



TOXICOLOGICAL REVIEW

OF

1,4-Dioxane

(CAS No. 123-91-1)

**In Support of Summary Information on the
Integrated Risk Information System (IRIS)**

May 2011

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LIST OF ABBREVIATIONS AND ACRONYMS

1	AIC	Akaike's Information Criterion
2	ALP	alkaline phosphatase
3	ALT	alanine aminotransferase
4	AST	aspartate aminotransferase
5	ATSDR	Agency for Toxic Substances and Disease Registry
6	BMC	<u>benchmark concentration</u>
7	BMCL	<u>benchmark concentration, lower 95% confidence limit</u>
8	BMCL₁₀	<u>benchmark concentration, lower 95% confidence limit at 10% extra risk</u>
9	BMD	benchmark dose
10	BMD₁₀	benchmark dose at 10% extra risk
11	BMD₃₀	benchmark dose at 30% extra risk
12	BMD₅₀	benchmark dose at 50% extra risk
13	BMDL	benchmark dose, lower 95% confidence limit
14	BMDL₁₀	benchmark dose, lower 95% confidence limit at 10% extra risk
15	BMDL₃₀	benchmark dose, lower 95% confidence limit at 30% extra risk
16	BMDL₅₀	benchmark dose, lower 95% confidence limit at 50% extra risk
17	BMDS	Benchmark Dose Software
18	BMR	benchmark response
19	BrdU	5-bromo-2'-deoxyuridine
20	BUN	blood urea nitrogen
21	BW(s)	body weight(s)
22	CASE	computer automated structure evaluator
23	CASRN	Chemical Abstracts Service Registry Number
24	CHO	Chinese hamster ovary (cells)
25	CI	confidence interval(s)
26	CNS	central nervous system
27	CPK	creatinine phosphokinase
28	CREST	antikinetochores
29	CSF	cancer slope factor
30	CV	concentration in venous blood
31	CYP450	cytochrome P450
32	DEN	diethylnitrosamine
33	FISH	fluorescence in situ hybridization
34	G-6-Pase	glucose-6-phosphatase
35	GC	gas chromatography
36	GGT	γ -glutamyl transpeptidase
37	GST-P	<u>glutathione S-transferase placental form</u>
38	HEAA	β -hydroxyethoxy acetic acid
39	HED(s)	human equivalent dose(s)
40	HPLC	high-performance liquid chromatography
41	HSDB	Hazardous Substances Data Bank
42	Hz	Hertz
43	IARC	International Agency for Research on Cancer
44	i.p.	intraperitoneal

1	i.v.	intravenous
2	IRIS	Integrated Risk Information System
3	JBRC	Japan Bioassay Research Center
4	k_e	1st order elimination rate of 1,4-dioxane
5	k_{INH}	1st order 1,4-dioxane inhalation rate constant
6	k_{LC}	1st order, non-saturable metabolism rate constant for 1,4-dioxane in the liver
7	K_m	Michaelis constant for metabolism of 1,4-dioxane in the liver
8	k_{me}	1st order elimination rate of HEAA (1,4-dioxane metabolite)
9	k_{OC}	soil organic carbon-water partitioning coefficient
10	LAP	leucine aminopeptidase
11	LD₅₀	median lethal dose
12	LDH	lactate dehydrogenase
13	LOAEL	lowest-observed-adverse-effect-level
14	<u>MCH</u>	<u>mean corpuscular hemoglobin</u>
15	MCV	mean corpuscular volume
16	MOA	mode of action
17	MS	mass spectrometry, multi-stage
18	MTD	maximum tolerated dose
19	MVK	Moolgavkar-Venzon-Knudsen (model)
20	NCE	normochromatic erythrocyte
21	NCI	National Cancer Institute
22	ND	no data, not detected
23	NE	not estimated
24	NOAEL	no-observed-adverse-effect-level
25	NRC	National Research Council
26	NTP	National Toxicology Program
27	OCT	ornithine carbamyl transferase
28	ODC	ornithine decarboxylase
29	OECD	Organization for Economic Co-operation and Development
30	PB	blood:air partition coefficient
31	PBPK	physiologically based pharmacokinetic
32	PC	partition coefficient
33	PCB	polychlorinated biphenyl
34	PCE	polychromatic erythrocyte
35	PFA	fat:air partition coefficient
36	PLA	liver:air partition coefficient
37	POD	point of departure
38	ppm	parts per million
39	PRA	rapidly perfused tissue:air partition coefficient
40	PSA	slowly perfused tissue:air partition coefficient
41	QCC	normalized cardiac output
42	QPC	normalized alveolar ventilation rate
43	RBC	red blood cell
44	RfC	inhalation reference concentration
45	RfD	oral reference dose
46	SCE	sister chromatid exchange

1	SDH	sorbitol dehydrogenase
2	SMR	standardized mortality ratio
3	SRC	Syracuse Research Corporation
4	TPA	12-O-tetradecanoylphorbol-13-acetate
5	TWA	time-weighted average
6	UF	uncertainty factor
7	UNEP	United Nations Environment Programme
8	U.S. EPA	U.S. Environmental Protection Agency
9	V	volts
10	VAS	visual analogue scale
11	V_d	volume of distribution
12	V_{max}	maximal rate of metabolism
13	V_{maxC}	normalized maximal rate of metabolism of 1,4-dioxane in liver
14	VOC(s)	volatile organic compound(s)
15	WBC	white blood cell
16	χ²	Chi-squared

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to 1,4-dioxane. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 1,4-dioxane.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration, and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of the data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

NOTE: New studies (Kasai et al., 2009; Kasai et al., 2008) regarding the toxicity of 1,4-dioxane through the inhalation route of exposure are available that were not included in the 1,4-dioxane assessment that was posted on the IRIS database in 2010 (U.S. EPA, 2010).

These studies have been incorporated into the previously posted assessment for review (U.S. EPA, 2010). Sections including new information can be identified by the red underlined text in the document. The entire document is provided for completeness.

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40 **EXTERNAL PEER REVIEWERS (INHALATION ASSESSMENT)**
41 **TO BE DETERMINED**
42

1. INTRODUCTION

1 This document presents background information and justification for the Integrated Risk
2 Information System (IRIS) Summary of the hazard and dose-response assessment of
3 1,4-dioxane. IRIS Summaries may include oral reference dose (RfD) and inhalation reference
4 concentration (RfC) values for chronic and subchronic exposure durations, and a carcinogenicity
5 assessment.

6 The RfD and RfC, if derived, provide quantitative information for use in risk assessments
7 for health effects known or assumed to be produced through a nonlinear (presumed threshold)
8 mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with
9 uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human
10 population (including sensitive subgroups) that is likely to be without an appreciable risk of
11 deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m³) is
12 analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The
13 inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for
14 effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference
15 values are generally derived for chronic exposures (up to a lifetime), but may also be derived for
16 acute (≤ 24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of
17 lifetime) exposure durations, all of which are derived based on an assumption of continuous
18 exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are
19 derived for chronic exposure duration.

20 The carcinogenicity assessment provides information on the carcinogenic hazard
21 potential of the substance in question and quantitative estimates of risk from oral and inhalation
22 exposure may be derived. The information includes a weight-of-evidence judgment of the
23 likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic
24 effects may be expressed. Quantitative risk estimates may be derived from the application of a
25 low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on
26 the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a
27 plausible upper bound on the estimate of risk per $\mu\text{g}/\text{m}^3$ air breathed.

28 Development of these hazard identification and dose-response assessments for
29 1,4-dioxane has followed the general guidelines for risk assessment as set forth by the National
30 Research Council ([NRC, 1983](#)). EPA guidelines and Risk Assessment Forum Technical Panel
31 Reports that may have been used in the development of this assessment include the following:

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#).

1 *Guidelines for the Health Risk Assessment of Chemical Mixtures* ([U.S. EPA, 1986c](#)), *Guidelines*
2 *for Mutagenicity Risk Assessment* ([U.S. EPA, 1986b](#)), *Recommendations for and Documentation*
3 *of Biological Values for Use in Risk Assessment* ([U.S. EPA, 1988](#)), *Guidelines for*
4 *Developmental Toxicity Risk Assessment* ([U.S. EPA, 1991](#)), *Interim Policy for Particle Size and*
5 *Limit Concentration Issues in Inhalation Toxicity* ([U.S. EPA, 1994a](#)), *Methods for Derivation of*
6 *Inhalation Reference Concentrations and Application of Inhalation Dosimetry* ([U.S. EPA,](#)
7 [1994b](#)), *Use of the Benchmark Dose Approach in Health Risk Assessment* ([U.S. EPA, 1995](#)),
8 *Guidelines for Reproductive Toxicity Risk Assessment* ([U.S. EPA, 1996](#)), *Guidelines for*
9 *Neurotoxicity Risk Assessment* ([U.S. EPA, 1998](#)), *Science Policy Council Handbook: Risk*
10 *Characterization* ([U.S. EPA, 2000b](#)), *Benchmark Dose Technical Guidance Document* (External
11 *Review Draft)* ([U.S. EPA, 2000a](#)), *Supplementary Guidance for Conducting Health Risk*
12 *Assessment of Chemical Mixtures* ([U.S. EPA, 2000c](#)), *A Review of the Reference Dose and*
13 *Reference Concentration Processes* ([U.S. EPA, 2002a](#)), *Guidelines for Carcinogen Risk*
14 *Assessment* ([U.S. EPA, 2005a](#)), *Supplemental Guidance for Assessing Susceptibility from Early-*
15 *Life Exposure to Carcinogens* ([U.S. EPA, 2005b](#)), *Science Policy Council Handbook: Peer*
16 *Review* ([U.S. EPA, 2006b](#)), and *A Framework for Assessing Health Risks of Environmental*
17 *Exposures to Children* ([U.S. EPA, 2006a](#)).

18 The literature search strategy employed for this compound was based on the Chemical
19 Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent
20 scientific information submitted by the public to the IRIS Submission Desk was also considered
21 in the development of this document. The relevant literature was reviewed through September
22 2009 [for the oral assessment and through March 2011 for the inhalation assessment.](#)

2. CHEMICAL AND PHYSICAL INFORMATION

1 1,4-Dioxane, a volatile organic compound (VOC), is a colorless liquid with a pleasant
2 odor ([Hawley & Lewis Rj Sr, 2001](#); [Lewis, 2000](#)). Synonyms include diethylene ether,
3 1,4-diethylene dioxide, diethylene oxide, dioxyethylene ether, and dioxane ([Hawley & Lewis
4 Rj Sr, 2001](#)). The chemical structure of 1,4-dioxane is shown in Figure 2-1. Selected chemical
5 and physical properties of this substance are listed in Table 2-1 below:

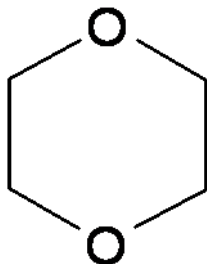


Figure 2-1. 1,4-Dioxane chemical structure.

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Table 2-1. Physical properties and chemical identity of 1,4-dioxane

CASRN:	123-91-1 (CRC, 2000)
Molecular weight:	88.10 (The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 2001)
Chemical formula:	C ₄ H ₈ O ₂ (The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 2001)
Boiling point:	101.1°C (The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 2001)
Melting point:	11.8°C (CRC, 2000)
Vapor pressure:	40 mmHg at 25°C (Lewis, 2000)
Density:	1.0337 g/mL at 20°C (CRC, 2000)
Vapor density:	3.03 (air = 1) (Lewis, 2000)
Water solubility:	Miscible with water (Hawley & Lewis Rj Sr, 2001)
Other solubilities:	Miscible with ethanol, ether, and acetone (CRC, 2000)
Log K _{ow} :	-0.27 (Hansch, Leo, & Hoekman, 1995)
Henry's Law constant:	4.80 × 10 ⁻⁶ atm·m ³ /molecule at 25°C (Park, Hussam, Couason, Fritz, & Carr, 1987)
OH reaction rate constant:	1.09 × 10 ⁻¹¹ cm ³ /molecule sec at 25°C (Atkinson, 1989)
K _{oc} :	17 (estimated using log K _{ow}) (ACS, 1990)
Bioconcentration factor:	0.4 (estimated using log K _{ow}) (Meylan et al., 1999)
Conversion factors (in air):	1 ppm = 3.6 mg/m ³ ; 1 mg/m ³ = 0.278 ppm (25°C and 1 atm) (HSDB, 2007)

1 1,4-Dioxane is produced commercially through the dehydration and ring closure of
2 diethylene glycol ([Surprenant, 2002](#)). Concentrated sulfuric acid is used as a catalyst
3 ([Surprenant, 2002](#)). This is a continuous distillation process with operating temperatures and
4 pressures of 130–200°C and 188–825 mmHg, respectively ([Surprenant, 2002](#)). During the years
5 1986 and 1990, the U.S. production of 1,4-dioxane reported by manufacturers was within the
6 range of 10–50 million pounds ([U.S. EPA, 2002b](#)). The production volume reported during the
7 years 1994, 1998, and 2002 was within the range of
8 1–10 million pounds ([U.S. EPA, 2002b](#)).

9 Historically, 1,4-dioxane has been used as a stabilizer for the solvent 1,1,1-trichloro-
10 ethane ([Surprenant, 2002](#)). However, this use is no longer expected to be important due to the
11 1990 Amendments to the Clean Air Act and the Montreal Protocol, which mandate the eventual
12 phase-out of 1,1,1-trichloroethane production in the U.S. ("[Amendments to the Clean Air Act,
13 Sec. 604. Phase-out of production and consumption of class I substances,](#)" 1990; [ATSDR, 2007](#);
14 [U.N. Environment Programme, 2000](#)). 1,4-Dioxane is a contaminant of some ingredients used in

1 the manufacture of personal care products and cosmetics. 1,4-Dioxane is also used as a solvent
2 for cellulose, organic products, lacquers, paints, varnishes, paint and varnish removers, resins,
3 oils, waxes, dyes, cements, fumigants, emulsions, and polishing compositions ([Hawley & Lewis](#)
4 [Rj Sr, 2001](#); [IARC, 1999](#); [The Merck Index: An Encyclopedia of Chemicals, Drugs, and](#)
5 [Biologicals, 2001](#)). 1,4-Dioxane has been used as a solvent in the formulation of inks, coatings,
6 and adhesives and in the extraction of animal and vegetable oil ([Surprenant, 2002](#)). Reaction
7 products of 1,4-dioxane are used in the manufacture of insecticides, herbicides, plasticizers, and
8 monomers ([Surprenant, 2002](#)).

9 When 1,4-dioxane enters the air, it will exist as a vapor, as indicated by its vapor pressure
10 ([HSDB, 2007](#)). It is expected to be degraded in the atmosphere through photooxidation with
11 hydroxyl radicals ([HSDB, 2007](#); [Surprenant, 2002](#)). The estimated half-life for this reaction is
12 6.7 hours ([HSDB, 2007](#)). It may also be broken down by reaction with nitrate radicals, although
13 this removal process is not expected to compete with hydroxyl radical photooxidation ([Grosjean,](#)
14 [1990](#)). 1,4-Dioxane is not expected to undergo direct photolysis ([Wolfe & Jeffers, 2000](#)).

15 1,4-Dioxane is primarily photooxidized to 2-oxodioxane and through reactions with nitrogen
16 oxides (NO_x) results in the formation of ethylene glycol diformate ([Platz, Sehested, Mogelberg,](#)
17 [Nielsen, & Wallington, 1997](#)). 1,4-Dioxane is expected to be highly mobile in soil based on its
18 estimated K_{oc} and is expected to leach to lower soil horizons and groundwater ([ACS, 1990](#);
19 [ATSDR, 2007](#)). This substance may volatilize from dry soil surfaces based on its vapor pressure
20 ([HSDB, 2007](#)). The estimated bioconcentration factor value indicates that 1,4-dioxane will not
21 bioconcentrate in aquatic or marine organisms ([Franke et al., 1994](#); [Meylan, et al., 1999](#)).
22 1,4-Dioxane is not expected to undergo hydrolysis or to biodegrade readily in the environment
23 ([ATSDR, 2007](#); [HSDB, 2007](#)). Therefore, volatilization is expected to be the dominant removal
24 process for moist soil and surface water. Based on a Henry's Law constant of 4.8×10^{-6}
25 atm-m³/mole, the half-life for volatilization of 1,4-dioxane from a model river is 5 days and that
26 from a model lake is 56 days ([ACS, 1990](#); [HSDB, 2007](#); [Park, et al., 1987](#)). 1,4-Dioxane may be
27 more persistent in groundwater where volatilization is hindered.

28 Recent environmental monitoring data for 1,4-dioxane are lacking. Existing data indicate
29 that 1,4-dioxane may leach from hazardous waste sites into drinking water sources located
30 nearby ([Lesage, Jackson, Priddle, & Riemann, 1990](#); [Yasuhara et al., 1997](#); [Yasuhara, Tanaka,](#)
31 [Tanabe, Kawata, & Katami, 2003](#)). 1,4-Dioxane has been detected in contaminated surface and
32 groundwater samples collected near hazardous waste sites and industrial facilities ([Derosa,](#)
33 [Wilbur, Holler, Richter, & Stevens, 1996](#)).

3. TOXICOKINETICS

1 Data for the toxicokinetics of 1,4-dioxane in humans are very limited. However,
2 absorption, distribution, metabolism, and elimination of 1,4-dioxane are well described in rats
3 exposed via the oral, inhalation, or intravenous (i.v.) routes. 1,4-Dioxane is extensively absorbed
4 and metabolized in humans and rats. The metabolite most often measured and reported is
5 β -hydroxyethoxy acetic acid (HEAA), which is predominantly excreted in the urine; however,
6 other metabolites have also been identified. Saturation of 1,4-dioxane metabolism has been
7 observed in rats and would be expected in humans; however, human exposure levels associated
8 with nonlinear toxicokinetics are not known.

9 Important data elements that have contributed to our current understanding of the
10 toxicokinetics of 1,4-dioxane are summarized in the following sections.

3.1. ABSORPTION

11 Absorption of 1,4-dioxane following inhalation exposure has been qualitatively
12 demonstrated in workers and volunteers. Workers exposed to a time-weighted average (TWA)
13 of 1.6 parts per million (ppm) of 1,4-dioxane in air for 7.5 hours showed a HEAA/1,4-dioxane
14 ratio of 118:1 in urine ([Young, Braun, Gehring, Horvath, & Daniel, 1976](#)). The authors assumed
15 lung absorption to be 100% and calculated an average absorbed dose of 0.37 mg/kg, although no
16 exhaled breath measurements were taken. In a study with four healthy male volunteers, Young
17 et al. ([1977](#)) reported 6-hour inhalation exposures of adult volunteers to 50 ppm of 1,4-dioxane
18 in a chamber, followed by blood and urine analysis for 1,4-dioxane and HEAA. The study
19 protocol was approved by a seven-member Human Research Review Committee of the Dow
20 Chemical Company, and written informed consent of study participants was obtained. At a
21 concentration of 50 ppm, uptake of 1,4-dioxane into plasma was rapid and approached steady-
22 state conditions by 6 hours. The authors reported a calculated absorbed dose of 5.4 mg/kg.
23 However, the exposure chamber atmosphere was kept at a constant concentration of 50 ppm and
24 exhaled breath was not analyzed. Accordingly, gas uptake could not be measured. As a result,
25 the absorbed fraction of inhaled 1,4-dioxane could not be accurately determined in humans. Rats
26 inhaling 50 ppm for 6 hours exhibited 1,4-dioxane and HEAA in urine with an HEAA to
27 1,4-dioxane ratio of over 3,100:1 ([J. D. Young, W. H. Braun, & P. J. Gehring, 1978a](#); [J. D.](#)
28 [Young, W. H. Braun, & P. J. Gehring, 1978b](#)). Plasma concentrations at the end of the 6-hour
29 exposure period averaged 7.3 $\mu\text{g/mL}$. The authors calculated an absorbed 1,4-dioxane dose of

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1 71.9 mg/kg; however, the lack of exhaled breath data and dynamic exposure chamber precluded
2 the accurate determination of the absorbed fraction of inhaled 1,4-dioxane.

3 No human data are available to evaluate the oral absorption of 1,4-dioxane.

4 Gastrointestinal absorption was nearly complete in male Sprague Dawley rats orally dosed with
5 10–1,000 mg/kg of [¹⁴C]-1,4-dioxane given as a single dose or as 17 consecutive daily doses
6 ([Young, et al., 1978a](#); [Young, et al., 1978b](#)). Cumulative recovery of radiolabel in the feces was
7 <1–2% of administered dose regardless of dose level or frequency.

8 No human data are available to evaluate the dermal absorption of 1,4-dioxane; however,
9 Bronaugh ([1982](#)) reported an in vitro study in which 1,4-dioxane penetrated excised human skin
10 10 times more under occluded conditions (3.2% of applied dose) than unoccluded conditions
11 (0.3% of applied dose). [¹⁴C]-1,4-Dioxane was dissolved in lotion, applied to the excised skin in
12 occluded and unoccluded diffusion cells, and absorption of the dose was recorded 205 minutes
13 after application. Bronaugh ([1982](#)) also reported observing rapid evaporation, which further
14 decreased the small amount available for skin absorption.

15 Dermal absorption data in animals are also limited. Dermal absorption in animals was
16 reported to be low following exposure of forearm skin of monkeys ([Marzulli, Anjo, & Maibach,](#)
17 [1981](#)). In this study, Rhesus monkeys were exposed to [¹⁴C]-1,4-dioxane in methanol or skin
18 lotion vehicle for 24 hours (skin was uncovered/unoccluded). Only 2–3% of the original
19 radiolabel was cumulatively recovered in urine over a 5-day period.

3.2. DISTRIBUTION

20 No data are available for the distribution of 1,4-dioxane in human tissues. No data are
21 available for the distribution of 1,4-dioxane in animals following oral or inhalation exposures.

22 Mikheev et al. ([1990](#)) studied the distribution of [¹⁴C]-1,4-dioxane in the blood, liver,
23 kidney, brain, and testes of rats (strain not reported) for up to 6 hours following intraperitoneal
24 (i.p.) injection of approximately one-tenth the median lethal dose (LD₅₀) (actual dose not
25 reported). While actual tissue concentrations were not reported, tissue:blood ratios were given
26 for each tissue at six time points ranging from 5 minutes to 6 hours. The time to reach maximum
27 accumulation of radiolabel was shorter for liver and kidney than for blood or the other tissues,
28 which the authors suggested was indicative of selective membrane transport. Tissue:blood ratios
29 were less than one for all tissues except testes, which had a ratio greater than one at the 6-hour
30 time point. The significance of these findings is questionable since the contribution of residual
31 blood in the tissues was unknown (though saline perfusion may serve to clear tissues of highly
32 water-soluble 1,4-dioxane), the tissue concentrations of radiolabel were not reported, and data
33 were collected from so few time points.

34 Woo et al. ([1977](#)) administered i.p. doses of [³H]-1,4-dioxane (5 mCi/kg body weight
35 [BW]) to male Sprague Dawley rats with and without pretreatment using mixed-function oxidase

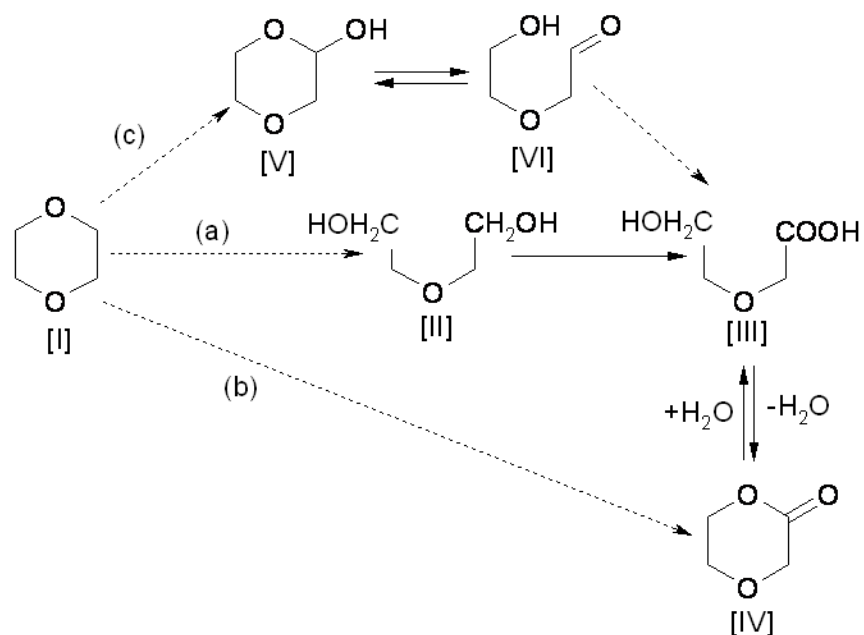
1 inducers (phenobarbital, 3-methylcholanthrene, or polychlorinated biphenyls [PCBs]). Liver,
2 kidney, spleen, lung, colon, and skeletal muscle tissues were collected from 1, 2, 6, and 12 hours
3 after dosing. Distribution was generally uniform across tissues, with blood concentrations higher
4 than tissues at all times except for 1 hour post dosing, when kidney levels were approximately
5 20% higher than blood. Since tissues were not perfused prior to analysis, the contribution of
6 residual blood to radiolabel measurements is unknown, though loss of 1,4-dioxane from tissues
7 would be unknown had saline perfusion been performed. Covalent binding reached peak
8 percentages at 6 hours after dosing in liver (18.5%), spleen (22.6%), and colon (19.5%). At
9 16 hours after dosing, peak covalent binding percentages were observed in whole blood (3.1%),
10 kidney (9.5%), lung (11.2%), and skeletal muscle (11.2%). Within hepatocytes, radiolabel
11 distribution at 6 hours after dosing was greatest in the cytosolic fraction (43.8%) followed by the
12 microsomal (27.9%), mitochondrial (16.6%), and nuclear (11.7%) fractions. While little
13 covalent binding of radiolabel was measured in the hepatic cytosol (4.6%), greater binding was
14 observed at 16 hours after dosing in the nuclear (64.8%), mitochondrial (45.7%), and
15 microsomal (33.4%) fractions. Pretreatment with inducers of mixed-function oxidase activity
16 did not significantly change the extent of covalent binding in subcellular fractions.

3.3. METABOLISM

17 The major product of 1,4-dioxane metabolism appears to be HEAA, although there is
18 one report that identified 1,4-dioxane-2-one as a major metabolite ([Woo, Arcos, et al., 1977](#)).
19 However, the presence of this compound in the sample was believed to result from the acidic
20 conditions (pH of 4.0–4.5) of the analytical procedures. The reversible conversion of HEAA and
21 p-1,4-dioxane-2-one is pH-dependent ([Braun & Young, 1977](#)). Braun and Young ([1977](#))
22 identified HEAA (85%) as the major metabolite, with most of the remaining dose excreted as
23 unchanged 1,4-dioxane in the urine of Sprague Dawley rats dosed with 1,000 mg/kg of
24 uniformly labeled 1,4-¹⁴C]dioxane. In fact, toxicokinetic studies of 1,4-dioxane in humans and
25 rats ([Young, et al., 1978a](#); [Young, et al., 1978b](#); [Young, et al., 1977](#)) employed an analytical
26 technique that converted HEAA to the more volatile 1,4-dioxane-2-one prior to gas
27 chromatography (GC); however, it is still unclear as to whether HEAA or 1,4-dioxane-2-one is
28 the major metabolite of 1,4-dioxane.

29 A proposed metabolic scheme for 1,4-dioxane metabolism ([Woo, Arcos, et al., 1977](#)) in
30 Sprague Dawley rats is shown in Figure 3-1. Oxidation of 1,4-dioxane to diethylene glycol
31 (pathway a), 1,4-dioxane-2-ol (pathway c), or directly to 1,4-dioxane-2-one (pathway b) could
32 result in the production of HEAA. 1,4-Dioxane oxidation appears to be cytochrome P450
33 (CYP450)-mediated, as CYP450 induction with phenobarbital or Aroclor 1254 (a commercial
34 PCB mixture) and suppression with 2,4-dichloro-6-phenylphenoxy ethylamine or cobaltous
35 chloride were effective in significantly increasing and decreasing, respectively, the appearance of

1 HEAA in the urine of male Sprague Dawley rats following 3 g/kg i.p. dose ([Woo, Argus, &](#)
 2 [Arcos, 1977a, 1978](#)). 1,4-Dioxane itself induced CYP450-mediated metabolism of several
 3 barbiturates in Hindustan mice given i.p. injections of 25 and 50 mg/kg 1,4-dioxane ([Mungikar](#)
 4 [& Pawar, 1978](#)). Of the three possible pathways proposed in this scheme, oxidation to
 5 diethylene glycol and HEAA appears to be the most likely, because diethylene glycol was found
 6 as a minor metabolite in Sprague Dawley rat urine following a single 1,000 mg/kg gavage dose
 7 of 1,4-dioxane ([Braun & Young, 1977](#)). Additionally, i.p. injection of 100–400 mg/kg
 8 diethylene glycol in Sprague Dawley rats resulted in urinary elimination of HEAA ([Woo, Argus,](#)
 9 [& Arcos, 1977b](#)).



Source: Adapted with permission from Elsevier Ltd., Woo et al. ([1977](#); [1977a](#)).

Figure 3-1. Suggested metabolic pathways of 1,4-dioxane in the rat.

I = 1,4-dioxane; II = diethylene glycol; III = β-hydroxyethoxy acetic acid (HEAA);

IV = 1,4-dioxane-2-one; V = 1,4-dioxane-2-ol; VI = β-hydroxyethoxy acetaldehyde.

Note: Metabolite [V] is a likely intermediate in pathway b as well as pathway c.

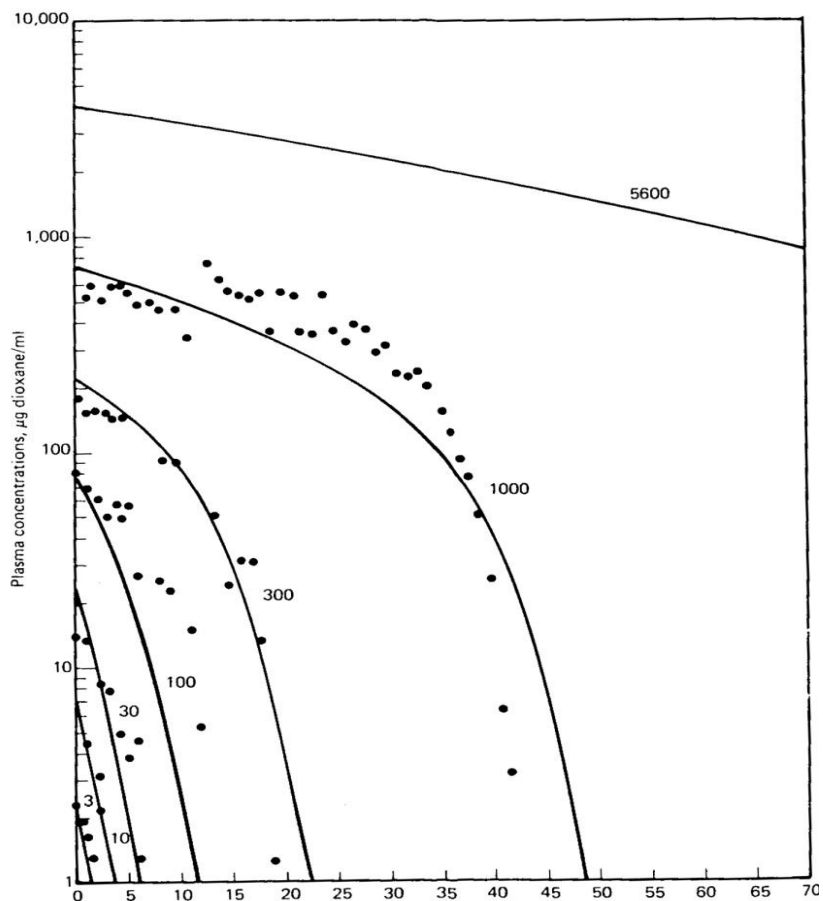
The proposed pathways are based on the metabolites identified; the enzymes responsible for each reaction have not been determined. The proposed pathways do not account for metabolite degradation to the labeled carbon dioxide (CO₂) identified in expired air after labeled 1,4-dioxane exposure.

10 Metabolism of 1,4-dioxane in humans is extensive. In a survey of 1,4-dioxane plant
 11 workers exposed to a TWA of 1.6 ppm of 1,4-dioxane for 7.5 hours, Young et al. ([1976](#)) found
 12 HEAA and 1,4-dioxane in the worker's urine at a ratio of 118:1. Similarly, in adult male
 13 volunteers exposed to 50 ppm for 6 hours ([Young, et al., 1977](#)), over 99% of inhaled 1,4-dioxane
 14 (assuming negligible exhaled excretion) appeared in the urine as HEAA. The linear elimination

1 of 1,4-dioxane in both plasma and urine indicated that 1,4-dioxane metabolism was a
2 nonsaturated, first-order process at this exposure level.

3 Like humans, rats extensively metabolize inhaled 1,4-dioxane, as HEAA content in urine
4 was over 3,000-fold higher than that of 1,4-dioxane following exposure to 50 ppm for 6 hours
5 ([Young, et al., 1978a](#); [Young, et al., 1978b](#)). 1,4-Dioxane metabolism in rats was a saturable
6 process, as exhibited by oral and i.v. exposures to various doses of [¹⁴C]-1,4-dioxane ([Young, et](#)
7 [al., 1978a](#); [Young, et al., 1978b](#)). Plasma data from Sprague Dawley rats given single i.v. doses
8 of 3, 10, 30, 100, 300, or 1,000 mg [¹⁴C]-1,4-dioxane/kg demonstrated a dose-related shift from
9 linear, first-order to nonlinear, saturable metabolism of 1,4-dioxane between plasma 1,4-dioxane
10 levels of 30 and 100 µg/mL (Figure 3-2). Similarly, in rats given, via gavage in distilled water,
11 10, 100, or 1,000 mg [¹⁴C]-1,4-dioxane/kg singly or 10 or 1,000 mg [¹⁴C]-1,4-dioxane/kg in
12 17 daily doses, the percent urinary excretion of the radiolabel decreased significantly with dose
13 while radiolabel in expired air increased. Specifically, with single [¹⁴C]-1,4-dioxane/kg doses,
14 urinary radiolabel decreased from 99 to 76% and expired 1,4-dioxane increased from <1 to 25%
15 as dose increased from 10 to 1,000 mg/kg. Likewise, with multiple daily doses 10 or 1,000 mg
16 [¹⁴C]-1,4-dioxane/kg, urinary radiolabel decreased from 99 to 82% and expired 1,4-dioxane
17 increased from 1 to 9% as dose increased. The differences between single and multiple doses in
18 urinary and expired radiolabel support the notion that 1,4-dioxane may induce its own
19 metabolism.

20 Induction of 1,4-dioxane metabolism is quantitatively illustrated by examining plasma
21 levels of the chemical in relationship to inhaled doses in a 13 week study by Kasai et al. (2008).
22 In this study, male and female F344 rats were exposed daily to concentrations of 0 (control),
23 100, 200, 400, 1,600, and 3,200 ppm. Plasma levels of 1,4-dioxane linearly increased with
24 increasing inhalation concentration, suggesting that metabolic saturation was not achieved during
25 the course of the experiments for plasma levels up to 730 and 1,054 µg/mL in male and female
26 rats, respectively, at the highest exposure concentration (3,200 ppm). In contrast, Young et al.
27 (1978a) single dose experiments showed possible saturation of metabolism at plasma levels of
28 100 µg/mL. Therefore, lack of the metabolic saturation of 1,4-dioxane found in the Kasai et al.
29 (2008) study is likely attributed to enhanced metabolism by the induction of P450 enzymes,
30 including CYP2E1, by 13 weeks of repeated inhalation exposure to 1,4-dioxane at concentrations
31 up to 3,200 ppm (Kasai, et al., 2008).



Source: Used with permission from Taylor and Francis, Young et al. (1978a).

Figure 3-2. Plasma 1,4-dioxane levels in rats following i.v. doses of 3-5,600 mg/kg [y-axis is plasma concentration of 1,4-dioxane (µg/mL) and x-axis is time (hr)]

1 1,4-Dioxane has been shown to induce several isoforms of CYP450 in various tissues
 2 following acute oral administration by gavage or drinking water (Nannelli, De Rubertis, Longo,
 3 & Gervasi, 2005). Male Sprague Dawley rats were exposed to either 2,000 mg/kg 1,4-dioxane
 4 via gavage for 2 consecutive days or by ingestion of a 1.5% 1,4-dioxane drinking water solution
 5 for 10 days. Both exposures resulted in significantly increased CYP2B1/2, CYP2C11, and
 6 CYP2E1 activities in hepatic microsomes. The gavage exposure alone resulted in increased
 7 CYP3A activity. The increase in 2C11 activity was unexpected, as that isoform has been
 8 observed to be under hormonal control and was typically suppressed in the presence of 2B1/2
 9 and 2E1 induction. In the male rat, hepatic 2C11 induction is associated with masculine pulsatile
 10 plasma profiles of growth hormone (compared to the constant plasma levels in the female),
 11 resulting in masculinization of hepatocyte function (Waxman, Pampori, Ram, Agrawal, &
 12 Shapiro, 1991). The authors postulated that 1,4-dioxane may alter plasma growth hormone
 13 levels, resulting in the observed 2C11 induction. However, growth hormone induction of 2C11

1 is primarily dependent on the duration between growth hormone pulses and secondarily on
2 growth hormone plasma levels ([Agrawal & Shapiro, 2000](#); [Waxman, et al., 1991](#)). Thus, the
3 induction of 2C11 by 1,4-dioxane may be mediated by changes in the time interval between
4 growth hormone pulses rather than changes in growth hormone levels. This may be
5 accomplished by 1,4-dioxane temporarily influencing the presence of growth hormone cell
6 surface binding sites ([Agrawal & Shapiro, 2000](#)). However, no studies are available to confirm
7 the influence of 1,4-dioxane on either growth hormone levels or changes in growth hormone
8 pulse interval.

9 In nasal and renal mucosal cell microsomes, CYP2E1 activity, but not CYP2B1/2
10 activity, was increased. Pulmonary mucosal CYP450 activity levels were not significantly
11 altered. Observed increases in 2E1 mRNA in rats exposed by gavage and i.p. injection suggest
12 that 2E1 induction in kidney and nasal mucosa is controlled by a transcriptional activation of
13 2E1 genes. The lack of increased mRNA in hepatocytes suggests that induction is regulated via
14 a post-transcriptional mechanism. Differences in 2E1 induction mechanisms in liver, kidney,
15 and nasal mucosa suggest that induction is controlled in a tissue-specific manner.

3.4. ELIMINATION

16 In workers exposed to a TWA of 1.6 ppm for 7.5 hours, 99% of 1,4-dioxane eliminated in
17 urine was in the form of HEAA ([Young, et al., 1976](#)). The elimination half-life was 59 minutes
18 in adult male volunteers exposed to 50 ppm 1,4-dioxane for 6 hours, with 90% of urinary
19 1,4-dioxane and 47% of urinary HEAA excreted within 6 hours of onset of exposure ([Young, et](#)
20 [al., 1977](#)). There are no data for 1,4-dioxane elimination in humans from oral exposures.

21 Elimination of 1,4-dioxane in rats ([Young, et al., 1978a](#); [Young, et al., 1978b](#)) was
22 primarily via urine. As comparably assessed in humans, the elimination half-life in rats exposed
23 to 50 ppm 1,4-dioxane for 6 hours was calculated to be 1.01 hours. In Sprague Dawley rats
24 given single daily doses of 10, 100, or 1,000 mg [¹⁴C]-1,4-dioxane/kg or multiple doses of 10 or
25 1,000 mg [¹⁴C]-1,4-dioxane/kg, urinary radiolabel ranged from 99% down to 76% of total
26 radiolabel. Fecal elimination was less than 2% for all doses. The effect of saturable metabolism
27 on expired 1,4-dioxane was apparent, as expired 1,4-dioxane in singly dosed rats increased with
28 dose from 0.4 to 25% while expired ¹⁴CO₂ changed little (between 2 and 3%) across doses. The
29 same relationship was seen in Sprague Dawley rats dosed i.v. with 10 or 1,000 mg
30 [¹⁴C]-1,4-dioxane/kg. Higher levels of ¹⁴CO₂ relative to 1,4-dioxane were measured in expired
31 air of the 10 mg/kg group, while higher levels of expired 1,4-dioxane relative to ¹⁴CO₂ were
32 measured in the 1,000 mg/kg group.

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

1 Physiologically based pharmacokinetic models (PBPK) models have been developed for
2 1,4-dioxane in rats ([Leung & Paustenbach, 1990](#); [Reitz, McCroskey, Park, Andersen, & Gargas,](#)
3 [1990](#); [Sweeney et al., 2008](#)), mice ([Sweeney, et al., 2008](#)), humans ([Leung & Paustenbach, 1990](#);
4 [Reitz, et al., 1990](#); [Sweeney, et al., 2008](#)), and lactating women ([Fisher, Mahle, Bankston,](#)
5 [Greene, & Gearhart, 1997](#)). Each of the models simulates the body as a series of compartments
6 representing tissues or tissue groups that receive blood from the central vascular compartment
7 (Figure 3-3). Modeling was conducted under the premise that transfers of 1,4-dioxane between
8 blood and tissues occur sufficiently fast to be effectively blood flow-limited, which is consistent
9 with the available data ([Ramsey & Andersen, 1984](#)). Blood time course and metabolite
10 production data in rats and humans suggest that absorption and metabolism are accomplished
11 through common mechanisms in both species ([1978a](#); [Young, et al., 1978b](#); [Young, et al., 1977](#)),
12 allowing identical model structures to be used for both species (and by extension, for mice as
13 well). In all three models, physiologically relevant, species-specific parameter values for tissue
14 volume, blood flow, and metabolism and elimination are used. The models and supporting data
15 are reviewed below, from the perspective of assessing their utility for predicting internal
16 dosimetry and for cross-species extrapolation of exposure-response relationships for critical
17 neoplastic and nonneoplastic endpoints (also see Appendix B).

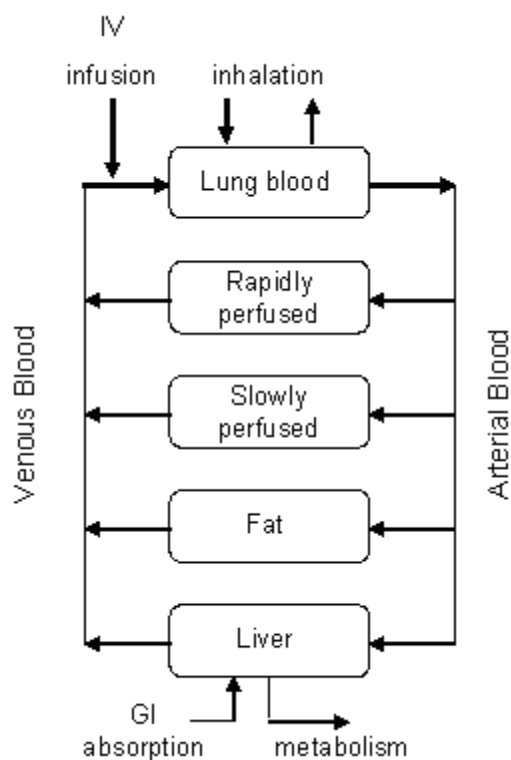


Figure 3-3. General PBPK model structure consisting of blood-flow limited tissue compartments connected via arterial and venous blood flows. Note: Orally administered chemicals are absorbed directly into the liver while inhaled and intravenously infused chemicals enter directly into the arterial and venous blood pools, respectively.

3.5.1. Available Pharmacokinetic Data

1 Animal and human data sets available for model calibration derive from Young et al.
 2 ([1978a](#); [1978b](#); [1977](#)), Mikheev et al. ([1990](#)), and Woo et al. ([1977](#); [1977b](#)). Young et al.
 3 ([1978a](#); [1978b](#)) studied the disposition of radiolabeled [¹⁴C]-1,4-dioxane in adult male
 4 Sprague Dawley rats following i.v., inhalation, and single and multiple oral gavage exposures.
 5 Plasma concentration-time profiles were reported for i.v. doses of 3, 10, 30, 100, and
 6 1,000 mg/kg. In addition, exhaled ¹⁴CO₂ and urinary 1,4-dioxane and HEAA profiles were
 7 reported following i.v. doses of 10 and 1,000 mg/kg. The plasma 1,4-dioxane concentration-time
 8 course, cumulative urinary 1,4-dioxane and cumulative urinary HEAA concentrations were
 9 reported following a 6-hour inhalation exposure to 50 ppm. Following oral gavage doses of 10–
 10 1,000 mg/kg, percentages of total orally administered radiolabel were measured in urine, feces,
 11 expired air, and the whole body.

12 Oral absorption of 1,4-dioxane was extensive, as only approximately 1% of the
 13 administered dose appeared in the feces within 72 hours of dosing ([Young, et al., 1978a](#))
 14 ([Young, et al., 1978b](#)). Although it may be concluded that the rate of oral absorption was high

1 enough to ensure nearly complete absorption by 72 hours, a more quantitative estimate of the
2 rate of oral absorption is not possible due to the absence of plasma time course data by oral
3 exposure.

4 Saturable metabolism of 1,4-dioxane was observed in rats exposed by either the i.v. or
5 oral routes ([Young, et al., 1978a](#); [Young, et al., 1978b](#)). Elimination of 1,4-dioxane from plasma
6 appeared to be linear following i.v. doses of 3-30 mg/kg, but was nonlinear following doses of
7 100–1,000 mg/kg. Accordingly, 10 mg/kg i.v. doses resulted in higher concentrations of ¹⁴CO₂
8 (from metabolized 1,4-dioxane) in expired air relative to unchanged 1,4-dioxane, while
9 1,000 mg/kg i.v. doses resulted in higher concentrations of expired 1,4-dioxane relative to ¹⁴CO₂.
10 Thus, at higher i.v. doses, a higher proportion of unmetabolized 1,4-dioxane is available for
11 exhalation. Taken together, the i.v. plasma and expired air data from Young et al. ([1978a](#);
12 [1978b](#)) corroborate previous studies describing the saturable nature of 1,4-dioxane metabolism in
13 rats ([1977](#); [Woo, Argus, et al., 1977b](#)) and are useful for optimizing metabolic parameters (V_{\max}
14 and K_m) in a PBPK model.

15 Similarly, increasing single or multiple oral doses of 10–1,000 mg/kg resulted in
16 increasing percentage of 1,4-dioxane in exhaled air and decreasing percentage of radiolabel
17 (either as 1,4-dioxane or a metabolite) in the urine, with significant differences in both metrics
18 being observed between doses of 10 and 100 mg/kg ([Young, et al., 1978a](#); [Young, et al., 1978b](#)).
19 These data identify the region (10–100 mg/kg) in which oral exposures will result in nonlinear
20 metabolism of 1,4-dioxane and can be used to test whether metabolic parameter value estimates
21 derived from i.v. dosing data are adequate for modeling oral exposures.

22 Post-exposure plasma data from a single 6-hour, 50 ppm inhalation exposure in rats were
23 reported ([Young, et al., 1978a](#); [Young, et al., 1978b](#)). The observed linear elimination of
24 1,4-dioxane after inhalation exposure suggests that, via this route, metabolism is in the linear
25 region at this exposure level.

26 The only human data adequate for use in PBPK model development ([Young, et al., 1977](#))
27 come from adult male volunteers exposed to 50 ppm 1,4-dioxane for 6 hours. Plasma
28 1,4-dioxane and HEAA concentrations were measured both during and after the exposure period,
29 and urine concentrations were measured following exposure. Plasma levels of 1,4-dioxane
30 approached steady-state at 6 hours. HEAA data were insufficient to describe the appearance or
31 elimination of HEAA in plasma. Data on elimination of 1,4-dioxane and HEAA in the urine up
32 to 24 hours from the beginning of exposure were reported. At 6 hours from onset of exposure,
33 approximately 90% and 47% of the cumulative (0–24 hours) urinary 1,4-dioxane and HEAA,
34 respectively, were measured in the urine. The ratio of HEAA to 1,4-dioxane in urine 24 hours
35 after onset of exposure was 192:1 (similar to the ratio of 118:1 observed by Young et al. ([1976](#))
36 in workers exposed to 1.6 ppm for 7.5 hours), indicating extensive metabolism of 1,4-dioxane
37 As with Sprague Dawley rats, the elimination of 1,4-dioxane from plasma was linear across all

1 observations (6 hours following end of exposure), suggesting that human metabolism of
2 1,4-dioxane is linear for a 50 ppm inhalation exposure to steady-state. Thus, estimation of
3 human V_{\max} and K_m from these data will introduce uncertainty into internal dosimetry performed
4 in the nonlinear region of metabolism.

5 Further data were reported for the tissue distribution of 1,4-dioxane in rats. Mikheev
6 et al. (1990) administered i.p. doses of [^{14}C]-1,4-dioxane to white rats (strain not reported) and
7 reported time-to-peak blood, liver, kidney, and testes concentrations. They also reported ratios
8 of tissue to blood concentrations at various time points after dosing. Woo et al. (1977; 1977b)
9 administered i.p. doses of [^{14}C]-1,4-dioxane to Sprague Dawley rats and measured radioactivity
10 levels in urine. However, since i.p. dosing is not relevant to human exposures, these data are of
11 limited use for PBPK model development.

3.5.2. Published PBPK Models for 1,4-Dioxane

3.5.2.1. *Leung and Paustenbach*

12 Leung and Paustenbach (1990) developed a PBPK model for 1,4-dioxane and its primary
13 metabolite, HEAA, in rats and humans. The model, based on the structure of a PBPK model for
14 styrene (Ramsey & Andersen, 1984), consists of a central blood compartment and four tissue
15 compartments: liver, fat, slowly perfused tissues (mainly muscle and skin), and richly perfused
16 tissues (brain, kidney, and viscera other than the liver). Tissue volumes were calculated as
17 percentages of total BW, and blood flow rates to each compartment were calculated as
18 percentages of cardiac output. Equivalent cardiac output and alveolar ventilation rates were
19 allometrically scaled to a power (0.74) of BW for each species. The concentration of
20 1,4-dioxane in alveolar blood was assumed to be in equilibrium with alveolar air at a ratio equal
21 to the experimentally measured blood:air partition coefficient. Transfers of 1,4-dioxane between
22 blood and tissues were assumed to be blood flow-limited and to achieve rapid equilibrium
23 between blood and tissue, governed by tissue:blood equilibrium partition coefficients. The latter
24 were derived from the quotient of blood:air and tissue:air partition coefficients, which were
25 measured in vitro (Leung & Paustenbach, 1990) for blood, liver, fat, and skeletal muscle (slowly
26 perfused tissue). Blood:air partition coefficients were measured for both humans and rats. Rat
27 tissue:air partition coefficients were used as surrogate values for humans, with the exception of
28 slowly perfused tissue:blood, which was estimated by optimization to the plasma time-course
29 data. Portals of entry included i.v. infusion (over a period of 36 seconds) into the venous blood,
30 inhalation by diffusion from the alveolar air into the lung blood at the rate of alveolar ventilation,
31 and oral administration via zero-order absorption from the gastrointestinal tract to the liver.
32 Elimination of 1,4-dioxane was accomplished through pulmonary exhalation and saturable
33 hepatic metabolism. Urinary excretion of HEAA was assumed to be instantaneous with the
34 generation of HEAA from the hepatic metabolism of 1,4-dioxane.

1 The parameter values for hepatic metabolism of 1,4-dioxane, V_{\max} and K_m , were
2 optimized and validated against plasma and/or urine time course data for 1,4-dioxane and HEAA
3 in rats following i.v. and inhalation exposures and humans following inhalation exposure ([1978a](#);
4 [1978b](#); [Young, et al., 1977](#)); the exact data (i.e., i.v., inhalation, or both) used for the
5 optimization and calibration were not reported. Although the liver and fat were represented by
6 tissue-specific compartments, no tissue-specific concentration data were available for model
7 development, raising uncertainty as the model's ability to adequately predict exposure to these
8 tissues. The human inhalation exposure of 50 ppm for 6 hours ([Young, et al., 1977](#)) was
9 reported to be in the linear range for metabolism; thus, uncertainty exists in the ability of the
10 allometrically-scaled value for the human metabolic V_{\max} to accurately describe 1,4-dioxane
11 metabolism from exposures resulting in metabolic saturation. Nevertheless, these values resulted
12 in the model producing good fits to the data. For rats, the values for V_{\max} had to be adjusted
13 upwards by a factor of 1.8 to reasonably simulate exposures greater than 300 mg/kg. The model
14 authors attributed this to metabolic enzyme induction by high doses of 1,4-dioxane.

3.5.2.2. *Reitz et al.*

15 Reitz et al. ([1990](#)) developed a model for 1,4-dioxane and HEAA in the mouse, rat, and
16 human. This model, also based on the styrene model of Ramsey and Andersen ([1984](#)), included
17 a central blood compartment and compartments for liver, fat, and rapidly and slowly perfused
18 tissues. Tissue volumes and blood flow rates were defined as percentages of total BW and
19 cardiac output, respectively. Physiological parameter values were similar to those used by
20 Andersen et al. ([1987](#)), except that flow rates for cardiac output and alveolar ventilation were
21 doubled in order to produce a better fit of the model to human blood level data ([Young, et al.,](#)
22 [1977](#)). Portals of entry included i.v. injection into the venous blood, inhalation, oral bolus
23 dosing, and oral dosing via drinking water. Oral absorption of 1,4-dioxane was simulated, in all
24 three species, as a first-order transfer to liver (halftime approximately 8 minutes).

25 Alveolar blood levels of 1,4-dioxane were assumed to be in equilibrium with alveolar air
26 at a ratio equal to the experimentally measured blood:air partition coefficient. Transfers of
27 1,4-dioxane between blood and tissues were assumed to be blood flow-limited and to achieve
28 rapid equilibrium between blood and tissue, governed by tissue:blood equilibrium partition
29 coefficients. These coefficients were derived by dividing experimentally measured ([Leung &](#)
30 [Paustenbach, 1990](#)) in vitro blood:air and tissue:air partition coefficients for blood, liver, fat.
31 Blood:air partition coefficients were measured for both humans and rats. The mouse blood:air
32 partition coefficient was different from rat or human values; the source of the partition
33 coefficient for blood in mice was not reported. Rat tissue:air partition coefficients were used as
34 surrogate values for humans. Rat tissue partition coefficient values were the same values as used
35 in the Leung and Paustenbach ([1990](#)) model (with the exception of slowly perfused tissues) and
36 were used in the models for all three species. The liver value was used for the rapidly perfused

1 tissues, as well as slowly perfused tissues. Although slowly perfused tissue:air partition
2 coefficients for rats were measured, the authors suggested that 1,4-dioxane in the muscle and air
3 may not have reached equilibrium in the highly gelatinous tissue homogenate ([Reitz, et al.,
4 1990](#)). Substitution of the liver value provided much closer agreement to the plasma data than
5 when the muscle value was used. Further, doubling of the measured human blood:air partition
6 coefficient improved the fit of the model to the human blood level data compared to the fit
7 resulting from the measured value ([Reitz, et al., 1990](#)). The Reitz et al. ([1990](#)) model simulated
8 three routes of 1,4-dioxane elimination: pulmonary exhalation, hepatic metabolism to HEAA,
9 and urinary excretion of HEAA. The elimination of HEAA was modeled as a first-order transfer
10 of 1,4-dioxane metabolite to urine.

11 Values for the metabolic rate constants, V_{\max} and K_m , were optimized to achieve
12 agreement with various observations. Reitz et al. ([1990](#)) optimized values for human V_{\max} and
13 K_m against the experimental human 1,4-dioxane inhalation data ([Young, et al., 1977](#)). As noted
14 previously, because the human exposures were below the level needed to exhibit nonlinear
15 kinetics, uncertainty exists in the ability of the optimized value of V_{\max} to simulate human
16 1,4-dioxane metabolism above the concentration that would result in saturation of metabolism.
17 Rat metabolic rate constants were obtained by optimization to simulated data from a two
18 compartment empirical pharmacokinetic model, which was fitted to i.v. exposure data ([Young, et
19 al., 1978a; Young, et al., 1978b](#)). As with the Leung and

20 The Leung and Paustenbach model ([1990](#)) and the Reitz et al. ([1990](#)) model included
21 compartments for the liver and fat, although no tissue-specific concentration data were available
22 to validate dosimetry for these organs. The derivations of human and rat HEAA elimination rate
23 constants were not reported. Since no pharmacokinetics data for 1,4-dioxane in mice were
24 available, mouse metabolic rate constants were allometrically scaled from rat and human values.

3.5.2.3. *Fisher et al.*

25 A PBPK model was developed by Fisher et al. ([1997](#)) to simulate a variety of volatile
26 organic compounds (VOCs, including 1,4-dioxane) in lactating humans. This model was similar
27 in structure to those of Leung and Paustenbach ([1990](#)) and Reitz et al. ([1990](#)) with the addition of
28 elimination of 1,4-dioxane to breast milk. Experimental measurements were made for blood:air
29 and milk:air partition coefficients. Other partition coefficient values were taken from Reitz et al.
30 ([1990](#)). The model was not optimized, nor was performance tested against experimental
31 exposure data. Thus, the ability of the model to simulate 1,4-dioxane exposure data is unknown.

3.5.2.4. *Sweeney et al.*

32 The Sweeney et al. (2008) model consisted of fat, liver, slowly perfused, and other well
33 perfused tissue compartments. Lung and stomach compartments were used to describe the route
34 of exposure, and an overall volume of distribution compartment was used for calculation of
35 urinary excretion levels of 1,4-dioxane and HEAA. Blood, saline, and tissue to air partition

1 coefficient values for 1,4-dioxane were experimentally determined for rats and mice. Average
2 values of the rat and mouse partition coefficients were used for humans. Metabolic constants
3 (V_{max}C and K_m) for the rat were derived by optimization of data from an i.v. exposure of 1,000
4 mg/kg (Young, et al., 1978a) for inducible metabolism. For uninduced V_{max}C estimation, data
5 generated by i.v. exposures to 3, 10, 30, and 100 mg/kg were used (Young, et al., 1978a).
6 Sweeney et al. (2008) determined best fit values for V_{max}C by fitting to blood data in the Young
7 et al. (1978a). The best fit V_{max}C values were 7.5, 10.8, and 12.7 mg/hr·kg^{0.75} for i.v. doses of 3
8 to 100, 300, and 1,000 mg/kg, suggesting a gradual dose dependent increase in metabolic rate
9 over i.v. doses ranging from 3 to 1,000 mg/kg. Although the Sweeney et al. (2008) model
10 utilized two values for V_{max}C (induced and uninduced), the PBPK model does not include a
11 dose-dependent function description of the change of V_{max} for i.v. doses between metabolic
12 induced and uninduced exposures. Mouse V_{max}C and absorption constants were derived by
13 optimizing fits to the blood 1,4-dioxane concentrations in mice administered nominal doses of
14 200 and 2,000 mg/kg 1,4-dioxane via gavage in a water vehicle (Young, et al., 1978a). The in
15 vitro V_{max} values for rats and mice were scaled to estimate in vivo rates. The scaled and
16 optimized rat V_{max}C values were very similar. The discrepancy between the scaled and
17 optimized mouse values was larger, which was attributed to possible induction in mice at the
18 lowest dose tested (200 mg/kg). The ratio of optimized/scaled values for the rat was used to
19 adjust the scaled human V_{max}C and K_m values to projected in vivo values.

