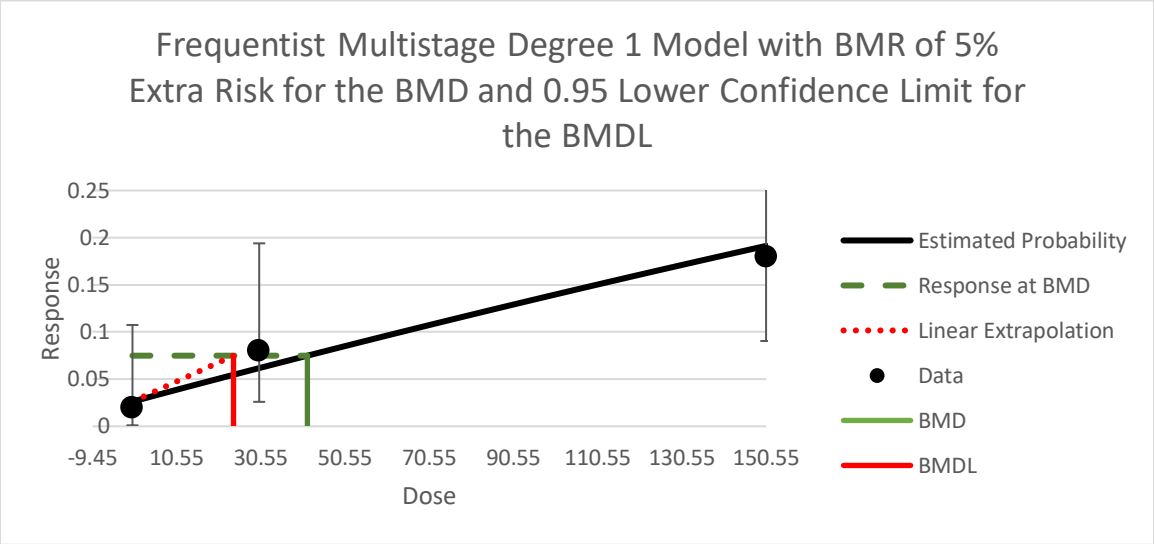
Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	7.61499E-07	0	50	-0.000873
30.11	0.002664086	0.13320431	0	50	-0.365459
150.55	0.013249586	0.662479309	0	50	-0.819375
752.76	0.064516294	3.225814676	4	50	0.4456635

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-13.93846859	4	-	-	NA
Fitted Model	-14.83145129	1	1.7859654	3	0.6179938
Reduced Model	-19.60782266	1	11.3387081	3	0.0100285

Figure E19. BMD modeling of subcutis fibromas with the high dose dropped from Kasai et al. (2009)



Model run output for Figure E19: Multistage cancer model for subcutis fibromas with the high dose dropped from Kasai et al. (2009)

Benchmark Dose

BMD	41.56077693
BMDL	24.01406536
BMDU	106.841607
AIC	89.20935214
P-value	0.524459708
D.O.F.	1
Chi^2	0.405114419
Slope Factor	0.002082113

Model Parameters

# of Parameters 2

Variable	Estimate	Std Error	Lower Conf	Upper Conf
g	0.026205403	5.41E-02	-0.0797685	0.13217932
b1	0.001234175	7.22E-02	-0.1403261	0.14279447

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.026205403	1.310270158	1	50	-0.274679
30.11	0.061728479	3.086423973	4	50	0.5368496
150.55	0.191326466	9.566323321	9	50	-0.203613

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-42.41009859	3	-	-	NA
Fitted Model	-42.60467607	2	0.38915496	1	0.5327439
Reduced Model	-46.52742704	1	8.23465689	2	0.016288

Model run output multisite multistage model for tumors in male F344 rats from Kasai et al., 2009

Benchmark Dose

BMD	11.40672986
BMDL	9.171713786
BMDU	14.55750783
Slope Factor	0.005451544
Combined Log-Likelihood	-274.5745935
Combined Log-Likelihood Constant	242.7138909

APPENDIX F. METHODOLOGY USED TO DERIVE CANCER SLOPE FACTORS

A multistage model was used to derive cancer potency estimates for 1,4-dioxane from the Kano et al. (2009) studies in male and female rats and male mice, and for the NCI (1978) studies in male and female mice. The data from the NCI (1978) study in female mice was modeled using poly-3 corrected incidences to account for differences in mortality that were significant but not severe between treated groups and the control group. A time-to-tumor extension of the multistage model (MSW, multistage Weibull) was used to derive a cancer potency estimate for the Kano et al. (2009) and JBRC (1998) study in female mice and the NCI (1978) studies in male and female rats. Based on consideration of the available mechanistic information on 1,4-dioxane, there is not enough evidence to assume a non-linear mode of action of carcinogenicity. There are no specific mechanistic data to suggest any deviation from the standard assumptions, including low-dose linearity, usually applied in cancer dose-response analysis. There are no principles or assumptions scientifically more appropriate, based on the available data, than the approach to the dose-response assessment of these studies described above, i.e., application of the multistage model and the time-to-tumor extension of that model.