20 The Sweeney et al. (2008) model outputs were compared, by visual inspection, with data
21 not used in fitting model parameters. The model predictions gave adequate match to the 1,4-
22 dioxane exhalation data in rats after a 1,000 mg/kg i.v. dose. 1,4-Dioxane exhalation was
23 overpredicted by a factor of about 3 after a 10 mg/kg i.v. dose. Similarly, the simulations of
24 exhaled 1,4-dioxane after oral dosing were adequate at 1,000 mg/kg and 100 mg/kg (within
25 50%), but poor at 10 mg/kg (model over predicted by a factor of 5). The fit of the model to the
26 human data (Young, et al., 1977) was problematic. Using physiological parameters of Brown et
27 al. (1997) and measured partitioning parameters (Leung & Paustenbach, 1990; Sweeney, et al.,
28 2008) with no metabolism, measured blood 1,4-dioxane concentrations reported by Young et al.
29 (1977) could not be achieved unless the estimated exposure concentration was increased by 2-
30 fold. As expected, inclusion of any metabolism resulted in a decrease in predicted blood
31 concentrations. If estimated metabolism rates were used with the reported exposure
32 concentration, urinary metabolite excretion was also underpredicted (Sweeney, et al., 2008).

3.5.3. Implementation of Published PBPK Models for 1,4-Dioxane

33 As previously described, several pharmacokinetic models have been developed to predict
34 the absorption, distribution, metabolism, and elimination of 1,4-dioxane in rats and humans.
35 Single compartment, empirical models for rats (Young, et al., 1978a; Young, et al., 1978b) and

1 humans ([Young, et al., 1977](#)) were developed to predict blood levels of 1,4-dioxane and urine
2 levels of the primary metabolite, HEAA. PBPK models that describe the kinetics of 1,4-dioxane
3 using biologically realistic flow rates, tissue volumes, enzyme affinities, metabolic processes,
4 and elimination behaviors were also developed ([Fisher, et al., 1997](#); [Leung & Paustenbach, 1990](#);
5 [Reitz, et al., 1990](#); [Sweeney, et al., 2008](#)).

6 In developing updated toxicity values for 1,4-dioxane the available PBPK models were
7 evaluated for their ability to predict observations made in experimental studies of rat and human
8 exposures to 1,4-dioxane (Appendix B). The Reitz et al. ([1990](#)) and Leung and Paustenbach
9 ([1990](#)) PBPK models were both developed from a PBPK model of styrene ([Ramsey & Andersen,
10 1984](#)), with the exception of minor differences in the use of partition coefficients and biological
11 parameters. The model code for Leung and Paustenbach ([1990](#)) was unavailable in contrast to
12 Reitz et al. ([1990](#)). The model of Reitz et al. ([1990](#)) was identified for further consideration to
13 assist in the derivation of toxicity values, and the Sweeney et al. ([2008](#)) PBPK model was also
14 evaluated.

15 The biological plausibility of parameter values in the Reitz et al. ([1990](#)) human model
16 were examined. The model published by Reitz et al. ([1990](#)) was able to predict the only
17 available human inhalation data (50 ppm 1,4-dioxane for 6 hours; Young et al., ([1977](#))) by
18 increasing (i.e., approximately doubling) the parameter values for human alveolar ventilation (30
19 L/hour/kg^{0.74}), cardiac output (30 L/hour/kg^{0.74}), and the blood:air partition coefficient (3,650)
20 above the measured values of 13 L/minute/kg^{0.74} ([Brown, et al., 1997](#)), 14 L/hour/kg^{0.74} ([Brown,
21 et al., 1997](#)), and 1,825 ([Leung & Paustenbach, 1990](#)), respectively. Furthermore, Reitz et al.
22 ([1990](#)) replaced the measured value for the slowly perfused tissue:air partition coefficient (i.e.,
23 muscle—value not reported in manuscript) with the measured liver value (1,557) to improve the
24 fit. Analysis of the Young et al. ([1977](#)) human data suggested that the apparent volume of
25 distribution (V_d) for 1,4-dioxane was approximately 10-fold higher in rats than humans,
26 presumably due to species differences in tissue partitioning or other process not represented in
27 the model. Based upon these observations, several model parameters (e.g.,
28 metabolism/elimination parameters) were re-calibrated using biologically plausible values for
29 flow rates and tissue:air partition coefficients.

30 Appendix B describes all activities that were conducted in the evaluation of the empirical
31 models and the re-calibration and evaluation of the Reitz et al. ([1990](#)) PBPK model to determine
32 the adequacy and preference for the potential use of the models.

33 The evaluation consisted of implementation of the Young et al. ([1978a](#); [1978b](#); [1977](#))
34 empirical rat and human models using the acsIXtreme simulation software, re-calibration of the
35 Reitz et al. ([1990](#)) human PBPK model, and evaluation of the model parameters published by
36 Sweeney et al. ([2008](#)). Using the model descriptions and equations given in Young et al. ([1978a](#);
37 [1978b](#); [1977](#)), model code was developed for the empirical models and executed, simulating the

1 reported experimental conditions. The model output was then compared with the model output
2 reported in Young et al. ([1978a](#); [1978b](#); [1977](#)).

3 The PBPK model of Reitz et al. ([1990](#)) was re-calibrated using measured values for
4 cardiac and alveolar flow rates and tissue:air partition coefficients. The predictions of blood and
5 urine levels of 1,4-dioxane and HEAA, respectively, from the re-calibrated model were
6 compared with the empirical model predictions of the same dosimeters to determine whether the
7 re-calibrated PBPK model could perform similarly to the empirical model. As part of the PBPK
8 model evaluation, EPA performed a sensitivity analysis to identify the model parameters having
9 the greatest influence on the primary dosimeter of interest, the blood level of 1,4-dioxane.

10 Variability data for the experimental measurements of the tissue:air partition coefficients were
11 incorporated to determine a range of model outputs bounded by biologically plausible values for
12 these parameters. Model parameters from Sweeney et al. ([2008](#)) were also tested to evaluate the
13 ability of the PBPK model to predict human data following exposure to 1,4-dioxane.

14 The rat and human empirical models of Young et al. ([1978a](#); [1978b](#); [1977](#)) were
15 successfully implemented in acslXtreme and perform identically to the models reported in the
16 published papers (Figures B-3 through B-7), with the exception of the lower predicted HEAA
17 concentrations and early appearance of the peak HEAA levels in rat urine. The early appearance
18 of peak HEAA levels cannot presently be explained, but may result from manipulations of k_{me} or
19 other parameters by Young et al. ([1978a](#); [1978b](#)) that were not reported. The lower predictions
20 of HEAA levels are likely due to reliance on a standard urine volume production rate in the
21 absence of measured (but unreported) urine volumes. While the human urinary HEAA
22 predictions were lower than observations, this is due to parameter fitting of Young et al. ([1977](#)).
23 No model output was published in Young et al. ([1977](#)) for comparison. The empirical models
24 were modified to allow for user-defined inhalation exposure levels. However, no modifications
25 were made to model oral exposures as adequate data to parameterize such modifications do not
26 exist for rats or humans. Further evaluations of the Young et al. (1977) modified model were
27 conducted against data from the Kasai et al. (2008) subchronic inhalation study. The results of
28 this evaluation are shown in Appendix B (Figure B-8). It shows that the Young et al. (1977)
29 inhalation empirical model failed to provide an adequate simulation of the 13 week inhalation
30 exposure blood data of Kasai et al. (2008). Since the Young et al. (1977) model consistently
31 overpredicted the Kasai et al. (2008) data, the lack of model fit is most likely due to the lack of
32 inclusion of other metabolic processes or parameters.

33 Several procedures were applied to the Reitz et al. ([1990](#)) human PBPK model to
34 determine if an adequate fit of the model to the empirical model output or experimental
35 observations could be attained using biologically plausible values for the model parameters. The
36 re-calibrated model predictions for blood 1,4-dioxane levels do not come within 10-fold of the
37 experimental values using measured tissue:air partition coefficients from Leung and Paustenbach

1 (1990) or Sweeney et al. (2008) (Figures B-9 and B-10). The utilization of a slowly perfused
2 tissue:air partition coefficient 10-fold lower than measured values produces exposure-phase
3 predictions that are much closer to observations, but does not replicate the elimination kinetics
4 (Figure B-11). Recalibration of the model with upper bounds on the tissue:air partition
5 coefficients results in predictions that are still six- to sevenfold lower than empirical model
6 prediction or observations (Figures B-13 and B-14). Exploration of the model space using an
7 assumption of zero-order metabolism (valid for the 50 ppm inhalation exposure) showed that an
8 adequate fit to the exposure and elimination data can be achieved only when unrealistically low
9 values are assumed for the slowly perfused tissue:air partition coefficient (Figure B-17).
10 Artificially low values for the other tissue:air partition coefficients are not expected to improve
11 the model fit, as these parameters are shown in the sensitivity analysis to exert less influence on
12 blood 1,4-dioxane than V_{maxC} and K_m . In the absence of actual measurements for the human
13 slowly perfused tissue:air partition coefficient, high uncertainty exists for this model parameter
14 value. Differences in the ability of rat and human blood to bind 1,4-dioxane may contribute to
15 the difference in V_d . However, this is expected to be evident in very different values for rat and
16 human blood:air partition coefficients, which is not the case (Table B-1). Therefore, some other,
17 as yet unknown, modification to model structure may be necessary.

18 Similarly, Sweeney et al. (2008) also evaluated the available PBPK models (Leung &
19 Paustenbach, 1990; Reitz, et al., 1990) for 1,4-dioxane. To address uncertainties and
20 deficiencies in these models, the investigators conducted studies to fill data gaps and reduce
21 uncertainties pertaining to the pharmacokinetics of 1,4-dioxane and HEAA in rats, mice, and
22 humans. The following studies were performed:

- Partition coefficients, including measurements for mouse blood and tissues (liver, kidney, fat, and muscle) and confirmatory measurements for human blood and rat blood and muscle.
- Blood time course measurements in mice conducted for gavage administration of nominal single doses (20, 200, or 2,000 mg/kg) of 1,4-dioxane administered in water.
- Metabolic rate constants for rat, mouse, and human liver based on incubations of 1,4-dioxane with rat, mouse, and human hepatocytes and measurement of HEAA.

23 The studies conducted by Sweeney et al. (2008) resulted in partition coefficients that
24 were consistent with previously measured values and those used in the Leung and Paustenbach
25 (1990) model. Of noteworthy significance, the laboratory results of Sweeney et al. (2008) did
26 not confirm the human blood:air partition coefficient Reitz et al. (1990) reported. Furthermore,
27 Sweeney et al. (2008) estimated metabolic rate constants (V_{maxC} and K_m) within the range used in
28 the previous models (Leung & Paustenbach, 1990; Reitz, et al., 1990). Overall, the Sweeney et
29 al. (2008) model utilized more rodent in vivo and in vitro data in model parameterization and
30 refinement; however, the model was still unable to adequately predict the human blood data from
31 Young et al. (1977).

1 Updated PBPK models were developed based on these new data and data from previous
2 kinetic studies in rats, workers, and human volunteers reported by Young et al. ([1978a](#); [1978b](#);
3 [1976](#); [1977](#)). The optimized rate of metabolism for the mouse was significantly higher than the
4 value previously estimated. The optimized rat kinetic parameters were similar to those in the
5 1990 models. Of the two available human studies ([Young, et al., 1976](#); [1977](#)), model predictions
6 were consistent with one study, but did not fit the second as well.

3.6. RAT NASAL EXPOSURE VIA DRINKING WATER

7 Sweeney et al. ([2008](#)) conducted a rat nasal exposure study to explore the potential for
8 direct contact of nasal tissues with 1,4-dioxane-containing drinking water under bioassay
9 conditions. Two groups of male Sprague Dawley rats (5/group) received drinking water in
10 45-mL drinking water bottles containing a fluorescent dye mixture (Cell Tracker
11 Red/FluoSpheres). The drinking water for one of these two groups also contained 0.5%
12 1,4-dioxane, a concentration within the range used in chronic toxicity studies. A third group of
13 five rats received tap water alone (controls). Water was provided to the rats overnight. The next
14 morning, the water bottles were weighed to estimate the amounts of water consumed. Rats were
15 sacrificed and heads were split along the midline for evaluation by fluorescence microscopy.
16 One additional rat was dosed twice by gavage with 2 mL of drinking water containing
17 fluorescent dye (the second dose was 30 minutes after the first dose; total of 4 mL administered)
18 and sacrificed 5 hours later to evaluate the potential for systemic delivery of fluorescent dye to
19 the nasal tissues.

20 The presence of the fluorescent dye mixture had no measurable impact on water
21 consumption; however, 0.5% 1,4-dioxane reduced water consumption by an average of 62% of
22 controls following a single, overnight exposure. Fluorescent dye was detected in the oral cavity
23 and nasal airways of each animal exposed to the Cell Tracker Red/FluoSpheres mixture in their
24 drinking water, including numerous areas of the anterior third of the nose along the nasal
25 vestibule, maxillary turbinates, and dorsal nasoturbinates. Fluorescent dye was occasionally
26 detected in the ethmoid turbinate region and nasopharynx. 1,4-Dioxane had no effect on the
27 detection of the dye. Little or no fluorescence at the wavelength associated with the dye mixture
28 was detected in control animals or in the single animal that received the dye mixture by oral
29 gavage. The investigators concluded that the findings indicate rat nasal tissues are exposed by
30 direct contact with drinking water under bioassay conditions.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS – EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

1 Case reports of acute occupational poisoning with 1,4-dioxane indicated that exposure to
2 high concentrations resulted in liver, kidney, and central nervous system (CNS) toxicity ([Barber,](#)
3 [1934](#); [Johnstone, 1959](#)). Barber ([1934](#)) described four fatal cases of hemorrhagic nephritis and
4 centrilobular necrosis of the liver attributed to acute inhalation exposure to high (unspecified)
5 concentrations of 1,4-dioxane. Death occurred within 5–8 days of the onset of illness. Autopsy
6 findings suggested that the kidney toxicity may have been responsible for lethality, while the
7 liver effects may have been compatible with recovery. Jaundice was not observed in subjects
8 and fatty change was not apparent in the liver. Johnstone ([1959](#)) presented the fatal case of one
9 worker exposed to high concentrations of 1,4-dioxane through both inhalation and dermal
10 exposure for a 1 week exposure duration. Measured air concentrations in the work environment
11 of this subject were 208–650 ppm, with a mean value of 470 ppm. Clinical signs that were
12 observed following hospital admission included severe epigastric pain, renal failure, headache,
13 elevation in blood pressure, agitation and restlessness, and coma. Autopsy findings revealed
14 significant changes in the liver, kidney, and brain. These included centrilobular necrosis of the
15 liver and hemorrhagic necrosis of the kidney cortex. Perivascular widening was observed in the
16 brain with small foci of demyelination in several regions (e.g., cortex, basal nuclei). It was
17 suggested that these neurological changes may have been secondary to anoxia and cerebral
18 edema.

19 Several studies examined the effects of acute inhalation exposure in volunteers. In a
20 study performed at the Pittsburgh Experimental Station of the U.S. Bureau of Mines, eye
21 irritation and a burning sensation in the nose and throat were reported in five men exposed to
22 5,500 ppm of 1,4-dioxane vapor for 1 minute ([Yant, Schrenk, Waite, & Patty, 1930](#)). Slight
23 vertigo was also reported by three of these men. Exposure to 1,600 ppm of 1,4-dioxane vapor
24 for 10 minutes resulted in similar symptoms with a reduced intensity of effect. In a study
25 conducted by the Government Experimental Establishment at Proton, England ([Fairley, Linton,](#)
26 [& Ford-Moore, 1934](#)), four men were exposed to 1,000 ppm of 1,4-dioxane for 5 minutes. Odor
27 was detected immediately and one volunteer noted a constriction in the throat. Exposure of six
28 volunteers to 2,000 ppm for 3 minutes resulted in no symptoms of discomfort. Wirth and
29 Klimmer ([1936](#)), of the Institute of Pharmacology, University of Wurzburg, reported slight
30 mucous membrane irritation in the nose and throat of several human subjects exposed to

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#).

1 concentrations greater than 280 ppm for several minutes. Exposure to approximately 1,400 ppm
2 for several minutes caused a prickling sensation in the nose and a dry and scratchy throat.
3 Silverman et al. ([1946](#)) exposed 12 male and 12 female subjects to varying air concentrations of
4 1,4-dioxane for 15 minutes. A 200 ppm concentration was reported to be tolerable, while a
5 concentration of 300 ppm caused irritation to the eyes, nose, and throat. The study conducted by
6 Silverman et al. ([1946](#)) was conducted by the Department of Industrial Hygiene, Harvard School
7 of Public Health, and was sponsored and supported by a grant from the Shell Development
8 Company. These volunteer studies published in the 1930s and 1940s ([Fairley, et al., 1934](#);
9 [Silverman, et al., 1946](#); [Wirth & Klimmer, 1936](#); [Yant, et al., 1930](#)) did not provide information
10 on the human subjects research ethics procedures undertaken in these studies; however, there is
11 no evidence that the conduct of the research was fundamentally unethical or significantly
12 deficient relative to the ethical standards prevailing at the time the research was conducted.

13 Young et al. ([1977](#)) exposed four healthy adult male volunteers to a 50-ppm
14 concentration of 1,4-dioxane for 6 hours. The investigators reported that the protocol of this
15 study was approved by a seven-member Human Research Review Committee of the Dow
16 Chemical Company and was followed rigorously. Perception of the odor of 1,4-dioxane
17 appeared to diminish over time, with two of the four subjects reporting inability to detect the
18 odor at the end of the exposure period. Eye irritation was the only clinical sign reported in this
19 study. The pharmacokinetics and metabolism of 1,4-dioxane in humans were also evaluated in
20 this study (see Section 3.3). Clinical findings were not reported in four workers exposed in the
21 workplace to a TWA concentration of 1.6 ppm for 7.5 hours ([Young, et al., 1976](#)).

22 Ernstgård et al. ([2006](#)) examined the acute effects of 1,4-dioxane vapor in male and
23 female volunteers. The study protocol was approved by the Regional Ethics Review Board in
24 Stockholm, and performed following informed consent and according to the Helsinki
25 declaration. In a screening study by these investigators, no self-reported symptoms (based on a
26 visual analogue scale (VAS) that included ratings for discomfort in eyes, nose, and throat,
27 breathing difficulty, headache, fatigue, nausea, dizziness, or feeling of intoxication) were
28 observed at concentrations up to 20 ppm; this concentration was selected as a tentative no-
29 observed-adverse-effect-level (NOAEL) in the main study. In the main study, six male and six
30 female healthy volunteers were exposed to 0 or 20 ppm 1,4-dioxane, at rest, for 2 hours. This
31 exposure did not significantly affect symptom VAS ratings, blink frequency, pulmonary function
32 or nasal swelling (measured before and at 0 and 3 hours after exposure), or inflammatory
33 markers in the plasma (C-reactive protein and interleukin-6) of the volunteers. Only ratings for
34 “solvent smell” were significantly increased during exposure.

35 Only two well documented epidemiology studies were available for occupational workers
36 exposed to 1,4-dioxane ([Buffler, Wood, Suarez, & Kilian, 1978](#); [Thiess, Tress, & Fleig, 1976](#)).

1 These studies did not provide evidence of effects in humans; however, the cohort size and
2 number of reported cases were small.

4.1.1. Thiess et al.

3 A cross-sectional survey was conducted by Thiess et al. (1976) in German workers
4 exposed to 1,4-dioxane. The study evaluated health effects in 74 workers, including 24 who
5 were still actively employed in 1,4-dioxane production at the time of the investigation,
6 23 previously exposed workers who were still employed by the manufacturer, and 27 retired or
7 deceased workers. The actively employed workers were between 32 and 62 years of age and had
8 been employed in 1,4-dioxane production for 5–41 years. Former workers (age range not given)
9 had been exposed to 1,4-dioxane for 3–38 years and retirees (age range not given) had been
10 exposed for 12–41 years. Air concentrations in the plant at the time of the study were
11 0.06–0.69 ppm. A simulation of previous exposure conditions (prior to 1969) resulted in air
12 measurements between 0.06 and 7.2 ppm.

13 Active and previously employed workers underwent a thorough clinical examination and
14 X-ray, and hematological and serum biochemistry parameters were evaluated. The examination
15 did not indicate pathological findings for any of the workers and no indication of malignant
16 disease was noted. Hematology results were generally normal. Serum transaminase levels were
17 elevated in 16 of the 47 workers studied; however, this finding was consistent with chronic
18 consumption of more than 80 g of alcohol per day, as reported for these workers. No liver
19 enlargement or jaundice was found. Renal function tests and urinalysis were normal in exposed
20 workers. Medical records of the 27 retired workers (15 living at the time of the study) were
21 reviewed. No symptoms of liver or kidney disease were reported and no cancer was detected.
22 Medical reasons for retirement did not appear related to 1,4-dioxane exposure (e.g., emphysema,
23 arthritis).

24 Chromosome analysis was performed on six actively employed workers and six control
25 persons (not characterized). Lymphocyte cultures were prepared and chromosomal aberrations
26 were evaluated. No differences were noted in the percent of cells with gaps or other
27 chromosome aberrations. Mortality statistics were calculated for 74 workers of different ages
28 and varying exposure periods. The proportional contribution of each of the exposed workers to
29 the total time of observation was calculated as the sum of man-years per 10-year age group.
30 Each person contributed one man-year per calendar year to the specific age group in which he
31 was included at the time. The expected number of deaths for this population was calculated from
32 the age-specific mortality statistics for the German Federal Republic for the years 1970–1973.
33 From the total of 1,840.5 person-years, 14.5 deaths were expected; however, only 12 deaths were
34 observed in exposed workers between 1964 and 1974. Two cases of cancer were reported,
35 including one case of lamellar epithelial carcinoma and one case of myelofibrosis leukemia.

1 These cancers were not considered to be the cause of death in these cases and other severe
2 illnesses were present. Standardized mortality ratios (SMRs) for cancer did not significantly
3 differ from the control population (SMR for overall population = 0.83; SMR for 65–75-year-old
4 men = 1.61; confidence intervals (CIs) were not provided).

4.1.2. Buffler et al.

5 Buffler et al. (1978) conducted a mortality study on workers exposed to 1,4-dioxane at a
6 chemical manufacturing facility in Texas. 1,4-Dioxane exposure was known to occur in a
7 manufacturing area and in a processing unit located 5 miles from the manufacturing plant.
8 Employees who worked between April 1, 1954, and June 30, 1975, were separated into two
9 cohorts based on at least 1 month of exposure in either the manufacturing plant (100 workers) or
10 the processing area (65 workers). Company records and follow-up techniques were used to
11 compile information on name, date of birth, gender, ethnicity, job assignment and duration, and
12 employment status at the time of the study. Date and cause of death were obtained from copies
13 of death certificates and autopsy reports (if available). Exposure levels for each job category
14 were estimated using the 1974 Threshold Limit Value for 1,4-dioxane (i.e., 50 ppm) and
15 information from area and personal monitoring. Exposure levels were classified as low
16 (<25 ppm), intermediate (50–75 ppm), and high (>75 ppm). Monitoring was not conducted prior
17 to 1968 in the manufacturing areas or prior to 1974 in the processing area; however, the study
18 authors assumed that exposures would be comparable, considering that little change had been
19 made to the physical plant or the manufacturing process during that time. Exposure to
20 1,4-dioxane was estimated to be below 25 ppm for all individuals in both cohorts.
21 Manufacturing area workers were exposed to several other additional chemicals and processing
22 area workers were exposed to vinyl chloride.

23 Seven deaths were identified in the manufacturing cohort and five deaths were noted for
24 the processing cohort. The average exposure duration was not greater for those workers who
25 died, as compared to those still living at the time of the study. Cancer was the underlying cause
26 of death for two cases from the manufacturing area (carcinoma of the stomach, alveolar cell
27 carcinoma) and one case from the processing area (malignant mediastinal tumor). The workers
28 from the manufacturing area were exposed for 28 or 38 months and both had a positive smoking
29 history (>1 pack/day). Smoking history was not available for processing area workers. The
30 single case of cancer in this area occurred in a 21-year-old worker exposed to 1,4-dioxane for
31 1 year. The mortality data for both industrial cohorts were compared to age-race-sex specific
32 death rates for Texas (1960–1969). Person-years of observation contributed by workers were
33 determined over five age ranges with each worker contributing one person-year for each year of
34 observation in a specific age group. The expected number of deaths was determined by applying
35 the Texas 1960–1969 death rate statistics to the number of person years calculated for each

1 cohort. The observed and expected number of deaths for overall mortality (i.e., all causes) was
2 comparable for both the manufacturing area (7 observed versus 4.9 expected) and the processing
3 area (5 observed versus 4.9 expected). No significant excess in cancer-related deaths was
4 identified for both areas of the facility combined (3 observed versus 1.7 expected). A separate
5 analysis was performed to evaluate mortality in manufacturing area workers exposed to
6 1,4-dioxane for more than 2 years. Six deaths occurred in this group as compared to
7 4.1 expected deaths. The use of a conditional Poisson distribution indicated no apparent excess
8 in mortality or death due to malignant neoplasms in this study. It is important to note that the
9 cohorts evaluated were limited in size. In addition, the mean exposure duration was less than
10 5 years (<2 years for 43% of workers) and the latency period for evaluation was less than
11 10 years for 59% of workers. The study authors recommended a follow-up investigation to
12 allow for a longer latency period; however, no follow-up study of these workers has been
13 published.

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS - ORAL AND INHALATION

14 The majority of the subchronic and chronic studies conducted for 1,4-dioxane were
15 drinking water studies. To date, there are only two subchronic inhalation studies (Fairley, et al.,
16 1934; Kasai, et al., 2008) and two chronic inhalation studies (Kasai, et al., 2009; Torkelson,
17 Leong, Kociba, Richter, & Gehring, 1974). The effects following oral and inhalation exposures
18 are described in detail below.

4.2.1. Oral Toxicity

4.2.1.1. Subchronic Oral Toxicity

19 Six rats and six mice (unspecified strains) were given drinking water containing 1.25%
20 1,4-dioxane for up to 67 days (Fairley, et al., 1934). Using reference BWs and drinking water
21 ingestion rates for rats and mice (U.S. EPA, 1988), it can be estimated that these rats and mice
22 received doses of approximately 1,900 and 3,300 mg/kg-day, respectively. Gross pathology and
23 histopathology were evaluated in all animals. Five of the six rats in the study died or were
24 sacrificed in extremis prior to day 34 of the study. Mortality was lower in mice, with five of six
25 mice surviving up to 60 days. Kidney enlargement was noted in 5/6 rats and 2/5 mice. Renal
26 cortical degeneration was observed in all rats and 3/6 mice. Large areas of necrosis were
27 observed in the cortex, while cell degeneration in the medulla was slight or absent. Tubular casts
28 were observed and vascular congestion and hemorrhage were present throughout the kidney.
29 Hepatocellular degeneration with vascular congestion was also noted in five rats and three mice.
30 For this assessment, EPA identified the tested doses of 1,900 mg/kg-day in rats and 3,300 mg/kg-

1 day in mice as the lowest-observed-adverse-effect-levels (LOAELs) for liver and kidney
2 degeneration in this study.

3 **4.2.1.1.1. Stoner et al.** 1,4-Dioxane was evaluated by Stoner et al. ([1986](#)) for its ability to induce
4 lung adenoma formation in A/J mice. Six- to 8-week-old male and female A/J mice
5 (16/sex/group) were given 1,4-dioxane by gavage or i.p. injection, 3 times/week for 8 weeks.
6 Total cumulative dose levels were given as 24,000 mg/kg (oral), and 4,800, 12,000, or
7 24,000 mg/kg (i.p.). Average daily dose estimates were calculated to be 430 mg/kg-day (oral),
8 and 86, 210, or 430 mg/kg-day (i.p.) by assuming an exposure duration of 56 days. The authors
9 indicated that i.p. doses represent the maximum tolerated dose (MTD), 0.5 times the MTD, and
10 0.2 times the MTD. Mice were killed 24 weeks after initiation of the bioassay, and lungs, liver,
11 kidney, spleen, intestines, stomach, thymus, salivary, and endocrine glands were examined for
12 gross lesions. Histopathology examination was performed if gross lesions were detected.
13 1,4-Dioxane did not induce lung tumors in male or female A/J mice in this study.

14 **4.2.1.1.2. Stott et al.** In the Stott et al. ([1981](#)) study, male Sprague Dawley rats (4–6/group)
15 were given average doses of 0, 10, or 1,000 mg/kg-day 1,4-dioxane (>99% pure) in their
16 drinking water, 7 days/week for 11 weeks. It should be noted that the methods description in this
17 report stated that the high dose was 100 mg/kg-day, while the abstract, results, and discussion
18 sections indicated that the high dose was 1,000 mg/kg-day. Rats were implanted with a [⁶-
19 ³H]thymidine loaded osmotic pump 7 days prior to sacrifice. Animals were sacrificed by
20 cervical dislocation and livers were removed, weighed, and prepared for histopathology
21 evaluation. [³H]-Thymidine incorporation was measured by liquid scintillation spectroscopy.

22 An increase in the liver to BW ratio was observed in rats from the high dose group
23 (assumed to be 1,000 mg/kg-day). Histopathological alterations, characterized as minimal
24 centrilobular swelling, were also seen in rats from this dose group (incidence values were not
25 reported). Hepatic DNA synthesis, measured by [³H]-thymidine incorporation, was increased
26 1.5-fold in high-dose rats. No changes relative to control were observed for rats exposed to
27 10 mg/kg-day. EPA found a NOAEL value of 10 mg/kg-day and a LOAEL value of
28 1,000 mg/kg-day for this study based on histopathological changes in the liver.

29 Stott et al. ([1981](#)) also performed several acute experiments designed to evaluate
30 potential mechanisms for the carcinogenicity of 1,4-dioxane. These experiments are discussed
31 separately in Section 4.5.2 (Mechanistic Studies).

32 **4.2.1.1.3. Kano et al.** In the Kano et al. ([2008](#)) study, groups of 6-week-old F344/DuCrj rats
33 (10/sex/group) and Crj:BDF1 mice (10/sex/group) were administered 1,4-dioxane (>99% pure)
34 in the drinking water for 13 weeks. The animals were observed daily for clinical signs of
35 toxicity. Food consumption and BWs were measured once per week and water consumption was

1 measured twice weekly. Food and water were available ad libitum. The concentrations of
2 1,4-dioxane in the water for rats and mice were 0, 640, 1,600, 4,000, 10,000, or 25,000 ppm.
3 The investigators used data from water consumption and BW changes to calculate a daily intake
4 of 1,4-dioxane by the male and female animals. Thus, male rats received doses of approximately
5 0, 52, 126, 274, 657, and 1,554 mg 1,4-dioxane/kg-day and female rats received 0, 83, 185, 427,
6 756, and 1,614 mg/kg-day. Male mice received 0, 86, 231, 585, 882, or 1,570 mg/kg-day and
7 female mice received 0, 170, 387, 898, 1,620, or 2,669 mg/kg-day.

8 No information was provided as to when the blood and urine samples were collected.
9 Hematology analysis included red blood cell (RBC) count, hemoglobin, hematocrit, mean
10 corpuscular volume (MCV), platelet count, white blood cell (WBC) count, and differential
11 WBCs. Serum biochemistry included total protein, albumin, bilirubin, glucose, cholesterol,
12 triglyceride (rat only), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate
13 dehydrogenase (LDH), leucine aminopeptidase (LAP), alkaline phosphatase (ALP), creatinine
14 phosphokinase (CPK) (rat only), urea nitrogen, creatinine (rat only), sodium, potassium,
15 chloride, calcium (rat only), and inorganic phosphorous (rat only). Urinalysis parameters were
16 pH, protein, glucose, ketone body, bilirubin (rat only), occult blood, and urobilinogen. Organ
17 weights (brain, lung, liver, spleen, heart, adrenal, testis, ovary, and thymus) were measured, and
18 gross necropsy and histopathologic examination of tissues and organs were performed on all
19 animals (skin, nasal cavity, trachea, lungs, bone marrow, lymph nodes, thymus, spleen, heart,
20 tongue, salivary glands, esophagus, stomach, small and large intestine, liver, pancreas, kidney,
21 urinary bladder, pituitary thyroid adrenal, testes, epididymis, seminal vesicle, prostate, ovary,
22 uterus, vagina, mammary gland, brain, spinal cord, sciatic nerve, eye, Harderian gland, muscle,
23 bone, and parathyroid). Dunnett's test and χ^2 test were used to assess the statistical significance
24 of changes in continuous and discrete variables, respectively.

25 Clinical signs of toxicity in rats were not discussed in the study report. One female rat in
26 the high dose group (1,614 mg/kg-day) group died, but cause and time of death were not
27 specified. Final BWs were reduced at the two highest dose levels in females (12 and 21%) and
28 males (7 and 21%), respectively. Food consumption was reduced 13% in females at
29 1,614 mg/kg-day and 8% in 1,554 mg/kg-day males. A dose-related decrease in water
30 consumption was observed in male rats starting at 52 mg/kg-day (15%) and in females starting at
31 185 mg/kg-day (12%). Increases in RBCs, hemoglobin, hematocrit, and neutrophils, and a
32 decrease in lymphocytes were observed in males at 1554 mg/kg-day. In females, MCV was
33 decreased at doses ≥ 756 mg/kg and platelets were decreased at 1,614 mg/kg-day. With the
34 exception of the 30% increase in neutrophils in high-dose male rats, hematological changes were
35 within 2–15% of control values. Total serum protein and albumin were significantly decreased
36 in males at doses ≥ 274 mg/kg-day and in females at doses ≥ 427 mg/kg-day. Additional
37 changes in high-dose male and female rats included decreases in glucose, total cholesterol,

1 triglycerides, and sodium (and calcium in females), and increases in ALT (males only), AST,
2 ALP, and LAP. Serum biochemistry parameters in treated rats did not differ more than twofold
3 from control values. Urine pH was decreased in males at ≥ 274 mg/kg-day and in females at
4 ≥ 756 mg/kg-day.

5 Kidney weights were increased in females at ≥ 185 mg/kg-day with a maximum increase
6 of 15% and 44% at 1,614 mg/kg-day for absolute and relative kidney weight, respectively. No
7 organ weight changes were noted in male rats. Histopathology findings in rats that were related
8 to exposure included nuclear enlargement of the respiratory epithelium, nuclear enlargement of
9 the olfactory epithelium, nuclear enlargement of the tracheal epithelium, hepatocyte swelling of
10 the centrilobular area of the liver, vacuolar changes in the liver, granular changes in the liver,
11 single cell necrosis in the liver, nuclear enlargement of the proximal tubule of the kidneys,
12 hydropic changes in the proximal tubule of the kidneys, and vacuolar changes in the brain. The
13 incidence data for histopathological lesions in rats are presented in Table 4-1. The effects that
14 occurred at the lowest doses were nuclear enlargement of the respiratory epithelium in the nasal
15 cavity and hepatocyte swelling in the central area of the liver in male rats. Based on these
16 histopathological findings the study authors identified the LOAEL as 126 mg/kg-day and the
17 NOAEL as 52 mg/kg-day.

Table 4-1. Incidence of histopathological lesions in F344/DuCrj rats exposed to 1,4-dioxane in drinking water for 13 weeks

Effect	Male dose (mg/kg-day) ^a					
	0	52	126	274	657	1,554
Nuclear enlargement; nasal respiratory epithelium	0/10	0/10	9/10 ^b	10/10 ^b	9/10 ^b	10/10 ^b
Nuclear enlargement; nasal olfactory epithelium	0/10	0/10	0/10	10/10 ^b	9/10 ^b	10/10 ^b
Nuclear enlargement; tracheal epithelium	0/10	0/10	0/10	10/10 ^b	10/10 ^b	10/10 ^b
Hepatocyte swelling	0/10	0/10	9/10 ^b	10/10 ^b	10/10 ^b	10/10 ^b
Vacuolic change; liver	0/10	0/10	0/10	0/10	10/10 ^b	10/10 ^b
Granular change; liver	0/10	0/10	0/10	5/10 ^c	2/10	10/10 ^b
Single cell necrosis; liver	0/10	0/10	0/10	5/10 ^c	2/10	10/10 ^b
Nuclear enlargement; renal proximal tubule	0/10	0/10	0/10	1/10	5/10 ^c	9/10 ^b
Hydropic change; renal proximal tubule	0/10	0/10	0/10	0/10	0/10	7/10 ^b
Vacuolic change; brain	0/10	0/10	0/10	0/10	0/10	10/10 ^b
	Female dose (mg/kg-day) ^a					
	0	83	185	427	756	1,614
Nuclear enlargement; nasal respiratory epithelium	0/10	0/10	5/10 ^c	10/10 ^b	10/10 ^b	8/9 ^b
Nuclear enlargement; nasal olfactory epithelium	0/10	0/10	0/10	9/10 ^b	10/10 ^b	8/9 ^b
Nuclear enlargement; tracheal epithelium	0/10	0/10	0/10	9/10 ^b	10/10 ^b	9/9 ^b
Hepatocyte swelling	0/10	0/10	0/10	0/10	9/10 ^b	9/9 ^b
Vacuolic change; liver	0/10	0/10	0/10	0/10	0/10	9/9 ^b
Granular change; liver	2/10	0/10	1/10	5/10 ^c	5/10 ^c	8/9 ^b
Single cell necrosis; liver	2/10	0/10	1/10	5/10	5/10	8/9 ^b
Nuclear enlargement; proximal tubule	0/10	0/10	0/10	0/10	8/10 ^b	9/9 ^b
Hydropic change; proximal tubule	0/10	0/10	0/10	0/10	0/10	5/9 ^c
Vacuolic change; brain	0/10	0/10	0/10	0/10	0/10	9/9 ^b

^aData are presented for sacrificed animals.

^b $p \leq 0.01$ by χ^2 test.

^c $p \leq 0.05$.

Source: Kano et al. (2008)

1 Clinical signs of toxicity in mice were not discussed in the study report. One male mouse
2 in the high-dose group (1,570 mg/kg-day) died, but no information was provided regarding cause
3 or time of death. Final BWs were decreased 29% in male mice at 1,570 mg/kg-day, but changed
4 less than 10% relative to controls in the other male dose groups and in female mice. Food
5 consumption was not significantly reduced in any exposure group. Water consumption was
6 reduced 14–18% in male mice exposed to 86, 231, or 585 mg/kg-day. Water consumption was
7 further decreased by 48 and 70% in male mice exposed to 882 and 1,570 mg/kg-day,
8 respectively. Water consumption was also decreased 31 and 57% in female mice treated with
9 1,620 and 2,669 mg/kg-day, respectively. An increase in MCV was observed in the two highest
10 dose groups in both male (882 and 1,570 mg/kg-day) and female mice (1,620 and
11 2,669 mg/kg-day). Increases in RBCs, hemoglobin, and hematocrit were also observed in high

1 dose males (1,570 mg/kg-day). Hematological changes were within 2–15% of control values.
2 Serum biochemistry changes in exposed mice included decreased total protein (at
3 1,570 mg/kg-day in males, \geq 1,620 mg/kg-day in females), decreased glucose (at
4 1,570 mg/kg-day in males, \geq 1,620 mg/kg-day in females), decreased albumin (at
5 1,570 mg/kg-day in males, 2,669 mg/kg-day in females), decreased total cholesterol
6 (\geq 585 mg/kg-day in males, \geq 1,620 mg/kg-day in females), increased serum ALT (at
7 1,570 mg/kg-day in males, \geq 620 mg/kg-day in females), increased AST (at 1,570 mg/kg-day in
8 males, 2,669 mg/kg-day in females), increased ALP (\geq 585 mg/kg-day in males, 2,669 mg/kg-
9 day in females), and increased LDH (in females only at doses \geq 1,620 mg/kg-day). With the
10 exception of a threefold increase in ALT in male and female mice, serum biochemistry
11 parameters in treated rats did not differ more than twofold from control values. Urinary pH was
12 decreased in males at \geq 882 mg/kg-day and in females at \geq 1,620 mg/kg-day.

13 Absolute and relative lung weights were increased in males at 1,570 mg/kg-day and in
14 females at 1,620 and 2,669 mg/kg-day. Absolute kidney weights were also increased in females
15 at 1,620 and 2,669 mg/kg-day and relative kidney weight was elevated at 2,669 mg/kg-day.
16 Histopathology findings in mice that were related to exposure included nuclear enlargement of
17 the respiratory epithelium, nuclear enlargement of the olfactory epithelium, eosinophilic change
18 in the olfactory epithelium, vacuolic change in the olfactory nerve, nuclear enlargement of the
19 tracheal epithelium, accumulation of foamy cells in the lung and bronchi, nuclear enlargement
20 and degeneration of the bronchial epithelium, hepatocyte swelling of the centrilobular area of the
21 liver, and single cell necrosis in the liver. The incidence data for histopathological lesions in
22 mice are presented in Table 4-2. Based on the changes in the bronchial epithelium in female
23 mice, the authors identified the dose level of 387 mg/kg-day as the LOAEL for mice; the
24 NOAEL was 170 mg/kg-day ([Kano, et al., 2008](#)).

Table 4-2. Incidence of histopathological lesions in Crj:BDF1 mice exposed to 1,4-dioxane in drinking water for 13 weeks

Effect	Male dose (mg/kg-day) ^a					
	0	86	231	585	882	1,570
Nuclear enlargement; nasal respiratory epithelium	0/10	0/10	0/10	2/10	5/10 ^b	0/9
Eosinophilic change; nasal respiratory epithelium	0/10	0/10	0/10	0/10	0/10	5/9 ^b
Nuclear enlargement; nasal olfactory epithelium	0/10	0/10	0/10	9/10 ^c	10/10 ^c	9/9 ^c
Eosinophilic change; nasal olfactory epithelium	0/10	0/10	0/10	0/10	0/10	6/9 ^c
Vacuolic change; olfactory nerve	0/10	0/10	0/10	0/10	0/10	9/9 ^c
Nuclear enlargement; tracheal epithelium	0/10	0/10	0/10	7/10 ^c	9/10 ^c	9/9 ^c
Accumulation of foamy cells; lung/bronchi	0/10	0/10	0/10	0/10	0/10	6/9 ^c
Nuclear enlargement; bronchial epithelium	0/10	0/10	0/10	9/10 ^c	9/10 ^c	9/9 ^c
Degeneration; bronchial epithelium	0/10	0/10	0/10	0/10	0/10	8/9 ^c
Hepatocyte swelling	0/10	0/10	0/10	10/10 ^c	10/10 ^c	9/9 ^c
Single cell necrosis; liver	0/10	0/10	0/10	5/10 ^b	10/10 ^c	9/9 ^c
	Female dose (mg/kg-day) ^a					
	0	170	387	898	1,620	2,669
Nuclear enlargement; nasal respiratory epithelium	0/10	0/10	0/10	3/10	3/10	7/10 ^c
Eosinophilic change; nasal respiratory epithelium	0/10	0/10	1/10	1/10	5/10 ^b	9/10 ^c
Nuclear enlargement; nasal olfactory epithelium	0/10	0/10	0/10	6/10 ^b	10/10 ^c	10/10 ^c
Eosinophilic change; nasal olfactory epithelium	0/10	0/10	0/10	1/10 ^c	6/10 ^b	6/10 ^b
Vacuolic change; olfactory nerve	0/10	0/10	0/10	0/10	2/10	8/10 ^c
Nuclear enlargement; tracheal epithelium	0/10	0/10	2/10	9/10 ^c	10/10 ^c	10/10 ^c
Accumulation of foamy cells; lung/bronchi	0/10	0/10	0/10	0/10	10/10 ^c	10/10 ^c
Nuclear enlargement; bronchial epithelium	0/10	0/10	10/10 ^c	10/10 ^c	10/10 ^c	10/10 ^c
Degeneration; bronchial epithelium	0/10	0/10	0/10	0/10	7/10 ^c	10/10 ^c
Hepatocyte swelling	0/10	1/10	1/10	10/10 ^c	10/10 ^c	9/10 ^b
Single cell necrosis; liver	0/10	0/10	0/10	7/10 ^c	10/10 ^c	9/10 ^c

^aData are presented for sacrificed animals.

^b $p \leq 0.01$ by χ^2 test.

^c $p \leq 0.05$.

Source: Kano et al (2008).

1 **4.2.1.1.4. Yamamoto et al.** Studies (Yamamoto et al., 1998; Yamamoto, Urano, & Nomura,
2 1998) in rasH2 transgenic mice carrying the human prototype c-Ha-ras gene have been
3 investigated as a bioassay model for rapid carcinogenicity testing. As part of validation studies
4 of this model, 1,4-dioxane was one of many chemicals that were evaluated. RasH2 transgenic
5 mice were F1 offspring of transgenic male C57BLr6J and normal female BALB/cByJ mice.
6 CB6F₁ mice were used as a nontransgenic control. Seven- to nine-week-old mice (10–15/group)

1 were exposed to 0, 0.5, or 1% 1,4-dioxane in drinking water for 26 weeks. An increase in lung
2 adenomas was observed in treated transgenic mice, as compared to treated nontransgenic mice.
3 The tumor incidence in transgenic animals, however, was not greater than that observed in
4 vehicle-treated transgenic mouse controls. Further study details were not provided.

4.2.1.2. Chronic Oral Toxicity and Carcinogenicity

5 **4.2.1.2.1. Argus et al.** Twenty-six adult male Wistar rats ([Argus, Arcos, & Hoch-Ligeti, 1965](#))
6 weighing between 150 and 200 g were exposed to 1,4-dioxane (purity not reported) in the
7 drinking water at a concentration of 1% for 64.5 weeks. A group of nine untreated rats served as
8 control. Food and water were available ad libitum. The drinking water intake for treated
9 animals was reported to be 30 mL/day, resulting in a dose/rat of 300 mg/day. Using a reference
10 BW of 0.462 kg for chronic exposure to male Wistar rats ([U.S. EPA, 1988](#)), it can be estimated
11 that these rats received daily doses of approximately 640 mg/kg-day. All animals that died or
12 were killed during the study underwent a complete necropsy. A list of specific tissues examined
13 microscopically was not provided; however, it is apparent that the liver, kidneys, lungs,
14 lymphatic tissue, and spleen were examined. No statistical analysis of the results was conducted.

15 Six of the 26 treated rats developed hepatocellular carcinomas, and these rats had been
16 treated for an average of 452 days (range, 448–455 days). No liver tumors were observed in
17 control rats. In two rats that died after 21.5 weeks of treatment, histological changes appeared to
18 involve the entire liver. Groups of cells were found that had enlarged hyperchromic nuclei. Rats
19 that died or were killed at longer intervals showed similar changes, in addition to large cells with
20 reduced cytoplasmic basophilia. Animals killed after 60 weeks of treatment showed small
21 neoplastic nodules or multifocal hepatocellular carcinomas. No cirrhosis was observed in this
22 study. Many rats had extensive changes in the kidneys often resembling glomerulonephritis,
23 however, incidence data was not reported for these findings. This effect progressed from
24 increased cellularity to thickening of the glomerular capsule followed by obliteration of the
25 glomeruli. One treated rat had an early transitional cell carcinoma in the kidney's pelvis; this rat
26 also had a large tumor in the liver. The lungs from many treated and control rats (incidence not
27 reported) showed severe bronchitis with epithelial hyperplasia and marked peribronchial
28 infiltration, as well as multiple abscesses. One rat treated with 1,4-dioxane developed leukemia
29 with infiltration of all organs, particularly the liver and spleen, with large, round, isolated
30 neoplastic cells. In the liver, the distribution of cells in the sinusoids was suggestive of myeloid
31 leukemia. The dose of 640 mg/kg-day tested in this study was a free-standing LOAEL,
32 identified by EPA, for glomerulonephritis in the kidney and histological changes in the liver
33 (hepatocytes with enlarged hyperchromic nuclei, large cells with reduced cytoplasmic
34 basophilia).

1 **4.2.1.2.2. *Argus et al.; Hoch-Ligeti et al.*** Five groups (28-32/dose group) of male
2 Sprague Dawley rats (2-3 months of age) weighing 110–230 g at the beginning of the experiment
3 were administered 1,4-dioxane (purity not reported) in the drinking water for up to 13 months at
4 concentrations of 0, 0.75, 1.0, 1.4, or 1.8% ([Argus, Sohal, Bryant, Hoch-Ligeti, & Arcos, 1973](#);
5 [Hoch-Ligeti, Argus, & Arcos, 1970](#)). The drinking water intake was determined for each group
6 over a 3-day measurement period conducted at the beginning of the study and twice during the
7 study (weeks were not specified). The rats were killed with ether at 16 months or earlier if nasal
8 tumors were clearly observable. Complete autopsies were apparently performed on all animals,
9 but only data from the nasal cavity and liver were presented and discussed. The nasal cavity was
10 studied histologically only from rats in which gross tumors in these locations were present;
11 therefore, early tumors may have been missed and pre-neoplastic changes were not studied. No
12 statistical analysis of the results was conducted. Assuming a BW of 0.523 kg for an adult male
13 Sprague Dawley rat ([U.S. EPA, 1988](#)) and a drinking water intake of 30 mL/day as reported by
14 the study authors, dose estimates were 0, 430, 574, 803, and 1,032 mg/kg-day. The progression
15 of liver tumorigenesis was evaluated by an additional group of 10 male rats administered 1%
16 1,4-dioxane in the drinking water (574 mg/kg-day), 5 of which were sacrificed after 8 months of
17 treatment and 5 were killed after 13 months of treatment. Liver tissue from these rats and control
18 rats was processed for electron microscopy examination.

19 Nasal cavity tumors were observed upon gross examination in six rats (1/30 in the 0.75%
20 group, 1/30 in the 1.0% group, 2/30 in the 1.4% group, and 2/30 in the 1.8% group). Gross
21 observation showed the tumors visible either at the tip of the nose, bulging out of the nasal
22 cavity, or on the back of the nose covered by intact or later ulcerated skin. As the tumors
23 obstructed the nasal passages, the rats had difficulty breathing and lost weight rapidly. No
24 neurological signs or compression of the brain were observed. In all cases, the tumors were
25 squamous cell carcinomas with marked keratinization and formation of keratin pearls. Bony
26 structure was extensively destroyed in some animals with tumors, but there was no invasion into
27 the brain. In addition to the squamous carcinoma, two adenocarcinomatous areas were present.
28 One control rat had a small, firm, well-circumscribed tumor on the back of the nose, which
29 proved to be subcutaneous fibroma. The latency period for tumor onset was 329–487 days.
30 Evaluation of the latent periods and doses received did not suggest an inverse relationship
31 between these two parameters.

32 *Argus et al.* ([1973](#)) studied the progression of liver tumorigenesis by electron microscopy
33 of liver tissues obtained following interim sacrifice at 8 and 13 months of exposure (5 rats/group,
34 574 mg/kg-day). The first change observed in the liver was an increase in the size of the nucleus
35 of the hepatocytes, mostly in the periportal area. Precancerous changes were characterized by
36 disorganization of the rough endoplasmic reticulum, an increase in smooth endoplasmic
37 reticulum, and a decrease in glycogen and increase in lipid droplets in hepatocytes. These

1 changes increased in severity in the hepatocellular carcinomas in rats exposed to 1,4-dioxane for
2 13 months.

3 Three types of liver nodules were observed in exposed rats at 13–16 months. The first
4 consisted of groups of cells with reduced cytoplasmic basophilia and a slightly nodular
5 appearance as viewed by light microscopy. The second type of circumscribed nodule was
6 described consisting of large cells, apparently filled and distended with fat. The third type of
7 nodule was described as finger-like strands, 2–3 cells thick, of smaller hepatocytes with large
8 hyperchromic nuclei and dense cytoplasm. This third type of nodule was designated as an
9 incipient hepatoma, since it showed all the histological characteristics of a fully developed
10 hepatoma. All three types of nodules were generally present in the same liver. Cirrhosis of the
11 liver was not observed. The numbers of incipient liver tumors and hepatomas in rats from this
12 study (treated for 13 months and observed at 13–16 months) are presented in Table 4-3.

Table 4-3. Number of incipient liver tumors and hepatomas in male Sprague-Dawley rats exposed to 1,4-dioxane in drinking water for 13 months

Dose (mg/kg-day) ^a	Incipient tumors	Hepatomas	Total
430	4	0	4
574	9	0	9
803	13	3	16
1,032	11	12	23

^aPrecise incidences cannot be calculated since the number of rats per group was reported as 28–32; incidence in control rats was not reported; no statistical analysis of the results was conducted in the study.

Source: Argus et al. (1973).

13 Treatment with all dose levels of 1,4-dioxane induced marked kidney alterations, but
14 quantitative incidence data were not provided. Qualitatively, the changes indicated
15 glomerulonephritis and pyelonephritis, with characteristic epithelial proliferation of Bowman's
16 capsule, periglomerular fibrosis, and distension of tubules. No kidney tumors were found. No
17 tumors were found in the lungs. One rat at the 1.4% treatment level showed early peripheral
18 adenomatous change of the alveolar epithelium and another rat in the same group showed
19 papillary hyperplasia of the bronchial epithelium. The lowest dose tested (430 mg/kg-day) was
20 considered a LOAEL by EPA for hepatic and renal effects in this study.

21 **4.2.1.2.3. Hoch-Ligeti and Argus.** Hoch-Ligeti and Argus (1970) provided a brief account of
22 the results of exposure of guinea pigs to 1,4-dioxane. A group of 22 male guinea pigs (neither
23 strain nor age provided) was administered 1,4-dioxane (purity not provided) in the drinking
24 water for at least 23 months and possibly up to 28 months. The authors stated that the
25 concentration of 1,4-dioxane was regulated so that normal growth of the guinea pigs was

1 maintained, and varied 0.5–2% (no further information provided). The investigators further
2 stated that the amount of 1,4-dioxane received by the guinea pigs over a 23-month period was
3 588–635 g. Using a reference BW of 0.89 kg for male guinea pigs in a chronic study ([U.S. EPA,
4 1988](#)) and assuming an exposure period of 700 days (23 months), the guinea pigs received doses
5 between 944 and 1,019 mg 1,4-dioxane/kg-day. A group of ten untreated guinea pigs served as
6 controls. All animals were sacrificed within 28 months, but the scope of the postmortem
7 examination was not provided.

8 Nine treated guinea pigs showed peri- or intrabronchial epithelial hyperplasia and nodular
9 mononuclear infiltration in the lungs. Also, two guinea pigs had carcinoma of the gallbladder,
10 three had early hepatomas, and one had an adenoma of the kidney. Among the controls, four
11 guinea pigs had peripheral mononuclear cell accumulation in the lungs, and only one had
12 hyperplasia of the bronchial epithelium. One control had formation of bone in the bronchus. No
13 further information was presented in the brief narrative of this study. Given the limited reporting
14 of the results, a NOAEL or LOAEL value was not provided for this study.

15 **4.2.1.2.4. *Kociba et al.*** Groups of 6–8-week-old Sherman rats (60/sex/dose level) were
16 administered 1,4-dioxane (purity not reported) in the drinking water at levels of 0 (controls),
17 0.01, 0.1, or 1.0% for up to 716 days ([Kociba, McCollister, Park, Torkelson, & Gehring, 1974](#)).
18 The drinking water was prepared twice weekly during the first year of the study and weekly
19 during the second year of the study. Water samples were collected periodically and analyzed for
20 1,4-dioxane content by routine gas liquid chromatography. Food and water were available ad
21 libitum. Rats were observed daily for clinical signs of toxicity, and BWs were measured twice
22 weekly during the first month, weekly during months 2–7, and biweekly thereafter. Water
23 consumption was recorded at three different time periods during the study: days 1–113, 114–
24 198, and 446–460. Blood samples were collected from a minimum of five male and five female
25 control and high-dose rats during the 4th, 6th, 12th, and 18th months of the study and at
26 termination. Each sample was analyzed for packed cell volume, total erythrocyte count,
27 hemoglobin, and total and differential WBC counts. Additional endpoints evaluated included
28 organ weights (brain, liver, kidney, testes, spleen, and heart) and gross and microscopic
29 examination of major tissues and organs (brain, bone and bone marrow, ovaries, pituitary, uterus,
30 mesenteric lymph nodes, heart, liver, pancreas, spleen, stomach, prostate, colon, trachea,
31 duodenum, kidneys, esophagus, jejunum, testes, lungs, spinal cord, adrenals, thyroid,
32 parathyroid, nasal turbinates, and urinary bladder). The number of rats with tumors, hepatic
33 tumors, hepatocellular carcinomas, and nasal carcinomas were analyzed for statistical
34 significance with Fisher’s Exact test (one-tailed), comparing each treatment group against the
35 respective control group. Survival rates were compared using χ^2 Contingency Tables and
36 Fisher’s Exact test. Student’s test was used to compare hematological parameters, body and
37 organ weights, and water consumption of each treatment group with the respective control group.

1 Male and female rats in the high-dose group (1% in drinking water) consumed slightly
2 less water than controls. BW gain was depressed in the high-dose groups relative to the other
3 groups almost from the beginning of the study (food consumption data were not provided).
4 Based on water consumption and BW data for specific exposure groups, Kociba et al. (1974)
5 calculated mean daily doses of 9.6, 94, and 1,015 mg/kg-day for male rats and 19, 148, and
6 1,599 mg/kg-day for female rats during days 114–198 for the 0.01, 0.1, and 1.0% concentration
7 levels, respectively. Treatment with 1,4-dioxane significantly increased mortality among high-
8 dose males and females beginning at about 2–4 months of treatment. These rats showed
9 degenerative changes in both the liver and kidneys. From the 5th month on, mortality rates of
10 control and treated groups were not different. There were no treatment-related alterations in
11 hematological parameters. At termination, the only alteration in organ weights noted by the
12 authors was a significant increase in absolute and relative liver weights in male and female high-
13 dose rats (data not shown). Histopathological lesions were restricted to the liver and kidney from
14 the mid- and high-dose groups and consisted of variable degrees of renal tubular epithelial and
15 hepatocellular degeneration and necrosis (no quantitative incidence data were provided). Rats
16 from these groups also showed evidence of hepatic regeneration, as indicated by hepatocellular
17 hyperplastic nodule formation and evidence of renal tubular epithelial regenerative activity
18 (observed after 2 years of exposure). These changes were not seen in controls or in low-dose
19 rats. The authors determined a LOAEL of 94 mg/kg-day based on the liver and kidney effects in
20 male rats. The corresponding NOAEL value was 9.6 mg/kg-day.

21 Histopathological examination of all the rats in the study revealed a total of 132 tumors in
22 114 rats. Treatment with 1% 1,4-dioxane in the drinking water resulted in a significant increase
23 in the incidence of hepatic tumors (hepatocellular carcinomas in six males and four females). In
24 addition, nasal carcinomas (squamous cell carcinoma of the nasal turbinates) occurred in one
25 high-dose male and two high-dose females. Since 128 out of 132 tumors occurred in rats from
26 the 12th to the 24th month, Kociba et al. (1974) assumed that the effective number of rats was
27 the number surviving at 12 months, which was also when the first hepatic tumor was noticed.
28 The incidences of liver and nasal tumors from Kociba et al. (1974) are presented in Table 4-4.
29 Tumors in other organs were not elevated when compared to control incidence and did not
30 appear to be related to 1,4-dioxane administration.

Table 4-4. Incidence of liver and nasal tumors in male and female Sherman rats (combined) treated with 1,4-dioxane in the drinking water for 2 years

Dose in mg/kg-day (average of male and female dose)	Effective number of animals ^a	Number of tumor- bearing animals	Number of animals		
			Hepatic tumors (all types)	Hepatocellular carcinomas	Nasal carcinomas
0	106	31	2	1	0
14	110	34	0	0	0
121	106	28	1	1	0
1307	66	21	12 ^b	10 ^c	3 ^d

^aRats surviving until 12 months on study.

^b $p = 0.00022$ by one-tailed Fisher's Exact test.

^c $p = 0.00033$ by one-tailed Fisher's Exact test.

^d $p = 0.05491$ by one-tailed Fisher's Exact test.

Source: Used with permission from Elsevier, Ltd., Kociba et al. (1974).

1 The high-dose level was the only dose that increased the formation of liver tumors over
 2 control (males 1,015 mg/kg-day; females 1,599 mg/kg-day) and also caused significant liver and
 3 kidney toxicity in these animals. The mid-dose group (males 94 mg/kg-day; females 148 mg/kg-
 4 day) experienced hepatic and renal degeneration and necrosis, as well as regenerative
 5 proliferation in hepatocytes and renal tubule epithelial cells. No increase in tumor formation was
 6 seen in the mid-dose group. No toxicity or tumor formation was observed in either sex in the
 7 low-dose (males 9.6 mg/kg-day; females 19 mg/kg-day) group of rats.

8 **4.2.1.2.5. National Cancer Institute (NCI).** Groups of Osborne-Mendel rats (35/sex/dose) and
 9 B6C3F₁ mice (50/sex/dose) were administered 1,4-dioxane ($\geq 99.95\%$ pure) in the drinking
 10 water for 110 or 90 weeks, respectively, at levels of 0 (matched controls), 0.5, or 1% (NCL,
 11 1978). Solutions of 1,4-dioxane were prepared with tap water. The report indicated that at
 12 105 weeks from the earliest starting date, a new necropsy protocol was instituted. This affected
 13 the male controls and high-dose rats, which were started a year later than the original groups of
 14 rats and mice. Food and water were available ad libitum. Endpoints monitored in this bioassay
 15 included clinical signs (twice daily), BWs (once every 2 weeks for the first 12 weeks and every
 16 month during the rest of the study), food and water consumption (once per month in 20% of the
 17 animals in each group during the second year of the study), and gross and microscopic
 18 appearance of all major organs and tissues (mammary gland, trachea, lungs and bronchi, heart,
 19 bone marrow, liver, bile duct, spleen, thymus, lymph nodes, salivary gland, pancreas, kidney,
 20 esophagus, thyroid, parathyroid, adrenal, gonads, brain, spinal cord, sciatic nerve, skeletal
 21 muscle, stomach, duodenum, colon, urinary bladder, nasal septum, and skin). Based on the
 22 measurements of water consumption and BWs, the investigators calculated average daily intakes

1 of 1,4-dioxane of 0, 240, and 530 mg/kg-day in male rats, 0, 350, and 640 mg/kg-day in female
 2 rats, 0, 720, and 830 mg/kg-day in male mice, and 0, 380, and 860 mg/kg-day in female mice.
 3 According to the report, the doses of 1,4-dioxane in high-dose male mice were only slightly
 4 higher than those of the low-dose group due to decreased fluid consumption in high-dose male
 5 mice.

6 During the second year of the study, the BWs of high-dose rats were lower than controls,
 7 those of low-dose males were higher than controls, and those of low-dose females were
 8 comparable to controls. The fluctuations in the growth curves were attributed to mortality by the
 9 investigators; quantitative analysis of BW changes was not done. Mortality was significantly
 10 increased in treated rats, beginning at approximately 1 year of study. Analysis of Kaplan-Meier
 11 curves (plots of the statistical estimates of the survival probability function) revealed significant
 12 positive dose-related trends ($p < 0.001$, Tarone test). In male rats, 33/35 (94%) in the control
 13 group, 26/35 (74%) in the mid-dose group, and 33/35 (94%) in the high-dose group were alive
 14 on week 52 of the study. The corresponding numbers for females were 35/35 (100%), 30/35
 15 (86%), and 29/35 (83%). Nonneoplastic lesions associated with treatment with 1,4-dioxane were
 16 seen in the kidneys (males and females), liver (females only), and stomach (males only). Kidney
 17 lesions consisted of vacuolar degeneration and/or focal tubular epithelial regeneration in the
 18 proximal cortical tubules and occasional hyaline casts. Elevated incidence of hepatocytomegaly
 19 also occurred in treated female rats. Gastric ulcers occurred in treated males, but none were seen
 20 in controls. The incidence of pneumonia was increased above controls in high-dose female rats.
 21 The incidence of nonneoplastic lesions in rats following drinking water exposure to 1,4-dioxane
 22 is presented in Table 4-5. EPA identified the LOAEL in rats from this study as 240 mg/kg-day
 23 for increased incidence of gastric ulcer and cortical tubular degeneration in the kidney in males;
 24 a NOAEL was not established.

Table 4-5. Incidence of nonneoplastic lesions in Osborne-Mendel rats exposed to 1,4-dioxane in drinking water

	Males (mg/kg-day)			Females (mg/kg-day)		
	0	240	530	0	350	640
Cortical tubule degeneration	0/31 ^a	20/31 ^b (65%)	27/33 ^b (82%)	0/31 ^a	0/34	10/32 ^b (31%)
Hepatocytomegaly	5/31 (16%)	3/32 (9%)	11/33 (33%)	7/31 ^a (23%)	11/33 (33%)	17/32 ^b (53%)
Gastric ulcer	0/30 ^a	5/28 ^b (18%)	5/30 ^b (17%)	0/31	1/33 (3%)	1/30 (3%)
Pneumonia	8/30 (27%)	15/31 (48%)	14/33 (42%)	6/30 ^a (20%)	5/34 (15%)	25/32 ^b (78%)

^aStatistically significant trend for increased incidence by Cochran-Armitage test ($p < 0.05$) performed for this review.

^bIncidence significantly elevated compared to control by Fisher's Exact test ($p < 0.05$) performed for this review.

Source: NCI (1978).

1 Neoplasms associated with 1,4-dioxane treatment were limited to the nasal cavity
2 (squamous cell carcinomas, adenocarcinomas, and one rhabdomyoma) in both sexes, liver
3 (hepatocellular adenomas) in females, and testis/epididymis (mesotheliomas) in males. The first
4 tumors were seen at week 52 in males and week 66 in females. The incidence of squamous cell
5 carcinomas in the nasal turbinates in male and female rats is presented in Table 4-6. Squamous
6 cell carcinomas were first seen on week 66 of the study. Morphologically, these tumors varied
7 from minimal foci of locally invasive squamous cell proliferation to advanced growths consisting
8 of extensive columns of epithelial cells projecting either into free spaces of the nasal cavity
9 and/or infiltrating into the submucosa. Adenocarcinomas of the nasal cavity were observed in
10 3 of 34 high-dose male rats, 1 of 35 low-dose female rats, and 1 of 35 high-dose female rats.
11 The single rhabdomyoma (benign skeletal muscle tumor) was observed in the nasal cavity of a
12 male rat from the low-dose group. A subsequent re-examination of the nasal tissue sections by
13 Goldsworthy et al. (1991) concluded that the location of the tumors in the nasal apparatus was
14 consistent with the possibility that the nasal tumors resulted from inhalation of water droplets by
15 the rats (see Section 4.5.2 for more discussion of Goldsworthy et al. (1991)).

Table 4-6. Incidence of nasal cavity squamous cell carcinoma and liver hepatocellular adenoma in Osborne-Mendel rats exposed to 1,4-dioxane in drinking water

Males (mg/kg-day) ^a			
	0	240 ^b	530
Nasal cavity squamous cell carcinoma	0/33 (0%)	12/33 (36%)	16/34 (47%) ^c
Hepatocellular adenoma	2/31 (6%)	2/32 (6%)	1/33 (3%)
Females (mg/kg-day) ^a			
	0	350	640
Nasal cavity squamous cell carcinoma	0/34 (0%) ^d	10/35 (29%) ^e	8/35 (23%) ^c
Hepatocellular adenoma	0/31 (0%) ^f	10/33 (30%) ^e	11/32 (34%) ^e

^aTumor incidence values were not adjusted for mortality.

^bGroup not included in statistical analysis by NCI because the dose group was started a year earlier without appropriate controls.

^c $p \leq 0.003$ by Fisher's Exact test pair-wise comparison with controls.

^d $p = 0.008$ by Cochran-Armitage test.

^e $p \leq 0.001$ by Fisher's Exact test pair-wise comparison with controls.

^f $p = 0.001$ by Cochran-Armitage test.

Source: NCI (1978).

16 The incidence of hepatocellular adenomas in male and female rats is presented in Table
17 4-6. Hepatocellular adenomas were first observed in high-dose females in week 70 of the study.
18 These tumors consisted of proliferating hepatic cells oriented as concentric cords. Hepatic cell
19 size was variable; mitoses and necrosis were rare. Mesothelioma of the vaginal tunics of the

1 testis/epididymis was seen in male rats (2/33, 4/33, and 5/34 in controls, low-, and high-dose
2 animals, respectively). The difference between the treated groups and controls was not
3 statistically significant. These tumors were characterized as rounded and papillary projections of
4 mesothelial cells, each supported by a core of fibrous tissue. Other reported neoplasms were
5 considered spontaneous lesions not related to treatment with 1,4-dioxane.

6 In mice, mean BWs of high-dose female mice were lower than controls during the second
7 year of the study, while those of low-dose females were higher than controls. In males, mean
8 BWs of high-dose animals were higher than controls during the second year of the study.
9 According to the investigators, these fluctuations could have been due to mortality; no
10 quantitative analysis of BWs was done. No other clinical signs were reported. Mortality was
11 significantly increased in female mice ($p < 0.001$, Tarone test), beginning at approximately
12 80 weeks on study. The numbers of female mice that survived to 91 weeks were 45/50 (90%) in
13 the control group, 39/50 (78%) in the low-dose group, and 28/50 (56%) in the high-dose group.
14 In males, at least 90% of the mice in each group were still alive at week 91. Nonneoplastic
15 lesions that increased significantly due to treatment with 1,4-dioxane were pneumonia in males
16 and females and rhinitis in females. The incidences of pneumonia were 1/49 (2%), 9/50 (18%),
17 and 17/47 (36%) in control, low-dose, and high-dose males, respectively; the corresponding
18 incidences in females were 2/50 (4%), 33/47 (70%), and 32/36 (89%). The incidences of rhinitis
19 in female mice were 0/50, 7/48 (14%), and 8/39 (21%) in control, low-dose, and high-dose
20 groups, respectively. Pair-wise comparisons of low-dose and high-dose incidences with controls
21 for incidences of pneumonia and rhinitis in females using Fisher's Exact test (done for this
22 review) yielded p -values < 0.001 in all cases. Incidences of other lesions were considered to be
23 similar to those seen in aging mice. The authors stated that hepatocytomegaly was commonly
24 found in dosed mice, but the incidences were not significantly different from controls and
25 showed no dose-response trend. EPA concluded the LOAEL for 1,4-dioxane in mice was
26 380 mg/kg-day based on the increased incidence of pneumonia and rhinitis in female mice; a
27 NOAEL was not established in this study.

28 As shown in Table 4-7, treatment with 1,4-dioxane significantly increased the incidence
29 of hepatocellular carcinomas or adenomas in male and female mice in a dose-related manner.
30 Tumors were first observed on week 81 in high-dose females and in week 58 in high-dose males.
31 Tumors were characterized by parenchymal cells of irregular size and arrangement, and were
32 often hypertrophic with hyperchromatic nuclei. Mitoses were seldom seen. Neoplasms were
33 locally invasive within the liver, but metastasis to the lungs was rarely observed.

Table 4-7. Incidence of hepatocellular adenoma or carcinoma in B6C3F₁ mice exposed to 1,4-dioxane in drinking water

Males (mg/kg-day) ^a			
	0	720	830
Hepatocellular carcinoma	2/49 (4%) ^b	18/50 (36%) ^c	24/47 (51%) ^c
Hepatocellular adenoma or carcinoma	8/49 (16%) ^b	19/50 (38%) ^d	28/47 (60%) ^c
Females (mg/kg-day) ^a			
	0	380	860
Hepatocellular carcinoma	0/50 (0%) ^b	12/48 (25%) ^c	29/37 (78%) ^c
Hepatocellular adenoma or carcinoma	0/50 (0%) ^b	21/48 (44%) ^c	35/37 (95%) ^c

^aTumor incidence values were not adjusted for mortality.

^b $p < 0.001$, positive dose-related trend (Cochran-Armitage test).

^c $p < 0.001$ by Fisher's Exact test pair-wise comparison with controls.

^d $p = 0.014$.

Source: NCI (1978).

1 In addition to liver tumors, a variety of other benign and malignant neoplasms occurred.
 2 However, the report (NCI, 1978) indicated that each type had been encountered previously as a
 3 spontaneous lesion in the B6C3F₁ mouse. The report further stated that the incidences of these
 4 neoplasms were unrelated by type, site, group, or sex of the animal, and hence, not attributable to
 5 exposure to 1,4-dioxane. There were a few nasal adenocarcinomas (1/48 in low-dose females
 6 and 1/49 in high-dose males) that arose from proliferating respiratory epithelium lining of the
 7 nasal turbinates. These growths extended into the nasal cavity, but there was minimal local
 8 tissue infiltration. Nasal mucosal polyps were rarely observed. The polyps were derived from
 9 mucus-secreting epithelium and were otherwise unremarkable. There was a significant negative
 10 trend for alveolar/bronchiolar adenomas or carcinomas of the lung in male mice, such that the
 11 incidence in the matched controls was higher than in the dosed groups. The report (NCI, 1978)
 12 indicated that the probable reason for this occurrence was that the dosed animals did not live as
 13 long as the controls, thus diminishing the possibility of the development of tumors in the dosed
 14 groups.

15 **4.2.1.2.6. Kano et al.; Japan Bioassay Research Center; Yamazaki et al.** The Japan
 16 Bioassay Research Center (JBRC) conducted a 2-year drinking water study determining the
 17 effects of 1,4-dioxane on both sexes of rats and mice. The study results have been reported
 18 several times: once as conference proceedings (Yamazaki et al., 1994), once as a laboratory
 19 report (JBRC, 1998), and most recently as a peer-reviewed manuscript (Kano et al., 2009). Dr.
 20 Yamazaki also provided some detailed information (Yamazaki, 2006). Variations in the data
 21 between these three reports were noted and included: (1) the level of detail on dose information
 22 reported; (2) categories for incidence data reported (e.g., all animals or sacrificed animals); and
 23 (3) analysis of non- and neoplastic lesions.

1 The 1,4-dioxane dose information provided in the reports varied. Specifically, Yamazaki
2 et al. (1994) only included drinking water concentrations for each dose group. In contrast, JBRC
3 (1998) included drinking water concentrations (ppm), in addition using body weights and water
4 consumption measurements to calculate daily chemical intake (mg/kg-day). JBRC (1998)
5 reported daily chemical intake for each dose group as a range. Thus, for the External Peer
6 Review draft of this *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2009b), the midpoint of
7 the range was used. Kano et al. (2009) also reported a calculation of daily chemical intake based
8 on body weight and water consumption measurements; however, for each dose group they
9 reported a mean and standard deviation estimate. Therefore, because the mean more accurately
10 represents the delivered dose than the midpoint of a range, the Kano et al. (2009) calculated
11 mean chemical intake (mg/kg-day) is used for quantitative analysis of this data.

12 The categories for which incidence rates were described also varied among the reports.
13 Yamazaki et al. (1994) and Kano et al. (2009) reported histopathological results for all animals,
14 including dead and moribund animals; however, the detailed JBRC laboratory findings (1998)
15 included separate incidence reports for dead and moribund animals, sacrificed animals, and all
16 animals.

17 Finally, the criteria used to evaluate some of the data were updated when JBRC published
18 the most recent manuscript by Kano et al. (2009). The manuscript by Kano et al. (2009) stated
19 that the lesions diagnosed in the earlier reports (JBRC, 1998; Yamazaki, et al., 1994) were re-
20 examined and recategorized as appropriate according to current pathological diagnostic criteria
21 (see references in Kano et al. (2009)).

22 Groups of F344/DuCrj rats (50/sex/dose level) were exposed to 1,4-dioxane (>99% pure)
23 in the drinking water at levels of 0, 200, 1,000, or 5,000 ppm for 2 years. Groups of Crj:BDF1
24 mice (50/sex/dose level) were similarly exposed in the drinking water to 0, 500, 2,000, or
25 8,000 ppm of 1,4-dioxane. The high doses were selected based on results from the Kano et al.
26 (2008) 13-week drinking water study so as not to exceed the maximum tolerated dose (MTD) in
27 that study. Both rats and mice were 6 weeks old at the beginning of the study. Food and water
28 were available ad libitum. The animals were observed daily for clinical signs of toxicity; and
29 BWs were measured once per week for 14 weeks and once every 2 weeks until the end of the
30 study. Food consumption was measured once a week for 14 weeks and once every 4 weeks for
31 the remainder of the study. The investigators used data from water consumption and BW to
32 calculate an estimate of the daily intake of 1,4-dioxane (mg/kg-day) by male and female rats and
33 mice. Kano et al. (2009) reported a calculated mean \pm standard deviation for the daily doses of
34 1,4-dioxane for the duration of the study. Male rats received doses of approximately 0, 11 ± 1 ,
35 55 ± 3 , or 274 ± 18 mg/kg-day and female rats received 0, 18 ± 3 , 83 ± 14 , or 429 ± 69 mg/kg-day.
36 Male mice received doses of 0, 49 ± 5 , 191 ± 21 , or 677 ± 74 mg/kg-day and female mice received 0,
37 66 ± 10 , 278 ± 40 , or 964 ± 88 mg/kg-day. For the remainder of this document, including the dose-

1 response analysis, the mean calculated intake values are used to identify dose groups. The Kano
2 et al. (2009) study was conducted in accordance with the Organization for Economic Co-
3 operation and Development (OECD) Principles for Good Laboratory Practice (GLP).

4 No information was provided as to when urine samples were collected. Blood samples
5 were collected only at the end of the 2-year study (Yamazaki, 2006). Hematology analysis
6 included RBCs, hemoglobin, hematocrit, MCV, platelets, WBCs and differential WBCs. Serum
7 biochemistry included total protein, albumin, bilirubin, glucose, cholesterol, triglyceride (rat
8 only), phospholipid, ALT, AST, LDH, LAP, ALP, γ -glutamyl transpeptidase (GGT), CPK, urea
9 nitrogen, creatinine (rat only), sodium, potassium, chloride, calcium, and inorganic phosphorous.
10 Urinalysis parameters were pH, protein, glucose, ketone body, bilirubin (rat only), occult blood,
11 and urobilinogen. Organ weights (brain, lung, liver, spleen, heart, adrenal, testis, ovary, and
12 thymus) were measured, and gross necropsy and histopathologic examination of tissues and
13 organs were performed on all animals (skin, nasal cavity, trachea, lungs, bone marrow, lymph
14 nodes, thymus, spleen, heart, tongue, salivary glands, esophagus, stomach, small and large
15 intestine, liver, pancreas, kidney, urinary bladder, pituitary, thyroid, adrenal, testes, epididymis,
16 seminal vesicle, prostate, ovary, uterus, vagina, mammary gland, brain, spinal cord, sciatic nerve,
17 eye, Harderian gland, muscle, bone, and parathyroid). Dunnett's test and χ^2 test were used to
18 assess the statistical significance of changes in continuous and discrete variables, respectively.

19 For rats, growth and mortality rates were reported in Kano et al. (2009) for the duration
20 of the study. Both male and female rats in the high dose groups (274 and 429 mg/kg-day,
21 respectively) exhibited slower growth rates and terminal body weights that were significantly
22 different ($p < 0.05$) compared to controls. A statistically significant reduction in terminal BWs
23 was observed in high-dose male rats (5%, $p < 0.01$) and in high-dose female rats (18%, $p < 0.01$)
24 (Kano, et al., 2009). Food consumption was not significantly affected by treatment in male or
25 female rats; however, water consumption in female rats administered 18 mg/kg-day was
26 significantly greater ($p < 0.05$).

27 All control and exposed rats lived at least 12 months following study initiation
28 (Yamazaki, 2006); however, survival at the end of the 2-year study in the high dose group of
29 male and female rats (274 and 429 mg/kg-day, respectively) was approximately 50%, which was
30 significantly different compared to controls. The investigators attributed these early deaths to the
31 increased incidence in nasal tumors and peritoneal mesotheliomas in male rats and nasal and
32 hepatic tumors in female rats. (Yamazaki, 2006).

33 Several hematological changes were noted in the JBRC report (1998): Decreases in RBC
34 (male rats only), hemoglobin, hematocrit, and MCV; and increases in platelets in high-dose
35 groups were observed (JBRC, 1998). These changes (except for MCV) also occurred in mid-
36 dose males. With the exception of a 23% decrease in hemoglobin in high-dose male rats and a
37 27% increase in platelets in high-dose female rats, hematological changes were within 15% of

1 control values. Significant changes in serum chemistry parameters occurred only in high-dose
2 rats (males: increased phospholipids, AST, ALT, LDH, ALP, GGT, CPK, potassium, and
3 inorganic phosphorus and decreased total protein, albumin, and glucose; females: increased total
4 bilirubin, cholesterol, phospholipids, AST, ALT, LDH, GGT, ALP, CPK, and potassium, and
5 decreased blood glucose) ([JBRC, 1998](#)). Increases in serum enzyme activities ranged from <2-
6 to 17-fold above control values, with the largest increases seen for ALT, AST, and GGT. Urine
7 pH was significantly decreased at 274 mg/kg-day in male rats (not tested at other dose levels)
8 and at 83 and 429 mg/kg-day in female rats ([JBRC, 1998](#)). Also, blood in the urine was seen in
9 female rats at 83 and 429 mg/kg-day ([JBRC, 1998](#)). In male rats, relative liver weights were
10 increased at 55 and 274 mg/kg-day ([Kano, et al., 2009](#)). In female rats, relative liver weight was
11 increased at 429 mg/kg-day ([Kano, et al., 2009](#)).

12 Microscopic examination of the tissues showed nonneoplastic alterations in the nasal
13 cavity, liver, and kidneys mainly in high-dose rats and, in a few cases, in mid-dose rats (Table s
14 4-8 and 4-9). Alterations in high-dose (274 mg/kg-day) male rats consisted of nuclear
15 enlargement and metaplasia of the olfactory and respiratory epithelia, atrophy of the olfactory
16 epithelium, hydropic changes and sclerosis of the lamina propria, adhesion, and inflammation.
17 In female rats, nuclear enlargement of the olfactory epithelium occurred at doses \geq 83 mg/kg-
18 day, and nuclear enlargement and metaplasia of the respiratory epithelium, squamous cell
19 hyperplasia, respiratory metaplasia of the olfactory epithelium, hydropic changes and sclerosis of
20 the lamina propria, adhesion, inflammation, and proliferation of the nasal gland occurred at
21 429 mg/kg-day. Alterations were seen in the liver at \geq 55 mg/kg-day in male rats (spongiosis
22 hepatitis, hyperplasia, and clear and mixed cell foci) and at 429 mg/kg-day in female rats
23 (hyperplasia, spongiosis hepatitis, cyst formation, and mixed cell foci). Nuclear enlargement of
24 the renal proximal tubule occurred in males at 274 mg/kg-day and in females at \geq 83 mg/kg-day
25 ([JBRC, 1998](#)).

Table 4-8. Incidence of histopathological lesions in male F344/DuCrj rats exposed to 1,4-dioxane in drinking water for 2 years

	Dose (mg/kg-day) ^{a,b}			
	0	11	55	274
Nuclear enlargement; nasal respiratory epithelium ^c	0/50	0/50	0/50	26/50 ^e
Squamous cell metaplasia; nasal respiratory epithelium ^c	0/50	0/50	0/50	31/50 ^e
Squamous cell hyperplasia; nasal respiratory epithelium ^c	0/50	0/50	0/50	2/50
Nuclear enlargement; nasal olfactory epithelium ^c	0/50	0/50	5/50 ^f	38/50 ^e
Respiratory metaplasia; nasal olfactory epithelium ^d	12/50	11/50	20/50	43/50
Atrophy; nasal olfactory epithelium ^d	0/50	0/50	0/50	36/50
Hydropic change; lamina propria ^d	0/50	0/50	0/50	46/50
Sclerosis; lamina propria ^d	0/50	0/50	1/50	44/50
Adhesion; nasal cavity ^d	0/50	0/50	0/50	48/50
Inflammation; nasal cavity ^d	0/50	0/50	0/50	13/50
Hyperplasia; liver ^d	3/50	2/50	10/50	24/50
Spongiosis hepatitis; liver ^d	12/50	20/50	25/50 ^f	40/50
Clear cell foci; liver ^c	3/50	3/50	9/50	8/50
Acidophilic cell foci; liver ^c	12/50	8/50	7/50	5/50
Basophilic cell foci; liver ^c	7/50	11/50	8/50	16/50 ^f
Mixed-cell foci; liver ^c	2/50	8/50	14/50 ^e	13/50 ^e
Nuclear enlargement; kidney proximal tubule ^d	0/50	0/50	0/50	50/50

^aData presented for all animals, including animals that became moribund or died before the end of the study.

^bDose levels from Kano et al. (2009).

^cData from Kano et al. (2009).

^dData from JBRC (1998). JBRC did not report statistical significance for the “All animals” comparison.

^e $p < 0.01$ by χ^2 test.

^f $p < 0.05$ by χ^2 test.

Sources: Kano et al. (2009) and JBRC (1998).

Table 4-9. Incidence of histopathological lesions in female F344/DuCrj rats exposed to 1,4-dioxane in drinking water for 2 years

	Dose (mg/kg-day) ^{a,b}			
	0	18	83	429
Nuclear enlargement; nasal respiratory epithelium ^c	0/50	0/50	0/50	13/50 ^e
Squamous cell metaplasia; nasal respiratory epithelium ^c	0/50	0/50	0/50	35/50 ^e
Squamous cell hyperplasia; nasal cavity ^c	0/50	0/50	0/50	5/50
Nuclear enlargement; nasal olfactory epithelium ^c	0/50	0/50	28/50 ^e	39/50
Respiratory metaplasia; nasal olfactory epithelium ^d	2/50	0/50	2/50	42/50
Atrophy; nasal olfactory epithelium ^d	0/50	0/50	1/50	40/50
Hydropic change; lamina propria ^d	0/50	0/50	0/50	46/50
Sclerosis; lamina propria ^d	0/50	0/50	0/50	48/50
Adhesion; nasal cavity ^d	0/50	0/50	0/50	46/50
Inflammation; nasal cavity ^d	0/50	0/50	1/50	15/50
Proliferation; nasal gland ^d	0/50	0/50	0/50	11/50
Hyperplasia; liver ^d	3/50	2/50	11/50 ^e	47/50
Spongiosis hepatitis; liver ^d	0/50	0/50	1/50	20/50
Cyst formation; liver ^d	0/50	1/50	1/50	8/50
Acidophilic cell foci; liver ^c	1/50	1/50	1/50	1/50
Basophilic cell foci; liver ^c	23/50	27/50	31/50	8/50 ^e
Clear cell foci; liver ^c	1/50	1/50	5/50	4/50
Mixed-cell foci; liver ^c	1/50	1/50	3/50	11/50 ^f
Nuclear enlargement; kidney proximal tubule ^d	0/50	0/50	6/50	39/50

^aData presented for all animals, including animals that became moribund or died before the end of the study.

^bDose levels from Kano et al. (2009).

^cData from Kano et al. (2009).

^dData from JBRC (1998). JBRC did not report statistical significance for the “All animals” comparison.

^e $p < 0.01$ by χ^2 test.

^f $p < 0.05$ by χ^2 test.

Sources: Kano et al. (2009) and JBRC (1998).

1 NOAEL and LOAEL values for rats in this study were identified by EPA as 55 and
2 274 mg/kg-day, respectively, based on toxicity observed in nasal tissue of male rats (i.e., atrophy
3 of olfactory epithelium, adhesion, and inflammation). Metaplasia and hyperplasia of the nasal
4 epithelium were also observed in high-dose male and female rats. These effects are likely to be
5 associated with the formation of nasal cavity tumors in these dose groups. Nuclear enlargement
6 was observed in the nasal olfactory epithelium and the kidney proximal tubule at a dose of
7 83 mg/kg-day in female rats; however, it is unclear whether these alterations represent adverse
8 toxicological effects. Hematological effects noted in male rats given 55 and 274 mg/kg-day
9 (decreased RBCs, hemoglobin, hematocrit, increased platelets) were within 20% of control
10 values. In female rats decreases in hematological effects were observed in the high dose group
11 (429 mg/kg-day). A reference range database for hematological effects in laboratory animals

1 ([Wolford et al., 1986](#)) indicates that a 20% change in these parameters may fall within a normal
2 range (10th–90th percentile values) and may not represent a treatment-related effect of concern.
3 Liver lesions were also seen at a dose of 55 mg/kg-day in male rats; these changes are likely to
4 be associated with liver tumorigenesis. Clear and mixed-cell foci are commonly considered
5 preneoplastic changes and would not be considered evidence of noncancer toxicity. The nature
6 of spongiosis hepatitis as a preneoplastic change is less well understood ([Bannasch, 2003](#); [Karbe
7 & Kerlin, 2002](#); [Stroebel, Mayer, Zerban, & Bannasch, 1995](#)). Spongiosis hepatitis is a cyst-like
8 lesion that arises from the perisinusoidal (Ito) cells (PSC) of the liver. It is commonly seen in
9 aging rats, but has been shown to increase in incidence following exposure to hepatocarcinogens.
10 Spongiosis hepatitis can be seen in combination with preneoplastic foci in the liver or with
11 hepatocellular adenoma or carcinoma and has been considered a preneoplastic lesion ([Bannasch,
12 2003](#); [Stroebel, et al., 1995](#)). This change can also be associated with hepatocellular hypertrophy
13 and liver toxicity and has been regarded as a secondary effect of some liver carcinogens ([Karbe
14 & Kerlin, 2002](#)). In the case of the JBRC ([1998](#)) study, spongiosis hepatitis was associated with
15 other preneoplastic changes in the liver (clear and mixed-cell foci). No other lesions indicative
16 of liver toxicity were seen in this study; therefore, spongiosis hepatitis was not considered
17 indicative of noncancer effects. Serum chemistry changes (increases in total protein, albumin,
18 and glucose; decreases in AST, ALT, LDH, and ALP, potassium, and inorganic phosphorous)
19 were observed in both male and female rats ([JBRC, 1998](#)) in the high dose groups, 274 and
20 429 mg/kg-day, respectively. These serum chemistry changes seen in terminal blood samples
21 from high-dose male and female rats are likely related to tumor formation in these dose groups.

22 Significantly increased incidences of liver tumors (adenomas and carcinomas) and tumors
23 of the nasal cavity occurred in high-dose male and female rats (Tables 4-10 and 4-11) treated
24 with 1,4-dioxane for 2 years ([Kano, et al., 2009](#)). The first liver tumor was seen at 85 weeks in
25 high-dose male rats and 73 weeks in high-dose female rats (vs. 101–104 weeks in lower dose
26 groups and controls) ([Yamazaki, 2006](#)). In addition, a significant increase ($p \leq 0.01$, Fisher's
27 Exact test) in mesotheliomas of the peritoneum was seen in high-dose males (28/50 versus 2/50
28 in controls). Mesotheliomas were the single largest cause of death among high-dose male rats,
29 accounting for 12 of 28 pretermination deaths ([Yamazaki, 2006](#)). Also, in males, there were
30 increasing trends in mammary gland fibroadenoma and fibroma of the subcutis, both statistically
31 significant ($p < 0.01$) by the Peto test of dose-response trend. Females showed a significant
32 increasing trend in mammary gland adenomas ($p < 0.01$ by Peto's test). The tumor incidence
33 values presented in Tables 4-10 and 4-11 were not adjusted for survival.

Table 4-10. Incidence of nasal cavity, peritoneum, and mammary gland tumors in F344/DuCrj rats exposed to 1,4-dioxane in drinking water for 2 years

Dose (mg/kg-day)	Males				Females			
	0	11	55	274	0	18	83	429
Nasal cavity								
Squamous cell carcinoma	0/50	0/50	0/50	3/50 ^a	0/50	0/50	0/50	7/50 ^{a,b}
Sarcoma	0/50	0/50	0/50	2/50	0/50	0/50	0/50	0/50
Rhabdomyosarcoma	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50
Esthesioneuroepithelioma	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
Peritoneum								
Mesothelioma	2/50	2/50	5/50	28/50 ^{a,b}	1/50	0/50	0/50	0/50
Mammary gland								
Fibroadenoma	1/50	1/50	0/50	4/50 ^a	3/50	2/50	1/50	3/50
Adenoma	0/50	1/50	2/50	2/50	6/50	7/50	10/50	16/50 ^{a,c}
Either adenoma or fibroadenoma	1/50	2/50	2/50	6/50 ^a	8/50	8/50	11/50	18/50 ^{a,c}

^aStatistically significant trend for increased tumor incidence by Peto's test ($p < 0.01$).

^bSignificantly different from control by Fisher's exact test ($p < 0.01$).

^cSignificantly different from control by Fisher's exact test ($p < 0.05$).

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Table 4-11. Incidence of liver tumors in F344/DuCrj rats exposed to 1,4-dioxane in drinking water for 2 years

Dose (mg/kg-day)	Males				Females			
	0	11	55	274	0	18	83	429
Hepatocellular adenoma	3/50	4/50	7/50	32/50 ^{a,b}	3/50	1/50	6/50	48/50 ^{a,b}
Hepatocellular carcinoma	0/50	0/50	0/50	14/50 ^{a,b}	0/50	0/50	0/50	10/50 ^{a,b}
Either adenoma or carcinoma	3/50	4/50	7/50	39/50 ^{a,b}	3/50	1/50	6/50	48/50 ^{a,b}

^aSignificantly different from control by Fisher's exact test ($p < 0.01$).

^bStatistically significant trend for increased tumor incidence by Peto's test ($p < 0.01$).

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

1 For mice, growth and mortality rates were reported in Kano et al. (2009) for the duration
2 of the study. Similar to rats, the growth rates of male and female mice were slower than controls
3 and terminal body weights were lower for the mid ($p < 0.01$ for males administered 191 mg/kg-
4 day and $p < 0.05$ for females administered 278 mg/kg-day) and high doses ($p < 0.05$ for males
5 and females administered 677 and 964 mg/kg-day, respectively). There were no differences in
6 survival rates between control and treated male mice; however, survival rates were significantly
7 decreased compared to controls for female mice in the mid (278 mg/kg-day, approximately 40%
8 survival) and high (964 mg/kg-day, approximately 20% survival) dose groups. The study

1 authors attributed these early female mouse deaths to the significant incidence of hepatic tumors,
2 and Kano et al. (2009) reported tumor incidence for all animals in the study (N=50), including
3 animals that became moribund or died before the end of the study. Additional data on survival
4 rates of mice were provided in a personal communication from Dr. Yamazaki (2006). Dr.
5 Yamazaki reported that the survival of mice was low in all male groups (31/50, 33/50, 25/50 and
6 26/50 in control, low-, mid-, and high-dose groups, respectively) and particularly low in high-
7 dose females (29/50, 29/50, 17/50, and 5/50 in control, low-, mid-, and high-dose groups,
8 respectively). These deaths occurred primarily during the second year of the study. Survival at
9 12 months in male mice was 50/50, 48/50, 50/50, and 48/50 in control, low-, mid-, and high-dose
10 groups, respectively. Female mouse survival at 12 months was 50/50, 50/50, 48/50, and 48/50 in
11 control, low-, mid-, and high-dose groups, respectively (Yamazaki, 2006). Furthermore, these
12 deaths were primarily tumor related. Liver tumors were listed as the cause of death for 31 of the
13 45 pretermination deaths in high-dose female Crj:BDF1 mice (Yamazaki, 2006). For mice,
14 growth and mortality rates were reported in Kano et al. (2009) for the duration of the study.
15 Similar to rats, the growth rates of male and female mice were slower than controls and terminal
16 body weights were lower for the mid ($p < 0.01$ for males administered 191 mg/kg-day and $p <$
17 0.05 for females administered 278 mg/kg-day) and high doses ($p < 0.05$ for males and females
18 administered 677 and 964 mg/kg-day, respectively).

19 Food consumption was not significantly affected, but water consumption was reduced
20 26% in high-dose male mice and 28% in high-dose female mice. Final BWs were reduced 43%
21 in high-dose male mice and 15 and 45% in mid- and high-dose female mice, respectively. Male
22 mice showed increases in RBC counts, hemoglobin, and hematocrit, whereas in female mice,
23 there was a decrease in platelets in mid- and high-dose rats. With the exception of a 60%
24 decrease in platelets in high-dose female mice, hematological changes were within 15% of
25 control values. Serum AST, ALT, LDH, and ALP activities were significantly increased in mid-
26 and high-dose male mice, whereas LAP and CPK were increased only in high-dose male mice.
27 AST, ALT, LDH, and ALP activities were increased in mid- and high-dose female mice, but
28 CPK activity was increased only in high-dose female mice. Increases in serum enzyme activities
29 ranged from less than two- to sevenfold above control values. Glucose and triglycerides were
30 decreased in high-dose males and in mid- and high-dose females. High-dose female mice also
31 showed decreases in serum phospholipid and albumin concentrations (not reported in males).
32 Blood calcium was lower in high-dose females and was not reported in males. Urinary pH was
33 decreased in high-dose males, whereas urinary protein, glucose, and occult blood were increased
34 in mid- and high-dose female mice. Relative and absolute lung weights were increased in high-
35 dose males and in mid- and high-dose females (JBRC, 1998). Microscopic examination of the
36 tissues for nonneoplastic lesions showed significant alterations in the epithelium of the
37 respiratory tract, mainly in high-dose animals, although some changes occurred in mid-dose mice

- 1 (Tables 4-12 and 4-13). Commonly seen alterations included nuclear enlargement, atrophy, and
- 2 inflammation of the epithelium. Other notable changes observed included nuclear enlargement
- 3 of the proximal tubule of the kidney and angiectasis in the liver in high-dose male mice.

Table 4-12. Incidence of histopathological lesions in male Crj:BDF1 mice exposed to 1,4-dioxane in drinking water for 2 years

	Dose (mg/kg-day) ^{a,b}			
	0	49	191	677
Nuclear enlargement; nasal respiratory epithelium ^c	0/50	0/50	0/50	31/50 ^e
Nuclear enlargement; nasal olfactory epithelium ^c	0/50	0/50	9/50 ^e	49/50 ^e
Atrophy; nasal olfactory epithelium ^d	0/50	0/50	1/50	48/50
Inflammation; nasal cavity ^d	1/50	2/50	1/50	25/50
Atrophy; tracheal epithelium ^d	0/50	0/50	0/50	42/50
Nuclear enlargement; tracheal epithelium ^d	0/50	0/50	0/50	17/50
Nuclear enlargement; bronchial epithelium ^d	0/50	0/50	0/50	41/50
Atrophy; lung/bronchial epithelium ^d	0/50	0/50	0/50	43/50
Accumulation of foamy cells; lung ^d	1/50	0/50	0/50	27/50
Angiectasis; liver ^d	2/50	3/50	4/50	16/50
Nuclear enlargement; kidney proximal tubule ^d	0/50	0/50	0/50	39/50

^aData presented for all animals, including animals that became moribund or died before the end of the study.

^bDose levels from Kano et al. (2009).

^cData from Kano et al. (2009).

^dData from JBRC (1998). JBRC did not report statistical significance for the “All animals” comparison.

^e $p < 0.01$ by χ^2 test.

Sources: Kano et al. (2009) and JBRC (1998).

Table 4-13. Incidence of histopathological lesions in female Crj:BDF1 mice exposed to 1,4-dioxane in drinking water for 2 years

	Dose (mg/kg-day) ^{a,b}			
	0	66	278	964
Nuclear enlargement; nasal respiratory epithelium ^c	0/50	0/50	0/50	41/50 ^e
Nuclear enlargement; nasal olfactory epithelium ^c	0/50	0/50	41/50 ^e	33/50 ^e
Atrophy; nasal olfactory epithelium ^d	0/50	0/50	1/50	42/50
Inflammation; nasal cavity ^d	2/50	0/50	7/50	42/50
Atrophy; tracheal epithelium ^d	0/50	0/50	2/50	49/50
Nuclear enlargement; bronchial epithelium ^d	0/50	1/50	22/50	48/50
Atrophy; lung/bronchial epithelium ^d	0/50	0/50	7/50	50/50
Accumulation of foamy cells; lung ^d	0/50	1/50	4/50	45/50

^aData presented for all animals, including animals that became moribund or died before the end of the study.

^bDose levels from Kano et al. (2009).

^cData from Kano et al. (2009).

^dData from JBRC (1998). JBRC did not report statistical significance for the “All animals” comparison.

^e $p < 0.01$ by χ^2 test.

Sources: Kano et al. (2009) and JBRC (1998).

1 NOAEL and LOAEL values for mice in this study were identified by EPA as 66 and
 2 278 mg/kg-day, respectively, based on nasal inflammation observed in female mice. Nuclear
 3 enlargement of the nasal olfactory epithelium and bronchial epithelium was also observed at a
 4 dose of 278 mg/kg-day in female mice; however, it is unclear whether these alterations represent
 5 adverse toxicological effects. The serum chemistry changes seen in terminal blood samples from
 6 male and female mice (mid- and high-dose groups) are likely related to tumor formation in these
 7 animals. Liver angiectasis, an abnormal dilatation and/or lengthening of a blood or lymphatic
 8 vessel, was seen in male mice given 1,4-dioxane at a dose of 677 mg/kg-day.

9 Treatment with 1,4-dioxane resulted in an increase in the formation of liver tumors
 10 (adenomas and carcinomas) in male and female mice. The incidence of hepatocellular adenoma
 11 was statistically increased in male mice in the mid-dose group only. The incidence of male mice
 12 with hepatocellular carcinoma or either tumor type (adenoma or carcinoma) was increased in the
 13 low, mid, and high-dose groups. The appearance of the first liver tumor occurred in male mice at
 14 64, 74, 63, and 59 weeks in the control, low- mid-, and high-dose groups, respectively
 15 (Yamazaki, 2006). In female mice, increased incidence was observed for hepatocellular
 16 carcinoma in all treatment groups, while an increase in hepatocellular adenoma incidence was
 17 only seen in the 66 and 278 mg/kg-day dose groups (Table 4-14). The appearance of the first
 18 liver tumor in female mice occurred at 95, 79, 71, and 56 weeks in the control, low-, mid-, and
 19 high-dose groups, respectively (Yamazaki, 2006). The tumor incidence data presented for male
 20 and female mice in Table 4-14 are based on reanalyzed sample data presented in Kano et al.

1 (2009) that included lesions in animals that became moribund or died prior to the completion of
 2 the 2-year study.

3 Katagiri et al. (1998) summarized the incidence of hepatocellular adenomas and
 4 carcinomas in control male and female BDF1 mice from ten 2-year bioassays at the JBRC. For
 5 female mice, out of 499 control mice, the incidence rates were 4.4% for hepatocellular adenomas
 6 and 2.0% for hepatocellular carcinomas. Kano et al. (2009) reported a 10% incidence rate for
 7 hepatocellular adenomas and a 0% incidence rate for hepatocellular carcinomas in control female
 8 BDF1. The background incidence rates for male BDF1 mice were 15% and 22.8% for
 9 hepatocellular adenomas and carcinomas, respectively, out of 500 control mice in ten 2-year
 10 bioassays (Katagiri, et al., 1998). Background rates for B6C3F₁ mice evaluated by the National
 11 Toxicology Program are similar (10.3% and 21.3% for hepatocellular adenomas and carcinomas
 12 in male mice, respectively; 4.0% and 4.1% for hepatocellular adenomas and carcinomas in
 13 female mice, respectively) to the BDF1 mice background rates observed by JBRC (Haseman,
 14 Huff, & Boorman, 1984). Thus, the BDF1 mouse is not particularly sensitive compared to the
 15 commonly used B6C3F₁ strain and indicates that the results obtained by JBRC are reasonable.

Table 4-14. Incidence of tumors in Crj:BDF1 mice exposed to 1,4-dioxane in drinking water for 2 years

Dose (mg/kg-day)	Males				Females			
	0	49	191	677	0	66	278	964
Nasal Cavity								
Adenocarcinoma	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/50
Esthesioneuroepithelioma	0/50	0/50	0/50	1/50	0/50	0/50	0/50	0/50
Liver								
Hepatocellular adenoma	9/50	17/50	23/50 ^a	11/50	5/50	31/50 ^a	20/50 ^a	3/50
Hepatocellular carcinoma	15/50	20/50	23/50	36/50 ^{a,b}	0/50	6/50 ^c	30/50 ^a	45/50 ^{a,b}
Either hepatocellular adenoma or carcinoma	23/50	31/50	37/50 ^c	40/50 ^{a,b}	5/50	35/50 ^a	41/50 ^a	46/50 ^{a,b}

^aSignificantly different from control by Fisher's exact test ($p < 0.01$).

^bStatistically significant trend for increased tumor incidence by Peto's test ($p < 0.01$).

^cSignificantly different from control by Fisher's exact test ($p < 0.05$).

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

16 A weight of evidence evaluation of the carcinogenicity studies presented in Section
 17 4.2.1.2 is located in Section 4.7 and Table 4-19.

4.2.2. Inhalation Toxicity

4.2.2.1. Subchronic Inhalation Toxicity

1 **4.2.2.1.1. Fairley et al.** Rabbits, guinea pigs, rats, and mice (3–6/species/group) were exposed
2 to 1,000, 2,000, 5,000, or 10,000 ppm of 1,4-dioxane vapor two-times a day for 1.5 hours
3 (3 hours/day) for 5 days/week and 1.5 hours on the 6th day (16.5 hours/week) ([Fairley, et al.,
4 1934](#)). Animals were exposed until death occurred or were sacrificed at varying time periods.
5 At the 10,000 ppm concentration, only one animal (rat) survived a 7-day exposure. The rest of
6 the animals (six guinea pigs, three mice, and two rats) died within the first five exposures.
7 Severe liver and kidney damage and acute vascular congestion of the lungs were observed in
8 these animals. Kidney damage was described as patchy degeneration of cortical tubules with
9 vascular congestion and hemorrhage. Liver lesions varied from cloudy hepatocyte swelling to
10 large areas of necrosis. At 5,000 ppm, mortality was observed in two mice and one guinea pig
11 following 15–34 exposures. The remaining animals were sacrificed following 49.5 hours
12 (3 weeks) of exposure (three rabbits) or 94.5 hours (5 weeks) of exposure (three guinea pigs).
13 Liver and kidney damage in both dead and surviving animals was similar to that described for
14 the 10,000 ppm concentration. Animals (four rabbits, four guinea pigs, six rats, and five mice)
15 were exposed to 2,000 ppm for 45–102 total exposure hours (approximately 2–6 weeks). Kidney
16 and liver damage was still apparent in animals exposed to this concentration. Animals exposed
17 to 1,000 ppm were killed at intervals with the total exposure duration ranging between 78 and
18 202.5 hours (approximately 4–12 weeks). Cortical kidney degeneration and hepatocyte
19 degeneration and liver necrosis were observed in these animals (two rabbits, three guinea pigs,
20 three rats, and four mice). The low concentration of 1,000 ppm was identified by EPA as a
21 LOAEL for liver and kidney degeneration in rats, mice, rabbits, and guinea pigs in this study.

22 **4.2.2.1.2. Kasai et al.** Male and female 6-week-old F344/DuCrj rats (10/sex/group) were
23 exposed to nominal concentrations of 0 (clean air), 100, 200, 400, 800, 1,600, 3,200, or 6,400
24 ppm (0, 360, 720, 1,400, 2,900, 5,800, 12000, and 23,000 mg/m³, respectively) of vaporized
25 1,4-dioxane (>99% pure) for 6 hours/day, 5 days/week, for 13 weeks in whole body inhalation
26 chambers (Kasai et al., 2008, 195044). Each inhalation chamber housed 20 individual cages for
27 10 males and 10 females. During exposure, the concentration of 1,4-dioxane vapor was
28 determined every 15 minutes by gas chromatography. In addition, during exposure, animals
29 received food and water ad libitum and the following data were collected: 1) clinical signs and
30 mortality (daily); 2) BW and food intake (weekly); 3) urinary parameters using Ames reagent
31 strips (measured during week 13 of the exposure); and 4) 1,4-dioxane content in plasma from
32 three rats of both sexes (measured on the third day of exposure during weeks 12 and 13 at 1 hour
33 postmortem). At the end of the 13 week exposure period or at the time of an animal's death
34 during exposure, all organs were collected, weighed, and evaluated for macroscopic lesions.

1 Histopathological evaluations of organs and tissues were conducted in accordance with the
2 OECD test guidelines, including all tissues of the respiratory tract. Hematological and clinical
3 chemistry parameters were also measured using blood collected from the abdominal aorta of rats
4 following an overnight fasting at the end of the 13-week exposure period. The measured
5 hematological and clinical chemistry parameters included: red blood cell count, hemoglobin,
6 hematocrit, MCV, AST, ALT, glucose, and triglyceride. Liver sections from male and female
7 rats exposed to 800, 1,600 and 3,200 ppm of 1,4-dioxane were also analyzed for foci (in the
8 absence of tumor formation) by immunohistochemical expression of glutathione S-transferase
9 placental form (GST-P). Statistically significant differences between 1,4-dioxane and clean air
10 exposed groups were determined using Dunnett's test or χ^2 test. A p-value of 0.05 was
11 considered the threshold for significance.

12 All rats exposed to 6,400 ppm of 1,4-dioxane died by the end of the first week of
13 exposure; the determined cause of death was renal failure and diagnosed as necrosis of the renal
14 tubules. At concentrations lower than 6,400 ppm, mortality was not observed and all exposed
15 rats were absent of clinical signs. Exposure-related effects on final BWs, organ weights,
16 hematology parameters, and histopathological lesions were reported as compared to controls.
17 Terminal BWs were significantly decreased in both sexes at 200 ppm (males, 6%, p <0.05;
18 female, 7%, p <0.01) and 3,200 ppm (males, 7%, p <0.01; female, 10%, p <0.01); and
19 additionally in females at 800 ppm (6%, p <0.01) and 1,600 ppm (8%, p <0.01). Statistically
20 significant increases in several organ weights were observed, including liver (\geq 800 ppm, both
21 sexes, p \leq 0.01; 800 ppm, males, p \leq 0.05), kidney (3,200 ppm, males, p \leq 0.01; \geq 800 ppm,
22 females, p \leq 0.01), and lung (\geq 1,600 ppm, males, p \leq 0.01; \geq 200 ppm, females, p \leq 0.05; 400
23 ppm, female, p \leq 0.05). Changes in hematological parameters were observed at 3,200 ppm
24 including increased levels of hemoglobin (both sexes, p \leq 0.05), ALT (males, p \leq 0.05; female, p \leq
25 0.01), RBC (both sexes, p \leq 0.01), AST (both sexes, p \leq 0.01), hematocrit (females, p \leq 0.5), and
26 MCV(both sexes, p \leq 0.1). In males only, at 3,200 ppm, decreased levels of glucose (p \leq 0.01)
27 and triglyceride (p \leq 0.05) were observed; and in females only, at 200 ppm, an increased AST
28 level in females (p \leq 0.05) was noted. At 3,200 ppm, in exposed male rats, urinary protein was
29 slightly decreased; however, this data was not shown in this study. In plasma, a linear increase
30 in 1,4-dioxane levels was detected at exposure concentrations of 400 ppm and above in both
31 sexes, and the highest blood levels were observed in females. Exposure and/or sex-related
32 histopathology findings included nuclear enlargement of the nasal respiratory, nasal olfactory,
33 tracheal, and bronchial epithelium; vacuolic change in the olfactory and bronchial epithelium;
34 atrophy of the nasal epithelium; hydropic change in the proximal tubules of the kidney; and
35 single-cell necrosis and centrilobular swelling in the liver (Table 4-15). Severity of these
36 histopathological lesions are noted in Table 4-15 as well. Further microscopic evaluation of liver

1 tissue revealed GST-P positive liver foci in both sexes at 3,200 ppm (3/10 males, 2/10 females)
 2 and in females at 1,600 ppm (4/10) (Table 4-15).

3 The study authors determined nuclear enlargement in the respiratory epithelium as the
 4 most sensitive lesion and a LOAEL value of 100 ppm was identified by the study authors based
 5 on the incidence data of this lesion in both male and female rats.

6 **Table 4-15. Incidence data of histopathological lesions in F344/DuCrj rats exposed**
 7 **to 1,4-dioxane vapor by whole-body inhalation for 13 weeks.**
 8

Effect^b	Males						
	1,4-dioxane vapor concentration (ppm)^a						
	0 (clean air)	100	200	400	800	1,600	3,200
<u>Nuclear enlargement; nasal respiratory epithelium</u>	<u>0/10</u>	<u>7/10^c</u> <u>(7, 1+)</u>	<u>9/10^c</u> <u>(9, 1+)</u>	<u>7/10^c</u> <u>(7, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>	<u>10/10^c</u> <u>(10, 2+)</u>	<u>10/10^c</u> <u>(10, 2+)</u>
<u>Nuclear enlargement; nasal olfactory epithelium</u>	<u>0/10</u>	<u>0/10</u>	<u>5/10</u> <u>(5, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>	<u>10/10^c</u> <u>(10, 2+)</u>	<u>10/10^c</u> <u>(10, 2+)</u>
<u>Nuclear enlargement; tracheal epithelium</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>1/10</u> <u>(1, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>
<u>Nuclear enlargement; bronchial epithelium</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>9/10^c</u> <u>(9, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>
<u>Vacuolic change; olfactory epithelium</u>	<u>0/10</u>	<u>1/10</u> <u>(1, 1+)</u>	<u>3/10</u> <u>(3, 1+)</u>	<u>6/10^d</u> <u>(6, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>
<u>Vacuolic change; bronchial epithelium</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>4/10</u> <u>(4, 1+)</u>	<u>6/10^d</u> <u>(6, 1+)</u>	<u>6/10^d</u> <u>(6, 1+)</u>
<u>Atrophy; olfactory epithelium^e</u>	<u>=</u>	<u>=</u>	<u>=</u>	<u>=</u>	<u>=</u>	<u>=</u>	<u>=</u>
<u>Hepatocyte centrilobular swelling</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>1/10</u> <u>(1, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>
<u>Hepatocyte single-cell necrosis</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>1/10</u> <u>(1, 1+)</u>	<u>8/10^c</u> <u>(8, 1+)</u>
<u>Hydropic change; renal proximal tubule^e</u>	<u>=</u>	<u>=</u>	<u>=</u>	<u>=</u>	<u>=</u>	<u>=</u>	<u>=</u>
Effect^b	Females						
	1,4-dioxane vapor concentration (ppm)^a						
	0 (clean air)	100	200	400	800	1,600	3,200
<u>Nuclear enlargement; nasal respiratory epithelium</u>	<u>0/10</u>	<u>5/10^d</u> <u>(5, 1+)</u>	<u>9/10^c</u> <u>(9, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>	<u>10/10^c</u> <u>(10, 2+)</u>	<u>10/10^c</u> <u>(10, 2+)</u>
<u>Nuclear enlargement; nasal olfactory epithelium</u>	<u>0/10</u>	<u>2/10</u> <u>(2, 1+)</u>	<u>6/10^d</u> <u>(6, 1+)</u>	<u>10/10^c</u> <u>(9, 1+;</u> <u>1, 2+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>	<u>10/10^c</u> <u>(7, 1+;</u> <u>3, 2+)</u>	<u>10/10^c</u> <u>(10, 2+)</u>
<u>Nuclear enlargement; tracheal epithelium</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>2/10</u> <u>(2, 1+)</u>	<u>7/10^c</u> <u>(7, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>
<u>Nuclear enlargement; bronchial epithelium</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>10/10^c</u> <u>(10, 1+)</u>
<u>Vacuolic change; olfactory epithelium</u>	<u>0/10</u>	<u>1/10</u> <u>(1, 1+)</u>	<u>2/10</u> <u>(2, 1+)</u>	<u>3/10</u> <u>(3, 1+)</u>	<u>7/10^c</u> <u>(7, 1+)</u>	<u>9/10^c</u> <u>(9, 1+)</u>	<u>10/10^c</u> <u>(10, 1+)</u>
<u>Vacuolic change; bronchial epithelium</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>1/10</u> <u>(1, 1+)</u>	<u>1/10</u> <u>(1, 1+)</u>	<u>3/10</u> <u>(3, 1+)</u>	<u>4/10</u> <u>(4, 1+)</u>
<u>Atrophy; olfactory epithelium</u>	<u>0/10</u>	<u>0/10</u>	<u>2/10</u> <u>(2, 1+)</u>	<u>3/10</u> <u>(3, 1+)</u>	<u>5/10^d</u> <u>(5, 1+)</u>	<u>5/10^d</u> <u>(5, 1+)</u>	<u>4/10</u> <u>(4, 1+)</u>
<u>Hepatocyte centrilobular swelling</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>0/10</u>	<u>1/10</u> <u>(1, 1+)</u>	<u>8/10^c</u> <u>(8, 1+)</u>

Hepatocyte single-cell necrosis	0/10	0/10	0/10	0/10	0/10	0/10	3/10 (3, 1+)
Hydropic change; renal proximal tubule	0/10	0/10	0/10	0/10	0/10	0/10	6/10^d (6, 1+)

^aData are presented for sacrificed animals.

^bValues listed are the number of animals with the indicated lesion. Values in parentheses, are the number of lesion bearing animals for a given grade of lesion severity. Severity key: 1+, slight and , 2+, moderate.

^c $p \leq 0.01$ by χ^2 test.

^d $p \leq 0.05$ by χ^2 test.

^eData were not reported for male rats.

Source: Kasai et al. (2008)

4.2.2.2. Chronic Inhalation Toxicity and Carcinogenicity

1 **4.2.2.2.1. Torkelson et al.** Whole body exposures of male and female Wistar rats (288/sex) to
2 1,4-dioxane vapors (99.9% pure) at a concentration of 0.4 mg/L (111 ppm), were carried out
3 7 hours/day, 5 days/week for 2 years ([Torkelson, et al., 1974](#)). The age of the animals at the
4 beginning of the study was not provided. The concentration of 1,4-dioxane vapor during
5 exposures was determined with infrared analyzers. Food and water were available ad libitum
6 except during exposures. Endpoints examined included clinical signs, eye and nasal irritation,
7 skin condition, respiratory distress, and tumor formation. BWs were determined weekly.
8 Standard hematological parameters were determined on all surviving animals after 16 and
9 23 months of exposure. Blood collected at termination was used also for determination of
10 clinical chemistry parameters (serum AST and ALP activities, blood urea nitrogen [BUN], and
11 total protein). Liver, kidneys, and spleen were weighed and the major tissues and organs were
12 processed for microscopic examination (lungs, trachea, thoracic lymph nodes, heart, liver,
13 pancreas, stomach, intestine, spleen, thyroid, mesenteric lymph nodes, kidneys, urinary bladder,
14 pituitary, adrenals, testes, ovaries, oviduct, uterus, mammary gland, lacrimal gland, lymph nodes,
15 brain, vagina, and bone marrow, and any abnormal growths). Nasal tissues were not obtained for
16 histopathological evaluation. Control and experimental groups were compared statistically using
17 Student's t test, Yates corrected χ^2 test, or Fisher's Exact test.

18 Exposure to 1,4-dioxane vapors had no significant effect on mortality or BW gain and
19 induced no signs of eye or nasal irritation or respiratory distress. Slight, but statistically
20 significant, changes in hematological and clinical chemistry parameters were within the normal
21 physiological limits and were considered to be of no toxicological importance by the
22 investigators. Altered hematological parameters included decreases in packed cell volume, RBC
23 count, and hemoglobin, and an increase in WBC count in male rats. Clinical chemistry changes
24 consisted of a slight decrease in both BUN (control— 23 ± 9.9 ; 111-ppm 1,4-dioxane— $19.8 \pm$
25 8.8) and ALP activity (control— 34.4 ± 12.1 ; 111-ppm 1,4-dioxane— 29.9 ± 9.2) and a small
26 increase in total protein (control— 7.5 ± 0.37 ; 111-ppm 1,4-dioxane— 7.9 ± 0.53) in male rats
27 (values are mean \pm standard deviation). Organ weights were not significantly affected.
28 Microscopic examination of organs and tissues did not reveal any treatment-related effects.

1 Based on the lack of significant effects on several endpoints, EPA identified the exposure
2 concentration of 0.4 mg/L (111 ppm) as a free standing NOAEL. The true NOAEL was likely to
3 be higher.