The lifetime probability of a tumor at a specific site given exposure to the chemical at dose d is modeled using the multistage polynomial:

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

where the background probability of tumor, β_0, is between 0 and 1 and the coefficients β_i, i = 1…j, are positive. The β_i are parameters of the model, which are taken to be constants and are estimated from the data. The parameter β_0 provides the basis for estimating the background lifetime probability of the tumor.

To derive a measure of the cancer response in studies where increases in treatment-related tumors were observed at a single site, as was observed in the NCI (1978) studies in male and female mice, the ‘Frequentist, Restricted’ multistage model in US EPA’s Benchmark Dose Software (BMDS version 3.2) can be used to estimate the lower bound on the dose associated with a 5% increased risk of developing a tumor. The ratio of the 5% risk level to that lower bound on dose is known as the “animal cancer slope factor (CSF_{animal}),” or “animal cancer potency.”

For carcinogens that induce tumors at multiple sites and/or in different cell types at the same site in a particular species and sex, as was observed in the Kano et al. (2009) and JBRC (1998) study in male and female rats and in male mice, the “Dichotomous – Multi-tumor (MS_Combo)” model in US EPA’s BMDS (version 3.2) can be used to derive maximum likelihood estimates (MLEs) for the parameters of the multisite carcinogenicity model by summing the MLEs for the individual multistage models for the different sites and/or cell types. This multisite model provides a basis for estimating the cumulative risk of treatment-related tumors. In order to derive a measure of the total cancer response in a given study, the dose associated with a 5% increased risk of developing a tumor at one or more of the sites of interest was calculated and the lower bound for this dose was estimated using the “Dichotomous – Multi-tumor

(MS_Combo)" model in BMDS. The ratio of the 5% risk level to that lower bound on dose is known as the multisite CSF_{animal}.

To account for the treatment-related intercurrent mortality observed in the NCI (1978) study in female mice, the poly-3 method was used to adjust the denominator (N) of tumor (i.e., hepatocellular adenoma or carcinoma) incidence as shown in Table 10. The differential mortality was accounted for by assigning a reduced contribution towards N, proportional to the third power of the fraction of time on study, only to animals lacking hepatocellular adenoma or carcinoma that died before terminal sacrifice (day 630 on study).[13] The equation is shown below:

$$\text{Contribution to N} = \left(\frac{\text{days on study}}{630 \text{ days}}\right)^3$$

When a large fraction of the animals dies before the end of the study, as occurred in the Kano et al. (2009) and JBRC (1998) study in female mice and the NCI (1978) studies in male and female rats, the multistage-in-dose Weibull-in-time (multistage Weibull) model can be used to estimate the cancer potency. The multistage Weibull model is an extension of the multistage polynomial model given above, with the lifetime probability of a tumor at a specific site by time t and given exposure to the chemical at dose d given as:

$$p(t,d) = 1 - \exp[-(\beta_0 + \beta_1 d + \beta_2 d^2 + ... + \beta_j d^j)(t - t_0)^c]$$

where the coefficients β_i, i = 0…j, are positive, $0 \leq t_0 < t$, where t_0 is commonly interpreted as the latency period,[14] and the age exponent, c, is restricted to be between 0 and 10. Carcinogenic potency for a given site is derived by applying a maximum likelihood modeling approach to estimate the model parameters (β_i, t_0, and c). Using the multistage Weibull model, the animal cancer potency (CSF_{animal}) is defined as the upper 95% confidence bound on β_1 estimated at the assumed standard lifetime of 104 weeks for rats and mice.

The multistage Weibull model defines the lifetime probability of tumor at a single site. Since 1,4-dioxane induced tumors at multiple sites in the NCI (1978) study in female rats, a multisite approach was used to derive a measure of the total cancer response. Statistical distributions were generated for the estimates of linear term (β_1) at each site. A Monte Carlo approach was used to find the upper 95% confidence bound on the sum of β_1s, which was taken as the multisite CSF_{animal} estimate for the NCI (1978) study in female rats.