4 Tumors, observed in all groups including controls, were characteristic of the rat strain
5 used and were considered unrelated to 1,4-dioxane inhalation. The most common tumors were
6 reticulum cell sarcomas and mammary tumors. Using Fisher's Exact test and a significance level
7 of $p < 0.05$, no one type of tumor occurred more frequently in treated rats than in controls. No
8 hepatic or nasal cavity tumors were seen in any rat.

9 4.2.2.2.2. *Kasai et al.* Groups of male 6-week-old F344/DuCrj rats (50/group) weighing $120 \pm$
10 5g (mean \pm SD) at the beginning of the study were exposed via inhalation to nominal
11 concentrations of 0 (clean air), 50, 250, and 1,250 ppm (0, 180, 900, and 4,500 mg/m³,
12 respectively) of vaporized 1,4-dioxane (>99% pure) for 6 hours/day, 5 days/week, for 104 weeks
13 (2 years) in whole body inhalation chambers (Kasai, et al., 2009). Each inhalation chamber
14 housed male rats individually in stainless-steel wire hanging cages. The authors stated female
15 counterparts were not exposed given data illustrating the absence of induced mesotheliomas
16 following exposure to 1,4-dioxane in drinking water (Yamazaki, et al., 1994). During exposure,
17 the concentration of 1,4-dioxane vapor was determined every 15 minutes by gas chromatography
18 and animals received food and water ad libitum. In addition, during the 2-year exposure period,
19 clinical signs and mortality were recorded daily. BW and food intake were measured once
20 weekly for the first 14 weeks of exposure, and thereafter, every 4 weeks. At the end of the 2-
21 year exposure period or at the time of an animal's death during exposure, all organs were
22 collected, weighed, and evaluated for macroscopic lesions. Additional examinations were
23 completed on rats sacrificed at the end of the 2-year exposure period. Endpoints examined
24 included: 1) histopathological evaluations of organs and tissues outlined in the OECD test
25 guideline which included all tissues of the respiratory tract; 2) measurement of urinary
26 parameters using Ames reagent strips during the last week of the exposure period; and 3)
27 measurement of hematological parameters using blood collected from the abdominal aorta of rats
28 following an overnight fasting at the end of the 2-year exposure period. Organs and tissues
29 collected for histopathological examination were fixed in 10% neutral buffered formalin with the
30 exception of nasal cavity samples. Nasal tissue was trimmed transversely at three levels after
31 decalcification and fixation in a formic acid-formalin solution. The levels were demarcated at
32 the following points: at the posterior edge of the upper incisor teeth (level 1), at the incisive
33 papilla (level 2), and at the anterior edge of the upper molar teeth (level 3). All tissue samples
34 were embedded in paraffin, and then sectioned (at 5 μ m thickness) and stained with hematoxylin
35 and eosin (H&E). For measured hematological parameters, analyses included: red blood cell
36 count, hemoglobin, hematocrit, MCV, mean corpuscular hemoglobin (MCH), AST, ALT, ALP,
37 and γ -GTP. Dunnett's test, χ^2 test, and Fisher's exact test were used to determine statistical

1 differences between 1,4-dioxane exposed and clean air exposed group data. A p-value of 0.05
2 was considered the threshold for significance.

3 Growth rates and survival rates were analyzed. Growth rates were not significantly
4 affected by 1,4-dioxane exposures, but a decreasing trend in growth was observed during the
5 latter half of the 2-year exposure period for all exposure doses (i.e., 50, 250, and 1,250 ppm).
6 Survival rates were significantly decreased following 91 weeks of exposure to 1,250 ppm of
7 1,4-dioxane. The authors attributed these deaths to increased incidences of peritoneal
8 mesotheliomas, but also noted that nasal tumors could be a contributing factor. Terminal
9 survival rates were 37/50, 37/50, 29/50, and 25/50 for 0, 50, 250, and 1,250 ppm exposed groups,
10 respectively. Statistically significant changes in body and organ weights were also observed.
11 Following exposure to 1,250 ppm of 1,4-dioxane, terminal body weights of rats were 6% less
12 than the control ($p < 0.05$) and relative liver and lung weights of rats were 27% ($p < 0.01$), and 2%
13 greater than the control, respectively. It is of note that the observed change in terminal body
14 weight was not an effect of food consumption, which was determined to be unaltered by the
15 study authors.

16 Deformity in the nose was the only clinical sign reported in this study. This deformity
17 was seen at exposure weeks 74 and 79 in one rat each, exposed to 250 ppm and 1,250 ppm of
18 1,4-dioxane, respectively. Both of these rats did not survive the 2-year exposure with deaths
19 caused by malignant nasal tumors. Altered hematological parameters were observed with
20 significant changes at 1,250 ppm. Altered endpoints included decreased hemoglobin ($p < 0.05$),
21 MCV ($p < 0.05$), and MCH ($p < 0.01$) and increased AST ($p < 0.01$), ALT ($p < 0.01$), ALP ($p <$
22 0.01), and γ -GTP ($p < 0.01$) levels. In addition, urine pH was decreased in 1,250 ppm exposed
23 rats ($p < 0.05$).

24 Histopathology findings of pre- and nonneoplastic lesions associated with 1,4-dioxane
25 treatment were seen in the nasal cavity, liver, and kidneys (Table 4-16). At the highest
26 concentration of 1,250 ppm, all pre- and nonneoplastic lesions were significantly increased, as
27 compared to controls, with the exception of clear and mixed cell foci in the liver. At the lowest
28 concentration of 50 ppm, nuclear enlargement of the respiratory epithelium was the most
29 sensitive lesion observed in the nasal cavity. Based on this finding, the study authors identified a
30 LOAEL of 50 ppm in male rats.

31 Tumor development was observed in the nasal cavity (squamous cell carcinoma), liver
32 (hepatocellular adenoma and carcinoma), peritoneum (peritoneal mesothelioma), kidney (renal
33 cell carcinoma), mammary gland (fibroadenoma and adenoma), Zymbal gland (adenoma), and
34 subcutaneous tissue (subcutis fibroma). Tumor incidences with a dose-dependent, statistically
35 significant positive trend (Peto's test) included nasal squamous cell carcinoma ($p < 0.01$),
36 hepatocellular adenoma ($p < 0.01$), peritoneal mesothelioma ($p < 0.01$), mammary gland
37 fibroadenoma ($p < 0.05$), and Zymbal gland adenoma ($p < 0.01$). Renal cell carcinoma was also

1 identified as statistically significant with a positive dose-dependent trend ($p \leq 0.01$); however, no
 2 tumor incidences were reported at 50 and 250 ppm. At 1,250 ppm, significant increases in nasal
 3 squamous cell carcinoma, hepatocellular adenoma, and peritoneal mesothelioma were observed.
 4 At 250 ppm, significant increases in peritoneum mesothelioma and subcutis fibroma were
 5 observed. Table 4-17 presents a summary of tumor incidences found in this study. Further
 6 characterizations of neoplasms revealed nasal squamous cell carcinoma occurred at the dorsal
 7 area of the nose (levels 1-3) marked by keratinization and the progression of growth into
 8 surrounding tissue. Peritoneal mesotheliomas were characterized by complex branching
 9 structures originating from the mesothelium of the scrotal sac. Invasive growth into surrounding
 10 tissues was occasionally observed for peritoneal mesotheliomas.

Table 4-16. Incidence of pre-and nonneoplastic lesions in male F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years.

<u>Effect</u>	<u>1,4-dioxane vapor concentration (ppm)</u>			
	<u>0 (clean air)</u>	<u>50</u>	<u>250</u>	<u>1,250</u>
<u>Nuclear enlargement; nasal respiratory epithelium</u>	<u>0/50</u>	<u>50/50^a</u>	<u>48/50^a</u>	<u>38/50^a</u>
<u>Squamous cell metaplasia; nasal respiratory epithelium</u>	<u>0/50</u>	<u>0/50</u>	<u>7/50^b</u>	<u>44/50^a</u>
<u>Squamous cell hyperplasia; nasal respiratory epithelium</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>	<u>10/50^a</u>
<u>Inflammation; nasal respiratory epithelium</u>	<u>13/50</u>	<u>9/50</u>	<u>7/50</u>	<u>39/50^a</u>
<u>Nuclear enlargement; nasal olfactory epithelium</u>	<u>0/50</u>	<u>48/50^a</u>	<u>48/50^a</u>	<u>45/50^a</u>
<u>Respiratory metaplasia; nasal olfactory epithelium</u>	<u>11/50</u>	<u>34/50^a</u>	<u>49/50^a</u>	<u>48/50^a</u>
<u>Atrophy; nasal olfactory epithelium</u>	<u>0/50</u>	<u>40/50^a</u>	<u>47/50^a</u>	<u>48/50^a</u>
<u>Inflammation; nasal olfactory epithelium</u>	<u>0/50</u>	<u>2/50</u>	<u>32/50^a</u>	<u>34/50^a</u>
<u>Hydropic change; lamina propria</u>	<u>0/50</u>	<u>2/50</u>	<u>36/50^a</u>	<u>49/50^a</u>
<u>Sclerosis; lamina propria</u>	<u>0/50</u>	<u>0/50</u>	<u>22/50^a</u>	<u>40/50^a</u>
<u>Proliferation; nasal gland</u>	<u>0/50</u>	<u>1/50</u>	<u>0/50</u>	<u>6/50^b</u>
<u>Nuclear enlargement; liver centrilobular</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>	<u>30/50^a</u>
<u>Necrosis; liver centrilobular</u>	<u>1/50</u>	<u>3/50</u>	<u>6/50</u>	<u>12/50^a</u>
<u>Spongiosis hepatitis; liver</u>	<u>7/50</u>	<u>6/50</u>	<u>13/50</u>	<u>19/50^a</u>
<u>Clear cell foci; liver</u>	<u>15/50</u>	<u>17/50</u>	<u>20/50</u>	<u>23/50</u>
<u>Basophilic cell foci; liver</u>	<u>17/50</u>	<u>20/50</u>	<u>15/50</u>	<u>44/50^a</u>
<u>Acidophilic cell foci; liver</u>	<u>5/50</u>	<u>10/50</u>	<u>12/50</u>	<u>25/50^a</u>
<u>Mixed-cell foci; liver</u>	<u>5/50</u>	<u>3/50</u>	<u>4/50</u>	<u>14/50</u>
<u>Nuclear enlargement; kidney proximal tubule</u>	<u>0/50</u>	<u>1/50</u>	<u>20/50^a</u>	<u>47/50^a</u>
<u>Hydropic change; kidney proximal tubule</u>	<u>0/50</u>	<u>0/50</u>	<u>5/50</u>	<u>6/50^a</u>

^a $p < 0.01$ by Fisher's exact test.

^b $p < 0.05$ by Fisher's exact test.

Source: Kasai et al. (2009).

Table 4-17. Incidence of tumors in male F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years.

<u>Effect</u>	<u>1,4-dioxane vapor concentration (ppm)</u>			
	<u>0 (clean air)</u>	<u>50</u>	<u>250</u>	<u>1,250</u>
<u>Nasal squamous cell carcinoma</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>	<u>6/50^{b,c}</u>
<u>Hepatocellular adenoma</u>	<u>1/50</u>	<u>2/50</u>	<u>3/50</u>	<u>21/50^{a,c}</u>
<u>Hepatocellular carcinoma</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>	<u>2/50</u>
<u>Renal cell carcinoma</u>	<u>0/50</u>	<u>0/50</u>	<u>0/50</u>	<u>4/50</u>
<u>Peritoneal mesothelioma</u>	<u>2/50</u>	<u>4/50</u>	<u>14/50^a</u>	<u>41/50^{a,c}</u>
<u>Mammary gland fibroadenoma</u>	<u>1/50</u>	<u>2/50</u>	<u>3/50</u>	<u>5/50^d</u>
<u>Mammary gland adenoma</u>	<u>0/50</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>
<u>Zymbal gland adenoma</u>	<u>0/50</u>	<u>0/50</u>	<u>0/50</u>	<u>4/50^c</u>
<u>Subcutis fibroma</u>	<u>1/50</u>	<u>4/50</u>	<u>9/50^a</u>	<u>5/50</u>

^ap< 0.01 by Fisher's exact test.

^bp< 0.05 by Fisher's exact test.

^cp< 0.01 by Peto's test for dose-related trend.

^dp< 0.05 by Peto's test for dose-related trend.

Source: Kasai et al. (2009).

4.2.3. Initiation/Promotion Studies

4.2.3.1. Bull et al.

1 Bull et al. (1986) tested 1,4-dioxane as a cancer initiator in mice using oral,
 2 subcutaneous, and topical routes of exposure. A group of 40 female SENCAR mice (6–8 weeks
 3 old) was administered a single dose of 1,000 mg/kg 1,4-dioxane (purity >99%) by gavage,
 4 subcutaneous injection, or topical administration (vehicle was not specified). A group of rats
 5 was used as a vehicle control (number of animals not specified). Food and water were provided
 6 ad libitum. Two weeks after administration of 1,4-dioxane, 12-O-tetradecanoylphorbol-13-
 7 acetate (TPA) (1.0 µg in 0.2 mL of acetone) was applied to the shaved back of mice
 8 3 times/week for a period of 20 weeks. The yield of papillomas at 24 weeks was selected as a
 9 potential predictor of carcinoma yields at 52 weeks following the start of the promotion
 10 schedule. Acetone was used instead of TPA in an additional group of 20 mice in order to
 11 determine whether a single dose of 1,4-dioxane could induce tumors in the absence of TPA
 12 promotion.

13 1,4-Dioxane did not increase the formation of papillomas compared to mice initiated with
 14 vehicle and promoted with TPA, indicating lack of initiating activity under the conditions of the
 15 study. Negative results were obtained for all three exposure routes. A single dose of
 16 1,4-dioxane did not induce tumors in the absence of TPA promotion.

4.2.3.2. *King et al.*

1 1,4-Dioxane was evaluated for complete carcinogenicity and tumor promotion activity in
2 mouse skin ([King, Shefner, & Bates, 1973](#)). In the complete carcinogenicity study, 0.2 mL of a
3 solution of 1,4-dioxane (purity not specified) in acetone was applied to the shaved skin of the
4 back of Swiss Webster mice (30/sex) 3 times/week for 78 weeks. Acetone was applied to the
5 backs of control mice (30/sex) for the same time period. In the promotion study, each animal
6 was treated with 50 µg of dimethylbenzanthracene 1 week prior to the topical application of the
7 1,4-dioxane solution described above (0.2 mL, 3 times/week, 78 weeks) (30 mice/sex). Acetone
8 vehicle was used in negative control mice (30/sex). Croton oil was used as a positive control in
9 the promotion study (30/sex). Weekly counts of papillomas and suspect carcinomas were made
10 by gross examination. 1,4-Dioxane was also administered in the drinking water (0.5 and 1%) to
11 groups of Osborne-Mendel rats (35/sex/group) and B6C3F₁ mice for 42 weeks (control findings
12 were only reported for 34 weeks).

13 1,4-Dioxane was negative in the complete skin carcinogenicity test using dermal
14 exposure. One treated female mouse had malignant lymphoma; however, no papillomas were
15 observed in male or female mice by 60 weeks. Neoplastic lesions of the skin, lungs, and kidney
16 were observed in mice given the promotional treatment with 1,4-dioxane. In addition, the
17 percentage of mice with skin tumors increased sharply after approximately 10 weeks of
18 promotion treatment. Significant mortality was observed when 1,4-dioxane was administered as
19 a promoter (only 4 male and 5 female mice survived for 60 weeks), but not as a complete
20 carcinogen (22 male and 25 female mice survived until 60 weeks). The survival of acetone-
21 treated control mice in the promotion study was not affected (29 male and 26 female mice
22 survived until 60 weeks); however, the mice treated with croton oil as a positive control
23 experienced significant mortality (0 male and 1 female mouse survived for 60 weeks). The
24 incidence of mice with papillomas was similar for croton oil and 1,4-dioxane; however, the
25 tumor multiplicity (i.e., number of tumors/mouse) was higher for the croton oil treatment.

26 Oral administration of 1,4-dioxane in drinking water caused appreciable mortality in rats,
27 but not mice, and increased weight gain in surviving rats and male mice. Histopathological
28 lesions (i.e., unspecified liver and kidney effects) were also reported in exposed male and female
29 rats; however, no histopathological changes were indicated for mice.

30 1,4-Dioxane was demonstrated to be a tumor promoter, but not a complete carcinogen in
31 mouse skin, in this study. Topical administration for 78 weeks following initiation with
32 dimethylbenzanthracene caused an increase in the incidence and multiplicity of skin tumors in
33 mice. Tumors were also observed at remote sites (i.e., kidney and lung), and survival was
34 affected. Topical application of 1,4-dioxane for 60 weeks in the absence of the initiating
35 treatment produced no effects on skin tumor formation or mortality in mice.

4.2.3.3. *Lundberg et al.*

1 Lundberg et al. ([1987](#)) evaluated the tumor promoting activity of 1,4-dioxane in rat liver.
2 Male Sprague Dawley rats (8/dose group, 19 for control group) weighing 200 g underwent a
3 partial hepatectomy followed 24 hours later by an i.p. injection of 30 mg/kg diethylnitrosamine
4 (DEN) (initiation treatment). 1,4-Dioxane (99.5% pure with 25 ppm butylated hydroxytoluene
5 as a stabilizer) was then administered daily by gavage (in saline vehicle) at doses of 0, 100, or
6 1,000 mg/kg-day, 5 days/week for 7 weeks. Control rats were administered saline daily by
7 gavage, following DEN initiation. 1,4-Dioxane was also administered to groups of rats that were
8 not given the DEN initiating treatment (saline used instead of DEN). Ten days after the last
9 dose, animals were sacrificed and liver sections were stained for GGT. The number and total
10 volume of GGT-positive foci were determined.

11 1,4-Dioxane did not increase the number or volume of GGT-foci in rats that were not
12 given the DEN initiation treatment. The high dose of 1,4-dioxane (1,000 mg/kg-day) given as a
13 promoting treatment (i.e., following DEN injection) produced an increase in the number of
14 GGT-positive foci and the total foci volume. Histopathological changes were noted in the livers
15 of high-dose rats. Enlarged, foamy hepatocytes were observed in the midzonal region of the
16 liver, with the foamy appearance due to the presence of numerous fat-containing cytoplasmic
17 vacuoles. These results suggest that cytotoxic doses of 1,4-dioxane may be associated with
18 tumor promotion of 1,4-dioxane in rat liver.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. *Giavini et al.*

19 Pregnant female Sprague Dawley rats (18–20 per dose group) were given 1,4-dioxane
20 (99% pure, 0.7% acetal) by gavage in water at concentrations of 0, 0.25, 0.5, or 1 mL/kg-day,
21 corresponding to dose estimates of 0, 250, 500, or 1,000 mg/kg-day (density of 1,4-dioxane is
22 approximately 1.03 g/mL) ([Giavini, Vismara, & Broccia, 1985](#)). The chemical was administered
23 at a constant volume of 3 mL/kg on days 6–15 of gestation. Food consumption was determined
24 daily and BWs were measured every 3 days. The dams were sacrificed with chloroform on
25 gestation day 21 and the numbers of corpora lutea, implantations, resorptions, and live fetuses
26 were recorded. Fetuses were weighed and examined for external malformations prior to the
27 evaluation of visceral and skeletal malformations (Wilson’s free-hand section method and
28 staining with Alizarin red) and a determination of the degree of ossification.

29 Maternal weight gain was reduced by 10% in the high-dose group (1,000 mg/kg-day).
30 Food consumption for this group was 5% lower during the dosing period, but exceeded control
31 levels for the remainder of the study. No change from control was observed in the number of
32 implantations, live fetuses, or resorptions; however, fetal birth weight was 5% lower in the

1 highest dose group ($p < 0.01$). 1,4-Dioxane exposure did not increase the frequency of major
2 malformations or minor anomalies and variants. Ossification of the sternebrae was reduced in
3 the 1,000 mg/kg-day dose group ($p < 0.05$). The study authors suggested that the observed delay
4 in sternebrae ossification combined with the decrease in fetal birth weight indicated a
5 developmental delay related to 1,4-dioxane treatment. NOAEL and LOAEL values of 500 and
6 1,000 mg/kg-day were identified from this study by EPA and based on delayed ossification of
7 the sternebrae and reduced fetal BWs.

4.4. OTHER DURATION OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute and Short-term Toxicity

8 The acute (≤ 24 hours) and short-term toxicity studies (<30 days) of 1,4-dioxane in
9 laboratory animals are summarized in Table 4-18. Several exposure routes were employed in
10 these studies, including dermal application, drinking water exposure, gavage, vapor inhalation,
11 and i.v. or i.p. injection.

4.4.1.1. Oral Toxicity

12 Mortality was observed in many acute high-dose studies, and LD50 values for
13 1,4-dioxane were calculated for rats, mice, and guinea pigs ([Laug, Calvery, Morris, & Woodard, 1939](#);
14 [Pozzani, Weil, & Carpenter, 1959](#); [Smyth Hf Jr, Seaton, & Fischer, 1941](#)). Clinical signs
15 of CNS depression were observed, including staggered gait, narcosis, paralysis, coma, and death
16 ([de Navasquez, 1935](#); [Laug, et al., 1939](#); [Nelson, 1951](#); [Schrenk & Yant, 1936](#)). Severe liver and
17 kidney degeneration and necrosis were often seen in acute studies ([David, 1964](#); [de Navasquez, 1935](#);
18 [JBRC, 1998](#); [Kesten, Mulinos, & Pomerantz, 1939](#); [Laug, et al., 1939](#); [Schrenk & Yant, 1936](#)).
19 JBRC (1998) additionally reported histopathological lesions in the nasal cavity and the
20 brain of rats following 2 weeks of exposure to 1,4-dioxane in the drinking water.

4.4.1.2. Inhalation Toxicity

21 Acute and short-term toxicity studies (all routes) are summarized in Table 4-18.
22 Mortality occurred in many high-concentration studies ([Nelson, 1951](#); [Pozzani, et al., 1959](#);
23 [Wirth & Klimmer, 1936](#)). Inhalation of 1,4-dioxane caused eye and nasal irritation, altered
24 respiration, and pulmonary edema and congestion ([Yant, et al., 1930](#)). Clinical signs of CNS
25 depression were observed, including staggered gait, narcosis, paralysis, coma, and death ([Nelson, 1951](#);
26 [Wirth & Klimmer, 1936](#)). Liver and kidney degeneration and necrosis were also seen in
27 acute and short-term inhalation studies ([Drew, Patel, & Lin, 1978](#); [Fairley, et al., 1934](#)).

Table 4-18. Acute and short-term toxicity studies of 1,4-dioxane

Animal	Exposure route	Test conditions	Results	Dose ^a	Reference
Oral studies					
Rat (inbred strain and gender unspecified)	Oral via drinking water	1–10 days of exposure	Ultrastructural changes in the kidney, degenerative nephrosis, hyaline droplet accumulation, crystal formation in mitochondria	11,000 mg/kg-day (5%)	David (1964)
Rat (strain and gender unspecified)	Oral via drinking water	5–12 days of exposure	Extensive degeneration of the kidney, liver damage, mortality in 8/10 animals by 12 days	11,000 mg/kg-day (5%)	Kesten et al. (1939)
F344/DuCrj rat	Oral via drinking water	14-Day exposure	Mortality, decreased BWs, histopathological lesions in the nasal cavity, liver, kidney, and brain	2,500 mg/kg-day (nuclear enlargement of olfactory epithelial cells), >7,500 mg/kg-day for all other effects	JBRC (1998)
Female Sprague Dawley rat	Gavage	0, 168, 840, 2550, or 4,200 mg/kg by gavage, 21 and 4 hours prior to sacrifice	Increased ODC activity, hepatic CYP450 content, and DNA single-strand breaks	840 mg/kg (ODC activity only)	Kitchin and Brown (1990)
Female Carworth Farms-Nelson rat	Gavage	Determination of a single dose LD ₅₀	Lethality	LD ₅₀ = 6,400 mg/kg (14,200 ppm)	Pozzani et al. (1959)
Male Wistar rat, guinea pig	Gavage	Single dose, LD ₅₀ determination	Lethality	LD ₅₀ (mg/kg): rat = 7,120 guinea pig = 3,150	Smyth et al. (1941)
Rat, mouse, guinea pig	Gavage	Single dose; several dose groups	Clinical signs of CNS depression, stomach hemorrhage, kidney enlargement, and liver and kidney degeneration	LD ₅₀ (mg/kg): mouse = 5,900 rat = 5,400 guinea pig = 4,030	Laug et al. (1939)
Rabbit	Gavage	Single gavage dose of 0, 207, 1,034, or 2,068 mg/kg-day	Clinical signs of CNS depression, mortality at 2068 mg/kg, renal toxicity (polyuria followed by anuria), histopathological changes in liver and kidneys	1,034 mg/kg-day	de Navasquez (1935)
Rat, rabbit	Gavage	Single dose; mortality after 2 weeks	Mortality and narcosis	3,160 mg/kg	Nelson (1951)

Animal	Exposure route	Test conditions	Results	Dose ^a	Reference
Crj:BDF1 mouse	Oral via drinking water	14-Day exposure	Mortality, decreased BWs, histopathological lesions in the nasal cavity, liver, kidney, and brain	10,800 mg/kg-day; hepatocellular swelling	JBRC (1998)
Dog	Drinking water ingestion	3–10 days of exposure	Clinical signs of CNS depression, and liver and kidney degeneration	11,000 mg/kg-day (5%)	Schrenk and Yant (1936)
Inhalation studies					
Male CD1 rat	Vapor inhalation	Serum enzymes measured before and after a single 4 hour exposure	Increase in ALT, AST, and OCT; no change in G-6-Pase	1,000 ppm	Drew et al. (1978)
Rat	Vapor inhalation	5 hours of exposure	Mortality and narcosis	6,000 ppm	Nelson (1951)
Female Carworth Farms-Nelson rat	Vapor inhalation	Determination of a 4-hour inhalation LC ₅₀	Lethality	LC ₅₀ = 51.3 mg/L	Pozzani et al. (1959)
Mouse, cat	Vapor inhalation	8 hours/day for 17 days	Paralysis and death	8,400 ppm	Wirth and Klimmer (1936)
Guinea pig	Vapor inhalation	8-Hour exposure to 0.1–3% by volume	Eye and nasal irritation, retching movements, altered respiration, narcosis, pulmonary edema and congestion, hyperemia of the brain	0.5% by volume	Yant et al. (1930)
Rabbit, guinea pig, rat, mouse	Vapor inhalation	3 hours exposure, for 5 days; 1.5 hour exposure for 1 day	Degeneration and necrosis in the kidney and liver, vascular congestion in the lungs	10,000 ppm	Fairley et al. (1934)
Other routes					
Male COBS/Wistar rat	Dermal	Nonoccluded technique using shaved areas of the back and flank; single application, 14-day observation	Negative; no effects noted	8,300 mg/kg	Clark et al. (1984)
Rabbit, cat	i.v. injection	Single injection of 0, 207, 1,034, 1,600 mg/kg-day	Clinical signs of CNS depression, narcosis at 1,034 mg/kg, mortality at 1,600 mg/kg	1,034 mg/kg-day	de Navasquez (1935)

Animal	Exposure route	Test conditions	Results	Dose ^a	Reference
Female Sprague Dawley rat	i.p. injection	Single dose; LD ₅₀ values determined 24 hours and 14 days after injection	Increased serum SDH activity at 1/16th of the LD ₅₀ dose; no change at higher or lower doses	LD ₅₀ (mg/kg): 24 hours = 4,848 14 days = 799	Lundberg et al. (1986)
CBA/J mouse	i.p. injection	Daily injection for 7 days, 0, 0.1, 1, 5, and 10%	Slightly lower lymphocyte response to mitogens	2,000 mg/kg-day (10%)	Thurman et al. (1978)

^aLowest effective dose for positive results/ highest dose tested for negative results.

ND = no data; OCT = ornithine carbamyl transferase; ODC = ornithine decarboxylase; SDH = sorbitol dehydrogenase

4.4.2. Neurotoxicity

1 Clinical signs of CNS depression have been reported in humans and laboratory animals
2 following high dose exposure to 1,4-dioxane (see Sections 4.1 and 4.2.1.1). Neurological
3 symptoms were reported in the fatal case of a worker exposed to high concentrations of
4 1,4-dioxane through both inhalation and dermal exposure ([Johnstone, 1959](#)). These symptoms
5 included headache, elevation in blood pressure, agitation and restlessness, and coma. Autopsy
6 findings demonstrated perivascular widening in the brain, with small foci of demyelination in
7 several regions (e.g., cortex, basal nuclei). It was suggested that these neurological changes may
8 have been secondary to anoxia and cerebral edema. In laboratory animals, the neurological
9 effects of acute high-dose exposure included staggered gait, narcosis, paralysis, coma, and death
10 ([de Navasquez, 1935](#); [Laug, et al., 1939](#); [Nelson, 1951](#); [Schrenk & Yant, 1936](#); [Yant, et al.,](#)
11 [1930](#)). The neurotoxicity of 1,4-dioxane was further investigated in several studies described
12 below ([Frantik, Hornychova, & Horvath, 1994](#); [Goldberg, Johnson, Pozzani, & Smyth, 1964](#);
13 [Kanada, Miyagawa, Sato, Hasegawa, & Honma, 1994](#); [Knoefel, 1935](#)).

4.4.2.1. *Frantik et al.*

14 The acute neurotoxicity of 1,4-dioxane was evaluated following a 4-hour inhalation
15 exposure to male Wistar rats (four per dose group) and a 2-hour inhalation exposure to female
16 H-strain mice (eight per dose group) ([Frantik, et al., 1994](#)). Three exposure groups and a control
17 group were used in this study. Exposure concentrations were not specified, but apparently were
18 chosen from the linear portion of the concentration-effect curve. The neurotoxicity endpoint
19 measured in this study was the inhibition of the propagation and maintenance of an electrically-
20 evoked seizure discharge. This endpoint has been correlated with the behavioral effects and
21 narcosis that occur following acute exposure to higher concentrations of organic solvents.
22 Immediately following 1,4-dioxane exposure, a short electrical impulse was applied through ear
23 electrodes (0.2 seconds, 50 hertz (Hz), 180 volts (V) in rats, 90 V in mice). Several time
24 characteristics of the response were recorded; the most sensitive and reproducible measures of

1 chemically-induced effects were determined to be the duration of tonic hind limb extension in
2 rats and the velocity of tonic extension in mice.

3 Linear regression analysis of the concentration-effect data was used to calculate an
4 isoeffective air concentration that corresponds to the concentration producing a 30% decrease in
5 the maximal response to an electrically-evoked seizure. The isoeffective air concentrations for
6 1,4-dioxane were $1,860 \pm 200$ ppm in rats and $2,400 \pm 420$ ppm in mice. A NOAEL value was
7 not identified from this study.

4.4.2.2. *Goldberg et al.*

8 Goldberg et al. ([1964](#)) evaluated the effect of solvent inhalation on pole climb
9 performance in rats. Female rats (Carworth Farms Elias strain) (eight per dose group) were
10 exposed to 0, 1,500, 3,000, or 6,000 ppm of 1,4-dioxane in air for 4 hours/day, 5 days/weeks, for
11 10 exposure days. Conditioned avoidance and escape behaviors were evaluated using a pole
12 climb methodology. Prior to exposure, rats were trained to respond to a buzzer or shock stimulus
13 by using avoidance/escape behavior within 2 seconds. Behavioral criteria were the abolishment
14 or significant deferment (>6 seconds) of the avoidance response (conditioned or buzzer response)
15 or the escape response (buzzer plus shock response). Behavioral tests were administered on day
16 1, 2, 3, 4, 5, and 10 of the exposure period. Rat BWs were also measured on test days.

17 1,4-Dioxane exposure produced a dose-related effect on conditioned avoidance behavior
18 in female rats, while escape behavior was generally not affected. In the 1,500 ppm group, only
19 one of eight rats had a decreased avoidance response, and this only occurred on days 2 and 5 of
20 exposure. A larger number of rats exposed to 3,000 ppm (two or three of eight) experienced a
21 decrease in the avoidance response, and this response was observed on each day of the exposure
22 period. The maximal decrease in the avoidance response was observed in the 6,000 ppm group
23 during the first 2 days of exposure (75–100% of the animals were inhibited in this response). For
24 exposure days 3–10, the percent of rats in the 6,000 ppm group with significant inhibition of the
25 avoidance response ranged from 37–62%. At the end of the exposure period (day 10), the BWs
26 for rats in the high exposure group were lower than controls.

4.4.2.3. *Kanada et al.*

27 Kanada et al. evaluated the effect of oral exposure to 1,4-dioxane on the regional
28 neurochemistry of the rat brain ([Kanada, et al., 1994](#)). 1,4-Dioxane was administered by gavage
29 to male Sprague Dawley rats (5/group) at a dose of 1,050 mg/kg, approximately equal to one-
30 fourth the oral LD50. Rats were sacrificed by microwave irradiation to the head 2 hours after
31 dosing, and brains were dissected into small brain areas. Each brain region was analyzed for the
32 content of biogenic amine neurotransmitters and their metabolites using high-performance liquid
33 chromatography (HPLC) or GC methods. 1,4-Dioxane exposure was shown to reduce the
34 dopamine and serotonin content of the hypothalamus. The neurochemical profile of all other
35 brain regions in exposed rats was similar to control rats.

4.4.2.4. *Knoefel*

1 The narcotic potency of 1,4-dioxane was evaluated following i.p. injection in rats and
2 gavage administration in rabbits ([Knoefel, 1935](#)). Rats were given i.p. doses of 20, 30, or
3 50 mmol/kg. No narcotic effect was seen at the lowest dose; however, rats given 30 mmol/kg
4 were observed to sleep approximately 8–10 minutes. Rats given the high dose of 50 mmol/kg
5 died during the study. Rabbits were given 1,4-dioxane at oral doses of 10, 20, 50, 75, or
6 100 mmol/kg. No effect on the normal erect animal posture was observed in rabbits treated with
7 less than 50 mmol/kg. At 50 and 75 mmol/kg, a semi-erect or staggering posture was observed;
8 lethality occurred at both the 75 and 100 mmol/kg doses.

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

4.5.1. Genotoxicity

9 The genotoxicity data for 1,4-dioxane are presented in Tables 4-[19](#) and 4-[20](#) for in vitro
10 and in vivo tests, respectively. 1,4-Dioxane has been tested for genotoxic potential using in vitro
11 assay systems with prokaryotic organisms, non-mammalian eukaryotic organisms, and
12 mammalian cells, and in vivo assay systems using several strains of rats and mice. In the large
13 majority of in vitro systems, 1,4-dioxane was not genotoxic. Where a positive genotoxic
14 response was observed, it was generally observed in the presence of toxicity. Similarly,
15 1,4-dioxane was not genotoxic in the majority of available in vivo studies. 1,4-Dioxane did not
16 bind covalently to DNA in a single study with calf thymus DNA. Several investigators have
17 reported that 1,4-dioxane caused increased DNA synthesis indicative of cell proliferation.
18 Overall, the available literature indicates that 1,4-dioxane is nongenotoxic or weakly genotoxic.

19 Negative findings were reported for mutagenicity in in vitro assays with the prokaryotic
20 organisms *Salmonella typhimurium*, *Escherichia coli*, and *Photobacterium phosphoreum*
21 (Mutatox assay) ([Haworth, Lawlor, Mortelmans, Speck, & Zeiger, 1983](#); [Hellmér & Bolcsfoldi, 1992](#);
22 [Khudoley, Mizgireuv, & Pliss, 1987](#); [Kwan, Dutka, Rao, & Liu, 1990](#); [Morita & Hayashi, 1998](#);
23 [Nestmann, Otson, Kowbel, Bothwell, & Harrington, 1984](#); [Stott, et al., 1981](#)). In in vitro
24 assays with nonmammalian eukaryotic organisms, negative results were obtained for the
25 induction of aneuploidy in yeast (*Saccharomyces cerevisiae*) and in the sex-linked recessive
26 lethal test in *Drosophila melanogaster* ([Yoon, Mason, Valencia, Woodruff, & Zimmering, 1985](#);
27 [Zimmermann, Mayer, Scheel, & Resnick, 1985](#)). In the presence of toxicity, positive results
28 were reported for meiotic nondisjunction in *Drosophila* ([Munoz & Barnett, 2002](#)).

29 The ability of 1,4-dioxane to induce genotoxic effects in mammalian cells in vitro has
30 been examined in model test systems with and without exogenous metabolic activation and in
31 hepatocytes that retain their xenobiotic-metabolizing capabilities. 1,4-Dioxane was reported as
32 negative in the mouse lymphoma cell forward mutation assay ([McGregor et al., 1991](#); [Morita &](#)

1 [Hayashi, 1998](#)). 1,4-Dioxane did not produce chromosomal aberrations or micronucleus
2 formation in Chinese hamster ovary (CHO) cells ([Galloway et al., 1987](#); [Morita & Hayashi,](#)
3 [1998](#)). Results were negative in one assay for sister chromatid exchange (SCE) in CHO ([Morita](#)
4 [& Hayashi, 1998](#)) and were weakly positive in the absence of metabolic activation in another
5 ([Galloway, et al., 1987](#)). In rat hepatocytes, 1,4-dioxane exposure in vitro caused single-strand
6 breaks in DNA at concentrations also toxic to the hepatocytes ([Sina, Bean, Dysart, Taylor, &](#)
7 [Bradley, 1983](#)) and produced a positive genotoxic response in a cell transformation assay with
8 BALB/3T3 cells also in the presence of toxicity ([Sheu, Moreland, Lee, & Dunkel, 1988](#)).

9 1,4-Dioxane was not genotoxic in the majority of available in vivo mammalian assays.
10 Studies of micronucleus formation following in vivo exposure to 1,4-dioxane produced mostly
11 negative results, including studies of bone marrow micronucleus formation in B6C3F₁, BALB/c,
12 CBA, and C57BL6 mice ([McFee, Abbott, Gulati, & Shelby, 1994](#); [Mirkova, 1994](#); [Tinwell &](#)
13 [Ashby, 1994](#)) and micronucleus formation in peripheral blood of CD1 mice ([Morita, 1994](#);
14 [Morita & Hayashi, 1998](#)). Mirkova ([1994](#)) reported a dose-related increase in the incidence of
15 bone marrow micronuclei in male and female C57BL6 mice 24 or 48 hours after administration
16 of 1,4-dioxane. At a sampling time of 24 hours, a dose of 450 mg/kg produced no change
17 relative to control, while doses of 900, 1,800, and 3,600 mg/kg increased the incidence of bone
18 marrow micronuclei by approximately two-, three-, and fourfold, respectively. A dose of
19 5,000 mg/kg also increased the incidence of micronuclei by approximately fourfold at 48 hours.
20 This compares with the negative results for BALB/c male mice tested in the same study at a dose
21 of 5,000 mg/kg and sampling time of 24 hours. Tinwell and Ashby ([1994](#)) could not explain the
22 difference in response in the mouse bone marrow micronucleus assay with C57BL6 mice
23 obtained in their laboratory (i.e., non-significant 1.6-fold increase over control) with the dose-
24 related positive findings reported by Mirkova ([Mirkova, 1994](#)) using the same mouse strain,
25 1,4-dioxane dose (3,600 mg/kg) and sampling time (24 hours). Morita and Hayashi ([1998](#))
26 demonstrated an increase in micronucleus formation in hepatocytes following 1,4-dioxane
27 dosing and partial hepatectomy to induce cellular mitosis. DNA single-strand breaks were
28 demonstrated in hepatocytes following gavage exposure to female rats ([Kitchin & Brown, 1990](#)).

29 Roy et al. ([2005](#)) examined micronucleus formation in male CD1 mice exposed to
30 1,4-dioxane to confirm the mixed findings from earlier mouse micronucleus studies and to
31 identify the origin of the induced micronuclei. Mice were administered 1,4-dioxane by gavage at
32 doses of 0, 1,500, 2,500, and 3,500 mg/kg-day for 5 days. The mice were also implanted with
33 5-bromo-2-deoxyuridine (BrdU)-releasing osmotic pumps to measure cell proliferation in the
34 liver and to increase the sensitivity of the hepatocyte assay. The frequency of micronuclei in the
35 bone marrow erythrocytes and in the proliferating BrdU-labeled hepatocytes was determined
36 24 hours after the final dose. Significant dose-related increases in micronuclei were seen in the
37 bone-marrow at all the tested doses ($\geq 1,500$ mg/kg-day). In the high-dose (3,500-mg/kg) mice,

1 the frequency of bone marrow erythrocyte micronuclei was about 10-fold greater than the control
2 frequency. Significant dose-related increases in micronuclei were also observed at the two
3 highest doses ($\geq 2,500$ mg/kg-day) in the liver. Antikinetochore (CREST) staining or
4 pancentromeric fluorescence in situ hybridization (FISH) was used to determine the origin of the
5 induced micronuclei. The investigators determined that 80–90% of the micronuclei in both
6 tissues originated from chromosomal breakage; small increase in micronuclei originating from
7 chromosome loss was seen in hepatocytes. Dose-related statistically significant decreases in the
8 ratio of bone marrow polychromatic erythrocytes (PCE):normochromatic erythrocytes (NCE), an
9 indirect measure of bone marrow toxicity, were observed. Decreases in hepatocyte proliferation
10 were also observed. Based on these results, the authors concluded that at high doses 1,4-dioxane
11 exerts genotoxic effects in both the mouse bone marrow and liver; the induced micronuclei are
12 formed primarily from chromosomal breakage; and 1,4-dioxane can interfere with cell
13 proliferation in both the liver and bone marrow. The authors noted that reasons for the
14 discrepant micronucleus assay results among various investigators was unclear, but could be
15 related to the inherent variability present when detecting moderate to weak responses using small
16 numbers of animals, as well as differences in strain, dosing regimen, or scoring criteria.

17 1,4-Dioxane did not affect in vitro or in vivo DNA repair in hepatocytes or in vivo DNA
18 repair in the nasal cavity ([Goldsworthy, et al., 1991](#); [Stott, et al., 1981](#)), but increased hepatocyte
19 DNA synthesis indicative of cell proliferation in several in vivo studies ([Goldsworthy, et al.,](#)
20 [1991](#); [Miyagawa, Shirotori, Tsuchitani, & Yoshikawa, 1999](#); [Stott, et al., 1981](#); [Uno et al., 1994](#)).
21 1,4-Dioxane caused a transient inhibition of RNA polymerase A and B in the rat liver ([Kurl,](#)
22 [Poellinger, Lund, & Gustafsson, 1981](#)), indicating a negative impact on the synthesis of
23 ribosomal and messenger RNA (DNA transcription). Intravenous administration of 1,4-dioxane
24 at doses of 10 or 100 mg/rat produced inhibition of both polymerase enzymes, with a quicker and
25 more complete recovery of activity for RNA polymerase A, the polymerase for ribosomal RNA
26 synthesis.

27 1,4-Dioxane did not covalently bind to DNA under in vitro study conditions ([Woo,](#)
28 [Argus, et al., 1977b](#)). DNA alkylation was also not detected in the liver 4 hours following a
29 single gavage exposure (1,000 mg/kg) in male Sprague Dawley rats ([Stott, et al., 1981](#)).

30 Rosenkranz and Klopman ([1992](#)) analyzed 1,4-dioxane using the computer automated
31 structure evaluator (CASE) structure activity method to predict its potential genotoxicity and
32 carcinogenicity. The CASE analysis is based on information contained in the structures of
33 approximately 3,000 chemicals tested for endpoints related to mutagenic/genotoxic and
34 carcinogenic potential. CASE selects descriptors (activating [biophore] or inactivating
35 [biophobe] structural fragments) from a learning set of active and inactive molecules. Using the
36 CASE methodology, Rosenkranz and Klopman ([1992](#)) predicted that 1,4-dioxane would be
37 inactive for mutagenicity in several in vitro systems, including Salmonella, induction of

1 chromosomal aberrations in CHO cells, and unscheduled DNA synthesis in rat hepatocytes.
 2 1,4-Dioxane was predicted to induce SCE in cultured CHO cells, micronuclei formation in rat
 3 bone marrow, and carcinogenicity in rodents.

4 Gene expression profiling in cultured human hepatoma HepG2 cells was performed using
 5 DNA microarrays to discriminate between genotoxic and other carcinogens ([van Delft et al.,](#)
 6 [2004](#)). Van Delft et al. ([2004](#)) examined this method using a training set of 16 treatments (nine
 7 genotoxins and seven nongenotoxins) and a validation set (three and three), with discrimination
 8 models based on Pearson correlation analyses for the 20 most discriminating genes. As reported
 9 by the authors ([van Delft, et al., 2004](#)), the gene expression profile for 1,4-dioxane indicated a
 10 classification of this chemical as a “nongenotoxic” carcinogen, and thus, 1,4-dioxane was
 11 included in the training set as a “nongenotoxic” carcinogen. The accuracy for carcinogen
 12 classification using this method ranged from 33 to 100%, depending on which chemical data sets
 13 and gene expression signals were included in the analysis.

Table 4-19. Genotoxicity studies of 1,4-dioxane; in vitro

Test system	Endpoint	Test conditions	Results ^a		Dose ^b	Source
			Without activation	With activation		
Prokaryotic organisms in vitro						
<i>S. typhimurium</i> strains TA98, TA100, TA1535, TA1537	Reverse mutation	Plate incorporation assay	–	–	10,000 µg/plate	Haworth et al. (1983)
<i>S. typhimurium</i> strains TA98, TA100, TA1530, TA1535, TA1537	Reverse mutation	Plate incorporation assay	–	–	ND	Khudoley et al. (1987)
<i>S. typhimurium</i> strains TA98, TA100, TA1535, TA1537	Reverse mutation	Plate incorporation and preincubation assays	–	–	5,000 µg/plate	Morita and Hayashi (1998)
<i>S. typhimurium</i> strains TA100, TA1535	Reverse mutation	Preincubation assay	–	–	103 mg	Nestmann et al. (1984)
<i>S. typhimurium</i> strains TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	Plate incorporation assay	–	–	103 mg	Stott et al. (1981)
<i>E. coli</i> K-12 uvrB/recA	DNA repair	Host mediated assay	–	–	1,150 mmol/L	Hellmer and Bolcsfoldi (1992)
<i>E. coli</i> WP2/WP2uvrA	Reverse mutation	Plate incorporation and preincubation assays	–	–	5,000 µg/plate	Morita and Hayashi (1998)
<i>P. phosphoreum</i> M169	Mutagenicity, DNA damage	Mutatox assay	–	ND	ND	Kwan et al. (1990)

Test system	Endpoint	Test conditions	Results ^a		Dose ^b	Source
			Without activation	With activation		
Nonmammalian eukaryotic organisms in vitro						
<i>S. cerevisiae</i> D61.M	Aneuploidy	Standard 16-hour incubation or cold-interruption regimen	-T	ND	4.75%	Zimmerman et al. (1985)
<i>D. melanogaster</i>	Meiotic nondisjunction	Oocytes were obtained for evaluation 24 and 48 hours after mating	+T ^c	ND ^d	2% in sucrose media	Munoz and Barnett (2002)
<i>D. melanogaster</i>	Sex-linked recessive lethal test	Exposure by feeding and injection	-	ND ^d	35,000 ppm in feed, 7 days or 50,000 ppm (5% in water) by injection	Yoon et al. (1985)
Mammalian cells in vitro						
Rat hepatocytes	DNA damage; single-strand breaks measured by alkaline elution	3-Hour exposure to isolated primary hepatocytes	+T ^e	ND ^d	0.3 mM	Sina et al. (1983)
Primary hepatocyte culture from male F344 rats	DNA repair	Autoradiography	-	ND ^d	1 mM	Goldsworthy et al. (1991)
L5178Y mouse lymphoma cells	Forward mutation assay	Thymidine kinase mutagenicity assay (trifluorothymidine resistance)	-	-	5,000 µg/mL	McGregor et al. (1991)
L5178Y mouse lymphoma cells	Forward mutation assay	Thymidine kinase mutagenicity assay (trifluorothymidine resistance)	-	-T	5,000 µg/mL	Morita and Hayashi (1998)
BALB/3T3 cells	Cell transformation	48-Hour exposure followed by 4 weeks incubation; 13 day exposure followed by 2.5 weeks incubation	+T ^f	ND ^d	0.5 mg/mL	Sheu et al. (1988)

Test system	Endpoint	Test conditions	Results ^a		Dose ^b	Source
			Without activation	With activation		
CHO cells	SCE	BrdU was added 2 hours after 1,4-dioxane addition; chemical treatment was 2 hours with S9 and 25 hours without S9	± ^g	—	10,520 µg/mL	Galloway et al. (1987)
CHO cells	Chromosomal aberration	Cells were harvested 8–12 hours or 18–26 hours after treatment (time of first mitosis)	—	—	10,520 µg/mL	Galloway et al. (1987)
CHO cells	SCE	3 hour pulse treatment; followed by continuous treatment of BrdU for 23 or 26 hours	—	—	5,000 µg/mL	Morita and Hayashi (1998)
CHO cells	Chromosomal aberration	5 hour pulse treatment, 20 hour pulse and continuous treatments, or 44 hour continuous treatment; cells were harvested 20 or 44 hours following exposure	—	—	5,000 µg/mL	Morita and Hayashi (1998)
CHO cells	Micronucleus formation	5 hour pulse treatment or 44 hour continuous treatment; cells were harvested 42 hours following exposure	—	—	5,000 µg/mL	Morita and Hayashi (1998)
Calf thymus DNA	Covalent binding to DNA	Incubation with microsomes from 3-methylcholanthrene treated rats	—	—	0.04 pmol/mg DNA (bound)	Woo et al. (1977b)

Test system	Endpoint	Test conditions	Results ^a		Dose ^b	Source
			Without activation	With activation		

^a+ = positive, ± = equivocal or weak positive, – = negative, T = toxicity. Endogenous metabolic activation is not applicable for in vivo studies.

^bLowest effective dose for positive results/highest dose tested for negative results; ND = no data.

^cRats were given doses of 0, 168, 840, 2,550, or 4,200 mg/kg at 4 and 21 hours prior to sacrifice. A 43 and 50% increase in the fraction of DNA eluted was observed for doses of 2,550 and 4,200 mg/kg, respectively. Alkaline elution of DNA was not significantly different from control in the two lowest dose groups (168 and 840 mg/kg).

^dA dose-related increase in the incidence of bone marrow micronuclei was observed in male and female C57BL6 mice 24 or 48 hours after administration of 1,4-dioxane. A dose of 450 mg/kg produced no change relative to control, while doses of 900, 1,800, 3,600, and 5,000 mg/kg increased the incidence of bone marrow micronuclei by approximately two-, three-, four- and fourfold, respectively.

^eA dose-related increase in the incidence of hepatocyte micronuclei was observed in partially hepatectomized mice 6 days after administration of 1,4-dioxane. A dose of 1,000 mg/kg produced no change relative to control, while doses of 2,000 and 3,000 mg/kg increased the incidence of hepatocyte micronuclei by 2.4- and 3.4-fold, respectively.

^fSignificant increases in the frequency of micronucleated erythrocytes were observed at each test dose of 1,4-dioxane (1,500, 2,500 and 3,500 mg/kg-day, 5 days/week).

^gA dose-related increase in the frequency of micronuclei was observed in proliferating cells with micronuclei at 2,500 and 3,500 mg/kg-day, 5 days/week. No increase in the frequency of micronuclei was seen in the non-proliferating cells.

^hNo increase in the hepatocyte labeling index was observed 24 or 48 hours following a single gavage exposure of 1,000 mg/kg. Continuous administration of 1% 1,4-dioxane in the drinking water for up to 2 weeks produced a twofold increase in the hepatocyte labeling index.

ⁱA similar pattern of RNA polymerase inhibition was observed at doses of 10 and 100 mg/rat. Inhibition was more pronounced at the higher dose.

^jHepatocyte viability was 86, 89, 87, 88, 78, and 86% 24 hours following exposure to 0, 1,000, 1,500, 2,000, or 4,000 mg/kg. The incidence (%) of replicative DNA synthesis was increased by 2.5-fold (1,000 mg/kg) or 4.5-fold (1,500 and 2,000 mg/kg). No increase in replicative DNA synthesis was observed at the highest dose (4,000 mg/kg).

^kReplicative DNA synthesis was measured 24, 39, and 48 hours following a single dose of 0, 1,000, or 2,000 mg/kg. Hepatocyte viability ranged from 71 to 82%. The only increase in replicative DNA synthesis was observed 24 hours after administration of 2,000 mg/kg (threefold increase). Cell viability for this group was 79%.

^lReplicative DNA synthesis was increased 1.5-fold in rats given 1,000 mg/kg of 1,4-dioxane for 11 weeks. No change from control was observed in rats exposed to 10 mg/kg for 11 weeks or rats acutely exposed to 10, 100, or 1,000 mg/kg.

Table 4-20. Genotoxicity studies of 1,4-dioxane; mammalian in vivo

Test system	Endpoint	Test Conditions	Results ^a	Dose ^b	Source
Female Sprague Dawley Rat	DNA damage; single-strand breaks measured by alkaline elution	Two gavage doses given 21 and 4 hours prior to sacrifice	+ ^c	2,550 mg/kg	Kitchin and Brown (1990)
Male Sprague Dawley Rat	DNA alkylation in hepatocytes	Gavage; DNA isolation and HPLC analysis 4 hours after dosing	–	1,000 mg/kg	Stott et al. (1981)
Male B6C3F ₁ Mouse	Micronucleus formation in bone marrow	i.p. injection; analysis of polychromatic erythrocytes 24 or 48 hours after dosing	–	Single dose of 4,000 mg/kg; 3 daily doses of 2,000	McFee et al. (1994)
Male and female C57BL6 Mouse; male BALB/c Mouse	Micronucleus formation in bone marrow	Gavage; analysis of polychromatic erythrocytes 24 or 48 hours after dosing	+ (C57BL6) ^d – (BALB/c)	900 mg/kg (C57BL6); 5,000 mg/kg (BALB/c)	Mirkova (1994)
Male CD1 Mouse	Micronucleus formation in peripheral blood	Two i.p. injections (1/day); micronucleated reticulocytes measured 24, 48, and 72 hours after the 2nd dose	–	3,200 mg/kg	Morita (1994)
Male CD1 Mouse	Micronucleus formation in hepatocytes	Gavage, partial hepatectomy 24 hours after dosing, hepatocytes analyzed 5 days after hepatectomy	+ ^e	2,000 mg/kg	Morita and Hayashi (1998)
Male CD1 Mouse	Micronucleus formation in peripheral blood	Gavage, partial hepatectomy 24 hours after dosing, peripheral blood obtained from tail vein 24 hours after hepatectomy	–	3,000 mg/kg	Morita and Hayashi (1998)
Male CBA and C57BL6 Mouse	Micronucleus formation in bone marrow	Gavage; analysis of polychromatic erythrocytes from specimens prepared 24 hours after dosing	–	3,600 mg/kg	Tinwell and Ashby (1994)
Male CD1 Mouse	Micronuclei formation in bone marrow	Gavage; analysis for micronucleated erythrocytes 24 hours after dosing	+ ^f	1,500 mg/kg-day for 5 days	Roy et al. (2005)
Male CD1 Mouse	Micronuclei formation in hepatocytes	Gavage; analysis for micronuclei 24 hours after dosing	+ ^g	2,500 mg/kg-day for 5 days	Roy et al. (2005)
Male Sprague Dawley Rat	DNA repair in hepatocytes	Drinking water; thymidine incorporation with hydroxyurea to repress normal DNA synthesis	–	1,000 mg/kg-day for 11 weeks	Stott et al. (1981)

Test system	Endpoint	Test Conditions	Results ^a	Dose ^b	Source
Male F344 Rat	DNA repair in hepatocytes (autoradiography)	Gavage and drinking water exposure; thymidine incorporation	–	1,000 mg/kg for 2 or 12 hours; 1,500 mg/kg-day for 2 weeks or 3,000 mg/kg-day for 1 week	Goldsworthy et al. (1991)
Male F344 Rat	DNA repair in nasal epithelial cells from the nasoturbinate or maxilloturbinate	Gavage and drinking water exposure; thymidine incorporation	–	1,500 mg/kg-day for 8 days + 1,000 mg/kg gavage dose 12 hours prior to sacrifice	Goldsworthy et al. (1991)
Male F344 Rat	Replicative DNA synthesis (i.e., cell proliferation) in hepatocytes	Gavage and drinking water exposure; thymidine incorporation	+ ^h (1–2-week exposure)	1,000 mg/kg for 24 or 48 hours; 1,500 mg/kg-day for 1 or 2 weeks	Goldsworthy et al. (1991)
Male F344 Rat	Replicative DNA synthesis (i.e., cell proliferation) in nasal epithelial cells	Drinking water exposure; thymidine incorporation	–	1,500 mg/kg-day for 2 weeks	Goldsworthy et al. (1991)
Male Sprague Dawley Rat	RNA synthesis; inhibition of RNA polymerase A and B	i.v. injection; activity measured in isolated hepatocytes	+ ⁱ	10 mg/rat	Kurl et al. (1981)
Male F344 Rat	DNA synthesis in hepatocytes	Gavage; thymidine and BrdU incorporation	+ ^j	1,000 mg/kg	Miyagawa (1999)
Male F344 Rat	DNA synthesis in hepatocytes	Thymidine incorporation	± ^k	2,000 mg/kg	Uno et al. (1994)
Male Sprague Dawley Rat	DNA synthesis in hepatocytes	Drinking water; thymidine incorporation	+ ^l	1,000 mg/kg-day for 11 weeks	Stott et al. (1981)

^a+ = positive, ± = equivocal or weak positive, – = negative, T = toxicity. Endogenous metabolic activation is not applicable for in vivo studies.

^bLowest effective dose for positive results/highest dose tested for negative results; ND = no data.

^cRats were given doses of 0, 168, 840, 2,550, or 4,200 mg/kg at 4 and 21 hours prior to sacrifice. A 43 and 50% increase in the fraction of DNA eluted was observed for doses of 2,550 and 4,200 mg/kg, respectively. Alkaline elution of DNA was not significantly different from control in the two lowest dose groups (168 and 840 mg/kg).

^dA dose-related increase in the incidence of bone marrow micronuclei was observed in male and female C57BL6 mice 24 or 48 hours after administration of 1,4-dioxane. A dose of 450 mg/kg produced no change relative to control, while doses of 900, 1,800, 3,600, and 5,000 mg/kg increased the incidence of bone marrow micronuclei by approximately two-, three-, four- and fourfold, respectively.

^eA dose-related increase in the incidence of hepatocyte micronuclei was observed in partially hepatectomized mice 6 days after administration of 1,4-dioxane. A dose of 1,000 mg/kg produced no change relative to control, while doses of 2,000 and 3,000 mg/kg increased the incidence of hepatocyte micronuclei by 2.4- and 3.4-fold, respectively.

^fSignificant increases in the frequency of micronucleated erythrocytes were observed at each test dose of 1,4-dioxane (1,500, 2,500 and 3,500 mg/kg-day, 5 days/week).

^gA dose-related increase in the frequency of micronuclei was observed in proliferating cells with micronuclei at 2,500 and 3,500 mg/kg-day, 5 days/week. No increase in the frequency of micronuclei was seen in the non-proliferating cells.

^hNo increase in the hepatocyte labeling index was observed 24 or 48 hours following a single gavage exposure of 1,000 mg/kg. Continuous administration of 1% 1,4-dioxane in the drinking water for up to 2 weeks produced a twofold increase in the hepatocyte labeling index.

ⁱA similar pattern of RNA polymerase inhibition was observed at doses of 10 and 100 mg/rat. Inhibition was more pronounced at the higher dose.

^jHepatocyte viability was 86, 89, 87, 88, 78, and 86% 24 hours following exposure to 0, 1,000, 1,500, 2,000, or 4,000 mg/kg. The incidence (%) of replicative DNA synthesis was increased by 2.5-fold (1,000 mg/kg) or 4.5-fold (1,500 and 2,000 mg/kg). No increase in replicative DNA synthesis was observed at the highest dose (4,000 mg/kg).

^kReplicative DNA synthesis was measured 24, 39, and 48 hours following a single dose of 0, 1,000, or 2,000 mg/kg. Hepatocyte viability ranged from 71 to 82%. The only increase in replicative DNA synthesis was observed 24 hours after administration of 2,000 mg/kg (threefold increase). Cell viability for this group was 79%.

^lReplicative DNA synthesis was increased 1.5-fold in rats given 1,000 mg/kg of 1,4-dioxane for 11 weeks. No change from control was observed in rats exposed to 10 mg/kg for 11 weeks or rats acutely exposed to 10, 100, or 1,000 mg/kg.

4.5.2. Mechanistic Studies

4.5.2.1. Free Radical Generation

1 Burmistrov et al. (2001) investigated the effect of 1,4-dioxane inhalation on free radical
2 processes in the rat ovary and brain. Female rats (6–9/group, unspecified strain) were exposed to
3 0, 10, or 100 mg/m³ of 1,4-dioxane vapor for 4 hours/day, 5 days/week, for 1 month. Rats were
4 sacrificed during the morning or evening following exposure and the ovaries and brain cortex
5 were removed and frozen. Tissue preparations were analyzed for catalase activity, glutathione
6 peroxidase activity, and protein peroxidation. Inhalation of 100 mg/m³ of 1,4-dioxane resulted in
7 a significant increase (p < 0.05) in glutathione peroxidase activity, and activation of free radical
8 processes were apparent in both the rat ovary and brain cortex. No change in catalase activity or
9 protein peroxidation was observed at either concentration. A circadian rhythm for glutathione
10 peroxidase activity was absent in control rats, but occurred in rat brain and ovary following
11 1,4-dioxane exposure.

4.5.2.2. Induction of Metabolism

12 The metabolism of 1,4-dioxane is discussed in detail in Section 3.3. 1,4-Dioxane has
13 been shown to induce its own metabolism (Young, et al., 1978a; Young, et al., 1978b). Nannelli
14 et al. (2005) (study details provided in Section 3.3) characterized the CYP450 isozymes that
15 were induced by 1,4-dioxane in the liver, kidney, and nasal mucosa of the rat. In the liver, the
16 activities of several CYP450 isozymes were increased (i.e., CYP2B1/2, CYP2E1, CYP2C11);
17 however, only CYP2E1 was inducible in the kidney and nasal mucosa. CYP2E1 mRNA was
18 increased approximately two- to threefold in the kidney and nasal mucosa, but mRNA levels
19 were not increased in the liver, suggesting that regulation of CYP2E1 is organ-specific.
20 Induction of hepatic CYPB1/2 and CYP2E1 levels by phenobarbital or fasting did not increase
21 the liver toxicity of 1,4-dioxane, as measured by hepatic glutathione content or serum ALT
22 activity. This result suggested that highly reactive and toxic intermediates did not play a large

1 role in the liver toxicity of 1,4-dioxane, even under conditions where metabolism was enhanced.
2 This finding is similar to an earlier conclusion by Kociba et al. (1975) who evaluated toxicity
3 from a chronic drinking water study alongside data providing a pharmacokinetic profile for
4 1,4-dioxane. Kociba et al. (1975) concluded that liver toxicity and eventual tumor formation
5 occurred only at doses where clearance pathways were saturated and elimination of 1,4-dioxane
6 from the blood was reduced. Nannelli et al. (2005) further suggested that a sustained induction
7 of CYP2E1 may lead to generation of reactive oxygen species contributing to target organ
8 toxicity and regenerative cell proliferation; however, no data were provided to support this
9 hypothesis.

4.5.2.3. *Mechanisms of Tumor Induction*

10 Several studies have been performed to evaluate potential mechanisms for the
11 carcinogenicity of 1,4-dioxane (Goldsworthy, et al., 1991; Kitchin & Brown, 1990; Stott, et al.,
12 1981). Stott et al. (1981) evaluated 1,4-dioxane in several test systems, including salmonella
13 mutagenicity in vitro, rat hepatocyte DNA repair activity in vitro, DNA synthesis determination
14 in male Sprague Dawley rats following acute gavage dosing or an 11-week drinking water
15 exposure (described in Section 4.2.1), and hepatocyte DNA alkylation and DNA repair following
16 a single gavage dose. This study used doses of 0, 10, 100, or 1,000 mg/kg-day, with the highest
17 dose considered to be a tumorigenic dose level. Liver histopathology and liver to BW ratios
18 were also evaluated in rats from acute gavage or repeated dose drinking water experiments.

19 The histopathology evaluation indicated that liver cytotoxicity (i.e., centrilobular
20 hepatocyte swelling) was present in rats from the 1,000 mg/kg-day dose group that received
21 1,4-dioxane in the drinking water for 11 weeks (Stott, et al., 1981). An increase in the liver to
22 BW ratio accompanied by an increase in hepatic DNA synthesis was also seen in this group of
23 animals. No effect on histopathology, liver weight, or DNA synthesis was observed in acutely
24 exposed rats or rats that were exposed to a lower dose of 10 mg/kg-day for 11 weeks.
25 1,4-Dioxane produced negative findings in the remaining genotoxicity assays conducted as part
26 of this study (i.e., Salmonella mutagenicity, in vitro and in vivo rat hepatocyte DNA repair, and
27 DNA alkylation in rat liver). The study authors suggested that the observed lack of genotoxicity
28 at tumorigenic and cytotoxic dose levels indicates an epigenetic mechanism for 1,4-dioxane
29 hepatocellular carcinoma in rats.

30 Goldsworthy et al. (1991) evaluated potential mechanisms for the nasal and liver
31 carcinogenicity of 1,4-dioxane in the rat. DNA repair activity was evaluated as a measure of
32 DNA reactivity and DNA synthesis was measured as an indicator of cell proliferation or
33 promotional activity. In vitro DNA repair was evaluated in primary hepatocyte cultures from
34 control and 1,4-dioxane-treated rats (1 or 2% in the drinking water for 1 week). DNA repair and
35 DNA synthesis were also measured in vivo following a single gavage dose of 1,000 mg/kg, a
36 drinking water exposure of 1% (1,500 mg/kg-day) for 1 week, or a drinking water exposure of

1 2% (3,000 mg/kg-day) for 2 weeks. Liver to BW ratios and palmitoyl CoA oxidase activity were
2 measured in the rat liver to determine whether peroxisome proliferation played a role in the liver
3 carcinogenesis of 1,4-dioxane. In vivo DNA repair was evaluated in rat nasal epithelial cells
4 derived from either the nasoturbinates or the maxilloturbinate of 1,4-dioxane-treated rats. These
5 rats received 1% 1,4-dioxane (1,500 mg/kg-day) in the drinking water for 8 days, followed by a
6 single gavage dose of 10, 100, or 1,000 mg/kg 12 hours prior to sacrifice. Archived tissues from
7 the NCI (1978) bioassay were reexamined to determine the primary sites for tumor formation in
8 the nasal cavity following chronic exposure in rats. Histopathology and cell proliferation were
9 determined for specific sites in the nasal cavity that were related to tumor formation. This
10 evaluation was performed in rats that were exposed to drinking water containing 1% 1,4-dioxane
11 (1,500 mg/kg-day) for 2 weeks.

12 1,4-Dioxane and its metabolite 1,4-dioxane-2-one did not affect in vitro DNA repair in
13 primary hepatocyte cultures (Goldsworthy, et al., 1991). In vivo DNA repair was also unaffected
14 by acute gavage exposure or ingestion of 1,4-dioxane in the drinking water for a 1- or 2-week
15 period. Hepatocyte cell proliferation was not affected by acute gavage exposure, but was
16 increased approximately twofold following a 1–2-week drinking water exposure. A 5-day
17 drinking water exposure to 1% 1,4-dioxane (1,500 mg/kg-day) did not increase the activity of
18 palmitoyl coenzyme A or the liver to BW ratio, suggesting that peroxisome proliferation did not
19 play a role in the hepatocarcinogenesis of 1,4-dioxane. Nannelli et al. (2005) also reported a lack
20 of hepatic palmitoyl CoA induction following 10 days of exposure to 1.5% 1,4-dioxane in the
21 drinking water (2,100 mg/kg-day).

22 Treatment of rats with 1% (1,500 mg/kg-day) 1,4-dioxane for 8 days did not alter DNA
23 repair in nasal epithelial cells (Goldsworthy, et al., 1991). The addition of a single gavage dose
24 of up to 1,000 mg/kg 12 hours prior to sacrifice also did not induce DNA repair. Reexamination
25 of tissue sections from the NCI (1978) bioassay suggested that the majority of nasal tumors were
26 located in the dorsal nasal septum or the nasoturbinates of the anterior portion of the dorsal
27 meatus (Goldsworthy, et al., 1991). No histopathological lesions were observed in nasal section
28 of rats exposed to drinking water containing 1% 1,4-dioxane (1,500 mg/kg-day) for 2 weeks and
29 no increase was observed in cell proliferation at the sites of highest tumor formation in the nasal
30 cavity.

31 Female Sprague Dawley rats (three to nine per group) were given 0, 168, 840, 2,550, or
32 4,200 mg/kg 1,4-dioxane (99% purity) by corn oil gavage in two doses at 21 and 4 hours prior to
33 sacrifice (Kitchin & Brown, 1990). DNA damage (single-strand breaks measured by alkaline
34 elution), ODC activity, reduced glutathione content, and CYP450 content were measured in the
35 liver. Serum ALT activity and liver histopathology were also evaluated. No changes were
36 observed in hepatic reduced glutathione content or ALT activity. Light microscopy revealed
37 minimal to mild vacuolar degeneration in the cytoplasm of hepatocytes from three of five rats

1 from the 2,550 mg/kg dose group. No histopathological lesions were seen in any other dose
2 group, including rats given a higher dose of 4,200 mg/kg. 1,4-Dioxane caused 43 and 50%
3 increases in DNA single-strand breaks at dose levels of 2,550 and 4,200 mg/kg, respectively.
4 CYP450 content was also increased at the two highest dose levels (25 and 66% respectively).
5 ODC activity was increased approximately two-, five-, and eightfold above control values at
6 doses of 840, 2,550, and 4,200 mg/kg, respectively. The results of this study demonstrated that
7 hepatic DNA damage can occur in the absence of significant cytotoxicity. Parameters associated
8 with tumor promotion (i.e., ODC activity, CYP450 content) were also elevated, suggesting that
9 promotion may play a role in the carcinogenesis of 1,4-dioxane.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

10 Liver and kidney toxicity were the primary noncancer health effects associated with
11 exposure to 1,4-dioxane in humans and laboratory animals. Several fatal cases of hemorrhagic
12 nephritis and centrilobular necrosis of the liver were related to occupational exposure (i.e.,
13 inhalation and dermal contact) to 1,4-dioxane ([Barber, 1934](#); [Johnstone, 1959](#)). Neurological
14 changes were also reported in one case; including, headache, elevation in blood pressure,
15 agitation and restlessness, and coma ([Johnstone, 1959](#)). Perivascular widening was observed in
16 the brain of this worker, with small foci of demyelination in several regions (e.g., cortex, basal
17 nuclei). Liver and kidney degeneration and necrosis were observed in acute oral and inhalation
18 studies ([David, 1964](#); [de Navasquez, 1935](#); [Drew, et al., 1978](#); [Fairley, et al., 1934](#); [JBRC, 1998](#);
19 [Kesten, et al., 1939](#); [Laug, et al., 1939](#); [Schrenk & Yant, 1936](#)). The results of subchronic and
20 chronic studies are discussed below.

4.6.1. Oral

21 Table 4-21 presents a summary of the noncancer results for the subchronic and chronic
22 oral studies of 1,4-dioxane toxicity in experimental animals. Liver and kidney toxicity were the
23 primary noncancer health effects of oral exposure to 1,4-dioxane in animals. Kidney damage at
24 high doses was characterized by degeneration of the cortical tubule cells, necrosis with
25 hemorrhage, and glomerulonephritis ([Argus, et al., 1965](#); [Fairley, et al., 1934](#); [Kociba, et al.,](#)
26 [1974](#); [NCI, 1978](#)). Renal cell degeneration generally began with cloudy swelling of cells in the
27 cortex ([Fairley, et al., 1934](#)). Nuclear enlargement of proximal tubule cells was observed at
28 doses below those producing renal necrosis ([JBRC, 1998](#); [Kano, et al., 2008](#)), but is of uncertain
29 toxicological significance. The lowest dose reported to produce kidney damage was 94 mg/kg-
30 day, which produced renal degeneration and necrosis of tubule epithelial cells in male rats in the
31 Kociba et al. ([1974](#)) study. Cortical tubule degeneration was seen at higher doses in the NCI
32 ([1978](#)) bioassay (240 mg/kg-day, male rats), and glomerulonephritis was reported for rats given
33 doses of ≥ 430 mg/kg-day ([Argus, et al., 1965](#); [1973](#)).

Table 4-21. Oral toxicity studies (noncancer effects) for 1,4-dioxane

Species	Dose/duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect	Reference
Subchronic studies					
Rat and mouse (6/species); unknown strain	Rats 0 or 1,900 mg/kg-day; mice 0 or 3,300 mg/kg-day for 67 days	NA	1,900 rats 3,300 mice	Renal cortical degeneration and necrosis, hemorrhage; hepatocellular degeneration	Fairley et al. (1934)
Male Sprague Dawley Rat (4–6/group)	0, 10, or 1,000 mg/kg-day for 11 weeks	10	1,000	Minimal centrilobular hepatocyte swelling; increased DNA synthesis	Stott et al. (1981)
F344/DuCrj rat (10/sex/group)	Males 0, 52, 126, 274, 657, or 1,554 mg/kg-day; females 0, 83, 185, 427, 756, or 1,614 mg/kg-day for 13 weeks	52	126	Nuclear enlargement of nasal respiratory epithelium; hepatocyte swelling	Kano et al. (2008)
Crj:BDF1 Mouse (10/sex/group)	Males 0, 86, 231, 585, 882, or 1,570 mg/kg-day; females 0, 170, 387, 898, 1,620, or 2,669 mg/kg-day for 13 weeks	170	387	Nuclear enlargement of bronchial epithelium	Kano et al. (2008)
Chronic studies					
Male Wistar Rat (26 treated, 9 controls)	0 or 640 mg/kg-day for 63 weeks	NA	640	Hepatocytes with enlarged hyperchromic nuclei; glomerulonephritis	Argus et al. (1965)
Male Sprague Dawley rats (30/group)	0, 430, 574, 803, or 1,032 mg/kg-day for 13 months	NA	430	Hepatocytomegaly; glomerulonephritis	Argus et al. (1973)
Sherman rat (60/sex/dose group)	Males 0, 9.6, 94, or 1,015 mg/kg-day; females 0, 19, 148, or 1,599 mg/kg-day for 2 years	9.6	94	Degeneration and necrosis of renal tubular cells and hepatocytes	Kociba et al. (1974)
Osborne-Mendel rat (35/sex/dose level)	Males 0, 240, or 530 mg/kg-day; females 0, 350, or 640 mg/kg-day for 110 weeks	NA	240	Pneumonia, gastric ulcers, and cortical tubular degeneration in the kidney	NCI (1978)
B6C3F ₁ mouse (50/sex/dose level)	Males 0, 720, or 830 mg/kg-day; females 0, 380, or 860 mg/kg-day for 90 weeks	NA	380	Pneumonia and rhinitis	NCI (1978)
F344/DuCrj rat (50/sex/dose level)	Males 0, 11, 55, or 274 mg/kg-day; females 0, 18, 83, or 429 mg/kg-day for 2 years	55	274	Atrophy of nasal olfactory epithelium; nasal adhesion and inflammation	JBRC (1998); Kano et al. (2009)
F344/DuCrj rat (50/sex/dose level)	Males 0, 11, 55, or 274 mg/kg-day; females 0, 18, 83, or 429 mg/kg-day for 2 years	11	55	Liver hyperplasia	JBRC (1998); Kano et al. (2009)

Species	Dose/duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect	Reference
F344/DuCrj rat (50/sex/dose level)	Males 0, 11, 55, or 274 mg/kg-day; females 0, 18, 83, or 429 mg/kg-day for 2 years	55	274	Increases in serum liver enzymes (GOT, GPT, LDH, and ALP)	JBRC (1998); Kano et al. (2009)
Crj:BDF1 mouse (50/sex/dose level)	Males 0, 49, 191 or 677 mg/kg-day; females 0, 66, 278, or 964 mg/kg-day for 2 years	66	278	Nasal inflammation	JBRC (1998); Kano et al. (2009)
Crj:BDF1 mouse (50/sex/dose level)	Males 0, 49, 191 or 677 mg/kg-day; females 0, 66, 278, or 964 mg/kg-day for 2 years	49	191	Increases in serum liver enzymes (GOT, GPT, LDH, and ALP)	JBRC (1998); Kano et al. (2009)
Developmental studies					
Sprague Dawley rat (18–20/group)	Pregnant dams 0, 250, 500, or 1,000 mg/kg-day on gestation days 6–15	500	1,000	Delayed ossification of the sternbrae and reduced fetal BWs	Giavani et al. (1985)

1 Liver effects included degeneration and necrosis, hepatocyte swelling, cells with
2 hyperchromic nuclei, spongiosis hepatitis, hyperplasia, and clear and mixed cell foci of the liver
3 ([Argus, et al., 1965](#); [Argus, et al., 1973](#); [Fairley, et al., 1934](#); [Kano, et al., 2008](#); [Kociba, et al.,](#)
4 [1974](#); [NCI, 1978](#)). Hepatocellular degeneration and necrosis were seen at high doses in a
5 subchronic study (1,900 mg/kg-day in rats) ([Fairley, et al., 1934](#)) and at lower doses in a chronic
6 study (94 mg/kg-day, male rats) ([Kociba, et al., 1974](#)). Argus et al. (1973) described a
7 progression of preneoplastic effects in the liver of rats exposed to a dose of 575 mg/kg-day.
8 Early changes (8 months exposure) were described as an increased nuclear size of hepatocytes,
9 disorganization of the rough endoplasmic reticulum, an increase in smooth endoplasmic
10 reticulum, a decrease in glycogen, an increase in lipid droplets in hepatocytes, and formation of
11 liver nodules. Spongiosis hepatitis, hyperplasia, and clear and mixed-cell foci were also observed
12 in the liver of rats (doses >55 mg/kg-day in male rats) ([JBRC, 1998](#); [Kano, et al., 2009](#)). Clear
13 and mixed-cell foci are commonly considered preneoplastic changes and would not be
14 considered evidence of noncancer toxicity when observed in conjunction with tumor formation.
15 If exposure to 1,4-dioxane had not resulted in tumor formation, these lesions could represent
16 potential noncancer toxicity. The nature of spongiosis hepatitis as a preneoplastic change is less
17 well understood ([Bannasch, 2003](#); [Karbe & Kerlin, 2002](#); [Stroebel, et al., 1995](#)). Spongiosis
18 hepatitis is a cyst-like lesion that arises from the perisinusoidal Ito cells of the liver. This change
19 is sometimes associated with hepatocellular hypertrophy and liver toxicity ([Karbe & Kerlin,](#)
20 [2002](#)), but may also occur in combination with preneoplastic foci, or hepatocellular adenoma or
21 carcinoma ([Bannasch, 2003](#); [Stroebel, et al., 1995](#)). In the case of the JBRC (1998) study,
22 spongiosis hepatitis was associated with other preneoplastic changes in the liver (hyperplasia,
23 clear and mixed-cell foci). No other lesions indicative of liver toxicity were seen in this study;
24 therefore, spongiosis hepatitis was not considered indicative of noncancer effects. The activity of

1 serum enzymes (i.e., AST, ALT, LDH, and ALP) was increased in rats and mice exposed to
2 1,4-dioxane, although only in groups with high incidence of liver tumors. Blood samples were
3 collected only at the end of the 2-year study, so altered serum chemistry may be associated with
4 the tumorigenic changes in the liver.

5 Hematological changes were reported in the JBRC (1998) study only. Mean doses are
6 reported based on information provided in Kano et al. (2009). Observed increases in RBCs,
7 hematocrit, hemoglobin in high-dose male mice (677 mg/kg-day) may be related to lower
8 drinking water consumption (74% of control drinking water intake). Hematological effects
9 noted in male rats given 55 mg/kg-day (decreased RBCs, hemoglobin, hematocrit, increased
10 platelets) were within 20% of control values. A reference range database for hematological
11 effects in laboratory animals (Wolford, et al., 1986) indicates that a 20% change in these
12 parameters may fall within a normal range (10th–90th percentile values) and may not represent a
13 treatment-related effect of concern.

14 Rhinitis and inflammation of the nasal cavity were reported in both the NCI (1978) (mice
15 only, dose \geq 380 mg/kg-day) and JBRC (1998) studies (\geq 274 mg/kg-day in rats, $>$ 278 mg/kg-
16 day in mice). The JBRC (1998) study also demonstrates atrophy of the nasal epithelium and
17 adhesion in rats and mice. Nasal inflammation may be a response to direct contact of the nasal
18 mucosa with drinking water containing 1,4-dioxane (Goldsworthy, et al., 1991; Sweeney, et al.,
19 2008) or could result from systemic exposure. Regardless, inflammation may indicate toxicity
20 due to 1,4-dioxane exposure. A significant increase in the incidence of pneumonia was reported
21 in mice from the NCI (1978) study. The significance of this effect is unclear, as it was not
22 observed in other studies that evaluated lung histopathology (JBRC, 1998; Kano, et al., 2008;
23 Kociba, et al., 1974). No studies were available regarding the potential for 1,4-dioxane to cause
24 immunological effects. Metaplasia and hyperplasia of the nasal epithelium were also observed in
25 high-dose male and female rats (JBRC, 1998); however, these effects are likely to be associated
26 with the formation of nasal cavity tumors in these dose groups. Nuclear enlargement of the nasal
27 olfactory epithelium was observed at a dose of 83 mg/kg-day in female rats (Kano, et al., 2009);
28 however, it is unclear whether this alteration represents an adverse toxicological effect. Nuclear
29 enlargement of the tracheal and bronchial epithelium and an accumulation of foamy cells in the
30 lung were also seen in male and female mice give 1,4-dioxane at doses of \geq 278 mg/kg for
31 2 years (JBRC, 1998).

4.6.2. Inhalation

32 Two subchronic (Fairley, et al., 1934; Kasai, et al., 2008) and two chronic inhalation
33 studies (Kasai, et al., 2009; Torkelson, et al., 1974) were identified. Nasal, liver, and kidney
34 toxicity were the primary noncancer health effects of inhalation exposure to 1,4-dioxane in

1 animals. Table 4-22 presents a summary of the noncancer results for the subchronic and chronic
2 inhalation studies of 1,4-dioxane toxicity in laboratory animals.

3 Of the inhalation studies, nasal tissue was only collected in rat studies conducted by
4 Kasai et al. (2009; 2008). Damage to nasal tissue was reported frequently in these studies and
5 statistically significant observations were noted as low as 50 ppm. Nasal effects included
6 deformity of the nose and histopathological lesions characterized by enlarged epithelial nuclei
7 (respiratory epithelium, olfactory epithelium, trachea, and bronchus), atrophy (olfactory
8 epithelium), vacuolic change (olfactory epithelium and bronchial epithelium), squamous cell
9 metaplasia and hyperplasia (respiratory epithelium), respiratory metaplasia (olfactory
10 epithelium), inflammation (respiratory and olfactory epithelium), hydropic change (lamina
11 propria), and sclerosis (lamina propria). In both studies, a concentration-dependent, statistically
12 significant change in enlarged nuclei of the respiratory epithelium was considered the most
13 severe nasal effect by the study authors; however, the toxicological significance of nuclear
14 enlargement is uncertain.

15 At high doses, liver damage was characterized by cell degeneration which varied from
16 swelling (Fairley, et al., 1934; Kasai, et al., 2008) to necrosis (Fairley, et al., 1934; Kasai, et al.,
17 2009; Kasai, et al., 2008), spongiosis hepatitis (Kasai, et al., 2009), nuclear enlargement of
18 centrilobular cells (Kasai, et al., 2009) and basophilic and acidophilic cell foci (Kasai, et al.,
19 2009). Altered cell foci are commonly considered preneoplastic changes and would not be
20 considered evidence of noncancer toxicity when observed in conjunction with tumor formation
21 (Bannasch, Moore, Klimek, & Zerban, 1982). Since exposure to 1,4-dioxane resulted in tumor
22 formation, these lesions are not considered potential noncancer toxicity.

23 At concentrations ranging from 200 ppm to 3,200 ppm, altered liver enzymes (i.e., AST,
24 ALT, ALP, and γ -GTP), increased liver weights, and induction of GST-P was also observed
25 (Kasai, et al., 2009; Kasai, et al., 2008). Changes in the activity of serum enzymes were mostly
26 observed in exposed rat groups of high 1,4-dioxane concentrations (Kasai, et al., 2009; Kasai, et
27 al., 2008). Induction of GST-P positive hepatocytes were observed in female rats at 1,600 ppm
28 and male and female rats at 3,200 ppm following 13 weeks of exposure to 1,4-dioxane. GST-P
29 is considered a good enzymatic marker for early detection of chemical hepatocarcinogenesis
30 (Sato, 1989). Although, GST-P positive liver foci were not observed in the 2 year bioassay, the
31 focally and proliferating GST-P positive hepatocytes noted in the 13 week study suggests
32 eventual progression to hepatocellular tumors after 2 years of exposure and therefore would not
33 be a potential noncancer effect.

34 The lowest concentration reported to produce liver lesions was 1,250 ppm, characterized
35 by necrosis of centrilobular cells, spongiosis hepatitis, and nuclear enlargement in the Kasai et al.
36 (2009) study. However, as previously stated, the toxicological significance of nuclear

enlargement lesions is uncertain; and altered cell foci may not be a potential noncancer effect given its observation in conjunction with tumor formation.

Kidney effects were reported less frequently in these inhalation studies and were generally observed at higher exposure concentrations than nasal and liver effects. Kidney damage was described as patchy degeneration of cortical tubules with vascular congestion and hemorrhage (Fairley, et al., 1934), hydropic change of proximal tubules (Kasai, et al., 2009; Kasai, et al., 2008), and as nuclear enlargement of proximal tubules cells (Kasai, et al., 2009). Changes in serum chemistry and urinalysis variables were also noted as evidence of renal damage. In a 13 week inhalation study of male and female rats (Kasai, et al., 2008) kidney toxicity was only observed in female rats exposed to 3,200 ppm of 1,4-dioxane (i.e. hydropic change in the renal proximal tubules), which suggests a possible increased susceptibility of female rats to renal damage following inhalation exposure to 1,4-dioxane.

Other noted noncancer effects in laboratory animals included acute vascular congestion of the lungs (Fairley, et al., 1934); changes in relative lung weights (Kasai, et al., 2008); and decrease in body weight gain (Kasai, et al., 2009; Kasai, et al., 2008). Following a 13-week exposure, higher 1,4-dioxane plasma levels were found in female rats as compared to male rats (Kasai, et al., 2008). 1,4-Dioxane was observed in plasma along with systemic effects following subchronic inhalation exposure to 1,4-dioxane in rats.

Table 4-22. Inhalation toxicity studies (noncancer effects) for 1,4-dioxane

<u>Species</u>	<u>Dose/duration</u>	<u>NOAEL (ppm)</u>	<u>LOAEL (ppm)</u>	<u>Effect</u>	<u>Reference</u>
<u>Subchronic studies</u>					
Rat, mouse, rabbit, and guinea pig (3-6/species/group); unknown strains	0, 1,000, 2,000, 5,000, or 10,000 ppm for 7 days. Days 1-5, two 1.5 hour exposures; day 6, one 1.5 hour exposure; and day 7, no exposure	NA	1,000	Renal cortical degeneration and hemorrhage; hepatocellular degeneration and necrosis	Fairley et al. (1934)
F344/DuCrj rat (10/sex/group)	0, 100, 200, 400, 800, 1,600, 3,200, or 6,400 ppm 6 hours/day 5 days/week, for 13 weeks	NA	100	Respiratory epithelium; nuclear enlargement of epithelial cells	Kasai et al. (2008)
<u>Chronic studies</u>					
Wistar rat (288/sex)	111 ppm for 7hours/day, 5days/week, for 2 years	111 (free standing)	NA	No significant effects were observed on BWs, survival, organ weights, hematology, clinical chemistry, or histopathology	Torkelson et al. (1974)
F344/DuCrj male rat (50/group)	0, 50, 250, or 1,250 ppm for 6 hours/day, 5 days/week for 2 years	N/A	50	Respiratory epithelium; nuclear enlargement of epithelial cells, atrophy, and metaplasia	Kasai et al. (2009)

19

4.6.3. Mode of Action Information

1 The metabolism of 1,4-dioxane in humans was extensive at low doses (<50 ppm). The
2 linear elimination of 1,4-dioxane in both plasma and urine indicated that 1,4-dioxane metabolism
3 was a nonsaturated, first-order process at this exposure level (1976; Young, et al., 1977). Like
4 humans, rats extensively metabolized inhaled 1,4-dioxane; however, plasma data from rats given
5 single i.v. doses of 3, 10, 30, 100, or 1,000 mg [¹⁴C]-1,4-dioxane/kg demonstrated a dose-related
6 shift from linear, first-order to nonlinear, saturable metabolism of 1,4-dioxane (Young, et al.,
7 1978a; Young, et al., 1978b). Conversely, using the Young et al. (1978a; 1978b) rat model, the
8 metabolism of 1,4-dioxane in rats that were exposed to 400, 800, 1,600, and 3,200 ppm via
9 inhalation for 13 weeks could not be accurately depicted due to a lack of knowledge on needed
10 model parameters and biological processes (See Section 3.5.3 and Appendix B). Metabolism
11 may be induced following prolonged inhalation exposure to 1,4-dioxane at concentrations up to
12 3,200 ppm (Appendix B).

13 1,4-Dioxane oxidation appeared to be CYP450-mediated, as CYP450 induction with
14 phenobarbital or Aroclor 1254 and suppression with 2,4-dichloro-6-phenylphenoxy ethylamine
15 or cobaltous chloride was effective in significantly increasing and decreasing, respectively, the
16 appearance of HEAA in the urine of rats (Woo, Argus, et al., 1977a; Woo, et al., 1978).
17 1,4-Dioxane itself induced CYP450-mediated metabolism of several barbiturates in Hindustan
18 mice given i.p. injections of 25 and 50 mg/kg of 1,4-dioxane (Mungikar & Pawar, 1978). The
19 differences between single and multiple doses in urinary and expired radiolabel support the
20 notion that 1,4-dioxane may induce its own metabolism. 1,4-Dioxane has been shown to induce
21 several isoforms of CYP450 in various tissues following acute oral administration by gavage or
22 drinking water (Nannelli, et al., 2005). In the liver, the activity of several CYP450 isozymes was
23 increased (i.e., CYP2B1/2, CYP2E1, CYPC11); however, only CYP2E1 was inducible in the
24 kidney and nasal mucosa. CYP2E1 mRNA was increased approximately two- to threefold in the
25 kidney and nasal mucosa, but mRNA levels were not increased in the liver, suggesting that
26 regulation of CYP2E1 was organ-specific.

27 Nannelli et al. (2005) investigated the role of CYP450 isozymes in the liver toxicity of
28 1,4-dioxane. Hepatic CYPB1/2 and CYP2E1 levels were induced by phenobarbital or fasting
29 and liver toxicity was measured as hepatic glutathione content or serum ALT activity. No
30 increase in glutathione content or ALT activity was observed, suggesting that highly reactive and
31 toxic intermediates did not play a large role in the liver toxicity of 1,4-dioxane, even under
32 conditions where metabolism was enhanced. Pretreatment with inducers of mixed-function
33 oxidases also did not significantly change the extent of covalent binding in subcellular fractions
34 (Woo, Argus, et al., 1977b). Covalent binding was measured in liver, kidney, spleen, lung,
35 colon, and skeletal muscle 1–12 hours after i.p. dosing with 1,4-dioxane. Covalent binding was

1 highest in liver, spleen, and colon. Within hepatocytes, 1,4-dioxane distribution was greatest in
2 the cytosolic fraction, followed by the microsomal, mitochondrial, and nuclear fractions.

3 The absence of an increase in toxicity following an increase in metabolism suggests that
4 accumulation of the parent compound may be related to 1,4-dioxane toxicity. This hypothesis is
5 supported by a comparison of the pharmacokinetic profile of 1,4-dioxane with the toxicology
6 data from a chronic drinking water study ([Kociba, et al., 1975](#)). This analysis indicated that liver
7 toxicity did not occur unless clearance pathways were saturated and elimination of 1,4-dioxane
8 from the blood was reduced. A dose-dependent increase of 1,4-dioxane accumulation in the
9 blood was seen, which correlated to the observed dose-dependent increase in incidences of nasal,
10 liver, and kidney toxicities ([Kasai, et al., 2008](#)). Alternative metabolic pathways (i.e., not
11 CYP450 mediated) may be present at high doses of 1,4-dioxane; however, the available studies
12 have not characterized these pathways or identified any possible reactive intermediates. Thus,
13 the mechanism by which 1,4-dioxane induces tissue damage is not known, nor is it known
14 whether the toxic moiety is 1,4-dioxane or a transient or terminal metabolite.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

15 Under the Guidelines for Carcinogen Risk Assessment ([U.S. EPA, 2005a](#)), 1,4-dioxane is
16 “likely to be carcinogenic to humans” based on evidence of carcinogenicity in several 2-year
17 bioassays conducted in four strains of rats, two strains of mice, and in guinea pigs ([Argus, et al.,](#)
18 [1965](#); [Argus, et al., 1973](#); [Hoch-Ligeti & Argus, 1970](#); [Hoch-Ligeti, et al., 1970](#); [JBRC, 1998](#);
19 [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Kociba, et al., 1974](#); [NCI, 1978](#); [Yamazaki, et al., 1994](#)).
20 Tissue sites where tumors have been observed in these laboratory animals due to exposure to
21 1,4-dioxane include, peritoneal ([JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Yamazaki,](#)
22 [et al., 1994](#)), mammary gland ([JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Yamazaki, et](#)
23 [al., 1994](#)), liver ([Kano, et al., 2009](#); [Kasai, et al., 2009](#)), kidney ([Kasai, et al., 2009](#)), Zymbal
24 gland ([Kasai, et al., 2009](#)), subcutaneous ([Kasai, et al., 2009](#)), nasal tissue ([Argus, et al., 1973](#);
25 [Hoch-Ligeti, et al., 1970](#); [JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Kociba, et al.,](#)
26 [1974](#); [NCI, 1978](#); [Yamazaki, et al., 1994](#)), and lung ([Hoch-Ligeti & Argus, 1970](#)). Studies in
27 humans are inconclusive regarding evidence for a causal link between occupational exposure to
28 1,4-dioxane and increased risk for cancer; however, only two studies were available and these
29 were limited by small cohort size and a small number of reported cancer cases ([Buffler, et al.,](#)
30 [1978](#); [Thiess, et al., 1976](#)).

31 The available evidence is inadequate to establish a mode of action (MOA) by which
32 1,4-dioxane induces liver tumors in rats and mice. A MOA hypothesis involving sustained
33 proliferation of spontaneously transformed liver cells has some support from data indicating that
34 1,4-dioxane acts as a tumor promoter in mouse skin and rat liver bioassays ([King, et al., 1973](#);

1 [Lundberg, et al., 1987](#)). Dose-response and temporal data support the occurrence of cell
2 proliferation and hyperplasia prior to the development of liver tumors ([JBRC, 1998](#); [Kociba, et](#)
3 [al., 1974](#)) in the rat model. However, the dose-response relationship for induction of hepatic cell
4 proliferation has not been characterized, and it is unknown if it would reflect the dose-response
5 relationship for liver tumors in the 2-year rat and mouse studies. Conflicting data from rat and
6 mouse bioassays ([JBRC, 1998](#); [Kociba, et al., 1974](#)) suggest that cytotoxicity may not be a
7 required precursor event for 1,4-dioxane-induced cell proliferation. Data regarding a plausible
8 dose response and temporal progression (see Table 4-21) from cytotoxicity and cell proliferation
9 to eventual liver tumor formation are not available.

10 For nasal tumors, a hypothesized MOA includes metabolic induction, cytotoxicity, and
11 regenerative cell proliferation. The induction of CYP450 has some support from data illustrating
12 that following acute oral administration of 1,4-dioxane by gavage or drinking water, CYP2E1
13 was inducible in nasal mucosa ([Nannelli, et al., 2005](#)). CYP2E1 mRNA was increased
14 approximately two- to threefold in nasal mucosa (and in the kidney, see section 3.3) in the
15 Nannelli et al. (2005) study. The possibility of the parent compound as a factor in the
16 development of nasal tumors also has some support. Following a 13-week inhalation study in
17 rats, a concentration-dependent accumulation of 1,4-dioxane in the blood was observed ([Kasai,](#)
18 et al., 2008). Studies have shown that water-soluble, gaseous irritants cause nasal injuries such
19 as squamous cell carcinomas ([Morgan, Patterson, & Gross, 1986](#)). Similarly, 1,4-dioxane, which
20 has been reported as a miscible compound ([Hawley & Lewis Rj Sr, 2001](#)), also caused nasal
21 injuries that were concentration-dependent, including nasal tumors ([Kasai, et al., 2009](#)). While
22 cell proliferation was observed following 1,4-dioxane exposure in both a 2-year inhalation study
23 in male rats (1,250 ppm) ([Kasai, et al., 2009](#)) and a 2-year drinking water study in male (274
24 mg/kg-day) and female rats (429 mg/kg-day), no evidence of cytotoxicity in the nasal cavity was
25 observed ([Kasai, et al., 2009](#)); therefore, cytotoxicity, as a key event, is not supported.

26 The MOA by which 1,4-dioxane produces liver, nasal, lung, peritoneal (mesotheliomas),
27 mammary gland, Zymbal gland, and subcutis tumors is unknown, and the available data do not
28 support any hypothesized carcinogenic MOA for 1,4-dioxane.

29 U.S. EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)) indicate that
30 for tumors occurring at a site other than the initial point of contact, the weight of evidence for
31 carcinogenic potential may apply to all routes of exposure that have not been adequately tested at
32 sufficient doses. An exception occurs when there is convincing information (e.g., toxicokinetic
33 data) that absorption does not occur by other routes. Information available on the carcinogenic
34 effects of 1,4-dioxane via the oral route demonstrates that tumors occur in tissues remote from
35 the site of absorption. In addition, information on the carcinogenic effects of 1,4-dioxane via the
36 inhalation route in animals also demonstrates that tumors occur at tissue sites distant from the
37 portal of entry. Information on the carcinogenic effects of 1,4-dioxane via the inhalation and

1 dermal routes in humans and [via the dermal route in](#) animals is absent. Based on the observance
2 of systemic tumors following oral [and inhalation exposure](#), it is assumed that an internal dose
3 will be achieved regardless of the route of exposure. Therefore, 1,4-dioxane is “likely to be
4 carcinogenic to humans” by all routes of exposure.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

5 Human studies of occupational exposure to 1,4-dioxane were inconclusive; in each case,
6 the cohort size [was limited](#) and number of reported cases were of limited size [was small](#) ([Buffler,](#)
7 [et al., 1978](#); [Thiess, et al., 1976](#)).

8 Several carcinogenicity bioassays have been conducted for 1,4-dioxane in mice, rats, and
9 guinea pigs ([Argus, et al., 1965](#); [Argus, et al., 1973](#); [Hoch-Ligeti & Argus, 1970](#); [Hoch-Ligeti, et](#)
10 [al., 1970](#); [JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Kociba, et al., 1974](#); [NCI, 1978](#);
11 [Torkelson, et al., 1974](#); [Yamazaki, et al., 1994](#)). Liver tumors have been observed following
12 drinking water exposure in male Wistar rats ([Argus, et al., 1965](#)), male guinea pigs ([Hoch-Ligeti](#)
13 [& Argus, 1970](#)), male Sprague Dawley rats ([Argus, et al., 1973](#); [Hoch-Ligeti, et al., 1970](#)), male
14 and female Sherman rats ([Kociba, et al., 1974](#)), female Osborne-Mendel rats ([NCI, 1978](#)), male
15 and female F344/DuCrj rats ([JBRC, 1998](#); [Kano, et al., 2009](#); [Yamazaki, et al., 1994](#)), male and
16 female B6C3F₁ mice ([NCI, 1978](#)), and male and female Crj:BDF₁ mice ([JBRC, 1998](#); [Kano, et](#)
17 [al., 2009](#); [Yamazaki, et al., 1994](#)); [and following inhalation exposure in male F344 rats \(Kasai, et](#)
18 [al., 2009\)](#). In the earliest cancer bioassays, the liver tumors were described as hepatomas ([Argus,](#)
19 [et al., 1965](#); [Argus, et al., 1973](#); [Hoch-Ligeti & Argus, 1970](#); [Hoch-Ligeti, et al., 1970](#)); however,
20 later studies made a distinction between hepatocellular carcinoma and hepatocellular adenoma
21 ([JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Kociba, et al., 1974](#); [NCI, 1978](#); [Yamazaki,](#)
22 [et al., 1994](#)). Both tumor types have been seen in rats and mice exposed to 1,4-dioxane [via](#)
23 [drinking water and inhalation](#). [Kociba et al. \(1974\)](#) noted evidence of liver toxicity at or below
24 the dose levels that produced liver tumors but did not report incidence data for these effects.
25 Hepatocellular degeneration and necrosis were observed in the mid- and high-dose groups of
26 male and female Sherman rats exposed to 1,4-dioxane, while tumors were only observed at the
27 highest dose. Hepatic regeneration was indicated in the mid- and high-dose groups by the
28 formation of hepatocellular hyperplastic nodules. [Kano et al., \(2009\)](#) also provided evidence of
29 liver hyperplasia in male F344/DuCrj rats at a dose level below the dose that induced a
30 statistically significant increase in tumor formation. [Kasai et al. \(2009\) noted evidence of liver](#)
31 [toxicity and tumor incidences \(i.e. hepatocellular adenoma\) in male F344/DuCrj rats following](#)
32 [inhalation exposures to 1,250 ppm. Increased liver toxicities included hepatocellular necrosis,](#)
33 [spongiosis hepatis, and acidophilic and basophilic cell foci.](#)

34 Nasal cavity tumors were also observed in Sprague Dawley rats ([Argus, et al., 1973](#);
35 [Hoch-Ligeti, et al., 1970](#)), Osborne-Mendel rats ([NCI, 1978](#)), Sherman rats ([Kociba, et al.,](#)

1 [1974](#)), and F344/DuCrj rats ([JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Yamazaki, et](#)
2 [al., 1994](#)). Most tumors were characterized as squamous cell carcinomas. Nasal tumors were
3 not elevated in B6C3F₁ or Crj:BDF₁ mice. [Kano et al. \(2009\) and Kasai et al. \(2009\) were](#) the
4 only studies that evaluated nonneoplastic changes in nasal cavity tissue following prolonged
5 exposure to 1,4-dioxane [via oral and inhalation routes, respectively](#). Histopathological lesions in
6 female F344/DuCrj rats were suggestive of toxicity and regeneration in this tissue (i.e., atrophy,
7 adhesion, inflammation, nuclear enlargement, and hyperplasia and metaplasia of respiratory and
8 olfactory epithelium). Some of these effects occurred at a lower dose (83 mg/kg-day) than that
9 shown to produce nasal cavity tumors (429 mg/kg-day) in female rats. Re-examination of tissue
10 sections from the NCI ([1978](#)) bioassay suggested that the majority of nasal tumors were located
11 in the dorsal nasal septum or the nasoturbinate of the anterior portion of the dorsal meatus.
12 [Histopathological lesions in male F344/DuCrj rats following exposure to 1,4-dioxane via](#)
13 [inhalation were also suggestive of toxicity and regeneration in the nasal cavity \(i.e. atrophy,](#)
14 [inflammation, nuclear enlargement, hyperplasia and metaplasia of the respiratory and olfactory](#)
15 [epithelium, and inflammation\). Some of these effects occurred at lower concentrations \(50 ppm](#)
16 [and 250 ppm\) than those shown to produce nasal cavity tumors \(1,250 ppm\) in male rats. Nasal](#)
17 [squamous cell carcinomas were observed in the dorsal area of levels 1-3 of the nasal cavity and](#)
18 [were characterized as well-differentiated and keratinized. In two cases, invasive growth into](#)
19 [adjacent tissue was noted, marked by carcinoma growth out of the nose and through a destroyed](#)
20 [nasal bone.](#)

21 Tumor initiation and promotion studies in mouse skin and rat liver suggested that
22 1,4-dioxane does not initiate the carcinogenic process, but instead acts as a tumor promoter
23 ([Bull, et al., 1986](#); [King, et al., 1973](#); [Lundberg, et al., 1987](#)) (see Section 4.2.3).

24 In addition to the liver and nasal tumors observed in several studies, a statistically
25 significant increase in mesotheliomas of the peritoneum was seen in male rats from the Kano et
26 al. ([2009](#)) study ([JBRC, 1998](#); [Yamazaki, et al., 1994](#)) [and the Kasai et al. \(2009\) study](#). Female
27 rats dosed with 429 mg/kg-day in drinking water for 2 years also showed a statistically
28 significant increase in mammary gland adenomas ([JBRC, 1998](#); [Kano, et al., 2009](#); [Yamazaki, et](#)
29 [al., 1994](#)). [In male rats, exposed via inhalation, a statistically significant positive trend of](#)
30 [mammary gland adenomas was observed by Kasai et al. \(2009\). A statistically significant](#)
31 [increase and/or trend of subcutis fibroma, Zymbal gland adenoma, and renal cell carcinoma](#)
32 [incidences was also observed in male rats exposed for 2 years via inhalation \(Kasai, et al., 2009\).](#)
33 A significant increase in the incidence of these tumors was not observed in other chronic oral [or](#)
34 [inhalation](#) bioassays of 1,4-dioxane ([Kociba, et al., 1974](#); [NCI, 1978](#); [Torkelson, et al., 1974](#)).

4.7.3. Mode of Action Information

1 The MOA by which 1,4-dioxane produces liver, nasal, peritoneal (mesotheliomas),
2 mammary gland, Zymbal gland, and subcutis tumors is unknown, and the available data do not
3 support any hypothesized mode of carcinogenic action for 1,4-dioxane. Available data also do
4 not clearly identify whether 1,4-dioxane or one of its metabolites is responsible for the observed
5 effects. The hypothesized MOAs for 1,4-dioxane carcinogenicity are discussed below within the
6 context of the modified Hill criteria of causality as recommended in the most recent Agency
7 guidelines ([U.S. EPA, 2005a](#)). MOA analyses were not conducted for peritoneal, mammary
8 gland, Zymbal gland, or subcutis tumors due to the absence of any chemical specific information
9 for these tumor types.

4.7.3.1. Identification of Key Events for Carcinogenicity

10 **4.7.3.1.1. Liver.** A key event in this MOA hypothesis is sustained proliferation of
11 spontaneously transformed liver cells, resulting in the eventual formation of liver tumors.
12 Precursor events in which 1,4-dioxane may promote proliferation of transformed liver cells are
13 uncertain. One study suggests that induced liver cytotoxicity may be a key precursor event to
14 cell proliferation leading to the formation of liver tumors ([Kociba, et al., 1974](#)), however, this
15 study did not report incidence data for these effects. Other studies suggest that cell proliferation
16 can occur in the absence of liver cytotoxicity. Liver tumors were observed in female rats and
17 female mice in the absence of lesions indicative of cytotoxicity ([JBRC, 1998](#); [Kano, et al., 2008](#);
18 [NCI, 1978](#)). Figure 4-1 presents a schematic representation of possible key events in the MOA
19 for 1,4-dioxane liver carcinogenicity. These include: (1) oxidation by CYP2E1 and CYP2B1/2
20 (i.e., detoxification pathway for 1,4-dioxane), (2) saturation of metabolism/clearance leading to
21 accumulation of the parent 1,4-dioxane, (3) liver damage followed by regenerative cell
22 proliferation, or (4) cell proliferation in the absence of cytotoxicity (i.e., mitogenesis),
23 (5) hyperplasia, and (6) tumor formation. It is suggested that liver toxicity is related to the
24 accumulation of the parent compound following metabolic saturation at high doses ([Kociba, et](#)
25 [al., 1975](#)); however, no in vivo or in vitro assays have examined the toxicity of metabolites
26 resulting from 1,4-dioxane to support this hypothesis. [Nannelli et al. \(2005\)](#) demonstrated that
27 an increase in the oxidative metabolism of 1,4-dioxane via CYP450 induction using
28 phenobarbital or fasting does not result in an increase in liver toxicity. This result suggested that
29 highly reactive and toxic intermediates did not play a large role in the liver toxicity of
30 1,4-dioxane, even under conditions where metabolism was enhanced. Alternative metabolic
31 pathways (e.g., not CYP450 mediated) may be present at high doses of 1,4-dioxane; although the
32 available studies have not characterized these pathways nor identified any possible reactive
33 intermediates. Tumor promotion studies in mouse skin and rat liver suggest that 1,4-dioxane
34 may enhance the growth of previously initiated cells ([King, et al., 1973](#); [Lundberg, et al., 1987](#)).

1 This is consistent with the increase in hepatocyte cell proliferation observed in several studies
2 ([Goldsworthy, et al., 1991](#); [Miyagawa, et al., 1999](#); [Stott, et al., 1981](#); [Uno, et al., 1994](#)). These
3 mechanistic studies provide evidence of cell proliferation, but do not indicate whether
4 mitogenesis or cytotoxicity is responsible for increased cell turnover.

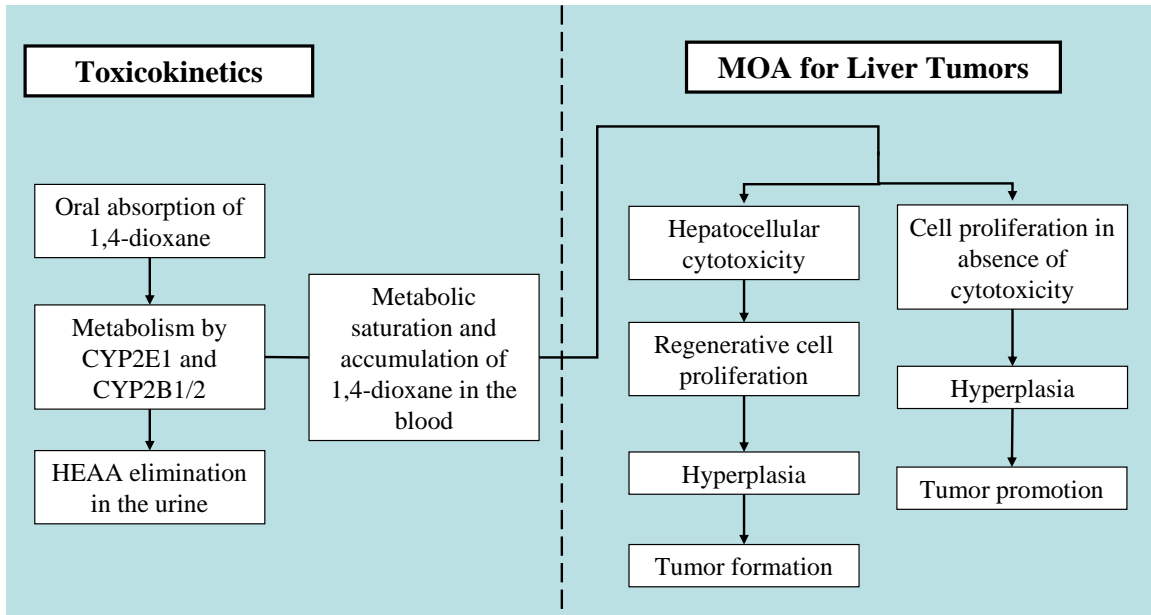


Figure 4-1. A schematic representation of the possible key events in the delivery of 1,4-dioxane to the liver and the hypothesized MOA(s) for liver carcinogenicity.

5 **4.7.3.1.2. Nasal cavity.** A possible key event in the MOA hypothesis for nasal tumors is
6 sustained proliferation of spontaneously transformed nasal epithelial cells, resulting in the
7 eventual formation of nasal cavity tumors. Cell proliferation was observed following
8 1,4-dioxane exposure in both a 2-year inhalation study in male rats (1,250 ppm) ([Kasai, et al.,](#)
9 2009) and a 2-year drinking water study in male (274 mg/kg-day) and female rats (429 mg/kg-
10 day); however, no evidence of cytotoxicity in the nasal cavity was observed ([Kasai, et al., 2009](#));
11 therefore, cytotoxicity as a key event is not supported. The [Kasai et al. \(2009; 2008\)](#) studies
12 suggest that nasal toxicity is related to the accumulation of the parent compound following
13 metabolic saturation at high doses; however no in vivo or in vitro assays have examined the
14 toxicity of metabolites resulting from 1,4-dioxane to support this hypothesis. [Nannelli et al.](#)
15 (2005) demonstrated that CYP2E1 was inducible in nasal mucosa following acute oral
16 administration of 1,4-dioxane by gavage or drinking water, which could potentially lead to an
17 increase in the oxidative metabolism of 1,4-dioxane and nasal toxicity. However, [Nannelli et al.](#)
18 (2005) did not characterize this pathway nor identify any possible reactive intermediates or nasal
19 toxicities.

20

4.7.3.2. *Strength, Consistency, Specificity of Association*

1 **4.7.3.2.1. *Liver.*** The plausibility of a MOA that would include liver cytotoxicity, with
2 subsequent reparative cell proliferation, as precursor events to liver tumor formation is
3 minimally supported by findings that nonneoplastic liver lesions occurred at exposure levels
4 lower than those resulting in significantly increased incidences of hepatocellular tumors ([Kociba,
5 et al., 1974](#)) and the demonstration of nonneoplastic liver lesions in subchronic ([Kano, et al.,
6 2008](#)) and acute and short-term oral studies (see Table 4-18). Because the incidence of
7 nonneoplastic lesions was not reported by Kociba et al. ([1974](#)), it is difficult to know whether the
8 incidence of liver lesions increased with increasing 1,4-dioxane concentration. Contradicting the
9 observations by Kociba et al. ([1974](#)), liver tumors were observed in female rats and female mice
10 in the absence of lesions indicative of cytotoxicity ([JBRC, 1998](#); [Kano, et al., 2008](#); [NCI, 1978](#)).
11 This suggests that cytotoxicity may not be a requisite step in the MOA for liver cancer.
12 Mechanistic and tumor promotion studies suggest that enhanced cell proliferation without
13 cytotoxicity may be a key event; however, data showing a plausible dose response and temporal
14 progression from cell proliferation to eventual liver tumor formation are not available (see
15 Sections 4.7.3.3 and 4.7.3.4). Mechanistic studies that demonstrated cell proliferation after
16 short-term exposure did not evaluate liver cytotoxicity ([Goldsworthy, et al., 1991](#); [Miyagawa, et
17 al., 1999](#); [Uno, et al., 1994](#)). Studies have not investigated possible precursor events that may
18 lead to cell proliferation in the absence of cytotoxicity (i.e., genetic regulation of mitogenesis).

19 **4.7.3.2.2. *Nasal cavity.*** Nasal cavity tumors have been demonstrated in several rat strains
20 ([JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Kociba, et al., 1974](#); [NCI, 1978](#); [Yamazaki,
21 et al., 1994](#)), but were not elevated in two strains of mice ([JBRC, 1998](#); [Kano, et al., 2009](#); [NCI,
22 1978](#); [Yamazaki, et al., 1994](#)). Chronic irritation was indicated by the observation of rhinitis
23 and/or inflammation of the nasal cavity in rats from the JBRC ([1998](#)) and [Kasai et al. \(2009;
24 2008\) studies](#). [The Kasai et al. \(2009; 2008\) studies](#) also showed atrophy of the nasal epithelium
25 [in rats](#), and [the JRBC \(1998\) study also observed atrophy of the nasal epithelium as well as](#)
26 adhesion in rats. Regeneration of the nasal epithelium is demonstrated by metaplasia and
27 hyperplasia observed in rats exposed to 1,4-dioxane ([JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et
28 al., 2009](#); [Yamazaki, et al., 1994](#)). [Oxidation of 1,4-dioxane metabolism by CYP450 is not
29 supported as a key event in the MOA of nasal tumors](#). [Nannelli et al. \(2005\) lacked details of
30 possible reactive intermediates and resulting nasal toxicity](#). [Accumulation of 1,4-dioxane in
31 blood as a precursor event of nasal tumor formation is also not supported because the parent
32 compound 1,4-dioxane was only measured in one subchronic study \(Kasai, et al., 2008\) and in
33 this subchronic study no evidence of nasal cytotoxicity, cell proliferation, or incidence of nasal
34 tumors were reported.](#)
35

4.7.3.3. Dose-Response Relationship

1 **4.7.3.3.1. Liver.** Table 4-23 presents the temporal sequence and dose-response relationship for
 2 possible key events in the liver carcinogenesis of 1,4-dioxane. Dose-response information
 3 provides some support for enhanced cell proliferation as a key event in the liver tumorigenesis of
 4 1,4-dioxane; however, the role of cytotoxicity as a required precursor event is not supported by
 5 data from more than one study. Kociba et al. (1974) demonstrated that liver toxicity and
 6 hepatocellular regeneration occurred at a lower dose level than tumor formation. Hepatocellular
 7 degeneration and necrosis were observed in the mid- and high-dose groups of Sherman rats
 8 exposed to 1,4-dioxane, although it is not possible to discern whether this effect was observed in
 9 both genders due to the lack of incidence data (Kociba, et al., 1974). Hepatic tumors were only
 10 observed at the highest dose (Kociba, et al., 1974). Hepatic regeneration was indicated in the
 11 mid- and high-dose group by the formation of hepatocellular hyperplastic nodules. Liver
 12 hyperplasia was also seen in rats from the JBRC (1998) study, at or below the dose level that
 13 resulted in tumor formation (Kano, et al., 2009); however, hepatocellular degeneration and
 14 necrosis were not observed. These results suggest that hepatic cell proliferation and hyperplasia
 15 may occur in the absence of significant cytotoxicity. Liver angiectasis (i.e., dilation of blood or
 16 lymphatic vessels) was observed in male mice at the same dose that produced liver tumors;
 17 however, the relationship between this vascular abnormality and tumor formation is unclear.