---

[13] Bailer AJ and Portier CJ (1988). Effects of treatment-induced mortality and tumor-induced mortality on test for carcinogenicity in small samples. Biometrics **44**(2):417-431

[14] When all tumors at a given site are considered incidental, as was the case with the liver tumors and nasal tumors observed in the Kano et al. (2009) and NCI (1978) studies, t_0 is not estimated and the probability of tumor (p(t,d)) by time t and lifetime dose rate d is given as: $p(t,d) = 1 - \exp[-(\beta_0 + \beta_1 d + \beta_2 d^2 + ... + \beta_j d^j)t^c]$

APPENDIX G. ADJUSTMENT FOR EARLY-IN-LIFE EXPOSURES

OEHHA accounts for the increased susceptibility of children and infants to carcinogens by applying age sensitivity factors (ASFs) to the cancer potency (OEHHA, 2009). Age-specific cancer risk is calculated for each age group by multiplying the cancer potency, age-specific exposure estimates (duration × intake rate), the concentration in drinking water and the ASF, where:

R	=	Default risk level of 10^{-6}
C	=	Concentration in water
CSF_{oral}	=	Oral cancer slope factor, in $(mg/kg\text{-}day)^{-1}$
CSF_{inh}	=	Inhalation cancer slope factor, in $(mg/kg\text{-}day)^{-1}$
ASF_1	=	Age sensitivity factor for 3rd trimester + infancy, value 10
ASF_2	=	Age sensitivity factor for childhood (ages 2-16), value 3
ASF_3	=	Age sensitivity factor for adult (ages 16-70), value 1.

For this calculation, the duration (d) of sensitive periods is expressed as fractions of the standard lifetime of 70 years as follows:

d_0	=	3rd trimester, value 0.25/70
d_1	=	infancy, value 2/40
d_2	=	childhood, value 14/70
d_3	=	adult, value 54/70.

The equivalent water exposure values (Daily Drinking Water Intake or DWI, expressed in terms of L_{eq}/kg-day) for each age range are expressed as follows:

DWI^o_1	=	Oral route, infancy
DWI^o_2	=	Oral route, childhood
DWI^o_3	=	Oral route, adult
DWI^i_2	=	Inhalation route, childhood
DWI^i_3	=	Inhalation route, adult.

For the risk equation, the overall lifetime risk is the sum of the cancer risk for each age bin and route. Note that for the third trimester of pregnancy, the ASF_1 for early-in-life exposures is applicable, but the consumption rate is assumed to be that of an adult (i.e., maternal consumption). Also, infants are assumed not to take showers, but mothers do. Then,

$$
\begin{aligned}
R = {} & (CSF_{oral} \times ASF_1 \times d_0 \times DWI^o_3 \times C) + \\
& (CSF_{oral} \times ASF_1 \times d_1 \times DWI^o_1 \times C) + \\
& (CSF_{oral} \times ASF_2 \times d_2 \times DWI^o_2 \times C) + \\
& (CSF_{oral} \times ASF_3 \times d_3 \times DWI^o_3 \times C) + \\
& (CSF_{inh} \times ASF_1 \times d_0 \times DWI^i_3 \times C) + \\
& (CSF_{inh} \times ASF_2 \times d_2 \times DWI^i_2 \times C) + \\
& (CSF_{inh} \times ASF_3 \times d_3 \times DWI^i_3 \times C) \qquad \text{Equation 1}
\end{aligned}
$$

This can be simplified by taking the common factor C outside a top-level bracket, and the common factors CSF_{oral} and CSF_{inh} can be taken outside second-level brackets:

$$R = C \times CSF_{oral} \times \left(\begin{array}{l} ASF_1 \times d_0 \times DWI^o_3 + \\ ASF_1 \times d_1 \times DWI^o_1 + \\ ASF_2 \times d_2 \times DWI^o_2 + \\ ASF_3 \times d_3 \times DWI^o_3 \end{array} \right) + CSF_{inh} \times \left(\begin{array}{l} ASF_1 \times d_0 \times DWI^i_3 + \\ ASF_2 \times d_2 \times DWI^i_2 + \\ ASF_3 \times d_3 \times DWI^i_3 \end{array} \right)$$

Equation 2

It is important to note that the calculation cannot be simplified further to any important degree since there are no other persistent common factors inside the second-level brackets. In other words, an accurate result cannot be achieved by summing consumption values and adjustment factors separately and then multiplying the results together.

Rearranging Equation 2:

$$C = \frac{R}{CSF_{oral} \times \left(\begin{array}{l} ASF_1 \times d_0 \times DWI^o_3 + \\ ASF_1 \times d_1 \times DWI^o_1 + \\ ASF_2 \times d_2 \times DWI^o_2 + \\ ASF_3 \times d_3 \times DWI^o_3 \end{array} \right) + CSF_{inh} \times \left(\begin{array}{l} ASF_1 \times d_0 \times DWI^i_3 + \\ ASF_2 \times d_2 \times DWI^i_2 + \\ ASF_3 \times d_3 \times DWI^i_3 \end{array} \right)}$$

Equation 3

The HPC is determined by solving Equation 3 for $R = 10^{-6}$.

Reference

OEHHA (2009). Technical Support Document for Cancer Potency Factors: Methodologies for Derivation, Listing of Available Values, and Adjustments to Allow for Early Life Stage Exposures. Appendix J. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.