Table 4-23. Temporal sequence and dose-response relationship for possible key events and liver tumors in rats and mice

Dose (mg/kg-day) or Exposure (ppm)	Key event (time →)				
	Metabolism 1,4-dioxane	Liver damage	Cell proliferation	Hyperplasia	Adenomas and/or carcinomas
Kociba et al., (1974)—Sherman rats (male and female combined)					
0 mg/kg-day	— ^a	— ^a	— ^a	— ^a	— ^a
14 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	— ^a
121 mg/kg-day	+ ^b	+ ^c	— ^a	+ ^c	— ^a
1,307 mg/kg-day	+ ^b	+ ^c	— ^a	+ ^c	+ ^c
NCI, (1978)—female Osborne-Mendel rats					
0 mg/kg-day	— ^a	— ^a	— ^a	— ^a	— ^a
350 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	+ ^c
640 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	+ ^c
NCI, (1978)—male B6C3F₁ mice					
0 mg/kg-day	— ^a	— ^a	— ^a	— ^a	— ^a
720 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	+ ^c
830 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	+ ^c
NCI, (1978)—female B6C3F₁ mice					

Dose (mg/kg-day) or Exposure (ppm)	Key event (time →)				
	Metabolism 1,4-dioxane	Liver damage	Cell proliferation	Hyperplasia	Adenomas and/or carcinomas
0 mg/kg-day	— ^a	— ^a	— ^a	— ^a	— ^a
380 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	+ ^c
860 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	+ ^c
Kano et al., (2009); JBRC, (1998)—male F344/DuCrj rats					
0 mg/kg-day	— ^a	— ^a	— ^a	— ^a	— ^a
11 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	— ^a
55 mg/kg-day	+ ^b	— ^a	— ^a	+ ^{c,e}	— ^a
274 mg/kg-day	+ ^b	+ ^{c,d}	— ^a	+ ^{c,e}	+ ^{c,e}
Kano et al., (2009); JBRC, (1998)—female F344/DuCrj rats					
0 mg/kg-day	— ^a	— ^a	— ^a	— ^a	— ^a
18 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	— ^a
83 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	— ^a
429 mg/kg-day	+ ^b	— ^a	— ^a	+ ^{c,e}	+ ^{c,e}
Kano et al., (2009); JBRC, (1998)—male Crj:BDF1 mice					
0 mg/kg-day	— ^a	— ^a	— ^a	— ^a	— ^a
49 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	+ ^{c,e}
191 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	+ ^{c,e}
677 mg/kg-day	+ ^b	+ ^{c,d}	— ^a	— ^a	+ ^{c,e}
Kano et al., (2009); JBRC, (1998)—female Crj:BDF1 mice					
0 mg/kg-day	— ^a	— ^a	— ^a	— ^a	— ^a
66 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	+ ^{c,e}
278 mg/kg-day	+ ^b	— ^a	— ^a	— ^a	+ ^{c,e}
964 mg/kg-day	+ ^b	+ ^{c,d}	— ^a	— ^a	+ ^{c,e}
Kasai et al. (2008)—F344 rats (male and female combined)					
0 ppm	— ^a	— ^a	— ^a	— ^a	— ^a
100 ppm	— ^a	— ^a	— ^a	— ^a	— ^a
200 ppm	— ^a	— ^a	— ^a	— ^a	— ^a
400 ppm	— ^a	— ^a	— ^a	— ^a	— ^a
800 ppm	— ^a	— ^a	— ^a	— ^a	— ^a
1,600 ppm	— ^a	— ^a	— ^a	— ^a	— ^a
3,200 ppm	— ^a	+ ^f	— ^a	— ^a	— ^a
6,400 ppm	— ^{a,g}	— ^{a,g}	— ^{a,g}	— ^{a,g}	— ^{a,g}

Dose (mg/kg-day) <u>or Exposure</u> <u>(ppm)</u>	Key event (time →)				
	Metabolism 1,4-dioxane	Liver damage	Cell proliferation	Hyperplasia	Adenomas and/or carcinomas
<u>Kasai et al., (2009)—male F344 rats</u>					
<u>0 ppm</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>50 ppm</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>250 ppm</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>1,250 ppm</u>	<u>—^a</u>	<u>+^h</u>	<u>—^a</u>	<u>—^a</u>	<u>+^h</u>

^a— No evidence demonstrating key event.

^b+ 1,4-dioxane metabolism was not evaluated as part of the chronic bioassays. Data from pharmacokinetic studies suggest that metabolism of 1,4-dioxane by CYP2E1 and CYP2B2 occurs immediately and continues throughout the duration of exposure at all exposure levels.

^c+ Evidence demonstrating key event.

^d+ Single cell necrosis was observed in a 13 week bioassay for male rats (274 mg/kg-day), male mice (585 mg/kg-day), and female mice (898 mg/kg-day) exposed to 1,4-dioxane in drinking water (Kano, et al., 2008).

^e+ Kano et al. (2009) reported incidence rates for hepatocellular adenomas and carcinomas; however, information from JBRC (1998) on incidence of liver hyperplasia was used to create this table.

^f+ Kasai et al. (2008) reported significant incidence rates for single cell necrosis in female rats only (3200 ppm) following a 2 year bioassay.

^gAll rats died during the first week of the 13-week bioassay (Kasai, et al., 2008).

^hKasai et al. (2009) reported incidence rates for centrilobular necrosis and hepatocellular adenomas in male rats (1,250 ppm).

1 **4.7.3.3.2. Nasal cavity.** Table 4-24 presents the temporal sequence and dose-response
2 relationship for possible key events in the nasal tissue carcinogenesis of 1,4-dioxane. Toxicity
3 and regeneration in nasal epithelium (i.e., atrophy, adhesion, inflammation, and hyperplasia and
4 metaplasia of respiratory and olfactory epithelium) was evident in one study at the same dose
5 levels that produced nasal cavity tumors (JBRC, 1998; Kano, et al., 2009). In another study,
6 dose-response information provided some support for nasal toxicity and regeneration in nasal
7 epithelium occurring before tumor development (Kasai, et al., 2009). However, the role of
8 cytotoxicity as a required precursor event is not supported by data from any of the reviewed
9 studies. The accumulation of parent 1,4-dioxane as a key event has some support since
10 concentration-dependent increases were noted for both 1,4-dioxane in plasma and toxicities
11 observed that are also other possible precursor events (i.e. regeneration in nasal
12 epithelium)(Kasai, et al., 2008). In a subsequent study by Kasai et al. (2009) some of these same
13 possible precursor events were observed at 50, 250, and 1,250 ppm with evidence of nasal
14 tumors at the highest concentration (1,250 ppm).

Table 4-24. Temporal sequence and dose-response relationship for possible key events and nasal tumors in rats and mice.

<u>Dose (mg/kg-day)</u> <u>or Exposure</u> <u>(ppm)</u>	<u>Key event (time →)</u>				<u>Adenomas</u> <u>and/or</u> <u>carcinomas</u>
	<u>Metabolism</u> <u>1,4-dioxane</u>	<u>Nasal</u> <u>cytotoxicity</u>	<u>Cell</u> <u>proliferation</u>	<u>Hyperplasia</u>	
<u>Kociba et al., (1974)—Sherman rats (male and female combined)</u>					
<u>0 mg/kg-day</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>14 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>121 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>1,307 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>NCI, (1978)—female Osborne-Mendel rats</u>					
<u>0 mg/kg-day</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>350 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>640 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>NCI, (1978)—male B6C3F₁ mice</u>					
<u>0 mg/kg-day</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>720 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>830 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>NCI, (1978)—female B6C3F₁ mice</u>					
<u>0 mg/kg-day</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>380 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>860 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>Kano et al., (2009); JBRC, (1998)—male F344/DuCrj rats</u>					
<u>0 mg/kg-day</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>11 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>55 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>274 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>+^{c,d}</u>	<u>+^{c,d}</u>
<u>Kano et al., (2009); JBRC, (1998)—female F344/DuCrj rats</u>					
<u>0 mg/kg-day</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>18 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>83 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>429 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>+^{c,d}</u>	<u>+^{c,d}</u>
<u>Kano et al., (2009); JBRC, (1998)—male Crj:BDF1 mice</u>					
<u>0 mg/kg-day</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>49 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>191 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>677 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>

<u>Dose (mg/kg-day)</u> <u>or Exposure</u> <u>(ppm)</u>	<u>Key event (time →)</u>				
	<u>Metabolism</u> <u>1,4-dioxane</u>	<u>Nasal</u> <u>cytotoxicity</u>	<u>Cell</u> <u>proliferation</u>	<u>Hyperplasia</u>	<u>Adenomas</u> <u>and/or</u> <u>carcinomas</u>
<u>Kano et al., (2009); JBRC, (1998)—female Crj:BDF1 mice</u>					
<u>0 mg/kg-day</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>66 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>278 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>964 mg/kg-day</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>Kasai et al. (2008)—F344 rats (male and female combined)</u>					
<u>0 ppm</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>100 ppm</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>200 ppm</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>400 ppm</u>	<u>+^c</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>800 ppm</u>	<u>+^c</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>1600 ppm</u>	<u>+^c</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>3200 ppm</u>	<u>+^c</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>6400 ppm</u>	<u>+^{a,b,f}</u>	<u>—^{a,f}</u>	<u>—^{a,f}</u>	<u>—^{a,f}</u>	<u>—^{a,f}</u>
<u>Kasai et al. (2009)—male F344 rats</u>					
<u>0 ppm</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>50 ppm</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>250 ppm</u>	<u>+^b</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>	<u>—^a</u>
<u>1,250 ppm</u>	<u>+^b</u>	<u>—^a</u>	<u>—^c</u>	<u>+^e</u>	<u>+^c</u>

^a— No evidence demonstrating key event.

^b+ 1,4-dioxane metabolism was not evaluated as part of these studies. Data from pharmacokinetic studies suggest that metabolism of 1,4-dioxane by CYP2E1 and CYP2B2 occurs immediately and continues throughout the duration of exposure at all exposure levels.

^c+ Evidence demonstrating key event.

^d+ Kano et al. (2009) reported incidence rates for squamous cell hyperplasia (respiratory epithelium) and squamous cell carcinomas (nasal cavity); however, information from JBRC (1998) on significant incidence of squamous cell hyperplasia was used to create this table.

^e+Kasai et al. (2009) reported incidence rates for squamous cell hyperplasia in male rats (1,250 ppm) following a 2 year bioassay.

^f+ All rats died during the first week of the 13 week bioassay (Kasai, et al., 2008).

4.7.3.4. Temporal Relationship

- 1 **4.7.3.4.1. Liver.** Available information regarding temporal relationships between the key event
- 2 (sustained proliferation of spontaneously transformed liver cells) and the eventual formation of
- 3 liver tumors is limited. A comparison of 13-week and 2-year studies conducted in F344/DuCrj
- 4 rats and Crj:BDF1 mice at the same laboratory revealed that tumorigenic doses of 1,4-dioxane
- 5 produced liver toxicity by 13 weeks of exposure (JBRC, 1998; Kano, et al., 2009; Kano, et al.,
- 6 2008). Hepatocyte swelling of the centrilobular area of the liver, vacuolar changes in the liver,
- 7 granular changes in the liver, and single cell necrosis in the liver were observed in mice and rats

1 given 1,4-dioxane in the drinking water for 13 weeks. Sustained liver damage may lead to
2 regenerative cell proliferation and tumor formation following chronic exposure. As discussed
3 above, histopathological evidence of regenerative cell proliferation has been seen following
4 long-term exposure to 1,4-dioxane ([JBRC, 1998](#); [Kociba, et al., 1974](#)). Tumors occurred earlier
5 at high doses in both mice and rats from this study ([Yamazaki, 2006](#)); however, temporal
6 information regarding hyperplasia or other possible key events was not available (i.e., interim
7 blood samples not collected, interim sacrifices were not performed). Argus et al. ([1973](#)) studied
8 the progression of tumorigenesis by electron microscopy of liver tissues obtained following
9 interim sacrifices at 8 and 13 months of exposure (five rats/group, 574 mg/kg-day). The first
10 change observed was an increase in the size of the nuclei of the hepatocytes, mostly in the
11 periportal area. Precancerous changes were characterized by disorganization of the rough
12 endoplasmic reticulum, increase in smooth endoplasmic reticulum, and decrease in glycogen and
13 increase in lipid droplets in hepatocytes. These changes increased in severity in the
14 hepatocellular carcinomas in rats exposed to 1,4-dioxane for 13 months.

15 Three types of liver nodules were observed in exposed rats at 13–16 months. The first
16 consisted of groups of these cells with reduced cytoplasmic basophilia and a slightly nodular
17 appearance as viewed by light microscopy. The second type of nodule was described consisting
18 of large cells, apparently filled and distended with fat. The third type of nodule was described as
19 finger-like strands, 2–3 cells thick, of smaller hepatocytes with large hyperchromic nuclei and
20 dense cytoplasm. This third type of nodule was designated as an incipient hepatoma, since it
21 showed all the histological characteristics of a fully developed hepatoma. All three types of
22 nodules were generally present in the same liver.

23 **4.7.3.4.2. Nasal cavity.** No information was available regarding the temporal relationship
24 between toxicity in the nasal epithelium and the formation of nasal cavity tumors. A comparison
25 of 13-week and 2-year studies conducted in F344/DuCrj rats could not be conducted since the
26 tumorigenic concentration of 1,4-dioxane was different from the concentration which produced
27 nasal toxicities by 13 weeks of exposure ([Kasai, et al., 2009](#); [Kasai, et al., 2008](#)). In addition,
28 severity data were only provided in the shorter term study. Sustained nasal damage may lead to
29 regenerative cell proliferation and tumor formation following chronic exposure. As discussed
30 above (Section 4.2.2.2.1), histopathological evidence of regenerative cell proliferation has been
31 seen following long-term exposure to 1,4-dioxane ([Kasai, et al., 2009](#); [Kasai, et al., 2008](#)), and
32 observations of nasal tumors were also noted at the highest exposure concentration. Other
33 incidences of nasal damage may have occurred before tumor formation; however, temporal
34 information regarding these events was not available (i.e., interim blood samples not collected,
35 interim sacrifices were not performed).

4.7.3.5. *Biological Plausibility and Coherence*

1 **4.7.3.5.1. *Liver.*** The hypothesis that sustained proliferation of spontaneously transformed liver
2 cells is a key event within a MOA is possible based on supporting evidence indicating that
3 1,4-dioxane is a tumor promoter of mouse skin and rat liver tumors ([Bull, et al., 1986](#); [King, et](#)
4 [al., 1973](#); [Lundberg, et al., 1987](#)). Further support for this hypothesis is provided by studies
5 demonstrating that 1,4-dioxane increased hepatocyte DNA synthesis, indicative of cell
6 proliferation ([Goldsworthy, et al., 1991](#); [Miyagawa, et al., 1999](#); [Stott, et al., 1981](#); [Uno, et al.,](#)
7 [1994](#)). In addition, the generally negative results for 1,4-dioxane in a number of genotoxicity
8 assays indicates the carcinogenicity of 1,4-dioxane may not be mediated by a mutagenic MOA.
9 The importance of cytotoxicity as a necessary precursor to sustained cell proliferation is
10 biologically plausible, but is not supported by the dose-response in the majority of studies of
11 1,4-dioxane carcinogenicity.

12 **4.7.3.5.2. *Nasal cavity.*** Sustained cell proliferation in response to cell death from toxicity may
13 be related to the formation of nasal cavity tumors; however, this MOA is also not established .
14 Nasal carcinogens are generally characterized as potent genotoxins ([Ashby, 1994](#)); however,
15 other MOAs have been proposed for nasal carcinogens that induce effects through other
16 mechanisms ([Green et al., 2000](#); [Kasper et al., 2007](#)).

17 The National Toxicological Program (NTP) database identified 12 chemicals from
18 approximately 500 bioassays as nasal carcinogens and 1,4-dioxane was the only identified nasal
19 carcinogen that showed little evidence of genotoxicity ([Haseman & Hailey, 1997](#)). Nasal tumors
20 were not observed in an inhalation study in Wistar rats exposed to 111 ppm for 5 days/week for
21 2 years ([Torkelson, et al., 1974](#)).

4.7.3.6. *Other Possible Modes of Action*

22 An alternate MOA could be hypothesized that 1,4-dioxane alters DNA, either directly or
23 indirectly, which causes mutations in critical genes for tumor initiation, such as oncogenes or
24 tumor suppressor genes. Following these events, tumor growth may be promoted by a number of
25 molecular processes leading to enhanced cell proliferation or inhibition of programmed cell
26 death. The results from in vitro and in vivo assays do not provide overwhelming support for the
27 hypothesis of a genotoxic MOA for 1,4-dioxane carcinogenicity. The genotoxicity data for
28 1,4-dioxane were reviewed in Section 4.5.1 and were summarized in Table 4-19. Negative
29 findings were reported for mutagenicity in *Salmonella typhimurium*, *Escherichia coli*, and
30 *Photobacterium phosphoreum* (Mutatox assay) ([Haworth, et al., 1983](#); [Hellmér & Bolcsfoldi,](#)
31 [1992](#); [Khudoley, et al., 1987](#); [Kwan, et al., 1990](#); [Morita & Hayashi, 1998](#); [Nestmann, et al.,](#)
32 [1984](#); [Stott, et al., 1981](#)). Negative results were also indicated for the induction of aneuploidy in
33 yeast (*Saccharomyces cerevisiae*) and the sex-linked recessive lethal test in *Drosophila*
34 *melanogaster* ([Zimmermann, et al., 1985](#)). In contrast, positive results were reported in assays

1 for sister chromatid exchange ([Galloway, et al., 1987](#)), DNA damage ([Kitchin & Brown, 1990](#)),
2 and in in vivo micronucleus formation in bone marrow ([Mirkova, 1994](#); [Roy, et al., 2005](#)), and
3 liver ([Morita & Hayashi, 1998](#); [Roy, et al., 2005](#)). Lastly, in the presence of toxicity, positive
4 results were reported for meiotic nondisjunction in drosophila ([Munoz & Barnett, 2002](#)), DNA
5 damage ([Sina, et al., 1983](#)), and cell transformation ([Sheu, et al., 1988](#)).

6 Additionally, 1,4-dioxane metabolism did not produce reactive intermediates that
7 covalently bound to DNA ([Stott, et al., 1981](#); [Woo, Argus, et al., 1977b](#)) and DNA repair assays
8 were generally negative ([Goldsworthy, et al., 1991](#); [Stott, et al., 1981](#)). No studies were
9 available to assess the ability of 1,4-dioxane or its metabolites to induce oxidative damage to
10 DNA.

4.7.3.7. *Conclusions About the Hypothesized Mode of Action*

11 **4.7.3.7.1. Liver.** The MOA by which 1,4-dioxane produces liver tumors is unknown, and
12 available evidence in support of any hypothetical mode of carcinogenic action for 1,4-dioxane is
13 inconclusive. A MOA hypothesis involving 1,4-dioxane induced cell proliferation is possible
14 but data are not available to support this hypothesis. Pharmacokinetic data suggest that
15 clearance pathways were saturable and target organ toxicity occurs after metabolic saturation.
16 Liver toxicity preceded tumor formation in one study ([Kociba, et al., 1974](#)) and a regenerative
17 response to tissue injury was demonstrated by histopathology. Liver hyperplasia and tumor
18 formation have also been observed in the absence of cytotoxicity ([JBRC, 1998](#); [Kano, et al.,
19 2009](#)). Cell proliferation and tumor promotion have been shown to occur after prolonged
20 exposure to 1,4-dioxane ([Bull, et al., 1986](#); [Goldsworthy, et al., 1991](#); [King, et al., 1973](#);
21 [Lundberg, et al., 1987](#); [Miyagawa, et al., 1999](#); [Stott, et al., 1981](#); [Uno, et al., 1994](#)).

22 **4.7.3.7.2. Nasal cavity.** The MOA for the formation of nasal cavity tumors is unknown, and
23 evidence in support of any hypothetical mode of carcinogenic action for 1,4-dioxane is
24 inconclusive. A MOA hypothesis involving nasal damage, cell proliferation, and hyperplasia is
25 possible, but data are not available to support this hypothesis. One or more of these events is
26 missing from the studies that examine nasal effects after exposure to 1,4-dioxane. Nasal cavity
27 tumors have been reported in the absence of cell proliferation ([Kasai, et al., 2009](#)) and
28 hyperplasia ([JBRC, 1998](#); [Kano, et al., 2009](#)).

4.7.3.8. *Relevance of the Mode of Action to Humans*

29 Several hypothesized MOAs for 1,4-dioxane induced tumors in laboratory animals have
30 been discussed along with the supporting evidence for each. As was stated, the MOA by which
31 1,4-dioxane produces liver, nasal, peritoneal, and mammary gland tumors is unknown. Some
32 mechanistic information is available to inform the MOA of the liver and nasal tumors but no
33 information exists to inform the MOA of the observed peritoneal or mammary gland tumors
34 ([JBRC, 1998](#); [Kano, et al., 2009](#); [Yamazaki, et al., 1994](#)).

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

1 There is no direct evidence to establish that certain populations and lifestages may be
2 susceptible to 1,4-dioxane. Changes in susceptibility with lifestage as a function of the presence
3 of microsomal enzymes that metabolize and detoxify this compound (i.e., CYP2E1 present in
4 liver, kidney, and nasal mucosa can be hypothesized). Vieira et al. (1996) reported that large
5 increases in hepatic CYP2E1 protein occur postnatally between 1 and 3 months in humans.
6 Adult hepatic concentrations of CYP2E1 are achieved sometime between 1 and 10 years. To the
7 extent that hepatic CYP2E1 levels are lower, children may be more susceptible to liver toxicity
8 from 1,4-dioxane than adults. CYP2E1 has been shown to be inducible in the rat fetus. The
9 level of CYP2E1 protein was increased by 1.4-fold in the maternal liver and 2.4-fold in the fetal
10 liver following ethanol treatment, as compared to the untreated or pair-fed groups (Carpenter,
11 Lasker, & Raucy, 1996). Pre- and postnatal induction of microsomal enzymes resulting from
12 exposure to 1,4-dioxane or other drugs or chemicals may reduce overall toxicity following
13 sustained exposure to 1,4-dioxane.

14 Genetic polymorphisms have been identified for the human CYP2E1 gene (Hayashi,
15 Watanabe, & Kawajiri, 1991; Watanabe, Hayashi, & Kawajiri, 1994) and were considered to be
16 possible factors in the abnormal liver function seen in workers exposed to vinyl chloride (Huang,
17 Huang, Cheng, Wang, & Hsieh, 1997). Individuals with a CYP2E1 genetic polymorphism
18 resulting in increased expression of this enzyme may be less susceptible to toxicity following
19 exposure to 1,4-dioxane.

20 Gender differences were noted in subchronic and chronic toxicity studies of 1,4-dioxane
21 in mice and rats (see Sections 4.6 and 4.7). No consistent pattern of gender sensitivity was
22 identified across studies. In a 13 week inhalation study of male and female rats (Kasai, et al.,
23 2008) kidney toxicity was observed in female rats exposed to 3,200 ppm of 1,4-dioxane (i.e.
24 hydropic change in the renal proximal tubules), but not male rats, which suggests a possible
25 increased susceptibility of female rats to renal damage following inhalation exposure to
26 1,4-dioxane.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Studies and Critical Effect with Rationale and Justification

1 Liver and kidney toxicity were the primary noncancer health effects associated with
2 exposure to 1,4-dioxane in humans and laboratory animals. Occupational exposure to
3 1,4-dioxane has resulted in hemorrhagic nephritis and centrilobular necrosis of the liver ([Barber,
4 1934](#); [Johnstone, 1959](#)). In animals, liver and kidney degeneration and necrosis were observed
5 frequently in acute oral and inhalation studies ([David, 1964](#); [de Navasquez, 1935](#); [Drew, et al.,
6 1978](#); [Fairley, et al., 1934](#); [JBRC, 1998](#); [Kesten, et al., 1939](#); [Laug, et al., 1939](#); [Schrenk & Yant,
7 1936](#)). Liver and kidney effects were also observed following chronic oral exposure to
8 1,4-dioxane in animals ([Argus, et al., 1965](#); [Argus, et al., 1973](#); [JBRC, 1998](#); [Kano, et al., 2009](#);
9 [Kociba, et al., 1974](#); [NCI, 1978](#); [Yamazaki, et al., 1994](#)) (see Table 4-21).

10 Liver toxicity in the available chronic studies was characterized by necrosis, spongiosis
11 hepatic, hyperplasia, cyst formation, clear foci, and mixed cell foci. Kociba et al. ([1974](#))
12 demonstrated hepatocellular degeneration and necrosis at doses of 94 mg/kg-day (LOAEL in
13 male rats) or greater. The NOAEL for liver toxicity was 9.6 mg/kg-day and 19 mg/kg-day in
14 male and female rats, respectively. No quantitative incidence data were provided in this study.
15 Argus et al. ([1973](#)) described early preneoplastic changes in the liver and JBRC ([1998](#))
16 demonstrated liver lesions that are primarily associated with the carcinogenic process. Clear and
17 mixed-cell foci in the liver are commonly considered preneoplastic changes and would not be
18 considered evidence of noncancer toxicity. In the JBRC ([1998](#)) study, spongiosis hepatitis was
19 associated with other preneoplastic changes in the liver (clear and mixed-cell foci) and no other
20 lesions indicative of liver toxicity were seen. Spongiosis hepatitis was therefore not considered
21 indicative of noncancer effects in this study. The activity of serum enzymes (i.e., AST, ALT,
22 LDH, and ALP) was increased in mice and rats chronically exposed to 1,4-dioxane ([JBRC,
23 1998](#)); however, these increases were seen only at tumorigenic dose levels. Blood samples were
24 collected at study termination and elevated serum enzymes may reflect changes associated with
25 tumor formation. Histopathological evidence of liver toxicity was not seen in rats from the
26 JBRC ([1998](#)) study. The highest non-tumorigenic dose levels for this study approximated the
27 LOAEL derived from the Kociba et al. ([1974](#)) study (94 and 148 mg/kg-day for male and female
28 rats, respectively).

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#).

1 Kidney damage in chronic toxicity studies was characterized by degeneration of the
2 cortical tubule cells, necrosis with hemorrhage, and glomerulonephritis ([Argus, et al., 1965](#);
3 [Argus, et al., 1973](#); [Fairley, et al., 1934](#); [Kociba, et al., 1974](#); [NCI, 1978](#)). Kociba et al. (1974)
4 described renal tubule epithelial cell degeneration and necrosis at doses of 94 mg/kg-day
5 (LOAEL in male rats) or greater, with a NOAEL of 9.6 mg/kg-day. No quantitative incidence
6 data were provided in this study ([Kociba, et al., 1974](#)). Doses of ≥ 430 mg/kg-day 1,4-dioxane
7 induced marked kidney alterations ([Argus, et al., 1973](#)). The observed changes included
8 glomerulonephritis and pyelonephritis, with characteristic epithelial proliferation of Bowman's
9 capsule, periglomerular fibrosis, and distension of tubules. Quantitative incidence data were not
10 provided in this study. In the NCI (1978) study, kidney lesions in rats consisted of vacuolar
11 degeneration and/or focal tubular epithelial regeneration in the proximal cortical tubules and
12 occasional hyaline casts. Kidney toxicity was not seen in rats from the JBRC (1998) study at any
13 dose level (highest dose was 274 mg/kg-day in male rats and 429 mg/kg-day in female rats).

14 Kociba et al. (1974) was chosen as the principal study for derivation of the RfD because
15 the liver and kidney effects in this study are considered adverse and represent the most sensitive
16 effects identified in the database (NOAEL 9.6 mg/kg-day, LOAEL 94 mg/kg-day in male rats).
17 Kociba et al. (1974) reported degenerative effects in the liver, while liver lesions reported in
18 other studies ([Argus, et al., 1973](#); [JBRC, 1998](#)) appeared to be related to the carcinogenic
19 process. Kociba et al. (1974) also reported degenerative changes in the kidney. NCI (1978) and
20 Argus et al. (1973) provided supporting data for this endpoint; however, kidney toxicity was
21 observed in these studies at higher doses. JBRC (1998) reported nasal inflammation in rats
22 (NOAEL 55 mg/kg-day, LOAEL 274 mg/kg-day) and mice (NOAEL 66 mg/kg-day, LOAEL
23 278 mg/kg-day).

24 Even though the study reported by Kociba et al. (1974) had one noteworthy weakness, it
25 had several noted strengths, including: (1) two-year study duration; (2) use of both male and
26 female rats and three dose levels, 10-fold apart, plus a control group; (3) a sufficient number of
27 animals per dose group (60 animals/sex/dose group); and (4) the authors conducted a
28 comprehensive evaluation of the animals including body weights and clinical observations, blood
29 samples, organ weights of all the major tissues, and a complete histopathological examination of
30 all rats. The authors did not report individual incidence data that would have allowed for a BMD
31 analysis of this robust dataset.

5.1.2. Methods of Analysis—Including Models (PBPK, BMD, etc.)

32 Several procedures were applied to the human PBPK model to determine if an adequate
33 fit of the model to the empirical model output or experimental observations could be attained
34 using biologically plausible values for the model parameters. The re-calibrated model
35 predictions for blood 1,4-dioxane levels did not come within 10-fold of the experimental values

1 using measured tissue:air partition coefficients of Leung and Paustenbach ([1990](#)) or Sweeney
2 et al. ([2008](#)) (Figures B-8 and B-9). The utilization of a slowly perfused tissue:air partition
3 coefficient 10-fold lower than measured values produces exposure-phase predictions that are
4 much closer to observations, but does not replicate the elimination kinetics (Figure B-10). Re-
5 calibration of the model with upper bounds on the tissue:air partition coefficients results in
6 predictions that are still six- to sevenfold lower than empirical model prediction or observations
7 (Figures B-12 and B-13). Exploration of the model space using an assumption of zero-order
8 metabolism (valid for the 50 ppm inhalation exposure) showed that an adequate fit to the
9 exposure and elimination data can be achieved only when unrealistically low values are assumed
10 for the slowly perfused tissue:air partition coefficient (Figure B-16). Artificially low values for
11 the other tissue:air partition coefficients are not expected to improve the model fit, as these
12 parameters are shown in the sensitivity analysis to exert less influence on blood 1,4-dioxane than
13 V_{maxC} and K_m . This suggests that the model structure is insufficient to capture the apparent 10-
14 fold species difference in the blood 1,4-dioxane between rats and humans. In the absence of
15 actual measurements for the human slowly perfused tissue:air partition coefficient, high
16 uncertainty exists for this model parameter value. Differences in the ability of rat and human
17 blood to bind 1,4-dioxane may contribute to the difference in V_d . However, this is expected to
18 be evident in very different values for rat and human blood:air partition coefficients, which is not
19 the case (Table B-1). Therefore, some other, as yet unknown, modification to model structure
20 may be necessary.

21 Kociba et al. ([1974](#)) did not provide quantitative incidence or severity data for liver and
22 kidney degeneration and necrosis. Benchmark dose (BMD) modeling could not be performed
23 for this study and the NOAEL for liver and kidney degeneration (9.6 mg/kg-day in male rats)
24 was used as the point of departure (POD) in deriving the RfD for 1,4-dioxane.

25 Alternative PODs were calculated using incidence data reported for cortical tubule
26 degeneration in male and female rats ([NCI, 1978](#)) and liver hyperplasia ([JBRC, 1998](#)). The
27 incidence data for cortical tubule cell degeneration in male and female rats exposed to
28 1,4-dioxane in the drinking water for 2 years are presented in Table 5-1. Details of the BMD
29 analysis of these data are presented in Appendix C. Male rats were more sensitive to the kidney
30 effects of 1,4-dioxane than females and the male rat data provided the lowest POD for cortical
31 tubule degeneration in the NCI ([1978](#)) study (BMDL₁₀ of 22.3 mg/kg-day) (Table 5-2).
32 Incidence data ([JBRC, 1998](#); [Kano, et al., 2009](#)) for liver hyperplasia in male and female rats
33 exposed to 1,4-dioxane in the drinking water for 2 years are presented in Table 5-3. Details of
34 the BMD analysis of these data are presented in Appendix C. Male rats were more sensitive to
35 developing liver hyperplasia due to exposure to 1,4-dioxane than females and the male rat data
36 provided the lowest POD for hyperplasia in the JBRC ([1998](#)) study (BMDL₁₀ of 23.8 mg/kg-
37 day) (Table 5-4). The BMDL₁₀ values of 22.3 mg/kg-day and 23.8 mg/kg-day from the NCI

- 1 (1978) and JBRC (1998) studies, respectively, are about double the NOAEL (9.6 mg/kg-day)
- 2 observed by Kociba et al. (1974).

Table 5-1. Incidence of cortical tubule degeneration in Osborne-Mendel rats exposed to 1,4-dioxane in drinking water for 2 years

Males (mg/kg-day)				Females (mg/kg-day)		
0	240	530		0	350	640
0/31 ^a	20/31 ^b	27/33 ^b		0/31 ^a	0/34	10/32 ^b

^aStatistically significant trend for increased incidence by Cochran-Armitage test ($p < 0.05$) performed for this review.

^bIncidence significantly elevated compared to control by Fisher's Exact test ($p < 0.001$) performed for this review.

Source: NCI (1978).

Table 5-2. BMD and BMDL values derived from BMD modeling of cortical tubule degeneration in male and female Osborne-Mendel rats exposed to 1,4-dioxane in drinking water for 2 years

	BMD ₁₀ (mg/kg-day)	BMDL ₁₀ (mg/kg-day)
Male rats	28.8	22.3
Female rats	596.4	452.4

Source: NCI (1978).

Table 5-3. Incidence of liver hyperplasia in F344/DuCrj rats exposed to 1,4-dioxane in drinking water for 2 years^a

Males (mg/kg-day)				Females (mg/kg-day)			
0	11	55	274	0	18	83	429
3/40	2/45	9/35 ^b	12/22 ^c	0/38 ^b	0/37	1/38	14/24 ^c

^aDose information from Kano et al. (2009) and incidence data for sacrificed animals from JBRC (1998).

^bStatistically significant compared to controls by the Dunnett's test ($p < 0.05$).

^cIncidence significantly elevated compared to control by χ^2 test ($p < 0.01$).

Sources: Kano et al. (2009); JBRC (1998).

Table 5-4. BMD and BMDL values derived from BMD modeling of liver hyperplasia in male and female F344/DuCrj rats exposed to 1,4-dioxane in drinking water for 2 years

	BMD ₁₀ (mg/kg-day)	BMDL ₁₀ (mg/kg-day)
Male rats	35.9	23.8
Female rats	137.3	88.5

Source: Kano et al. (2009); JBRC (1998).

5.1.3. RfD Derivation - Including Application of Uncertainty Factors (UFs)

1 The RfD of 3×10^{-2} mg/kg-day is based on liver and kidney toxicity in rats exposed to
 2 1,4-dioxane in the drinking water for 2 years (Kociba, et al., 1974). The Kociba et al. (1974)
 3 study was chosen as the principal study because it provides the most sensitive measure of
 4 adverse effects by 1,4-dioxane. The incidence of liver and kidney lesions was not reported for
 5 each dose group. Therefore, BMD modeling could not be used to derive a POD. The RfD for
 6 1,4-dioxane is derived by dividing the NOAEL of 9.6 mg/kg-day (Kociba, et al., 1974) by a
 7 composite UF of 300, as follows:

$$\begin{aligned}
 \text{RfD} &= \text{NOAEL} / \text{UF} \\
 &= 9.6 \text{ mg/kg-day} / 300 \\
 &= 0.03 \text{ or } 3 \times 10^{-2} \text{ mg/kg-day}
 \end{aligned}$$

11 The composite UF of 300 includes factors of 10 for animal-to-human extrapolation and
 12 for interindividual variability, and an UF of 3 for database deficiencies.

13 A default interspecies UF of 10 was used to account for pharmacokinetic and
 14 pharmacodynamic differences across species. Existing PBPK models could not be used to derive
 15 an oral RfD for 1,4-dioxane (Appendix B).

16 A default interindividual variability UF of 10 was used to account for variation in
 17 sensitivity within human populations because there is limited information on the degree to which
 18 humans of varying gender, age, health status, or genetic makeup might vary in the disposition of,
 19 or response to, 1,4-dioxane.

20 An UF of 3 for database deficiencies was applied due to the lack of a multigeneration
 21 reproductive toxicity study. A single oral prenatal developmental toxicity study in rats was
 22 available for 1,4-dioxane (Giavini, et al., 1985). This developmental study indicates that the
 23 developing fetus may be a target of toxicity.

24 An UF to extrapolate from a subchronic to a chronic exposure duration was not necessary
 25 because the RfD was derived from a study using a chronic exposure protocol.

26 An UF to extrapolate from a LOAEL to a NOAEL was not necessary because the RfD
 27 was based on a NOAEL. Kociba et al. (1974) was a well-conducted, chronic drinking water

1 study with an adequate number of animals. Histopathological examination was performed for
2 many organs and tissues, but clinical chemistry analysis was not performed. NOAEL and
3 LOAEL values were derived by the study authors based on liver and kidney toxicity; however
4 quantitative incidence data was not reported. Several additional oral studies (acute/short-term,
5 subchronic, and chronic durations) were available that support liver and kidney toxicity as the
6 critical effect ([Argus, et al., 1973](#); [JBRC, 1998](#); [Kano, et al., 2008](#); [NCI, 1978](#)) (Tables 4-15 and
7 4-17). Although degenerative liver and kidney toxicity was not observed in rats from the JBRC
8 ([1998](#)) study at doses at or below the LOAEL in the Kociba et al. ([1974](#)) study, other endpoints
9 such as metaplasia and hyperplasia of the nasal epithelium, nuclear enlargement, and
10 hematological effects, were noted.

5.1.4. RfD Comparison Information

11 PODs and sample oral RfDs based on selected studies included in Table 4-18 are arrayed
12 in Figures 5-1 to 5-3, and provide perspective on the RfD supported by Kociba et al. ([1974](#)).
13 These figures should be interpreted with caution because the PODs across studies are not
14 necessarily comparable, nor is the confidence in the data sets from which the PODs were derived
15 the same. PODs in these figures may be based on a NOAEL, LOAEL, or BMDL (as indicated),
16 and the nature, severity, and incidence of effects occurring at a LOAEL are likely to vary. To
17 some extent, the confidence associated with the resulting sample RfD is reflected in the
18 magnitude of the total UF applied to the POD (i.e., the size of the bar); however, the text of
19 Sections 5.1.1 and 5.1.2 should be consulted for a more complete understanding of the issues
20 associated with each data set and the rationale for the selection of the critical effect and principal
21 study used to derive the RfD.

22 The predominant noncancer effect of chronic oral exposure to 1,4-dioxane is
23 degenerative effects in the liver and kidney. Figure 5-1 provides a graphical display of effects
24 that were observed in the liver following chronic oral exposure to 1,4-dioxane. Information
25 presented includes the PODs and UFs that could be considered in deriving the oral RfD. As
26 discussed in Sections 5.1.1 and 5.1.2, among those studies that demonstrated liver toxicity, the
27 study by Kociba et al. ([1974](#)) provided the data set most appropriate for deriving the RfD. For
28 degenerative liver effects resulting from 1,4-dioxane exposure, the Kociba et al. ([1974](#)) study
29 represents the most sensitive effect and dataset observed in a chronic bioassay (Figure 5-1).

30 Kidney toxicity as evidenced by glomerulonephritis ([Argus, et al., 1965](#); [Argus, et al.,](#)
31 [1973](#)) and degeneration of the cortical tubule ([Kociba, et al., 1974](#); [NCI, 1978](#)) has also been
32 observed in response to chronic exposure to 1,4-dioxane. As was discussed in Sections 5.1 and
33 5.2, degenerative effects were observed in the kidney at the same dose level as effects in the liver
34 ([Kociba, et al., 1974](#)). A comparison of the available datasets from which an RfD could
35 potentially be derived is presented in Figure 5-2.

1 Rhinitis and inflammation of the nasal cavity were reported in both the NCI (1978) (mice
2 only, dose ≥ 380 mg/kg-day) and JBRC (1998) studies (≥ 274 mg/kg-day in rats, >278 mg/kg-
3 day in mice). JBRC (1998) reported nasal inflammation in rats (NOAEL 55 mg/kg-day, LOAEL
4 274 mg/kg-day) and mice (NOAEL 66 mg/kg-day, LOAEL 278 mg/kg-day). A comparison of
5 the available datasets from which an RfD could potentially be derived is presented in Figure 5-3.

6 Figure 5-4 displays PODs for the major targets of toxicity associated with oral exposure
7 to 1,4-dioxane. Studies in experimental animals have also found that relatively high doses of
8 1,4-dioxane (1,000 mg/kg-day) during gestation can produce delayed ossification of the
9 sternebrae and reduced fetal BWs (Giavini, et al., 1985). This graphical display (Figure 5-4)
10 compares organ specific toxicity for 1,4-dioxane, including a single developmental study. The
11 most sensitive measures of degenerative liver are and kidney effects. The sample RfDs for
12 degenerative liver and kidney effects are identical since they were derived from the same study
13 and dataset (Kociba, et al., 1974) and are presented for completeness.

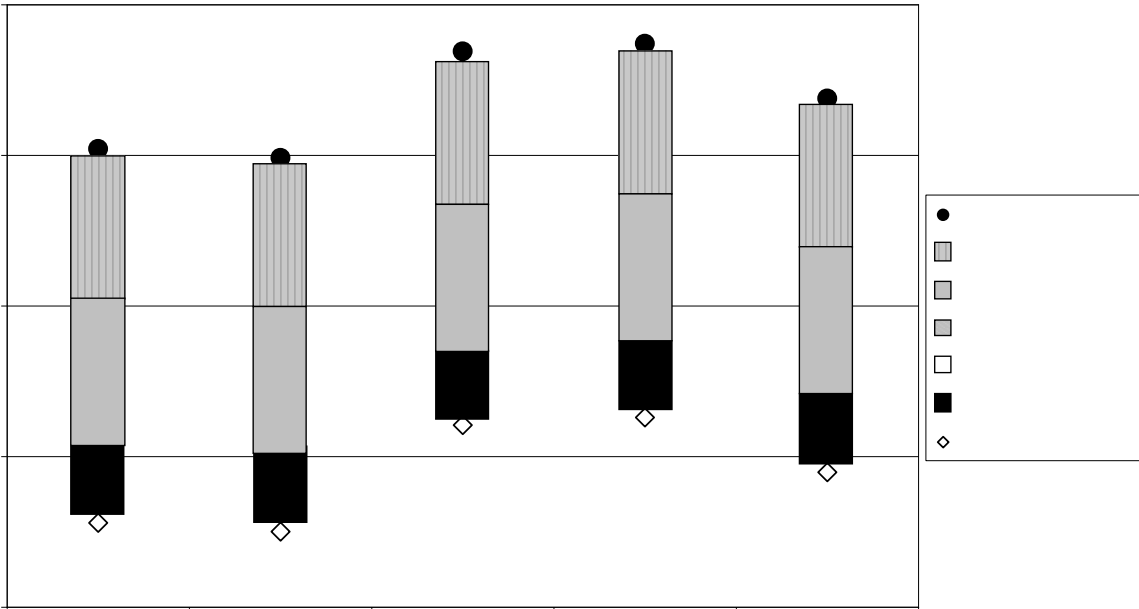


Figure 5-1. Potential points of departure (POD) for liver toxicity endpoints with corresponding applied uncertainty factors and derived RfDs following oral exposure to 1,4-dioxane.

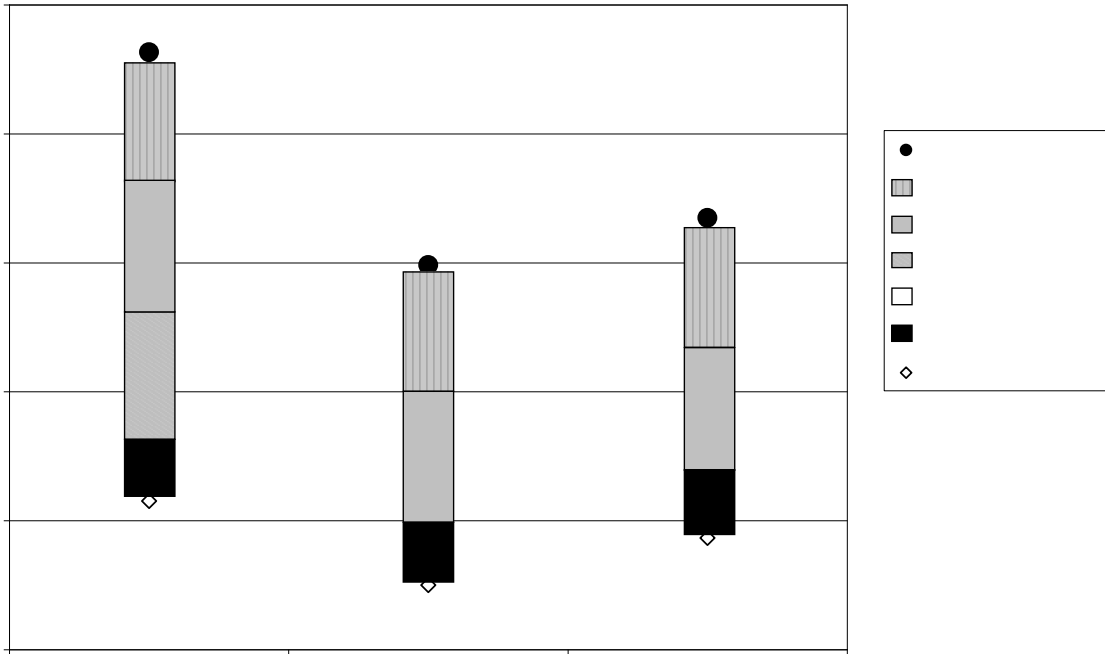


Figure 5-2. Potential points of departure (POD) for kidney toxicity endpoints with corresponding applied uncertainty factors and derived RfDs following oral exposure to 1,4-dioxane.

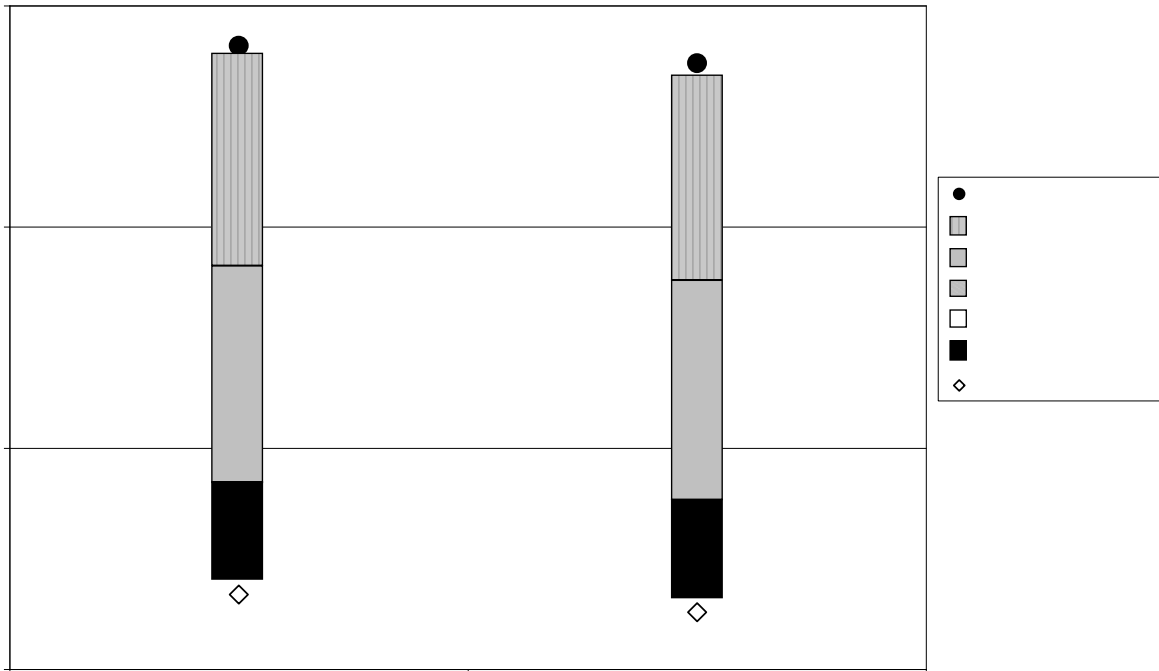


Figure 5-3. Potential points of departure (POD) for nasal inflammation with corresponding applied uncertainty factors and derived sample RfDs following oral exposure to 1,4-dioxane.

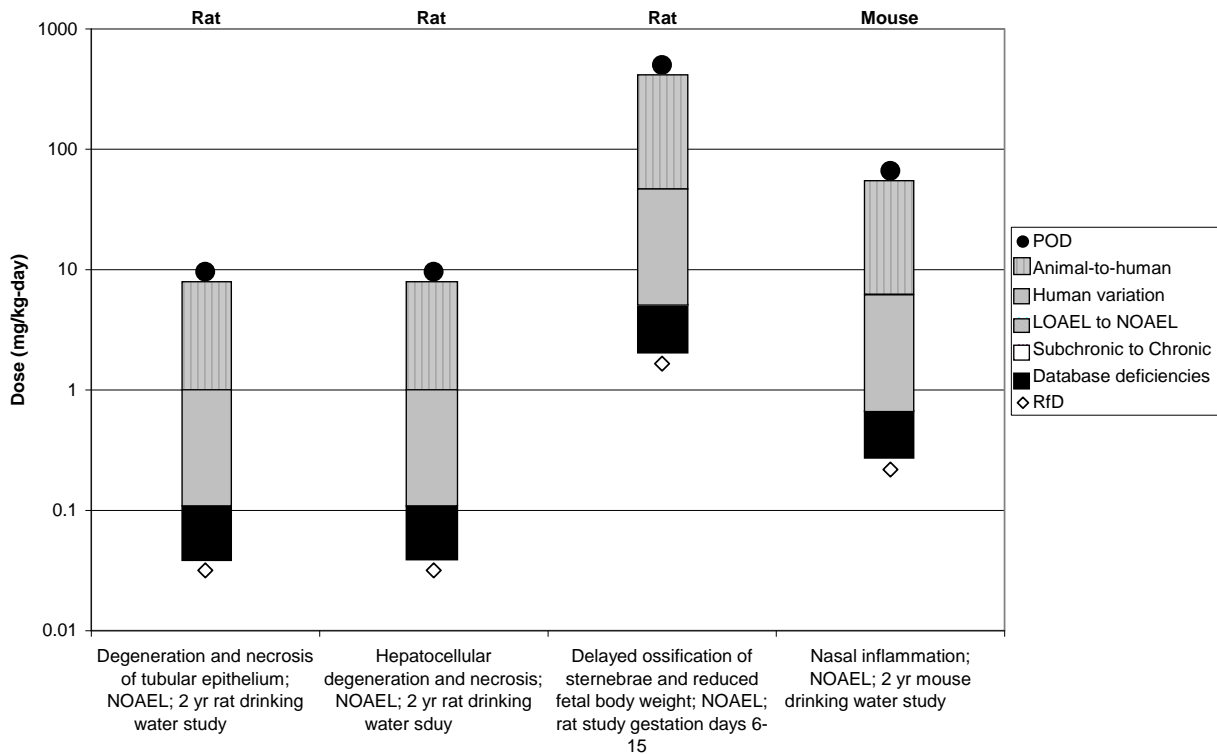


Figure 5-4. Potential points of departure (POD) for organ specific toxicity endpoints with corresponding applied uncertainty factors and derived sample RfDs following oral exposure to 1,4-dioxane.

5.1.5. Previous RfD Assessment

1 An assessment for 1,4-dioxane was previously posted on the IRIS database in 1988. An
 2 oral RfD was not developed as part of the 1988 assessment.

5.2. INHALATION REFERENCE CONCENTRATION (RFC)

5.2.1. Choice of Principal Studies and Critical Effect(s) with Rationale and Justification

3 Two human studies of occupational exposure to 1,4-dioxane have been published
 4 (Buffler, et al., 1978; Thiess, et al., 1976); however, neither study provides sufficient information
 5 and data to quantify subchronic or chronic noncancer effects. In each study, findings were
 6 inconclusive and the cohort size and number of reported cases were limited (Buffler, et al., 1978;
 7 Thiess, et al., 1976).

8 Four inhalation studies in animals were identified in the literature; two, 13-week
 9 subchronic studies in laboratory animals (Fairley, et al., 1934; Kasai, et al., 2008) and two, 2-
 10 year chronic studies in rats (Kasai, et al., 2009; Torkelson, et al., 1974).

1 In the subchronic study by Fairley et al. (1934) rabbits, guinea pigs, rats, and mice
2 (3-6/species/group) were exposed to 1,000, 2,000, 5,000, or 10,000 ppm of 1,4-dioxane vapor for
3 1.5 hours two times a day for 5 days, 1.5 hours for one day, and no exposure on the seventh day.
4 Animals were exposed until death occurred or were sacrificed after various durations of exposure
5 (3-202.5 hours). Detailed dose-response information was not provided; however, severe liver
6 and kidney damage and acute vascular congestion of the lungs were observed at concentrations ≥
7 1,000 ppm. Kidney damage was described as patchy degeneration of cortical tubules with
8 vascular congestion and hemorrhage. Liver lesions varied from cloudy hepatocyte swelling to
9 large areas of necrosis. In this study, a LOAEL of 1,000 ppm for liver and kidney degeneration
10 in rats, mice, rabbits, and guinea pigs was identified by the EPA.

11 In the subchronic study by Kasai et al. (2008) male and female rats (10/group/sex) were
12 exposed to 0, 100, 200, 400, 800, 1,600, 3,200, and 6,400 ppm of 1,4-dioxane for 6 hours/day, 5
13 days/week for 13 weeks. This study observed a range of 1,4-dioxane induced nonneoplastic
14 effects across several organ systems including the liver and respiratory tract (from the nose to the
15 bronchus region) in both sexes and the kidney in females. Detailed dose-response information
16 was provided, illustrating a concentration-dependent increase of nuclear enlargement of nasal
17 (respiratory and olfactory), trachea, and bronchus epithelial cells (both sexes); vacuolic change
18 of nasal and bronchial epithelial cells (both sexes), necrosis and centrilobular swelling of
19 hepatocytes (both sexes); and hydropic change in the proximal tubules of the kidney (females).
20 The study authors determined nuclear enlargement of the nasal respiratory epithelium as the
21 most sensitive lesion and a LOAEL of 100 ppm was identified based on this effect.

22 Torkelson et al. (1974) performed a chronic inhalation study in which male and female
23 Wistar rats (288/sex) were exposed to 111 ppm 1,4-dioxane vapor for 7 hours/day, 5 days/week
24 for 2 years. Control rats (192/sex) were exposed to filtered air. No significant effects were
25 observed on BWs, survival, organ weights, hematology, clinical chemistry, or histopathology.
26 A free standing NOAEL of 111 ppm was identified in this study by EPA.

27 Kasai et al. (2009) reported data for groups of male F344 rats (50/group) exposed to 0,
28 50, 250, and 1,250 ppm of 1,4-dioxane for 6 hours/day, 5 days/week, for 2 years. In contrast to
29 the subchronic Kasai et al. (2008) study, this 2-year bioassay reported more nonneoplastic effects
30 in multiple organ systems. Additional noted incidences included: (1) inflammation of nasal
31 respiratory and olfactory epithelium, (2) squamous cell metaplasia and hyperplasia of nasal
32 respiratory epithelium, (3) atrophy and respiratory metaplasia of olfactory epithelium, (4)
33 hydropic change and sclerosis in lamina propria of nasal cavity, (5) nuclear enlargement in
34 proximal tubules of kidney and in centrilobular of liver, (6) centrilobular necrosis in the liver,
35 and (7) spongiosis hepatis. Some of these histopathological lesions were significantly increased
36 compared to controls at the lowest exposure level (50 ppm), including nuclear enlargement of
37 respiratory and olfactory epithelium; and atrophy and respiratory metaplasia of olfactory

1 epithelium. Many of these histopathological lesions were increased in a concentration-dependent
2 manner.

3 Because Fairley et al. (1934) did not present the statistics of the dose response data, and
4 Torkelson et al. (1974) identified a free-standing NOAEL only, neither study was sufficient to
5 characterize the inhalation risks of 1,4-dioxane. A route extrapolation from oral toxicity data
6 was not performed because 1,4-dioxane inhalation causes direct effects on the respiratory tract
7 (i.e., respiratory irritation in humans, pulmonary congestion in animals) (Fairley, et al., 1934;
8 Wirth & Klimmer, 1936; Yant, et al., 1930), which would not be accounted for in a cross-route
9 extrapolation. In addition, available kinetic models are not suitable for this purpose (Appendix
10 B).

11 The chronic Kasai et al. (2009) study was selected as the principal study for the
12 derivation of the RfC. Based on the noncancer database for 1,4-dioxane, this study demonstrated
13 exposure concentration-related effects for histopathological lesions at lower doses as compared
14 to the subchronic Kasai et al. study (2008). In addition, the Kasai et al. (2009) 2-year bioassay
15 study utilized 50 animals per exposure group, a range of exposure concentrations which were
16 based on the results of the subchronic study (2008) and thoroughly examined toxicity of 1-
17 4,dioxane in multiple organ systems. This 2-year bioassay (Kasai, et al., 2009) did not observe
18 effects in both sexes, but the use of only male rats was proposed by the study authors as justified
19 by data illustrating the absence of induced mesotheliomas in female rats following exposure to
20 1,4-dioxane in drinking water (Yamazaki, et al., 1994).

21 All systemic and portal-of-entry nonneoplastic lesions from the Kasai et al. (2009) study
22 that were statistically increased at the low- or mid- exposure concentration (50 or 250 ppm)
23 compared to controls, or the lesions that demonstrated a dose-response relationship in the
24 absence of statistical significance were considered candidates for the critical effect. The
25 candidate endpoints included centrilobular necrosis of the liver, spongiosis hepatitis, squamous
26 cell metaplasia of nasal respiratory epithelium, squamous cell hyperplasia of nasal respiratory
27 epithelium, respiratory metaplasia of nasal olfactory epithelium, sclerosis in lamina propria of
28 nasal cavity, and two degenerative nasal lesions, that is, atrophy of nasal olfactory epithelium
29 and hydropic change in the lamina propria (Table 5-5). Despite statistical increases at the low-
30 and mid exposure concentrations, incidences of nuclear enlargement of respiratory epithelium
31 (nasal cavity), olfactory epithelium (nasal cavity), and proximal tubule (kidney) were not
32 considered candidates for the critical effect given that the toxicological significance of nuclear
33 enlargement is uncertain (See Section 4.6.2 and Table 4-22).

Table 5-5. Incidences of nonneoplastic lesions resulting from chronic exposure (ppm) to 1,4-dioxane considered for identification of a critical effect.

<u>Species/Strain</u>	<u>Tissue</u>	<u>Endpoint</u>	<u>Concentration (ppm)</u>			
			<u>0</u>	<u>50</u>	<u>250</u>	<u>1250</u>
<u>Rat/ F344 (male)</u>	<u>Liver</u>	<u>Centrilobular necrosis</u>	<u>1/50</u>	<u>3/50</u>	<u>6/50</u>	<u>12/50^a</u>
		<u>Spongiosis hepatitis</u>	<u>7/50</u>	<u>6/50</u>	<u>13/50</u>	<u>19/50^a</u>
	<u>Nasal</u>	<u>Squamous cell metaplasia; respiratory epithelium</u>	<u>0/50</u>	<u>0/50</u>	<u>7/50^b</u>	<u>44/50^a</u>
		<u>Squamous cell hyperplasia; respiratory epithelium</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>	<u>10/50^a</u>
		<u>Respiratory metaplasia; olfactory epithelium</u>	<u>11/50</u>	<u>34/50^a</u>	<u>49/50^a</u>	<u>48/50^a</u>
		<u>Atrophy; olfactory epithelium</u>	<u>0/50</u>	<u>40/50^a</u>	<u>47/50^a</u>	<u>48/50^a</u>
		<u>Hydropic change; lamina propria</u>	<u>0/50</u>	<u>2/50</u>	<u>36/50^a</u>	<u>49/50^a</u>
		<u>Sclerosis; lamina propria</u>	<u>0/50</u>	<u>0/50</u>	<u>22/50^a</u>	<u>40/50^a</u>

^ap ≤ 0.01 by Fisher's exact test.

^bp ≤ 0.05 by Fisher's exact test.

Source: Kasai et al. (2009).

5.2.2. Methods of Analysis

1 Benchmark dose (BMD) modeling methodology (U.S. EPA, 2000a) was used to analyze
2 the candidate endpoints identified for 1,4-dioxane. Use of BMD methods involves fitting
3 mathematical models to the observed dose-response data and provides a BMD and its 95% lower
4 confidence limit (BMDL) associated with a predetermined benchmark response (BMR). The
5 suitability of these methods to determine a POD is dependent on the nature of the toxicity
6 database for a specific chemical. For 1,4-dioxane, the selected datasets in Table 5-5 were
7 analyzed using BMD modeling. Information regarding the degree of change in the selected
8 endpoints that is considered biologically significant was not available. Therefore, a BMR of
9 10% extra risk was selected under the assumption that it represents a minimally biologically
10 significant response level (U.S. EPA, 2000a).

11 BMD model results were inadequate (poor fit and/or substantial model uncertainty – see
12 Appendix F) for the following nasal lesions: atrophy (olfactory epithelium), respiratory
13 metaplasia (olfactory epithelium), and sclerosis (lamina propria). Considering the datasets for
14 atrophy and respiratory metaplasia, in which the first non-control dose had a response level
15 substantially above the desired BMR, the use of BMD methods included substantial model
16 uncertainty (Appendix F). The detailed results of the BMDS analysis are provided in Appendix

1 F. Consequently, NOAELs and LOAELs were used as potential PODs for the endpoints not
 2 suitable for BMD modeling.

3 **5.2.3. Exposure Duration and Dosimetric Adjustments**

3 Because an RfC is a measure that assumes continuous human exposure over a lifetime,
 4 data derived from animal studies need to be adjusted to account for the noncontinuous exposure
 5 protocols used in animal studies. In the Kasai et al. (2009) study, rats were exposed to 1,4-
 6 dioxane for 6 hours/day, 5 days/week for 2 years. Therefore, the duration-adjusted PODs for
 7 nasal and systemic lesions in rats were calculated as follows:

8
$$\text{POD}_{\text{ADJ}}(\text{ppm}) = \text{POD}(\text{ppm}) \times \frac{\text{hours exposed per day}}{24\text{hours}} \times \frac{\text{days exposed per week}}{7\text{days}}$$

9 RfCs are typically expressed in units of mg/m³; so PODADJ (ppm) values were
 10 converted using the chemical specific conversion factor of 1 ppm = 3.6 mg/m³ for 1,4-dioxane
 11 (Table 2-1). The following calculation was used:

12
$$\text{POD}_{\text{ADJ}}(\text{mg/m}^3) = \text{POD}_{\text{ADJ}}(\text{ppm}) \times \frac{3.6 \text{ mg/m}^3}{1\text{ppm}}$$

13 The calculated POD_{ADJ} (mg/m³) values for all considered endpoints are presented in the
 14 last column of Table 5-6.

Table 5-6. Duration adjusted POD estimates for best fitting BMDS models or NOAEL/LOAEL from chronic exposure to 1,4-dioxane

<u>Endpoint</u>	<u>NOAEL^a (ppm)</u>	<u>LOAEL^b (ppm)</u>	<u>Model</u>	<u>BMR</u>	<u>BMD (ppm)</u>	<u>BMDL (ppm)</u>	<u>POD_{ADJ} (mg/m³)</u>
<i>Nasal Effects</i>							
<u>Squamous cell metaplasia; respiratory epithelium</u>	<u>50</u>	<u>250</u>	<u>Log-probit</u>	<u>10</u>	<u>218</u>	<u>160</u>	<u>103</u>
<u>Squamous cell hyperplasia; respiratory epithelium</u>	<u>250</u>	<u>1250</u>	<u>Log-probit</u>	<u>10</u>	<u>756</u>	<u>561</u>	<u>361</u>
<u>Respiratory metaplasia; olfactory epithelium</u>	<u>--</u>	<u>50</u>	<u>--^c</u>	<u>--</u>	<u>--</u>	<u>--</u>	<u>32.2</u>
<u>Atrophy; olfactory epithelium</u>	<u>--</u>	<u>50</u>	<u>--^c</u>	<u>--</u>	<u>--</u>	<u>--</u>	<u>32.2</u>
<u>Hydropic change; lamina propria</u>	<u>50</u>	<u>250</u>	<u>Log-logistic</u>	<u>10</u>	<u>69</u>	<u>47</u>	<u>30.2</u>
<u>Sclerosis; lamina propria</u>	<u>50</u>	<u>250</u>	<u>--^c</u>	<u>--</u>	<u>--</u>	<u>--</u>	<u>32.2</u>
<i>Systemic Effects</i>							
<u>Centrilobular necrosis; Liver</u>	<u>250</u>	<u>1250</u>	<u>Dichotomous-Hill</u>	<u>10</u>	<u>220</u>	<u>60</u>	<u>38.6</u>
<u>Spongiosis hepatitis; Liver</u>	<u>250</u>	<u>1250</u>	<u>Log-logistic^d</u>	<u>10</u>	<u>314</u>	<u>172</u>	<u>111</u>

^aNOAEL is identified as the highest tested exposure dose at which there is no statistically significant effect in the exposed group as compared to control.

^bLOAEL is identified as the lowest tested exposure dose at which there is a statistically significant effect in the exposed group as compared to control.

^cBMDs model results are not adequate for use to derive a POD. Therefore, NOAEL/LOAEL approach is recommended to determine a POD_{ADJ} for these endpoints. BMDs analysis for these endpoints is included in Appendix F.

^dDichotomous Hill model had lowest BMDL, but model output warned that the BMDL estimate was “imprecise at best”.

1 Based on analysis of data in Table 5-5, hydropic change, atrophy, and respiratory
2 metaplasia were a considered for selection of the critical effect. Typically, chemical-induced
3 nasal effects include atrophy and/or necrosis, cell proliferation/hyperplasia, and metaplasia
4 depending on the nature of the tissue damage and exposure (Boorman, Morgan, & Uriah, 1990;
5 Gaskell, 1990; Harkema, Carey, & Wagner, 2006). These effects are often accompanied by an
6 inflammatory response. BMD analysis indicated hydropic change of lamina propria was the
7 most sensitive endpoint. Hydropic change and atrophy were the two degenerative nasal lesions
8 observed (Table 5-5); however, because hydropic change has a NOAEL (i.e., 50 ppm) and a
9 calculated BMDL (i.e., 47 ppm) at an exposure concentration equivalent to the LOAEL (i.e., 50
10 ppm) designated for other nasal lesions (i.e., atrophy and respiratory metaplasia), hydropic
11 change was not selected as the critical effect. Therefore, in this assessment the LOAEL approach
12 is the preferred methodology for selection of the critical effect and POD.

13 Using the LOAEL approach, atrophy and respiratory metaplasia of the olfactory
14 epithelium are identified as the most sensitive endpoints with a POD_{ADJ} of 32.2 mg/m³. Since
15 atrophy of the olfactory epithelium had an increased incidence rate at the LOAEL compared to
16 respiratory metaplasia and is likely to occur earlier in the continuum of pathological events
17 associated with respiratory tract effects, it is selected as the critical effect in this assessment.

18 For the derivation of a RfC based upon an animal study, the selected POD must be
19 adjusted to reflect the human equivalent concentration (HEC). The HEC was calculated by the
20 application of the appropriate dosimetric adjustment factor (DAF), in accordance with the U.S.
21 EPA RfC methodology (U.S. EPA, 1994b). DAFs are ratios of animal and human physiologic
22 parameters, and are dependent on the nature of the contaminant (particle or gas) and the target
23 site (e.g., respiratory tract or remote to the portal-of-entry) (U.S. EPA, 1994b).

24 1,4-Dioxane is miscible with water and has a high blood:air partition coefficient.
25 Typically, highly water-soluble and directly reactive chemicals (i.e. Category 1 gases) partition
26 greatly into the upper respiratory tract, induce portal-of-entry effects, and do not accumulate
27 significantly in the blood. 1,4-Dioxane induces both systemic and portal of entry effects and has
28 been measured in the blood after inhalation exposure (Kasai, et al., 2008). The observations of
29 systemic (i.e., nonrespiratory) effects and measured blood levels resulting from 1,4-dioxane
30 exposure clearly indicate that this compound is absorbed into the bloodstream and distributed

1 throughout the body. Furthermore, the lack of an anterior to posterior gradient for the nasal
2 effects induced by 1,4-dioxane is not typical of chemicals which are predominantly directly
3 reactive. Thus, 1,4-dioxane might be best described as a water-soluble and non-directly reactive
4 gas. Gases such as these are readily taken up into respiratory tract tissues and can also diffuse
5 into the blood capillaries (Medinsky & Bond, 2001). The effects in the olfactory epithelium may
6 be the result of the metabolism of 1,4-dioxane to an acid metabolite; however, for the reasons
7 stated above it is unclear whether or not these effects are solely the result of portal-of-entry or
8 systemic delivery. A similar pattern of systemic effects (i.e., respiratory tract effects) were
9 observed after oral exposure to 1,4-dioxane .

10 Consequently, for dosimetric purposes, the human equivalent concentration (HEC) for
11 1,4-dioxane was calculated by the application of the appropriate dosimetric adjustment factor
12 (DAF) for systemic acting gases (i.e. Category 3 gases), in accordance with the U.S. EPA RfC
13 methodology (U.S. EPA, 1994b) as follows:

$$14 \quad \text{DAF} = (\text{Hb/g})_A / (\text{Hb/g})_H$$

15 where:

16 (Hb/g)_A = the animal blood:air partition coefficient = 1861 (Sweeney, et al., 2008)

17 (Hb/g)_H = the human blood:air partition coefficient = 1666 (Sweeney, et al., 2008)

$$18 \quad \text{DAF} = 1861 / 1666$$

$$19 \quad \text{DAF} = 1.12$$

20
21
22 Given that the animal blood:air partition coefficient is higher than the human value
23 resulting in a DAF > 1, a default value of 1 is substituted in accordance with the U.S. EPA RfC
24 methodology (U.S. EPA, 1994b). Analysis of the existing inhalation dosimetry modeling
25 database supports the application of a DAF of 1 to be appropriate (U.S. EPA, 2009a).
26 Application of these models to gases that have similar physicochemical properties and induce
27 similar nasal effects as 1,4-dioxane estimate DAFs ≥ 1.

28 Utilizing a DAF of 1, the HEC for atrophy of the olfactory epithelium in male
29 F344/DuCrj rats is calculated as follows:

$$30 \quad \begin{aligned} 31 \quad \text{POD}_{\text{HEC}} (\text{mg}/\text{m}^3) &= \text{POD}_{\text{ADJ}} (\text{mg}/\text{m}^3) \times \text{DAF} \\ 32 \quad &= \text{POD}_{\text{ADJ}} (\text{mg}/\text{m}^3) \times 1.0 \\ 33 \quad &= 32.2 \text{ mg}/\text{m}^3 \times 1.0 \\ 34 \quad &= 32.2 \text{ mg}/\text{m}^3 \end{aligned}$$

35
36 Therefore, the POD_{HEC} of 32.2 mg/m³ for the critical effect of atrophy of the olfactory
37 epithelium is used for the derivation of a RfC for 1,4-dioxane.

5.2.4. RfC Derivation- Including Application of Uncertainty Factors (UFs)

1 The RfC of 3×10^{-2} mg/m³ is based on atrophy of the olfactory epithelium in male rats
2 exposed to 1,4-dioxane via inhalation for 2 years (Kasai, et al., 2009). The RfC for 1,4-dioxane is
3 derived by dividing the POD_{HEC} for 1,4-dioxane by a composite UF of 1000.

$$\begin{aligned} \text{RfC} &= \text{POD}_{\text{HEC}} / \text{UF} \\ &= 32.2 \text{ mg/m}^3 / 1000 \\ &= 0.0322 \text{ or } 3 \times 10^{-2} \text{ mg/m}^3 \end{aligned}$$

4
5
6
7
8
9 The composite UF of 1000 includes factors of 10 for LOAEL-to-NOAEL extrapolation
10 and for human interindividual variability, and factors of 3 were used for animal-to-human
11 extrapolation and for database deficiencies.

12 An UF of 10 was used to extrapolate from a LOAEL to a NOAEL given significant
13 incidence data reported at the lowest tested concentration for this endpoint. A NOAEL for
14 atrophy of the olfactory epithelium was not identified in this study. Adequate BMD model
15 estimates were not available for derivation of the RfC, and since a NOAEL was not identified for
16 atrophy of the olfactory epithelium, the LOAEL (lowest dose tested in the study by Kasai et al.
17 (2009)) was chosen as the POD.

18 A default interindividual variability UF of 10 was used to account for variation in
19 sensitivity within human populations because there is limited information on the degree to which
20 humans of varying gender, age, health status, or genetic makeup might vary in the disposition of,
21 or response to, 1,4-dioxane.

22 An UF of 3 was used to for animal-to-human extrapolation to account for
23 pharmacodynamic differences between species. This uncertainty factor for animal-to-human
24 extrapolation is comprised of two separate and equal areas of uncertainty to account for
25 difference in the toxicokinetics and toxicodynamics of animals and humans. In this assessment,
26 the toxicokinetic uncertainty was accounted for by the calculation of a HEC by the application of
27 a dosimetric adjustment factor as outlined in the RfC methodology (U.S. EPA, 1994b). As the
28 toxicokinetic differences are thus accounted for, only the toxicodynamic uncertainties remain,
29 and an UF of 3 is retained to account for this uncertainty.

30 An UF of 3 for database deficiencies was applied due to the lack of a multigeneration
31 reproductive toxicity study. The oral toxicity database included a single prenatal developmental
32 study that indicated the developing fetus may be a target of toxicity (Giavini, et al., 1985)

33 An UF to extrapolate from a subchronic to a chronic exposure duration was not necessary
34 because the RfC was derived from a study using a chronic exposure protocol.

5.2.5. RfC Comparison Information

1 Figure 5-5 presents PODs, applied UFs, and derived sample RfCs for possible endpoints
2 from the chronic inhalation Kasai et al. (2009) in male rats. The PODs are based on the BMDL₁₀,
3 NOAEL, or LOAEL and appropriate unit conversion and duration and dosimetric adjustments
4 were applied before applications of uncertainty factors.

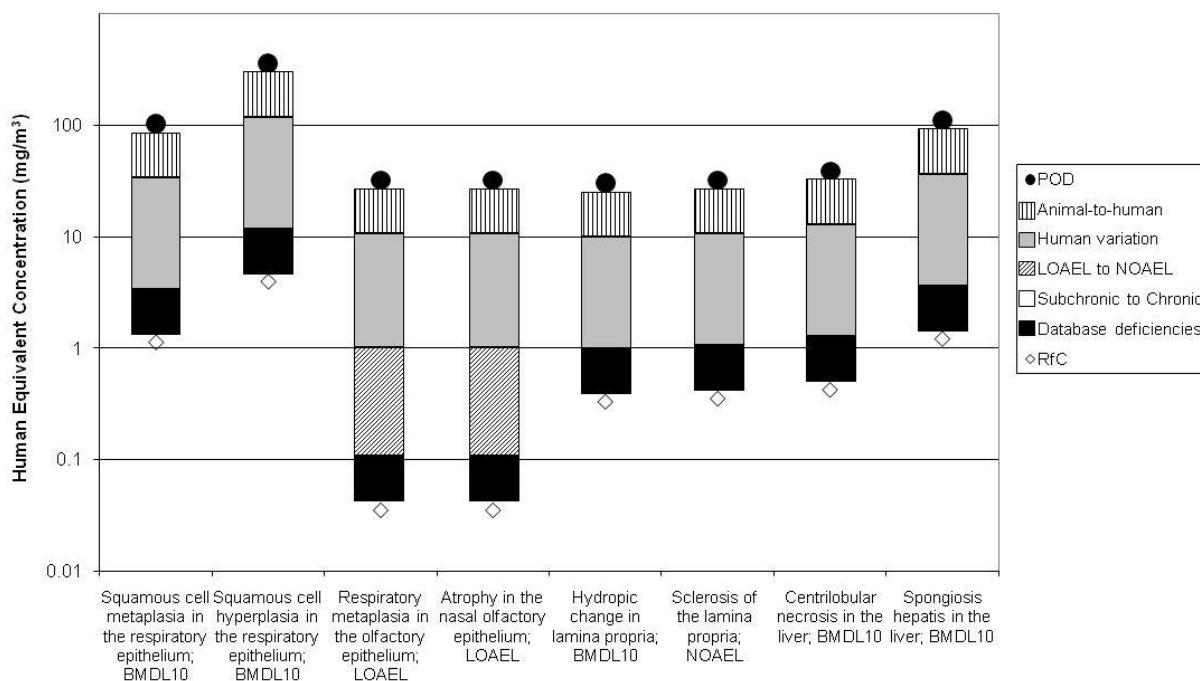


Figure 5-5. Potential points of departure (POD) for candidate endpoints with corresponding applied uncertainty factors and derived sample RfCs following inhalation exposure to 1,4-dioxane.

Source: Kasai et al. (2009)

5.2.6. Previous RfC Assessment

5 An assessment for 1,4-dioxane was previously posted on the IRIS database in 1988 and
6 2010. An inhalation RfC was not developed as part of either the 1988 or 2010 assessment.

5.3. UNCERTAINTIES IN THE ORAL REFERENCE DOSE AND INHALATION REFERENCE CONCENTRATION

7 Risk assessments need to portray associated uncertainty. The following discussion
8 identifies uncertainties associated with the RfD and RfC for 1,4-dioxane. As presented earlier in
9 this section (see Sections 5.1.2, 5.1.3 for the RfD and Sections 5.2.2, and 5.2.3 for the RfC), the
10 uncertainty factor approach (U.S. EPA, 1994b, 2002a) was used to derive the RfD and RfC for

1 1,4-dioxane. Using this approach, the POD was divided by a set of factors to account for
2 uncertainties associated with a number of steps in the analysis, including extrapolation from
3 LOAEL to NOAEL exposure and responses observed in animal bioassays to humans, a diverse
4 population of varying susceptibilities, and to account for database deficiencies. Because
5 information specific to 1,4-dioxane was unavailable to fully inform many of these extrapolations,
6 default factors were generally applied.

7 An adequate range of animal toxicology data are available for the hazard assessment of
8 1,4-dioxane, as described throughout the previous section (Section 4). The database of oral
9 toxicity studies includes chronic drinking water studies in rats and mice, multiple subchronic
10 drinking water studies conducted in rats and mice, and a developmental study in rats. Toxicity
11 associated with oral exposure to 1,4-dioxane is observed predominately in the liver and kidney.
12 The database of inhalation toxicity studies in animals includes two subchronic bioassays in
13 rabbits, guinea pigs, mice, and rats, and two chronic inhalation bioassays in rats. Toxicity
14 associated with inhalation exposure to 1,4-dioxane was observed predominately in the liver and
15 nasal cavity. In addition to oral and inhalation data, there are PBPK models and genotoxicity
16 studies of 1,4-dioxane. Critical data gaps have been identified and uncertainties associated with
17 data deficiencies of 1,4-dioxane are more fully discussed below.

18 Consideration of the available dose-response data led to the selection of the two-year
19 drinking water bioassay in Sherman rats ([Kociba, et al., 1974](#)) as the principal study and
20 increased liver and kidney degeneration as the critical effects for deriving the RfD for
21 1,4-dioxane. The dose-response relationship for oral exposure to 1,4-dioxane and cortical tubule
22 degeneration in Osborne-Mendel rats ([NCI, 1978](#)) was also suitable for deriving a RfD, but it is
23 associated with higher a POD and potential RfD compared to Kociba et al. ([1974](#)).

24 The RfD was derived by applying UFs to a NOAEL for degenerative liver and kidney
25 effects. The incidence data for the observed effects were not reported in the principal study
26 ([Kociba, et al., 1974](#)), precluding modeling of the dose-response. However confidence in the
27 NOAEL can be derived from additional studies ([Argus, et al., 1965](#); [Argus, et al., 1973](#); [JBRC,](#)
28 [1998](#); [NCI, 1978](#)) that observed effects on the same organs at comparable dose levels and by the
29 BMDL generated by modeling of the kidney dose-response data from the chronic NCI ([1978](#))
30 study.

31 The RfC was derived by applying UFs to a LOAEL for atrophy of the olfactory
32 epithelium. The incidence data for the observed effects were not appropriate for BMD modeling
33 for this endpoint (see Appendix F). The LOAEL for this effect was less than or equal to the
34 LOAEL or NOAEL for other effects observed in the same study.

35 Extrapolating from animals to humans embodies further issues and uncertainties. The
36 effect and the magnitude associated with the dose at the POD in rodents are extrapolated to
37 human response. Pharmacokinetic models are useful to examine species differences in

1 pharmacokinetic processing; however, it was determined that dosimetric adjustment using
2 pharmacokinetic modeling to reduce uncertainty following oral exposure to 1,4-dioxane was not
3 supported. Insufficient information was available to quantitatively assess toxicokinetic or
4 toxicodynamic differences between animals and humans, so a 10-fold UF was used to account
5 for uncertainty in extrapolating from laboratory animals to humans in the derivation of the RfD.
6 A DAF was used to account for pharmacokinetic differences between rodents and humans in the
7 derivation of the RfC; however, there was no information to inform pharmacodynamic
8 differences between species, so a reduced UF of 3 was used in derivation of the RfC to account
9 for these uncertainties.

10 Heterogeneity among humans is another uncertainty associated with extrapolating doses
11 from animals to humans. Uncertainty related to human variation needs consideration. In the
12 absence of 1,4-dioxane-specific data on human variation, a factor of 10 was used to account for
13 uncertainty associated with human variation in the derivation of the RfD and RfC. Human
14 variation may be larger or smaller; however, 1,4-dioxane-specific data to examine the potential
15 magnitude of over- or under-estimation are unavailable.

16 Uncertainties in the assessment of the health hazards of 1,4-dioxane are associated with
17 deficiencies in reproductive toxicity information. The oral and inhalation databases lack a
18 multigeneration reproductive toxicity study. A single oral prenatal developmental toxicity study
19 in rats was available for 1,4-dioxane ([Giavini, et al., 1985](#)). This developmental study indicates
20 that the developing fetus may be a target of toxicity. The database of inhalation studies also
21 lacks a developmental toxicity study.

5.4. CANCER ASSESSMENT

5.4.1. Choice of Study/Data – with Rationale and Justification

5.4.1.1. Oral Study/Data

22 Three chronic drinking water bioassays provided incidence data for liver tumors in rats
23 and mice, and nasal cavity, peritoneal, and mammary gland tumors in rats only ([JBRC, 1998](#);
24 [Kano, et al., 2009](#); [Kociba, et al., 1974](#); [NCI, 1978](#); [Yamazaki, et al., 1994](#)). The dose-response
25 data from each of these studies are summarized in Table 5-7. With the exception of the NCI
26 ([1978](#)) study, the incidence of nasal cavity tumors was generally lower than the incidence of liver
27 tumors in exposed rats. The Kano et al. ([2009](#)) drinking water study was chosen as the principal
28 study for derivation of an oral cancer slope factor (CSF) for 1,4-dioxane. This study used three
29 dose groups in addition to controls and characterized the dose-response relationship at lower
30 exposure levels, as compared to the high doses employed in the NCI ([1978](#)) bioassay (Table 5-
31 [7](#)). The Kociba et al. ([1974](#)) study also used three dose groups and low exposures; however, the
32 study authors only reported the incidence of hepatocellular carcinoma, which may underestimate

1 the combined incidence of rats with adenoma or carcinoma. In addition to increased incidence of
 2 liver tumors, chosen as the most sensitive target organ for tumor formation, the Kano et al.
 3 (2009) study also noted increased incidence of peritoneal and mammary gland tumors. Nasal
 4 cavity tumors were also seen in high-dose male and female rats; however, the incidence of nasal
 5 tumors was much lower than the incidence of liver tumors in both rats and mice.

6 In a personal communication, Dr. Yamazaki (2006) provided that the survival of mice
 7 was low in all male groups (31/50, 33/50, 25/50 and 26/50 in control, low-, mid-, and high-dose
 8 groups, respectively) and particularly low in high-dose females (29/50, 29/50, 17/50, and 5/50 in
 9 control, low-, mid-, and high-dose groups, respectively). These deaths occurred primarily during
 10 the second year of the study. Survival at 12 months in male mice was 50/50, 48/50, 50/50, and
 11 48/50 in control, low-, mid-, and high-dose groups, respectively. Female mouse survival at
 12 12 months was 50/50, 50/50, 48/50, and 48/50 in control, low-, mid-, and high-dose groups,
 13 respectively (Yamazaki, 2006). Furthermore, these deaths were primarily tumor related. Liver
 14 tumors were listed as the cause of death for 31 of the 45 pretermination deaths in high-dose
 15 female Crj:BDF1 mice (Yamazaki, 2006). Thus, the high mortality rates in the female mice
 16 were still considered to be relevant for this analysis.

Table 5-7. Incidence of liver, nasal cavity, peritoneal, and mammary gland tumors in rats and mice exposed to 1,4-dioxane in drinking water for 2 years (based on survival to 12 months)

Study	Species/strain/gender	Animal dose (mg/kg-day)	Tumor Incidence			
			Liver	Nasal cavity	Peritoneal	Mammary gland
Kociba et al. (1974)	Sherman rats, male and female combined ^{a,b}	0	1/106 ^h	0/106 ^h	NA	NA
		14	0/110	0/110	NA	NA
		121	1/106	0/106	NA	NA
		1,307	10/66 ⁱ	3/66	NA	NA
NCI (1978)	Male Osborne-Mendel rats ^b	0	NA	0/33 ^h	NA	NA
		240	NA	12/26	NA	NA
		530	NA	16/33 ⁱ	NA	NA
	Female Osborne-Mendel rats ^{b,c}	0	0/31 ^h	0/34 ^h	NA	NA
		350	10/30 ⁱ	10/30 ⁱ	NA	NA
		640	11/29 ⁱ	8/29 ⁱ	NA	NA
	Male B6C3F ₁ mice ^d	0	8/49 ^h	NA	NA	NA
		720	19/50 ⁱ	NA	NA	NA
		830	28/47 ⁱ	NA	NA	NA
	Female B6C3F ₁ mice ^d	0	0/50 ^h	NA	NA	NA
		380	21/48 ⁱ	NA	NA	NA
		860	35/37 ⁱ	NA	NA	NA

Kano et al. (2009)	Male F344/DuCrj rats ^{d,e,f,g}	0	3/50	0/50	2/50	1/50
		11	4/50	0/50	2/50	2/50
		55	7/50	0/50	5/50	2/50
		274	39/50 ^{i,k}	7/50 ^k	28/50 ^{j,k}	6/50 ^k
	Female F344/DuCrj rats ^{d,e,f,g}	0	3/50	0/50	1/50	8/50
		18	1/50	0/50	0/50	8/50
		83	6/50	0/50	0/50	11/50
		429	48/50 ^{i,k}	8/50 ^{j,k}	0/50	18/50 ^{i,k}
	Male Crj:BDF1 mice ^d	0	23/50	0/50	NA	NA
		49	31/50	0/50	NA	NA
		191	37/50 ⁱ	0/50	NA	NA
		677	40/50 ^{i,k}	1/50	NA	NA
	Female Crj:BDF1 mice ^d	0	5/50	0/50	NA	NA
		66	35/50 ^j	0/50	NA	NA
		278	41/50 ^j	0/50	NA	NA
		964	46/50 ^{i,k}	1/50	NA	NA

^aIncidence of hepatocellular carcinoma.

^bIncidence of nasal squamous cell carcinoma.

^cIncidence of hepatocellular adenoma.

^dIncidence of hepatocellular adenoma or carcinoma.

^eIncidence (sum) of all nasal tumors including squamous cell carcinoma, sarcoma, rhabdomyosarcoma, and esthesioneuroepithelioma.

^fIncidence of peritoneal tumors (mesothelioma).

^gIncidence of mammary gland tumors (fibroadenoma or adenoma)

^h $p < 0.05$; positive dose-related trend (Cochran-Armitage or Peto's test).

ⁱSignificantly different from control at $p < 0.05$ by Fisher's Exact test.

^jSignificantly different from control at $p < 0.01$ by Fisher's Exact test.

^k $p < 0.01$; positive dose-related trend (Peto's test).

NA = data were not available for modeling (no significant change from controls)

5.4.1.2. Inhalation Study/Data

1 Epidemiological studies of populations exposed to 1,4-dioxane are not adequate for dose-
2 response analysis and derivation of an inhalation unit risk (IUR). However, two chronic
3 inhalation studies in animals are available and were evaluated for the potential to estimate an
4 IUR (Table 5-8). The chronic inhalation study conducted by Torkelson et al. (1974) in rats did
5 not find any treatment-related tumors; however, only a single exposure concentration was used
6 (111 ppm 1,4-dioxane vapor for 7 hours/day, 5 days/week for 2 years). A chronic bioassay of
7 1,4-dioxane by the inhalation route reported by Kasai et al. (2009) provides data adequate for
8 dose-response modeling and was subsequently chosen as the principal study for the derivation of
9 an IUR for 1,4-dioxane. In this bioassay, groups of 50 male F344 rats were exposed to either 0,
10 50, 250 or 1,250 ppm 1,4-dioxane, 6 hours/day, 5 days/week, for 2 years (104-weeks). In male
11 F344 rats, 1,4-dioxane produced a statistically significant increase in incidence and/or a
12 statistically significant dose-response trend for the following tumor types: hepatomas, nasal
13 squamous cell carcinomas, renal cell carcinomas, peritoneal mesotheliomas, mammary gland

1 fibroadenomas, Zymbal gland adenomas, and subcutis fibromas (Kasai, et al., 2009). It is
 2 important to note that the incidence of adenomas and the incidence of carcinomas within a dose
 3 group at a site or tissue (i.e., liver) in rodents are sometimes combined. This practice is based
 4 upon the hypothesis that adenomas may develop into carcinomas if exposure at the same dose
 5 was continued (McConnell, Solleveld, Swenberg, & Boorman, 1986; U.S. EPA, 2005a).
 6 Consistent with the oral cancer assessment (Appendix D), the incidence of hepatic adenomas and
 7 carcinomas was summed without double-counting, to calculate the combined incidence of either
 8 a hepatocellular carcinoma or adenoma in rodents (See Table 5-8).

Table 5-8. Incidence of liver, nasal cavity, kidney, peritoneal, and mammary gland, Zymbal gland, and subcutis tumors in rats exposed to 1,4-dioxane vapors for 2 years.

Study	Species/ strain/ gender	Animal Exposure (ppm)	Tumor Incidence						
			Liver ^c	Nasal cavity ^d	Kidney ^e	Peritoneal ^f	Mammary gland	Zymbal gland ^g	Subcutis ^h
Torkelson et al. (1974) ^a	Male	0	0/150	0/150	0/150 ⁱ	NA	NA	NA	0/150
	Wistar rats	111	0/206	0/206	1/206 ⁱ	NA	NA	NA	2/206
	Female	0	0/139	0/139	1/139 ^j	NA	11/139 ^k	NA	0/139
	Wistar rats	111	0/217	0/217	0/217 ^j	NA	29/217 ^k	NA	0/217
Kasai et al. (2009) ^b	Male	0	1/50	0/50	0/50	2/50	1/50 ^l	0/50	1/50
	F344	50	2/50	0/50	0/50	4/50	2/50 ^l	0/50	4/50
	rats	250	4/50	1/50	0/50	14/50 ⁿ	3/50 ^l	0/50	9/50 ⁿ
		1,250	22/50	6/50 ^m	4/50	41/50 ⁿ	5/50 ^l	4/50	5/50

^aIncidence reported based on survival to 9 months.

^bIncidence reported based on survival to 12 months.

^cIncidence of hepatocellular adenoma or carcinoma. For Kasai et al. (2009) incidence data was provided via personal communication from Dr. Tatsuya Kasai to Dr. Reeder Sams on 12/23/2008 (2008). Statistics were not reported. Individual incidence rates for adenomas and carcinomas are in Table 5-10.

^dIncidence of nasal squamous cell carcinoma.

^eIncidence of renal cell carcinoma.

^fIncidence of peritoneal mesothelioma.

^gIncidence of Zymbal gland adenoma.

^hIncidence of subcutis fibroma.

ⁱIncidence of kidney fibroma.

^jIncidence of kidney adenocarcinoma

^kIncidence of mammary gland adenoma.

^lIncidence of mammary gland fibroadenoma.

^mTumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.05$).

ⁿTumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.01$).

NA = data are not available

5.4.2. Dose-Response Data

5.4.2.1. Oral Data

9 Table 5-9 summarizes the incidence of hepatocellular adenoma or carcinoma in rats and
 10 mice from the Kano et al. (2009) 2-year drinking water study. There were statistically

1 significant increasing trends in tumorigenic response for males and females of both species. The
 2 dose-response curve for female mice is steep, with 70% incidence of liver tumors occurring in
 3 the low-dose group (66 mg/kg-day). Exposure to 1,4-dioxane increased the incidence of these
 4 tumors in a dose-related manner.

5 A significant increase in the incidence of peritoneal mesothelioma was observed in high-
 6 dose male rats only (28/50 rats, Table 5-7). The incidence of peritoneal mesothelioma was lower
 7 than the observed incidence of hepatocellular adenoma or carcinoma in male rats (Table 5-9);
 8 therefore, hepatocellular adenoma or carcinoma data were used to derive an oral CSF for
 9 1,4-dioxane.

Table 5-9. Incidence of hepatocellular adenoma or carcinoma in rats and mice exposed to 1,4-dioxane in drinking water for 2 years

Species/strain/gender	Animal dose (mg/kg-day)	Incidence of liver tumors ^a
Male F344/DuCrj rats	0	3/50
	11	4/50
	55	7/50
	274	39/50 ^{b,c}
Female F344/DuCrj rats	0	3/50
	18	1/50
	83	6/50
	429	48/50 ^{b,c}
Male Crj:BDF1 mice	0	23/50
	49	31/50
	191	37/50 ^d
	677	40/50 ^{b,c}
Female Crj:BDF1 mice	0	5/50
	66	35/50 ^c
	278	41/50 ^c
	964	46/50 ^{b,c}

^aIncidence of either hepatocellular adenoma or carcinoma.

^b $p < 0.05$; positive dose-related trend (Peto's test).

^cSignificantly different from control at $p < 0.01$ by Fisher's Exact test.

^dSignificantly different from control at $p < 0.01$ by Fisher's Exact test.

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

5.4.2.2. ***Inhalation Data***

10 Multi-tumor dose-response modeling was performed for all tumor responses from the
 11 Kasai et al. (2009) bioassay. Kasai et al. (2009) reported tumor incidence data for male F344
 12 rats exposed via inhalation to 0, 50, 250, or 1,250 ppm 1,4-dioxane for 6 hours/day, 5days/week,
 13 for 2 years (104-weeks). Statistically significant dose-response trends for the increase in tumors

1 with increasing dose was observed for the nasal cavity squamous cell carcinomas, hepatomas,
 2 renal cell carcinomas, peritoneal mesotheliomas, mammary gland fibroadenomas, and Zymbal
 3 gland adenomas. Following 250 ppm 1,4-dioxane exposure statistically elevated tumor
 4 incidences were found in two tissue types (peritoneal mesothelioma and subcutis fibroma)
 5 compared to controls. Tumor incidences following 1,250 ppm inhalation exposure to 1,4-
 6 dioxane were statistically elevated compared to controls in three tissues (nasal cavity squamous
 7 cell carcinoma, hepatomas, and peritoneal mesothelioma). Incidence data for the tumor types
 8 reported by Kasai et al. (2009) are summarized in Table 5-10.

Table 5-10. Incidence of tumors in F344 male rats exposed to 1,4-dioxane for 104 weeks (6 hours/day, 5 days/week)

<u>Tumor Type</u>	<u>Animal Exposure (ppm)</u>			
	<u>0</u>	<u>50</u>	<u>250</u>	<u>1,250</u>
<u>Nasal cavity squamous cell carcinoma</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>	<u>6/50^{a,b}</u>
<u>Hepatocellular adenoma</u>	<u>1/50</u>	<u>2/50</u>	<u>3/50</u>	<u>21/50^{a,c}</u>
<u>Hepatocellular carcinoma</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>	<u>2/50</u>
<u>Hepatocellular adenoma or carcinoma^c</u>	<u>1/50</u>	<u>2/50</u>	<u>4/50</u>	<u>22/50^{a,c}</u>
<u>Renal cell carcinoma</u>	<u>0/50</u>	<u>0/50</u>	<u>0/50</u>	<u>4/50^a</u>
<u>Peritoneal mesothelioma</u>	<u>2/50</u>	<u>4/50</u>	<u>14/50^c</u>	<u>41/50^{a,c}</u>
<u>Mammary gland fibroadenoma</u>	<u>1/50</u>	<u>2/50</u>	<u>3/50</u>	<u>5/50^d</u>
<u>Mammary gland adenoma</u>	<u>0/50</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>
<u>Zymbal gland adenoma</u>	<u>0/50</u>	<u>0/50</u>	<u>0/50</u>	<u>4/50^a</u>
<u>Subcutis fibroma</u>	<u>1/50</u>	<u>4/50</u>	<u>9/50^c</u>	<u>5/50</u>

^aStatistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).

^bTumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.05$).

^cTumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.01$).

^dStatistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.05$).

^eProvided via personal communication from Dr. Tatsuya Kasai to Dr. Reeder Sams on 12/23/2008 (2008).

Statistics were not reported for these data by study authors, so statistical analyses were conducted by EPA.

Source: Kasai et al. (2009) and Kasai personal communication(2008)

5.4.3. Dose Adjustments and Extrapolation Method(s)

5.4.3.1. Oral

9 Human equivalent doses (HEDs) were calculated from the administered animal doses
 10 using a BW scaling factor ($BW^{0.75}$). This was accomplished using the following equation:

$$11 \quad \text{HED} = \text{animal dose (mg/kg)} \times \left[\frac{\text{animal BW (kg)}}{\text{human BW (kg)}} \right]^{0.25}$$

12 For all calculations, a human BW of 70 kg was used. HEDs for the principal study ([Kano, et al.,](#)
 13 [2009](#)) are given in Table 5-[11](#). HEDs were also calculated for supporting studies ([Kociba, et al.,](#)
 14 [1974](#); [NCI, 1978](#)) and are also shown in Table 5-[11](#).

Table 5-11. Calculated HEDs for the tumor incidence data used for dose-response modeling

Study	Species/strain/gender	Animal BW (g) TWA	Animal dose (mg/kg-day)	HED (mg/kg-day) ^d
Kano et al. (2009)	Male F344/DuCrj rats	432 ^a	11	3.1
		432 ^a	81	23
		432 ^a	398	112
	Female F344/DuCrj rats	267 ^a	18	4.5
		267 ^a	83	21
		267 ^a	429	107
	Male Crj:BDF1 mice	47.9 ^a	49	7.9
		47.9 ^a	191	31
		47.9 ^a	677	110
	Female Crj:BDF1 mice	35.9 ^a	66	10
		35.9 ^a	278	42
		35.9 ^a	964	145
Kociba et al. (1974)	Male and female (combined) Sherman rats	325 ^b	14	3.7
		325 ^b	121	32
		285 ^c	1,307	330
NCI (1978)	Male Osborne-Mendel rats	470 ^b	240	69
		470 ^b	530	152
	Female Osborne-Mendel rats	310 ^b	350	90
		310 ^b	640	165
	Male B6C3F ₁ mice	32 ^b	720	105
		32 ^b	830	121
	Female B6C3F ₁ mice	30 ^b	380	55
		30 ^b	860	124

^a TWA BWs were determined from BW growth curves provided for each species and gender.

^bTWA BWs were determined from BW curve provided for control animals.

^cBWs of high dose male and female rats were significantly lower than controls throughout the study. TWA represents the mean of TWA for male and females (calculated separately from growth curves).

^dHEDs are calculated as $HED = (\text{animal dose}) \times (\text{animal BW} / \text{human BW})^{0.25}$.

Sources: Kano et al. (2009); Kociba et al. (1974); and NCI (1978).

1 The U.S. EPA *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#))
2 recommend that the method used to characterize and quantify cancer risk from a chemical is
3 determined by what is known about the mode of action of the carcinogen and the shape of the
4 cancer dose-response curve. The linear approach is recommended if the mode of action of
5 carcinogenicity is not understood ([U.S. EPA, 2005a](#)). In the case of 1,4-dioxane, the mode of
6 carcinogenic action for peritoneal, mammary, nasal, and liver tumors is unknown. Therefore, a
7 linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated
8 with 1,4-dioxane exposure.

1 However, several of the external peer review panel members (Appendix A: Summary of
2 External Peer Review and Public Comments and Disposition) recommended that the mode of
3 action data support the use of a nonlinear extrapolation approach to estimate human carcinogenic
4 risk associated with exposure to 1,4-dioxane and that such an approach should be presented in
5 the Toxicological Review. As discussed in Section 4.7.3., numerous short-term in vitro and a
6 few in vivo tests were nonpositive for 1,4-dioxane-induced genotoxicity. Results from two-stage
7 mouse skin tumor bioassays demonstrated that 1,4-dioxane does not initiate mouse skin tumors,
8 but it is a promoter of skin tumors initiated by DMBA ([King, et al., 1973](#)). These data suggest
9 that a potential mode of action for 1,4-dioxane-induced tumors may involve proliferation of cells
10 initiated spontaneously, or by some other agent, to become tumors ([Bull, et al., 1986](#);
11 [Goldsworthy, et al., 1991](#); [King, et al., 1973](#); [Lundberg, et al., 1987](#); [Miyagawa, et al., 1999](#);
12 [Stott, et al., 1981](#); [Uno, et al., 1994](#)). However, key events related to the promotion of tumor
13 formation by 1,4-dioxane are unknown. Therefore, under the U.S. EPA *Guidelines for*
14 *Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)), EPA concluded that the available information
15 does not establish a plausible mode of action for 1,4-dioxane and data are insufficient to establish
16 significant biological support for a nonlinear approach. EPA determined that there are no data
17 available to inform the low-dose region of the dose response, and thus, a nonlinear approach was
18 not included.

19 Accordingly, the CSF for 1,4-dioxane was derived via a linear extrapolation from the
20 POD calculated by curve fitting the experimental dose-response data. The POD is the 95%
21 lower confidence limit on the dose associated with a benchmark response (BMR) near the lower
22 end of the observed data. The BMD modeling analysis used to estimate the POD is described in
23 detail in Appendix D and is summarized below in Section 5.4.4.

24 Model estimates were derived for all available bioassays and tumor endpoints (Appendix
25 D); however, the POD used to derive the CSF is based on the most sensitive species and target
26 organ in the principal study ([Kano, et al., 2009](#)).

27 The oral CSF was calculated using the following equation:

$$28 \qquad \text{CSF} = \frac{\text{BMR}}{\text{BMDL}}$$

29 **5.4.3.2. Inhalation**

30 In accordance with the U.S. EPA (1994b) RfC methodology, the HEC values for the
31 various tumors were calculated by the application of DAFs. As discussed in Section 5.2.3. since
32 1,4-dioxane is miscible with water, has a high partition coefficient, and systemic and portal of
33 entry effects are observed, a DAF of 1.0 was applied. The lifetime continuous inhalation risk for
34 humans is defined as the slope of the line from the POD, the lower 95% bound on the exposure
associated with a level of extra risk near the low end of the data range.

1 All PODs were converted to equivalent continuous exposure levels by multiplying by [(6
2 hours)/(24 hours)] ×[(5 days)/(7 days)], or 0/178, under the assumption of equal cumulative
3 exposures leading to equivalent outcomes.

4 Given the multiplicity of tumor sites, basing the IUR on one tumor site may
5 underestimate the carcinogenic potential of 1,4-dioxane. Simply pooling the counts of animals
6 with one or more tumors (i.e., counts of tumor bearing animals) would tend to underestimate the
7 overall risk when tumors are independent across sites and ignores potential differences in the
8 dose-response relationships across the sites (Bogen, 1990; Spurgeon, Hopkin, & Jones, 1994).
9 NRC (1994) also noted that the assumption of independence across tumor types is not likely to
10 produce substantial error in the risk estimates unless tumors are known to be biologically
11 dependent.

12 Kopylev et al. (2009) describe a Markov Chain Monte Carlo (MCMC) computational
13 approach to calculating the dose associated with a specified composite risk under assumption of
14 independence of tumors. The *Guidelines for Carcinogen Risk Assessment* recommend
15 calculation of an upper bound to account for uncertainty in the estimate (U.S. EPA, 2005a). For
16 uncertainty characterization, MCMC methods have the advantage of providing information about
17 the full distribution of risk and/or benchmark dose, which can be used in generating a confidence
18 bound. This MCMC approach building on the re-sampling approach recommended by Bogen
19 (1990), which also provides a distribution of the combined potency across sites. The Bayesian
20 MCMC computations were conducted using WinBugs (Spiegelhalter, Thomas, & Best, 2003)
21 and additional details of this analysis are included in Appendix G. In addition, the best fitting
22 BMDs multistage model was determined for each individual tumor type as shown in Section
23 5.4.4.2 and Appendix G.

24 IUR estimates based were calculated using the following equation:

$$25 \quad \underline{\underline{IUR = BMR / HEC}}$$

5.4.4. Oral Slope Factor and Inhalation Unit Risk

5.4.4.1. *Oral Slope Factor*

26 The dichotomous models available in the Benchmark Dose Software (BMDS, version
27 2.1.1) were fit to the incidence data for “either hepatocellular carcinoma or adenoma” in rats and
28 mice, as well as mammary and peritoneal tumors in rats exposed to 1,4-dioxane in the drinking
29 water (Kano, et al., 2009; Kociba, et al., 1974; NCI, 1978) (Table 5-7). Animal doses are used
30 for BMD modeling and HED BMD and BMDL values are calculated using the animal TWAs
31 (Table 5-12) and a human BW of 70kg. Doses associated with a BMR of 10% extra risk were
32 calculated. BMDs and BMDLs from all models are reported, and the output and plots
33 corresponding to the best-fitting model are shown (Appendix D). When the best-fitting model is
34 not a multistage model, the multistage model output and plot are also provided (Appendix D). A

1 summary of the BMDS model predictions for the Kano et al. (2009), NCI (1978), and Kociba
 2 et al. (1974) studies is shown in Table 5-12.

Table 5-12. BMD_{HED} and BMDL_{HED} values from models fit to tumor incidence data for rats and mice exposed to 1,4-dioxane in drinking water for 2 years and corresponding oral CSFs

Study	Gender/strain/species	Tumor type	BMD _{HED} ^a (mg/kg-day)	BMDL _{HED} ^a (mg/kg-day)	Oral CSF (mg/kg-day) ⁻¹
Kano et al. (2009)	Male F344/DuCrj rats ^b	Hepatocellular adenoma or carcinoma	17.43	14.33	7.0 x 10 ⁻³
	Female F344/DuCrj rats ^c		19.84	14.43	6.9 x 10 ⁻³
	Male Crj:BDF1 mice ^d		5.63	2.68	3.7 x 10 ⁻²
	Female Crj:BDF1 mice ^d		0.83	0.55	0.18
	Female Crj:BDF1 mice ^{d,e}		3.22 ^e	2.12 ^e	0.14
	Female Crj:BDF1 mice ^{d,f}		7.51 ^f	4.95 ^f	0.10
	Female F344/DuCrj rats ^g	Nasal squamous cell carcinoma	94.84	70.23	1.4 x 10 ⁻³
	Male F344/DuCrj rats ^g		91.97	68.85	1.5 x 10 ⁻³
	Male F344/DuCrj rats ^b	Peritoneal mesothelioma	26.09	21.39	4.7 x 10 ⁻³
Female F344/DuCrj rats ^d	Mammary gland adenoma	40.01	20.35	4.9 x 10 ⁻³	
Kociba et al. (1974)	Male and female (combined) Sherman rats ^g	Nasal squamous cell carcinomas	448.24	340.99	2.9 x 10 ⁻⁴
	Male and female (combined) Sherman rats ^b	Hepatocellular carcinoma	290.78	240.31	4.2 x 10 ⁻⁴
NCI (1978)	Male Osborne Mendel rats ^d	Nasal squamous cell carcinomas	16.10	10.66	9.4 x 10 ⁻³
	Female Osborne Mendel rats ^d		40.07	25.82	3.9 x 10 ⁻³
	Female Osborne Mendel rats ^d	Hepatocellular adenoma	28.75	18.68	5.4 x 10 ⁻³
	Female B6C3F ₁ mice ^c	Hepatocellular adenoma or carcinoma	23.12	9.75	1.0 x 10 ⁻²
	Male B6C3F ₁ mice ^h		87.98	35.67	2.8 x 10 ⁻³

^aValues associated with a BMR of 10% unless otherwise noted.

^bProbit model, slope parameter not restricted.

^cMultistage model, degree of polynomial = 2.

^dLog-logistic model, slope restricted ≥ 1.

^eValues associated with a BMR of 30%.

^fValues associated with a BMR of 50%.

^gMultistage model, degree of polynomial = 3.

^hGamma model.

3 The multistage model did not provide an adequate fit (as determined by AIC, *p*-value
 4 < 0.1, and $\chi^2 p > |0.1|$) to the data for the incidence of hepatocellular adenoma or carcinoma in
 5 female mice (Appendix D). The high dose was dropped for the female mouse liver tumor dataset
 6 in an attempt to achieve an adequate fit; however, an adequate fit was still not achieved.

1 Because the female mice were clearly the most sensitive group tested, other BMD models were
2 applied to the female mouse liver tumor dataset to achieve an adequate fit. The log-logistic
3 model was the only model that provided adequate fit for this data set due to the steep rise in the
4 dose-response curve (70% incidence at the low dose) followed by a plateau at near maximal
5 tumor incidence in the mid- and high-dose regions (82 and 92% incidence, respectively). The
6 predicted BMD₁₀ and BMDL₁₀ for the female mouse data are presented in Table 5-12, as well as
7 BMD_{HED} and BMDL_{HED} values associated with BMRs of 30 and 50% .

8 The multistage model also did not provide an adequate fit to mammary tumor incidence
9 data for the female rat or male rat peritoneal tumors. The predicted BMD₁₀ and BMDL₁₀ for
10 female rat mammary tumors and male peritoneal tumors obtained from the log-logistic and
11 probit models, respectively, are presented in Table 5-12.

12 A comparison of the model estimates derived for rats and mice from the Kano et al.
13 (2009), NCI (1978), and Kociba et al. (1974) studies (Table 5-12) indicates that female mice are
14 more sensitive to liver carcinogenicity induced by 1,4-dioxane compared to other species or
15 tumor types. The BMDL_{50 HED} for the female mouse data was chosen as the POD and the CSF of
16 0.10 (mg/kg-day)⁻¹ was calculated as follows:

$$17 \quad \text{CSF} = \frac{0.50}{4.95 \text{ mg/kg} \cdot \text{day (BMDL}_{50 \text{ HED}} \text{ for female mice)}} = 0.10 \text{ (mg/kg} \cdot \text{day)}^{-1}$$

18 Calculation of a CSF for 1,4-dioxane is based upon the dose-response data for the most
19 sensitive species and gender.

5.4.4.2. Inhalation Unit Risk

20 Inhalation unit risk estimates were based on the multiple carcinogenic effects of
21 1,4-dioxane observed in rats via the inhalation route.

22 The multistage cancer models available in the Benchmark Dose Software (BMDS,
23 version 2.1.1) were fit to the incidence data for each tumor type observed in rats exposed to
24 1,4-dioxane via inhalation (Kasai, et al., 2009) to determine the degree (e.g., 1st, 2nd, or 3rd) of the
25 multistage model that best fit the data (details in Appendix G). A Bayesian MCMC analysis was
26 performed using WinBUGS to calculate the total tumor risk. For comparative purposes only, a
27 total tumor analysis was also performed with the BMDS (version 2.2Beta) MSCombo model and
28 yielded similar results (See Appendix G). MSCombo is a new addition to BMDS that allows for
29 multi-tumor analysis. A summary of the BMDS model predictions for the Kasai et al. (2009)
30 study is shown in Table 5-13. Animal exposure concentrations were used for BMD modeling
31 and continuous human equivalent exposures were calculated by adjusting for duration of
32 exposure (Table 5-13) and applying an appropriate DAF (see Section 5.2.3). In accordance with
33 the U.S. EPA (2005a) Cancer Guidelines, the BMCL₁₀ (lower bound on the concentration
34 estimated to produce a 10% increase in tumor incidence over background) was estimated for the
35 dichotomous incidence data and the results of the model that best characterized the cancer

1 incidences were selected. BMCs and BMCLs from all models are reported, and the output and
 2 plots corresponding to the best-fitting model are shown (Appendix G).

3 The IUR estimates are provided in Table 5-13. Human equivalent risks estimated from
 4 the individual rat tumor sites ranged from 1.5×10^{-7} to 2.4×10^{-6} ($\mu\text{g}/\text{m}^3$)⁻¹ (or rounded to one
 5 significant figure, 2×10^{-7} to 2×10^{-6} ($\mu\text{g}/\text{m}^3$)⁻¹). The highest IUR (2.4×10^{-6} ($\mu\text{g}/\text{m}^3$)⁻¹)
 6 corresponded to peritoneal mesotheliomas in male rats, and the lowest IUR (1.5×10^{-7} ($\mu\text{g}/\text{m}^3$)⁻¹)
 7 corresponded to renal cell carcinoma and Zymbal gland adenomas in male rats.

Table 5-13. Dose-response modeling summary results for male rat tumors associated with inhalation exposure to 1,4-dioxane for 2 years

<u>Tumor Type^a</u>	<u>Multistage Model Degree^b</u>	<u>Point of Departure^c</u>				<u>IUR Estimate^e ($\mu\text{g}/\text{m}^3$)⁻¹</u>
		<u>Bioassay Exposure Concentration (ppm)</u>		<u>HEC (mg/m^3)^d</u>		
		<u>BMC₁₀</u>	<u>BMCL₁₀</u>	<u>BMC₁₀</u>	<u>BMCL₁₀</u>	
<u>Nasal cavity squamous cell carcinoma</u>	<u>1</u>	<u>1107</u>	<u>629.9</u>	<u>712.3</u>	<u>405.3</u>	<u>2.5×10^{-7}</u>
<u>Hepatocellular adenoma or carcinoma</u>	<u>1</u>	<u>252.8</u>	<u>182.3</u>	<u>162.7</u>	<u>117.3</u>	<u>8.5×10^{-7}</u>
<u>Renal cell carcinoma</u>	<u>3</u>	<u>1355</u>	<u>1016</u>	<u>872</u>	<u>653.7</u>	<u>1.5×10^{-7}</u>
<u>Peritoneal mesothelioma</u>	<u>1</u>	<u>82.21</u>	<u>64.38</u>	<u>52.89</u>	<u>41.42</u>	<u>2.4×10^{-6}</u>
<u>Mammary gland fibroadenoma</u>	<u>1</u>	<u>1635</u>	<u>703.0</u>	<u>1052</u>	<u>452.4</u>	<u>2.2×10^{-7}</u>
<u>Zymbal gland adenoma</u>	<u>3</u>	<u>1355</u>	<u>1016</u>	<u>872</u>	<u>653.7</u>	<u>1.5×10^{-7}</u>
<u>Subcutis fibroma</u>	<u>1</u>	<u>141.8</u>	<u>81.91</u>	<u>91.21</u>	<u>52.70</u>	<u>1.9×10^{-6}</u>
<u>Bayesian Total Tumor Analysis^f</u>		<u>39.2</u>	<u>31.4</u>	<u>25.2</u>	<u>20.2</u>	<u>5.0×10^{-6}</u>

^aTumor incidence data from Kasai et al. (2009).

^bBest-fitting multistage model degree ($p > 0.1$, lowest AIC). See Appendix G for modeling details.

^cBMC = Concentration at specified extra risk (benchmark dose); BMCL = 95% lower bound on concentration at specified extra risk.

^dHuman continuous equivalent estimated by multiplying exposures by [(6 hours)/(24 hours) × (5 days)/(7 days) × molecular weight of 1,4-dioxane]/ 24.45.

^eThe inhalation unit risk ($\mu\text{g}/\text{m}^3$)⁻¹ was derived from the BMCL₁₀, the 95% lower bound on the concentration associated with a 10% extra cancer risk. Specifically, by dividing the BMR (0.10) by the BMCL₁₀. Thus, representing an upper bound, continuous lifetime exposure estimate of cancer potency.

^fResults in this table are from the Bayesian analysis using WinBUGS. Additionally, for comparative purposes only, total tumor analysis was performed with the draft BMDS (version 2.2Beta) MSCCombo model and yielded similar results (See Appendix G).

8 Given the multiplicity of tumor sites, basing the inhalation unit risk on one tumor site
 9 may underestimate the carcinogenic potential of 1,4-dioxane. Consistent with recommendations
 10 of the NRC (1994) and the current *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,
 11 2005a) for the assessment of total risk, and upper bound risk for all tumor sites in male F344 rats
 12 was estimated. This estimate of total risk describes the risk of developing any combination of
 13 the tumor types considered, not just the risk of developing all simultaneously. As shown in
 14 Table 5-13, the resulting total inhalation unit risk for all tumor types for male F344 rats was $5 \times$
 15 10^{-6} ($\mu\text{g}/\text{m}^3$)⁻¹. Overall, the consideration of the other tumor sites approximately doubled the unit

1 risk compared to the highest unit risk associated with any individual tumor type, 2×10^{-6}
2 $(\mu\text{g}/\text{m}^3)^{-1}$ for male peritoneal mesotheliomas.

3 The HEC BMCL₁₀ for the male rat combined tumor estimate was chosen as the POD and
4 the IUR of $5 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$ was calculated as follows:

$$\text{IUR } (\text{mg}/\text{m}^3)^{-1} \quad \frac{0.10}{20.2 \text{ mg}/\text{m}^3} \quad 0.005 (\text{mg}/\text{m}^3)^{-1}$$

5 $\text{IUR } (\mu\text{g}/\text{m}^3)^{-1} \quad 0.005 (\text{mg}/\text{m}^3)^{-1} \quad \frac{1\mu\text{g}}{10^3 \text{ mg}} \quad 5 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$

$$\text{IUR } (\mu\text{g}/\text{m}^3)^{-1} \quad 5 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$$

6 Based on the analysis discussed above, the recommended upper bound estimate on
7 human extra cancer risk from continuous lifetime exposure to 1,4-dioxane is $5 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$.
8 The recommended unit risk estimate reflects the exposure-response relationships for the multiple
9 tumor sites in male F344 rats.

5.4.5. Previous Cancer Assessment

10 A previous cancer assessment was posted for 1,4-dioxane on IRIS in 1988. 1,4-Dioxane
11 was classified as a Group B2 Carcinogen (probable human carcinogen; sufficient evidence from
12 animal studies and inadequate evidence or no data from human epidemiology studies ([U.S. EPA,](#)
13 [1986a](#))) based on the induction of nasal cavity and liver carcinomas in multiple strains of rats,
14 liver carcinomas in mice, and gall bladder carcinomas in guinea pigs. An oral CSF of 0.011
15 $(\text{mg}/\text{kg}\text{-day})^{-1}$ was derived from the tumor incidence data for nasal squamous cell carcinoma in
16 male rats exposed to 1,4-dioxane in drinking water for 2 years ([NCI, 1978](#)). The linearized
17 multistage extra risk procedure was used for linear low dose extrapolation. An inhalation unit
18 risk was not previously derived.

5.5. UNCERTAINTIES IN CANCER RISK VALUES

19 As in most risk assessments, extrapolation of study data to estimate potential risks to
20 human populations from exposure to 1,4-dioxane has engendered some uncertainty in the results.
21 Several types of uncertainty may be considered quantitatively, but other important uncertainties
22 cannot be considered quantitatively. Thus an overall integrated quantitative uncertainty analysis
23 is not presented. In addition, the use of the assumptions, particularly those underlying the
24 Guidelines for Carcinogen Risk Assessment ([U.S. EPA, 2005a](#)) is explained and the decision
25 concerning the preferred approach is given and justified. Principal uncertainties are summarized
26 below and in Table 5-14.

5.5.1. Sources of Uncertainty

5.5.1.1. Choice of Low-Dose Extrapolation Approach

1 The range of possibilities for the low-dose extrapolation of tumor risk for exposure to
2 1,4-dioxane, or any chemical, ranges from linear to nonlinear, but is dependent upon a plausible
3 MOA(s) for the observed tumors. The MOA is a key consideration in clarifying how risks
4 should be estimated for low-dose exposure. Exposure to 1,4-dioxane has been observed in
5 animal models to induce multiple tumor types, including liver adenomas and carcinomas, nasal
6 carcinomas, mammary adenomas and fibroadenomas, and mesotheliomas of the peritoneal cavity
7 ([JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Kociba, et al., 1974](#); [NCI, 1978](#)). MOA
8 information that is available for the carcinogenicity of 1,4-dioxane has largely focused on liver
9 adenomas and carcinomas, with little or no MOA information available for the remaining tumor
10 types. In Section 4.7.3, hypothesized MOAs were explored for 1,4-dioxane. Information that
11 would provide sufficient support for any MOA is not available. In the absence of a MOA(s) for
12 the observed tumor types, a linear low-dose extrapolation approach was used to estimate human
13 carcinogenic risk associated with 1,4-dioxane exposure.

14 It is not possible to predict how additional MOA information would impact the dose-
15 response assessment for 1,4-dioxane because of the variety of tumors observed and the lack of
16 data on how 1,4-dioxane or a metabolite thereof, interacts with cells starting the progression to
17 the observed tumors.

18 In general, the Agency has preferred to use the multistage model for analyses of tumor
19 incidence and related endpoints because they have a generic biological motivation based on
20 long-established mathematical models such as the Moolgavkar-Venzon-Knudsen (MVK) model.

21 The MVK model does not necessarily characterize all modes of tumor formation, but it is
22 a starting point for most investigations and, much more often than not, has provided at least an
23 adequate description of tumor incidence data.

24 The multistage cancer model provided good fits for the tumor incidence data following a
25 2-year inhalation exposure to 1,4-dioxane by male rats ([Kasai, et al., 2009](#)). However, in the
26 studies evaluated for the oral cancer assessment ([Kano, et al., 2009](#); [Kociba, et al., 1974](#); [NCI,](#)
27 [1978](#)), the multistage model provided good descriptions of the incidence of a few tumor types in
28 male (nasal cavity) and female (hepatocellular and nasal cavity) rats and in male mice
29 (hepatocellular) exposed to 1,4-dioxane (Appendix D for details). The multistage model did not
30 provide an adequate fit for the female mouse liver tumor dataset based upon the following ([U.S.](#)
31 [EPA, 2000a](#)):

- Goodness-of-fit p -value was not greater than 0.10;
- Akaike's Information Criterion (AIC) was larger than other acceptable models;

- Data deviated from the fitted model, as measured by their χ^2 residuals (values were greater than an absolute value of one).

1 BMD software typically implements the guidance in the external peer review draft
2 BMD technical guidance document ([U.S. EPA, 2000a](#)) by imposing constraints on the values of
3 certain parameters of the models. When these constraints were imposed, the multistage model
4 and most other models did not fit the incidence data for female mouse liver adenomas or
5 carcinomas.

6 The log-logistic model was selected because it provides an adequate fit for the female
7 mouse data ([Kano, et al., 2009](#)). A BMR of 50% was used because it is proximate to the
8 response at the lowest dose tested and the $BMDL_{50\text{ HED}}$ was derived by applying appropriate
9 parameter constraints, consistent with recommended use of BMD software in the BMD technical
10 guidance document ([U.S. EPA, 2000a](#)).

11 The human equivalent oral CSFs estimated from tumor datasets with statistically
12 significant increases ranged from 4.2×10^{-4} to 0.18 per mg/kg-day (Table 5-12), a range of about
13 three orders of magnitude, with the extremes coming from the combined male and female rat
14 data for hepatocellular carcinomas ([Kociba, et al., 1974](#)) and the female mouse combined liver
15 adenoma and carcinomas ([Kano, et al., 2009](#)).

5.5.1.2. *Dose Metric*

16 1,4-Dioxane is known to be metabolized in vivo. However, it is unknown whether a
17 metabolite or the parent compound, or some combination of parent compound and metabolites, is
18 responsible for the observed toxicity. If the actual carcinogenic moiety is proportional to
19 administered exposure, then use of administered exposure as the dose metric is the least biased
20 choice. On the other hand, if this is not the correct dose metric, then the impact on the CSF is
21 unknown.

5.5.1.3. *Cross-Species Scaling*

22 For the oral cancer assessment, an adjustment for cross-species scaling ($BW^{0.75}$) was
23 applied to address toxicological equivalence of internal doses between each rodent species and
24 humans, consistent with the 2005 Guidelines for Carcinogen Risk Assessment ([U.S. EPA,](#)
25 [2005a](#)). It is assumed that equal risks result from equivalent constant lifetime exposures.

26 Differences in the anatomy of the upper respiratory tract and resulting differences in
27 absorption or in local respiratory system effects are sources of uncertainty in the inhalation
28 cancer assessment.

5.5.1.4. *Statistical Uncertainty at the POD*

29 Parameter uncertainty can be assessed through confidence intervals. Each description of
30 parameter uncertainty assumes that the underlying model and associated assumptions are valid.
31 For the log-logistic model applied to the female mouse data following oral exposure, there is a

1 reasonably small degree of uncertainty at the 10% excess incidence level (the POD for linear
2 low-dose extrapolation). For the multistage model applied for the male rat inhalation dataset,
3 there is a reasonable small degree of uncertainty at the 10% extra risk level (the POD for linear
4 low-dose extrapolation).

5.5.1.5. *Bioassay Selection*

5 The study by Kano et al. (2009) was used for development of an oral CSF. This was a
6 well-designed study, conducted in both sexes in two species (rats and mice) with a sufficient
7 number (N=50) of animals per dose group. The number of test animals allocated among three
8 dose levels and an untreated control group was adequate, with examination of appropriate
9 toxicological endpoints in both sexes of rats and mice. Alternative bioassays (Kociba, et al.,
10 1974; NCI, 1978) were available and were fully considered for the derivation of the oral CSF.

11 The study by Kasai et al. (2009) was used for derivation of an inhalation unit risk. This
12 was a well-designed and peer reviewed study, conducted in male rats with a sufficient number
13 (N=50) of animals per dose group. Three dose levels plus an untreated control group were
14 examined following exposure to 1,4-dioxane via inhalation for 2 years. Other bioassays (Kasai,
15 et al., 2008; Torkelson, et al., 1974) were available and were considered for the derivation of the
16 inhalation unit risk.

5.5.1.6. *Choice of Species/Gender*

17 The oral CSF for 1,4-dioxane was quantified using the tumor incidence data for the
18 female mouse, which was shown to be more sensitive than male mice or either sex of rats to the
19 carcinogenicity of 1,4-dioxane. While all data, both species and sexes reported from the Kano
20 et al. (2009) study, were suitable for deriving an oral CSF, the female mouse data represented the
21 most sensitive indicator of carcinogenicity in the rodent model. The lowest exposure level
22 (66 mg/kg-day or 10 mg/kg-day [HED]) resulted in a considerable and significant increase in
23 combined liver adenomas and carcinomas observed. Additional testing of doses within the range
24 of control and the lowest dose (66 mg/kg-day or 10 mg/kg-day [HED]) could refine and reduce
25 uncertainty for the oral CSF.

26 A personal communication from Dr. Yamazaki (2006) provided that the survival of mice
27 was particularly low in high-dose females (29/50, 29/50, 17/50, and 5/50 in control, low-, mid-,
28 and high-dose groups, respectively). These deaths occurred primarily during the second year of
29 the study. Female mouse survival at 12 months was 50/50, 50/50, 48/50, and 48/50 in control,
30 low-, mid-, and high-dose groups, respectively (Yamazaki, 2006). Furthermore, these deaths
31 were primarily tumor related. Liver tumors were listed as the cause of death for 1/21, 2/21, 8/33,
32 and 31/45 of the pretermination deaths in control, low-, mid- and, high-dose female Crj:BDF1
33 mice (Yamazaki, 2006). Therefore, because a number of the deaths in female mice were
34 attributed to liver tumors, this endpoint and species was still considered to be relevant for this
35 analysis; however, the high mortality rate does contribute uncertainty.

1 Additionally, the incidence of hepatocellular adenomas and carcinomas in historical
2 controls was evaluated with the data from Kano et al. (2009). Katagiri et al. (1998) summarized
3 the incidence of hepatocellular adenomas and carcinomas in control male and female BDF1 mice
4 from ten 2-year bioassays at the JBRC. For female mice, out of 499 control mice, the incidence
5 rates were 4.4% for hepatocellular adenomas and 2.0% for hepatocellular carcinomas. Kano et
6 al. (2009) reported a 10% incidence rate for hepatocellular adenomas and a 0% incidence rate for
7 hepatocellular carcinomas in control female BDF1. These incidence rates are near the historical
8 control values and thus are appropriate for consideration in this assessment.

9 Male F344 rat data were used to estimate risk following inhalation of 1,4-dioxane. Kasai
10 et al. (2008) showed that male rats were more sensitive than female rats to the effects of 1,4-
11 dioxane following inhalation; therefore, male rats were chosen to be studies in the 2-year
12 bioassay conducted by the same laboratory (Kasai, et al., 2009).

5.5.1.7. *Relevance to Humans*

13 The derivation of the oral CSF is derived using the tumor incidence in the liver of female
14 mice. A thorough review of the available toxicological data available for 1,4-dioxane provides
15 no scientific justification to propose that the liver adenomas and carcinomas observed in animal
16 models due to exposure to 1,4-dioxane are not relevant to humans. As such, liver adenomas and
17 carcinomas were considered relevant to humans due to exposure to 1,4-dioxane.

18 The derivation of the inhalation unit risk is based on the tumor incidence at multiple sites
19 in male rats. There is no information on 1,4-dioxane to indicate that the observed rodent tumors
20 are not relevant to humans. Further, no data exist to guide quantitative adjustment for
21 differences in sensitivity among rodents and humans.

5.5.1.8. *Human Population Variability*

22 The extent of inter-individual variability in 1,4-dioxane metabolism has not been
23 characterized. A separate issue is that the human variability in response to 1,4-dioxane is also
24 unknown. Data exploring whether there is differential sensitivity to 1,4-dioxane carcinogenicity
25 across life stages are unavailable. This lack of understanding about potential differences in
26 metabolism and susceptibility across exposed human populations thus represents a source of
27 uncertainty. Also, the lack of information linking a MOA for 1,4-dioxane to the observed
28 carcinogenicity is a source of uncertainty.

Table 5-14. Summary of uncertainty in the 1,4-dioxane cancer risk estimation

Consideration/ approach	Potential Impact	Decision	Justification
Low-dose extrapolation procedure	Departure from EPA's <i>Guidelines for Carcinogen Risk Assessment</i> POD paradigm, if justified, could ↓ or ↑ unit risk an unknown extent	Log-logistic model to determine POD, for OSF ; Bayesian multistage modeling for IUR ; linear low-dose extrapolation from POD	A linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,4-dioxane exposure. Where data are insufficient to ascertain the MOA, EPA's 2005 Guidelines for Carcinogen Risk Assessment recommend application of a linear low-dose extrapolation approach.
Dose metric	Alternatives could ↑ or ↓ CSF by an unknown extent	Used administered exposure	Experimental evidence supports a role for metabolism in toxicity, but it is unclear if the parent compound, metabolite or both contribute to 1,4-dioxane toxicity.
Cross-species scaling	Alternatives could ↓ or ↑ CSF [e.g., 3.5-fold ↓ (scaling by BW) or ↑ twofold (scaling by $BW^{0.67}$)]	$BW^{0.75}$ (default approach)	There are no data to support alternatives. $BW^{0.75}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks. PBPK modeling was conducted but not deemed suitable for interspecies extrapolation.
Bioassay	Alternatives could ↑ or ↓ cancer potency by an unknown extent	OSF (Kano, et al., 2009) ; IUR (Kasai, et al., 2009)	Alternative bioassays were available and considered for derivation of oral CSF and inhalation UR .
Species /gender combination	Human risk could ↓ or ↑, depending on relative sensitivity	Female mouse	There are no MOA data to guide extrapolation approach for any choice. It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. Calculation of the CSF for 1,4-dioxane was based on dose-response data from the most sensitive species and gender. The carcinogenic response occurs across species.
Human relevance of mouse tumor data	If rodent tumors proved not to be relevant to humans, unit risk would not apply i.e., could ↓ CSF	Mouse liver adenomas and carcinomas are relevant to humans (basis for OSF) . Rat tumors at multiple sites are relevant to humans (basis for IUR)	1,4-dioxane is a multi-site carcinogen in rodents and the MOA(s) is unknown; carcinogenicity observed in the rodent studies is considered relevant to human exposure.
Human population variability in metabolism and response/ sensitive subpopulations	Low-dose risk ↑ or ↓ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity, including whether children are more sensitive.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

1 1,4-Dioxane is absorbed rapidly following oral and inhalation exposure, with much less
2 absorption occurring from the dermal route. 1,4-Dioxane is primarily metabolized to HEAA,
3 which is excreted in the urine. Liver, kidney, and nasal toxicity are the primary noncancer health
4 effects associated with exposure to 1,4-dioxane in humans and laboratory animals. Several fatal
5 cases of hemorrhagic nephritis and centrilobular necrosis of the liver were related to
6 occupational exposure (i.e., inhalation and dermal contact) to 1,4-dioxane ([Barber, 1934](#);
7 [Johnstone, 1959](#)). Neurological changes were also reported in one case, including headache,
8 elevation in blood pressure, agitation and restlessness, and coma ([Johnstone, 1959](#)). Perivascular
9 widening was observed in the brain of this worker, with small foci of demyelination in several
10 regions (e.g., cortex, basal nuclei). Severe liver and kidney degeneration and necrosis were
11 observed frequently in acute oral and inhalation studies ($\geq 1,000$ mg/kg-day oral, $\geq 1,000$ ppm
12 inhalation) ([David, 1964](#); [de Navasquez, 1935](#); [Drew, et al., 1978](#); [Fairley, et al., 1934](#); [JBRC,](#)
13 [1998](#); [Kesten, et al., 1939](#); [Laug, et al., 1939](#); [Schrenk & Yant, 1936](#)).

14 Liver and kidney toxicity were the primary noncancer health effects of subchronic and
15 chronic oral exposure to 1,4-dioxane in animals. Hepatocellular degeneration and necrosis were
16 observed ([Kociba, et al., 1974](#)) and preneoplastic changes were noted in the liver following
17 chronic administration of 1,4-dioxane in drinking water ([Argus, et al., 1973](#); [JBRC, 1998](#); [Kano,](#)
18 [et al., 2009](#)). Liver and kidney toxicity appear to be related to saturation of clearance pathways
19 and an increase in the 1,4-dioxane concentration in the blood ([Kociba, et al., 1974](#)). Kidney
20 damage was characterized by degeneration of the cortical tubule cells, necrosis with hemorrhage,
21 and glomerulonephritis ([Argus, et al., 1965](#); [Argus, et al., 1973](#); [Fairley, et al., 1934](#); [Kociba, et](#)
22 [al., 1974](#); [NCI, 1978](#)). In chronic inhalation studies conducted in rats, nasal and liver toxicity
23 were the primary noncancer health effects. Degeneration of nasal tissue (i.e. metaplasia,
24 hyperplasia, atrophy, hydropic change, and vacuolic change) and preneoplastic cell proliferation
25 were observed in the nasal cavity following 2 years of 1,4-dioxane exposure via inhalation
26 ([Kasai, et al., 2009](#)). Liver toxicity was described as necrosis of the centrilobular region and
27 preneoplastic changes were noted as well.

28 Several carcinogenicity bioassays have been conducted for 1,4-dioxane in mice, rats, and
29 guinea pigs ([Argus, et al., 1965](#); [Argus, et al., 1973](#); [Hoch-Ligeti & Argus, 1970](#); [Hoch-Ligeti, et](#)
30 [al., 1970](#); [JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Kociba, et al., 1974](#); [NCI, 1978](#);

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#).

1 [Torkelson, et al., 1974](#)). Liver tumors (hepatocellular adenomas and carcinomas) have been
2 observed following drinking water exposure in several species and strains of rats, mice, and
3 guinea pigs [and following inhalation exposure in rats](#). Nasal (squamous cell carcinomas),
4 peritoneal, mammary, [Zymbal gland, and subcutaneous](#) tumors were also observed in rats, but
5 were not seen in mice. With the exception of the NCI ([1978](#)) study, the incidence of nasal cavity
6 tumors was generally lower than that of tumors [observed in other tissues of](#) the same study
7 population.

8 Under the *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)), 1,4-dioxane is
9 “likely to be carcinogenic to humans” based on evidence of [multiple tissue](#) carcinogenicity in
10 several 2-year bioassays conducted in three strains of rats, two strains of mice, and in guinea pigs
11 ([Argus, et al., 1965](#); [Argus, et al., 1973](#); [Hoch-Ligeti & Argus, 1970](#); [Hoch-Ligeti, et al., 1970](#);
12 [JBRC, 1998](#); [Kano, et al., 2009](#); [Kasai, et al., 2009](#); [Kociba, et al., 1974](#); [NCI, 1978](#)). Studies in
13 humans found no conclusive evidence for a causal link between occupational exposure to
14 1,4-dioxane and increased risk for cancer; however, only two studies were available and these
15 were limited by small cohort size and a small number of reported cancer cases ([Buffler, et al.,](#)
16 [1978](#); [Thiess, et al., 1976](#)).

17 The available evidence is inadequate to establish a MOA by which 1,4-dioxane induces
18 tumors in rats and mice. The genotoxicity data for 1,4-dioxane is generally characterized as
19 negative, although several studies may suggest the possibility of genotoxic effects ([Galloway, et](#)
20 [al., 1987](#); [Kitchin & Brown, 1990](#); [Mirkova, 1994](#); [Morita & Hayashi, 1998](#); [Roy, et al., 2005](#)).
21 A MOA hypothesis [for liver tumors](#) involving sustained proliferation of spontaneously
22 transformed liver cells has some support by evidence that suggests 1,4-dioxane is a tumor
23 promoter in mouse skin and rat liver bioassays ([King, et al., 1973](#); [Lundberg, et al., 1987](#)). Some
24 dose-response and temporal evidence support the occurrence of cell proliferation and hyperplasia
25 prior to the development of liver tumors ([JBRC, 1998](#); [Kociba, et al., 1974](#)). However, the dose-
26 response relationship for the induction of hepatic cell proliferation has not been characterized,
27 and it is unknown if it would reflect the dose-response relationship for liver tumors in the 2-year
28 rat and mouse studies. Conflicting data from rat and mouse bioassays ([JBRC, 1998](#); [Kociba, et](#)
29 [al., 1974](#)) suggest that cytotoxicity is not a required precursor event for 1,4-dioxane-induced cell
30 proliferation. Liver tumors were observed in female rats and female mice in the absence of
31 lesions indicative of cytotoxicity ([JBRC, 1998](#); [Kano, et al., 2009](#); [NCI, 1978](#)). Data regarding a
32 plausible dose response and temporal progression from cytotoxicity to cell proliferation and
33 eventual liver tumor formation are not available. [Hypothesized MOAs by which 1,4-dioxane](#)
34 [induces tumors in other organ systems such as the respiratory system are uncertain \(See Section](#)
35 [4.7.3\).](#)

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

1 The RfD of 3×10^{-2} mg/kg-day was derived based on liver and kidney toxicity in rats
2 exposed to 1,4-dioxane in the drinking water for 2 years ([Kociba, et al., 1974](#)). This study was
3 chosen as the principal study because it provides the most sensitive measure of adverse effects
4 by 1,4-dioxane. The incidence of liver and kidney lesions was not reported for each dose group.
5 Therefore, BMD modeling could not be used to derive a POD. Instead, the RfD is derived by
6 dividing the NOAEL of 9.6 mg/kg-day by a composite UF of 300 (factors of 10 for animal-to-
7 human extrapolation and interindividual variability, and an UF of 3 for database deficiencies).
8 Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences
9 between animals and humans and the potential variability in human susceptibility; thus, the
10 interspecies and intraspecies uncertainty factors of 10 were applied. In addition, a threefold
11 database uncertainty factor was applied due to the lack of information addressing the potential
12 reproductive toxicity associated with 1,4-dioxane.

13 The overall confidence in the RfD is medium. Confidence in the principal study ([Kociba,
14 et al., 1974](#)) is medium. Confidence in the database is medium due to the lack of a
15 multigeneration reproductive toxicity study. Reflecting medium confidence in the principal
16 study and medium confidence in the database, confidence in the RfD is medium.

6.2.2. Noncancer/Inhalation

17 The RfC of 3×10^{-2} mg/m³ was derived based on olfactory epithelium atrophy in rats
18 exposed for 2 years to 1,4-dioxane via inhalation ([Kasai, et al., 2009](#)). This study was chosen as
19 the principal study because it provides the adequate study design and most sensitive measure of
20 adverse effects by 1,4-dioxane. The POD was derived using the LOAEL for olfactory atrophy in
21 male rats from the [Kasai et al. \(2009\)](#) study. A composite UF of 1000 was applied, consisting
22 of factors of 10 for a LOAEL-to NOAEL extrapolation and for interindividual variability, and 3
23 for animal-to-human extrapolation and for database deficiencies.

24 The overall confidence in the RfC is medium. Confidence in the principal study ([Kasai,
25 et al., 2009](#)) is medium. Confidence in the database is medium due to the lack of supporting
26 studies and a multigeneration reproductive toxicity study. Reflecting medium confidence in the
27 principal study and medium confidence in the database, the confidence in the RfC is medium.

6.2.3. Cancer

28 Under EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)),
29 1,4-dioxane is "likely to be carcinogenic to humans" by all routes of exposure. This descriptor is
30 based on evidence of carcinogenicity from animal studies.

6.2.3.1. Oral

1 An oral CSF for 1,4-dioxane of $0.10 \text{ (mg/kg-day)}^{-1}$ was based on liver tumors in female
2 mice from a chronic study ([Kano, et al., 2009](#)). The available data indicate that the MOA(s) by
3 which 1,4-dioxane induces peritoneal, mammary, or nasal tumors in rats and liver tumors in rats
4 and mice is unknown (see Section 4.7.3 for a more detailed discussion of 1,4-dioxane's
5 hypothesized MOAs). Therefore, based on the U.S. EPA's *Guidelines for Carcinogen Risk*
6 *Assessment* ([U.S. EPA, 2005a](#)), a linear low dose extrapolation was used. The POD was
7 calculated by curve fitting the animal experimental dose-response data from the range of
8 observation and converting it to a HED ($\text{BMDL}_{50 \text{ HED}}$ of 4.95 mg/kg-day).

9 The uncertainties associated with the quantitation of the oral CSF are discussed below.

6.2.3.2. Inhalation

10 An inhalation unit risk (IUR) for 1,4-dioxane of $5 \times 10^{-6} \text{ (}\mu\text{g/m}^3\text{)}^{-1}$ was based on a chronic
11 inhalation study conducted by Kasai et al. (2009). Statistically significant increases in tumor
12 incidence and positive dose-response trends were observed at multiple sites in the male rat
13 including the nasal cavity (squamous cell carcinoma), liver (adenoma), peritoneal
14 (mesothelioma), and the subcutis (fibroma). Statistically significant dose-response trends were
15 also observed in the kidney (carcinoma), mammary gland (fibroadenoma), and the Zymbal gland
16 (adenoma). The available data indicate that the MOA(s) by which 1,4-dioxane induces tumors in
17 rats is unknown (see Section 4.7.3 for a more detailed discussion of 1,4-dioxane's hypothesized
18 MOAs). Therefore, based on the U.S. EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S.](#)
19 [EPA, 2005a](#)), a linear low dose extrapolation was used. A Bayesian approach (see Section
20 5.4.3.2 and Appendix G for details) was used to calculate the POD for the total tumor risk
21 following inhalation of 1,4-dioxane. The POD was calculated by curve fitting the animal
22 experimental dose-response data from the range of observation and converting it to a continuous
23 human equivalent exposure.

24 The uncertainties associated with the quantitation of the IUR are discussed below.

6.2.3.3. *Choice of Low-Dose Extrapolation Approach*

25 The range of possibilities for the low-dose extrapolation of tumor risk for exposure to
26 1,4-dioxane, or any chemical, ranges from linear to nonlinear, but is dependent upon a plausible
27 MOA(s) for the observed tumors. The MOA is a key consideration in clarifying how risks
28 should be estimated for low-dose exposure. Exposure to 1,4-dioxane has been observed in
29 animal models to induce multiple tumor types, including liver adenomas and carcinomas, nasal
30 carcinomas, mammary adenomas and fibroadenomas, and mesotheliomas of the peritoneal cavity
31 ([Kano, et al., 2009](#)). MOA information that is available for the carcinogenicity of 1,4-dioxane
32 has largely focused on liver adenomas and carcinomas, with little or no MOA information
33 available for the remaining tumor types. In Section 4.7.3, hypothesized MOAs were explored
34 for 1,4-dioxane. Data are not available to support a carcinogenic MOA for 1,4-dioxane. In the

1 absence of a MOA(s) for the observed tumor types associated with exposure to 1,4-dioxane, a
2 linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated
3 with 1,4-dioxane exposure.

4 In general, the Agency has preferred to use the multistage model for analyses of tumor
5 incidence and related endpoints because they have a generic biological motivation based on
6 long-established mathematical models such as the MVK model. The MVK model does not
7 necessarily characterize all modes of tumor formation, but it is a starting point for most
8 investigations and, much more often than not, has provided at least an adequate description of
9 tumor incidence data.

10 The multistage cancer model did provide good fits for the tumor incidence data following
11 a 2-year inhalation exposure to 1,4-dioxane by male rats (Kasai, et al., 2009). However, in the
12 studies evaluated for the oral cancer assessment (Kano, et al., 2009; Kociba, et al., 1974; NCI,
13 1978) the multistage model provided good descriptions of the incidence of a few tumor types in
14 male (nasal cavity) and female (hepatocellular and nasal cavity) rats and in male mice
15 (hepatocellular) exposed to 1,4-dioxane (see Appendix D for details). However, the multistage
16 model did not provide an adequate fit for female mouse liver tumor dataset based upon the
17 following (U.S. EPA, 2000a):

- Goodness-of-fit *p*-value was not greater than 0.10;
- AIC was larger than other acceptable models;
- Data deviated from the fitted model, as measured by their χ^2 residuals (values were greater than an absolute value of one).

18 BMDS software typically implements the guidance in the BMD technical guidance
19 document (U.S. EPA, 2000a) by imposing constraints on the values of certain parameters of the
20 models. When these constraints were imposed, the multistage model and most other models did
21 not fit the incidence data for female mouse liver adenomas or carcinomas.

22 The log-logistic model was selected because it provides an adequate fit for the female
23 mouse data (Kano, et al., 2009). A BMR of 50% was used because it is proximate to the
24 response at the lowest dose tested and the BMDL₅₀ was derived by applying appropriate
25 parameter constraints, consistent with recommended use of BMDS in the BMD technical
26 guidance document (U.S. EPA, 2000a).

27 The human equivalent oral CSF estimated from liver tumor datasets with statistically
28 significant increases ranged from 4.2×10^{-4} to 1.0×10^{-1} per mg/kg-day, a range of about three
29 orders of magnitude, with the extremes coming from the combined male and female data for
30 hepatocellular carcinomas (Kociba, et al., 1974) and the female mouse liver adenoma and
31 carcinoma dataset (Kano, et al., 2009).

6.2.3.4. *Dose Metric*

1 1,4-Dioxane is known to be metabolized in vivo. However, evidence does not exist to
2 determine whether the parent compound, metabolite(s), or a combination of the parent compound
3 and metabolites is responsible for the observed toxicity following exposure to 1,4-dioxane. If the
4 actual carcinogenic moiety is proportional to administered exposure, then use of administered
5 exposure as the dose metric is the least biased choice. On the other hand, if this is not the correct
6 dose metric, then the impact on the CSF is unknown.

6.2.3.5. *Cross-Species Scaling*

7 For the oral cancer assessment, an adjustment for cross-species scaling ($BW^{0.75}$) was
8 applied to address toxicological equivalence of internal doses between each rodent species and
9 humans, consistent with the *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)). It is
10 assumed that equal risks result from equivalent constant lifetime exposures.

11 Differences in the anatomy of the upper respiratory tract and resulting differences in
12 absorption or in local respiratory system effects are sources of uncertainty in the inhalation
13 cancer assessment.

6.2.3.6. *Statistical Uncertainty at the POD*

14 Parameter uncertainty can be assessed through confidence intervals. Each description of
15 parameter uncertainty assumes that the underlying model and associated assumptions are valid.
16 For the log-logistic model applied to the female mouse data following oral exposure, there is a
17 reasonably small degree of uncertainty at the 50% excess incidence level (the POD for linear
18 low-dose extrapolation). For the multistage model applied for the male rat inhalation dataset,
19 there is a reasonable small degree of uncertainty at the 10% extra risk level (the POD for linear
20 low-dose extrapolation).

6.2.3.7. *Bioassay Selection*

21 The study by Kano et al. ([2009](#)) was used for development of an oral CSF. This was a
22 well-designed study, conducted in both sexes in two species (rats and mice) with a sufficient
23 number (N=50) of animals per dose group. The number of test animals allocated among three
24 dose levels and an untreated control group was adequate, with examination of appropriate
25 toxicological endpoints in both sexes of rats and mice. Alternative bioassays ([Kociba, et al.,](#)
26 [1974](#); [NCI, 1978](#)) were available and were fully considered for the derivation of the oral CSF.

27 The study by Kasai et al. (2009) was used for derivation of an inhalation unit risk. This
28 was a well-designed and peer reviewed study, conducted in male rats with a sufficient number
29 (N=50) of animals per dose group. Three dose levels plus an untreated control group were
30 examined following exposure to 1,4-dioxane via inhalation for 2 years. Other bioassays (Kasai,
31 et al., 2008; Torkelson, et al., 1974) were available and were fully considered for the derivation
32 of the inhalation unit risk.

6.2.3.8. *Choice of Species/Gender*

1 The oral CSF for 1,4-dioxane was derived using the tumor incidence data for the female
2 mouse, which was thought to be more sensitive than male mice or either sex of rats to the
3 carcinogenicity of 1,4-dioxane. While all data, from both species and sexes reported from the
4 Kano et al. (2009) study, were suitable for deriving an oral CSF, the female mouse data
5 represented the most sensitive indicator of carcinogenicity in the rodent model. The lowest
6 exposure level (66 mg/kg-day [animal dose] or 10 mg/kg-day [HED]) observed a considerable
7 and significant increase in combined liver adenomas and carcinomas. Additional testing of doses
8 within the range of control and the lowest dose (66 mg/kg-day [animal dose] or 10 mg/kg-day
9 [HED]) could refine and reduce uncertainty for the oral CSF.

10 Male F344 rat data were used to estimate risk following inhalation of 1,4-dioxane. Kasai
11 et al. (2008) showed that male rats were more sensitive than female rats to the effects of 1,4-
12 dioxane following inhalation; therefore, male rats were chosen to be studied in the 2-year
13 bioassay conducted by the same laboratory (Kasai, et al., 2009).

6.2.3.9. *Relevance to Humans*

14 The oral CSF was derived using the tumor incidence in the liver of female mice. A
15 thorough review of the available toxicological data available for 1,4-dioxane provides no
16 scientific justification to propose that the liver adenomas and carcinomas observed in animal
17 models following exposure to 1,4-dioxane are not plausible in humans. Liver adenomas and
18 carcinomas were considered plausible outcomes in humans due to exposure to 1,4-dioxane.

19 The derivation of the inhalation unit risk is based on the tumor incidence at multiple sites
20 in male rats. There is no information on 1,4-dioxane to indicate that the observed rodent tumors
21 are not relevant to humans. Further, no data exist to guide quantitative adjustment for
22 differences in sensitivity among rodents and humans.

6.2.3.10. *Human Population Variability*

23 The extent of inter-individual variability in 1,4-dioxane metabolism has not been
24 characterized. A separate issue is that the human variability in response to 1,4-dioxane is also
25 unknown. Data exploring whether there is differential sensitivity to 1,4-dioxane carcinogenicity
26 across life stages is unavailable. This lack of understanding about potential differences in
27 metabolism and susceptibility across exposed human populations thus represents a source of
28 uncertainty. Also, the lack of information linking a MOA for 1,4-dioxane to the observed
29 carcinogenicity is a source of uncertainty.

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Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#).

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27

APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

1 Note: The comments and responses in this appendix were in regards to the oral
2 assessment previously reviewed. A summary of external peer review and public comments and
3 disposition following review of the inhalation assessment for 1,4-dioxane will be included when
4 they become available.

5 The *Toxicological Review of 1,4-Dioxane* has undergone formal external peer review
6 performed by scientists in accordance with EPA guidance on peer review ([U.S. EPA, 2000b,](#)
7 [2006b](#)). The external peer reviewers were tasked with providing written answers to general
8 questions on the overall assessment and on chemical-specific questions in areas of scientific
9 controversy or uncertainty. A summary of significant comments made by the external reviewers
10 and EPA's responses to these comments arranged by charge question follow. In many cases the
11 comments of the individual reviewers have been synthesized and paraphrased for development of
12 Appendix A. The majority of the specific observations (in addition to EPA's charge questions)
13 made by the peer reviewers were incorporated into the document and are not discussed further in
14 this Appendix. Public comments that were received are summarized and addressed following the
15 peer-reviewers' comments and disposition.

A.1. EXTERNAL PEER REVIEW PANEL COMMENTS

16 The reviewers made several editorial suggestions to clarify portions of the text. These
17 changes were incorporated in the document as appropriate and are not discussed further.

18 In addition, the external peer reviewers commented on decisions and analyses in the
19 *Toxicological Review of 1,4-Dioxane* under multiple charge questions, and these comments were
20 organized and summarized under the most appropriate charge question.

A.1.1. General Charge Questions

21 1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and
22 objectively represented and synthesized the scientific evidence for noncancer and cancer
23 hazards?

24 **Comment:** All reviewers found the *Toxicological Review* to be logical, clear, and concise.
25 One reviewer remarked that it was an accurate, open-minded and balanced analysis of the
26 literature. Most reviewers found that the scientific evidence was presented objectively
27 and transparently; however, one reviewer suggested two things to improve the objectivity

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#).

1 and transparency (1) provide a clear description of the mode of action and how it feeds
2 into the choice of the extrapolation for the cancer endpoint and (2) provide a presentation
3 of the outcome if internal dose was used in the cancer and noncancer assessments.

4 One reviewer commented that conclusions could not be evaluated in a few places
5 where dose information was not provided (Sections 3.2, 3.3 and 4.5.2.2). The same
6 reviewer found the MOA schematics, key event temporal sequence/dose-response table,
7 and the POD plots to be very helpful in following the logic employed in the assessment.

8
9 **Response:** The mode of action analysis and how conclusions from that analysis fed into
10 the choice of extrapolation method for the cancer assessment are discussed further under
11 charge questions C2 and C5. Because of the decision not to utilize the PBPK models,
12 internal doses were not calculated and thus were not included as alternatives to using the
13 external dose as the POD for the cancer and noncancer assessments.

14 In the sections noted by the reviewer (3.2, 3.3, and 4.5.2.2) dose information was
15 added as available. In Section 3.2, Mikheev et al. (1990) did not report actual doses,
16 which is noted in this section. All other dose information in this section was found to be
17 present after further review by the Agency. In Section 3.3, dose information for Woo
18 et al. (1977a; 1978) was added to the paragraph. In Section 4.5.2.2, study details for
19 Nannelli et al. (2005) were provided earlier in Section 3.3 and a statement referring the
20 reader to this section was added.

- 21
22 2. Please identify any additional studies that should be considered in the assessment of the
23 noncancer and cancer health effects of 1,4-dioxane.

24 **Comment:** Five reviewers stated they were unaware of any additional studies available to
25 add to the oral toxicity evaluation of 1,4-dioxane. These reviewers also acknowledged
26 the Kasai et al. (2009; 2008) publications that may be of use to derive toxicity values
27 following inhalation of 1,4-dioxane.

- 28 a. Kasai T; Saito H; Senoh Y; et al. (2008) Thirteen-week inhalation toxicity of
29 1,4-dioxane in rats. *Inhal Toxicol* 20: 961-971.
30 b. Kasai T; Kano Y; Umeda T; et al. (2009) Two-year inhalation study of
31 carcinogenicity and chronic toxicity of 1,4-dioxane in male rats. *Inhal Toxicol in*
32 *press*.

33 Other references suggested by reviewers include:

- 34 c. California Department of Health Services (1989) Risk Specific Intake Levels for
35 the Proposition 65 Carcinogen 1, 4-dioxane. Reproductive and Cancer Hazard
36 Assessment Section. Office of Environmental Health Hazard Assessment

- 1 d. National Research Council ([2009](#)) Science and Decisions: Advancing Risk
2 Assessment. Committee on Improving Risk Analysis Approaches Used by the
3 U.S. EPA. Washington, D.C., National Academy Press.
- 4 e. ATSDR ([2007](#)) Toxicological Profile for 1,4-dioxane. Agency for Toxic
5 Substances and Disease Registry. Atlanta, GA.
- 6 f. Stickney JA; Sager SL; Clarkson JR; et al. ([2003](#)) An updated evaluation of the
7 carcinogenic potential of 1,4-dioxane. Regul Toxicol Pharmacol 38: 183-195.
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9 study of 1,4-dioxane in R344 rats by multiple-route exposure (drinking water and
10 inhalation). J Toxicol Sci 25: 347.

11
12 **Response:** The references a-b above will be evaluated for derivation of an RfC and IUR,
13 which will follow as an update to this oral assessment. References c and e noted above
14 were considered during development of this assessment as to the value they added to the
15 cancer and noncancer analyses. Reference g listed above is an abstract from conference
16 proceedings from the 27th Annual Meeting of the Japanese Society of Toxicology;
17 abstracts are not generally considered in the development of an IRIS assessment.
18 Reference d reviews EPA's current risk assessment procedures and provides no specific
19 information regarding 1,4-dioxane. The Stickney et al. ([2003](#)) reference was a review
20 article and no new data were presented, thus it was not referenced in this Toxicological
21 Review but the data were considered during the development of this assessment.

22 Following external peer review (as noted above) Kano et al. ([2009](#)) was added to
23 the assessment, which was an update and peer-reviewed published manuscript of the
24 JBRC ([1998](#)) report.

- 25
26 3. Please discuss research that you think would be likely to increase confidence in the database
27 for future assessments of 1,4-dioxane.

28 **Comment:** All reviewers provided suggestions for additional research that would
29 strengthen the assessment and reduce uncertainty in several areas. The following is a
30 brief list of questions that were identified that could benefit from further research. What
31 are the mechanisms responsible for the acute and chronic nephrotoxicity? Is the acute
32 kidney injury (AKI) multifactorial? Are there both tubular and glomerular/vascular
33 toxicities that result in cortical tubule degeneration and evidence for glomerulonephritis?
34 What are the functional correlates of the histologic changes in terms of assessment of
35 renal function? What is the exposure in utero and risk to the fetus and newborn? What are
36 the concentrations in breast milk following maternal exposure to 1,4-dioxane? What is
37 the risk for use of contaminated drinking water to reconstitute infant formula? What are

1 the exposures during early human development? What is the pharmacokinetic and
2 metabolic profile of 1,4-dioxane during development? What are the susceptible
3 populations (e.g., individuals with decreased renal function or chronic renal disease,
4 obese individuals, gender, age)?

5 Additional suggestions for future research include: evaluation of potential
6 epigenetic mechanisms of carcinogenicity, additional information on sources of exposure
7 and biological concentrations as well as human toxicokinetic data for derivation of
8 parameter to refine PBPK model, studies to determine toxic moiety, focused studies to
9 inform mode of action, additional inhalation studies and a multigeneration reproductive
10 toxicity study.

11 One reviewer suggested additional analyses of the existing data including a
12 combined analysis of the multiple datasets and outcomes for cancer and non-cancer
13 endpoints, evaluation of the dose metrics relevant to the MOA to improve confidence in
14 extrapolation approach and uncertainty factors, and complete a Bayesian analysis of
15 human pharmacokinetic data to estimate human variability in key determinants of
16 toxicity (e.g., metabolic rates and partition coefficients).

17
18 **Response:** A number of research suggestions were provided for further research that may
19 enhance future health assessments of 1,4-dioxane. Regarding the suggested additional
20 analyses for the existing data, EPA did not identify a MOA in this assessment, thus
21 combined analysis of the cancer and non-cancer endpoints as well as application of
22 various dose metrics to a MOA is not applicable. Because the human PBPK model was
23 not implemented in this assessment for oral exposure to 1,4-dioxane a Bayesian analysis
24 was not completed. No additional changes to the *Toxicological Review of 1,4-Dioxane*
25 were made in response to these research recommendations.

- 26
27 4. Please comment on the identification and characterization of sources of uncertainty in
28 Sections 5 and 6 of the assessment document. Please comment on whether the key sources of
29 uncertainty have been adequately discussed. Have the choices and assumptions made in the
30 discussion of uncertainty been transparently and objectively described? Has the impact of the
31 uncertainty on the assessment been transparently and objectively described?

32 **Comment:** Six reviewers stated Sections 5 and 6 adequately discussed and characterized
33 uncertainty, in a succinct, and transparent manner. One reviewer suggested adding
34 additional discussion of uncertainty relating to the critical study used in the cancer
35 assessment and another reviewer suggested adding more discussion around the
36 uncertainty of the toxic moiety.

1 One reviewer made specific comments on uncertainty surrounding the Kociba et
2 al. (1974) study as used for derivation of the RfD, choice of the non-cancer dose metric,
3 and use of a 10% BMR as the basis for the CSF derivation. These comments and
4 responses are summarized below under their appropriate charge question.

5
6 **Response:** The majority of the reviewers thought the amount of uncertainty discussion
7 was appropriate. Since the external review, Kano et al. (2009) was published and this
8 assessment was updated accordingly (previously JBRC (1998)). It is assumed the
9 uncertainty referred to by the reviewer was addressed by the published Kano et al. (2009)
10 paper.

11 Clarification regarding the uncertainty surrounding the identification of the toxic
12 moiety was added to Section 4.6.3 stating that the mechanism by which 1,4-dioxane
13 induces tissue damage is not known, nor is it known whether the toxic moiety is
14 1,4-dioxane or a metabolite of 1,4-dioxane. Additional text was added to Section 4.7.3
15 clarifying that available data also do not clearly identify whether 1,4-dioxane or one of its
16 metabolites is responsible for the observed effects. The impact of the lack of evidence to
17 clearly identify a toxic moiety related to 1,4-dioxane exposure was summarized in
18 Sections 5.5.1.2 and 6.2.3.2.

A.1.2. Oral reference dose (RfD) for 1,4-dioxane

- 19 1. A chronic RfD for 1,4-dioxane has been derived from a 2-year drinking water study (Kociba,
20 et al., 1974) in rats and mice. Please comment on whether the selection of this study as the
21 principal study has been scientifically justified. Has the selection of this study been
22 transparently and objectively described in the document? Are the criteria and rationale for
23 this selection transparently and objectively described in the document? Please identify and
24 provide the rationale for any other studies that should be selected as the principal study.

25 **Comment:** Seven of the reviewers agreed that the use of the Kociba et al. (1974) study
26 was the best choice for the principal study.

27 One reviewer stated that Kociba et al. (1974) was not the best choice because it
28 reported only NOAEL and LOAELs without providing incidence data for the endpoints.
29 This reviewer also stated that the study should not have been selected based on sensitivity
30 of the endpoints, but rather study design and adequacy of reporting of the study results.
31 Additionally, this reviewer suggested a better principal study would be either the NCI
32 (1978) or JBRC (1998) study.

33
34 **Response:** The reviewer is correct that Kociba et al. (1974) did not provide incidence
35 data; however, Kociba et al. (1974) identified a NOAEL (9.6 mg/kg-day) and LOAEL
36 (94 mg/kg-day) within the text of the manuscript. Kociba et al. (1974) was a well

1 conducted chronic bioassay (four dose levels, including controls, with 60 rats/sex/group)
2 and seven of the peer reviewers found this study to be appropriate as the basis for the
3 RfD. Further support for the selection of the Kociba et al. (1974) as the principal study
4 comes from comparison of the liver and kidney toxicity data reported by JBRC (1998)
5 and NCI (1978), which was presented in Section 5.1. The effects reported by JBRC
6 (1998) and NCI (1978) were consistent with what was observed by Kociba et al. (1974)
7 and within a similar dose range. Derivation of an RfD from these datasets resulted in a
8 similar value (Section 5.1.).
9

- 10 2. Degenerative liver and kidney effects were selected as the critical effect. Please comment on
11 whether the rationale for the selection of this critical effect has been scientifically justified.
12 Are the criteria and rationale for this selection transparently and objectively described in the
13 document? Please provide a detailed explanation. Please comment on whether EPA's
14 rationale regarding adversity of the critical effect for the RfD has been adequately and
15 transparently described and is scientifically supported by the available data. Please identify
16 and provide the rationale for any other endpoints that should be considered in the selection of
17 the critical effect.

18 **Comment:** Five of the reviewers agreed with the selection of liver and kidney effects as
19 the critical effect. One of these reviewers suggested analyzing all datasets following dose
20 adjustment (e.g., body weight scaling or PBPK model based) to provide a better rationale
21 for selection of a critical effect.

22 One reviewer stated that 1,4-dioxane causing liver and kidney organ specific
23 effects is logical; however, with regards to nephrotoxicity, the models and limited human
24 data have not addressed the mechanisms of injury or the clinical correlates to the
25 histologic data. Also, advances in the field of biomarkers have not yet been used for the
26 study of 1,4-dioxane.

27 One reviewer found the selection of these endpoints to be 'without merit' because
28 of the lack of incidence data to justify the NOAEL and LOAEL values identified in the
29 study. This reviewer suggested selecting the most sensitive endpoint(s) from the NCI
30 (NCI, 1978) or JBRC (1998) studies for the basis of the RfD, but did not provide a
31 suggestion as to what effect should be selected.
32

33 **Response:** The liver and kidney effects from Kociba et al. (1974) was supported as the
34 critical effect by most of the reviewers. PBPK model adjustment was not performed
35 because the PBPK model was found to be inadequate for use in the assessment. EPA
36 acknowledges that neither the mechanisms of injury nor the clinical correlates to

1 histologic data exist for 1,4-dioxane. This type of information could improve future
2 health assessments of 1,4-dioxane.

3 As stated above, Kociba et al. ([1974](#)) identified a NOAEL (9.6 mg/kg-day) and
4 LOAEL (94 mg/kg-day) within the text of the manuscript and was a well conducted
5 chronic bioassay (four dose levels, including controls, with 60 rats/sex/group).
6

- 7 3. Kociba et al. ([1974](#)) derived a NOAEL based upon the observation of degenerative liver and
8 kidney effects and these data were utilized to derive the point of departure (POD) for the
9 RfD. Please provide comments with regard to whether the NOAEL approach is the best
10 approach for determining the POD. Has the approach been appropriately conducted and
11 objectively and transparently described? Please identify and provide rationales for any
12 alternative approaches for the determination of the POD and discuss whether such
13 approaches are preferred to EPA's approach.

14 **Comment:** Seven reviewers agreed with the NOAEL approach described in the
15 document. One of these reviewers also questioned whether any attempt was made to
16 “semi-qualitatively represent the histopathological observations to facilitate a quantitative
17 analysis”.

18 One reviewer stated that data were not used to derive the POD, but rather a claim
19 by the authors of Kociba et al. ([1974](#)) of the NOAEL and LOAEL for the endpoints. This
20 reviewer preferred the use of a BMD approach for which data include the reported
21 incidence rather than a study reported NOAEL or LOAEL.
22

23 **Response:** The suggestion to “semi-qualitatively represent the histopathological
24 observations to facilitate a quantitative analysis” was not incorporated into the document
25 because it is unclear how this would be conducted since Kociba et al. ([1974](#)) did not
26 provide incidence data and the reviewer did not illustrate their suggested approach. See
27 responses to B1 and B2 regarding the NOAEL and LOAEL approach. The Agency
28 agrees that a Benchmark Dose approach is preferred over the use of a NOAEL or
29 LOAEL for the POD if suitable data (e.g., reflecting the most sensitive sex, species, and
30 endpoint identified) are available for modeling and, if suitable data are not available, then
31 NOAEL and LOAEL values are utilized. In this case, the data were not suitable for
32 BMD modeling and the LOAEL or NOAEL approach was used.
33

- 34 4. EPA evaluated the PBPK and empirical models available to describe kinetics following
35 inhalation of 1,4-dioxane ([Reitz, et al., 1990](#); [Young, et al., 1978a](#); [Young, et al., 1978b](#);
36 [Young, et al., 1977](#)). EPA concluded that the use of existing, revised, and recalibrated PBPK
37 models for 1,4-dioxane were not superior to default approaches for the dose-extrapolation

1 between species. Please comment on whether EPA's rationale regarding the decision to not
2 utilize existing or revised PBPK models has been adequately and transparently described and
3 is supported by the available data. Please identify and provide the rationale for any
4 alternative approaches that should be considered or preferred to the approach presented in the
5 toxicological review.

6 **Comment:** Six reviewers found the decision not to utilize the available PBPK models to
7 be appropriate and supported by available data. One of these reviewers suggested
8 presenting as part of the uncertainty evaluation an adjustment of the experimental doses
9 based on metabolic saturation. Another reviewer stated Appendix B was hard to follow
10 and that the main document should include a more complete description of the model
11 refinement effort performed by Sweeney et al. ([2008](#)).

12 Two reviewers noted a complete evaluation of the models was evident; one of the
13 reviewers questioned the decision not to use the models on the basis that they were
14 unable to fit the human blood PK data for 1,4-dioxane. This reviewer suggested the rat
15 model might fit the human blood PK data, thus raising concern in the reliance on the
16 human blood PK data to evaluate the PBPK model for 1,4-dioxane. Instead, the reviewer
17 suggested the human urinary metabolite data may be sufficient to give confidence in the
18 model. One other reviewer also questioned the accuracy of the available human data.
19 One reviewer commented that the rationale for not using the PBPK model to extrapolate
20 from high to low dose was questioned. In addition, the reviewer suggested that two
21 aspects of the model code for Reitz et al. ([1990](#)) need to be verified:

- 22 a. In the document, KLC is defined as a first-order rate constant and is scaled by
23 $BW^{0.7}$. This is inconsistent when multiplied by concentration does not result
24 in units of mg/hr. However, if the parameter is actually considered a
25 clearance constant (zero-order rate constant) then the scaling rule used, as well
26 as the interpretations provided, would be acceptable.
- 27 b. It is unclear as to why AM is calculated on the basis of RAM and not RMEX.
28 RMEX seems to represent the amount metabolized per unit time.

29
30 **Response:** The USEPA performed a rigorous evaluation of the PBPK models available
31 for 1,4-dioxane. This effort was extensively described in Section 3.5 and in Appendix B.
32 In short, several procedures were applied to the human PBPK model to determine if an
33 adequate fit of the model to the empirical model output or experimental observations
34 could be attained using biologically plausible values for the model parameters. The re-
35 calibrated model predictions for blood 1,4-dioxane levels did not come within 10-fold of
36 the experimental values using measured tissue:air partition coefficients of ([Leung &](#)
37 [Paustenbach, 1990](#)) or ([Sweeney, et al., 2008](#)) (Figures B-8 and B-9). The utilization of a

1 slowly perfused tissue:air partition coefficient 10-fold lower than measured values
2 produces exposure-phase predictions that are much closer to observations, but does not
3 replicate the elimination kinetics (Figure B-10). Re-calibration of the model with upper
4 bounds on the tissue:air partition coefficients results in predictions that are still six- to
5 sevenfold lower than empirical model prediction or observations (Figures B-12 and B-
6 13). Exploration of the model space using an assumption of first-order metabolism (valid
7 for the 50 ppm inhalation exposure) showed that an adequate fit to the exposure and
8 elimination data can be achieved only when unrealistically low values are assumed for
9 the slowly perfused tissue:air partition coefficient (Figure B-16). Artificially low values
10 for the other tissue:air partition coefficients are not expected to improve the model fit, as
11 these parameters are shown in the sensitivity analysis to exert less influence on blood
12 1,4-dioxane than $V_{\max}C$ and K_m . In the absence of actual measurements for the human
13 slowly perfused tissue:air partition coefficient, high uncertainty exists for this model
14 parameter value. Differences in the ability of rat and human blood to bind 1,4-dioxane
15 may contribute to the difference in V_d . However, this is expected to be evident in very
16 different values for rat and human blood:air partition coefficients, which is not the case
17 (Table B-1). Therefore, some other, as yet unknown, modification to model structure
18 may be necessary.

19 The results of USEPA's model evaluation were confirmed by other investigators
20 ([Sweeney, et al., 2008](#)). Sweeney et al. (2008) concluded that the available PBPK model
21 with refinements resulted in an under-prediction of human blood levels for 1,4-dioxane
22 by six- to seven fold. It is anticipated that the high uncertainty in predictions of the
23 PBPK model for 1,4-dioxane would not result in a more accurate derivation of human
24 health toxicity values.

25 Because it is unknown whether the parent or the metabolite is the toxic moiety,
26 analyses were not conducted to adjust the experimental doses on the basis of metabolic
27 saturation.

28 The discussion of Sweeney et al. (2008) was expanded in the main document in
29 Section 3.5.3. In the absence of evidence to the contrary, the Agency cannot discount the
30 human blood kinetic data published by Young et al. (1977). Even though the PBPK
31 model provided satisfactory fits to the rodent kinetic data, it was not used to extrapolate
32 from high dose to low dose in the animal because an internal dose metric was not
33 identified and external doses were utilized in derivation of the toxicity values.

34 KLC was implemented by USEPA during the evaluation of the model and should
35 have been described as a clearance constant (zero-order rate constant) with units of
36 $L/hr/kg^{0.70}$. These corrections have been made in the document; however, this does not

1 impact the model predictions because it was in reference to the terminology used to
2 describe this constant.

3 The reviewer is correct that RMEX is the rate of metabolism of 1,4-dioxane per
4 unit time; however an amount of 1,4-dioxane metabolized was not calculated in the Reitz
5 et al. (1990) model code. Thus, AM is the amount of the metabolite (i.e., HEAA) in the
6 body rather than the amount metabolized of 1,4-dioxane. RAM was published by Reitz
7 et al. (1990) as equation 2 for the change in the amount of metabolite in the body per unit
8 time. AMEX is the amount of the metabolite excreted in the urine. While the variables
9 used are confusing, the code describes the metabolism of 1,4-dioxane as published in the
10 manuscripts. The comments in the model code were updated to make this description
11 more clear (Appendix B).

12
13 5. Please comment on the selection of the uncertainty factors applied to the POD for the
14 derivation of the RfD. For instance, are they scientifically justified and transparently and
15 objectively described in the document? If changes to the selected uncertainty factors are
16 proposed, please identify and provide a rationale(s). Please comment specifically on the
17 following uncertainty factors:

- 18 • An interspecies uncertainty factor of 10 was used to account for uncertainties in
19 extrapolating from laboratory animals to humans because a PBPK model to support
20 interspecies extrapolation was not suitable.
- 21 • An intraspecies (human variability) uncertainty factor of 10 was applied in deriving the
22 RfD because the available information on the variability in human response to
23 1,4-dioxane is considered insufficient to move away from the default uncertainty factor
24 of 10.
- 25 • A database uncertainty factor of 3 was used to account for lack of adequate
26 reproductive toxicity data for 1,4-dioxane, and in particular absence of a
27 multigeneration reproductive toxicity study. Has the rationale for the selection of these
28 uncertainty factors been transparently and objectively described in the document?
29 Please comment on whether the application of these uncertainty factors has been
30 scientifically justified.

31
32 **Comment:**

33 One reviewer noted the uncertainty factors appear to be the standard default choices and
34 had no alternatives to suggest.

- 35 ○ Five reviewers agreed that the use of an uncertainty factor of 10 for the interspecies
36 extrapolation is fully supportable. One reviewer suggested using $BW^{3/4}$ scaling
37 rather than an uncertainty factor of 10 for animal to human extrapolation. Along

1 the same lines, one reviewer suggested a steady-state quantitative analysis to
2 determine the importance of pulmonary clearance and hepatic clearance and stated
3 that if hepatic clearance scales to body surface and pulmonary clearance is
4 negligible, then an adjusted uncertainty factor based on body surface scaling would
5 be more appropriate.

- 6 ○ Seven reviewers stated that the uncertainty factor of 10 for interindividual
7 variability (intraspecies) is fully supportable.
- 8 ○ Six reviewers commented that the uncertainty factor of 3 for database deficiencies
9 is fully justifiable. One reviewer suggested adding text to clearly articulate the
10 science policy for the use of a factor of 3 for database deficiencies.

11
12 **Response:** The preferred approach to interspecies scaling is the use of a PBPK model;
13 however, the PBPK models available for 1,4-dioxane are not suitable for use in this
14 health assessment as outlined elsewhere. Another approach that has been commonly
15 implemented in the cancer assessments is the use of body weight scaling based on body
16 surface area ($BW^{3/4}$ scaling). It is not standard practice to apply $BW^{3/4}$ scaling in
17 noncancer assessments at this time. The current default approach used by the Agency
18 when PBPK models are not available for extrapolation is the application of an UF_A of 10,
19 which was implemented in this assessment.

20 The absence of a multigenerational reproductive study is why the uncertainty
21 factor for database deficiencies (UFD) was retained; however, it was reduced from 10 to
22 3. In the text in Section 5.1.3 text was included to clearly state that because of the
23 absence of a multigenerational reproductive study for 1,4-dioxane an uncertainty factor of
24 3 was used for database deficiencies. No other changes regarding the use of the
25 uncertainty factors were made to the document.

A.1.3. Carcinogenicity of 1,4-dioxane

- 26 1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment
27 (www.epa.gov/iris/backgr-d.htm), the Agency concluded that 1,4-dioxane is likely to be
28 carcinogenic to humans. Please comment on the cancer weight of evidence characterization.
29 Has the scientific justification for the weight of evidence descriptor been sufficiently,
30 transparently and objectively described? Do the available data for both liver tumors in rats
31 and mice and nasal, mammary, and peritoneal tumors in rats support the conclusion that
32 1,4-dioxane is a likely human carcinogen?

33 **Comment:** All reviewers agreed with the Agency's conclusion that 1,4-dioxane is "likely
34 to be carcinogenic to humans". However, two reviewers also thought 1,4-dioxane could
35 be categorized as a potential human carcinogen, since low-dose environmental exposures
36 would be unlikely to result in cancer. One reviewer also suggested providing a brief

1 recapitulation of the guidance provided by the 2005 Guidelines for Carcinogen Risk
2 Assessment regarding classification of a compound as likely to be carcinogenic to
3 humans and how a chemical falls into this category.
4

5 **Response:** The document includes a weight-of-evidence approach to categorize the
6 carcinogenic potential of 1,4-dioxane. This was included in Section 4.7.1 based upon
7 U.S. EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)). 1,4-Dioxane
8 can be described as likely to be carcinogenic to humans based on evidence of liver
9 carcinogenicity in several 2-year bioassays conducted in three strains of rats, two strains
10 of mice, and in guinea pigs. Additionally, tumors in other organs and tissues have been
11 observed in rats due to exposure to 1,4-dioxane.
12

- 13 2. Evidence indicating the mode of action of carcinogenicity of 1,4-dioxane was considered.
14 Several hypothesized MOAs were evaluated within the Toxicological Review and EPA
15 reached the conclusion that a MOA(s) could not be supported for any tumor types observed
16 in animal models. Please comment on whether the weight of the scientific evidence supports
17 this conclusion. Please comment on whether the rationale for this conclusion has been
18 transparently and objectively described. Please comment on data available for 1,4-dioxane
19 that may provide significant biological support for a MOA beyond what has been described
20 in the Toxicological Review. Considerations should include the scientific support regarding
21 the plausibility for the hypothesized MOA(s), and the characterization of uncertainty
22 regarding the MOA(s).

23 **Comment:** Three reviewers commented that the weight of evidence clearly supported the
24 conclusion that a mode of action could not be identified for any of the tumor sites. One
25 reviewer commented that there is inadequate evidence to support a specific MOA with
26 any confidence and low-dose linear extrapolation is necessary; this reviewer also pointed
27 out that EPA should not rule out a metabolite as the toxic moiety.

28 One reviewer stated this was outside of his/her area of expertise but indicated that
29 the discussion was too superficial and suggested adding statements as to what the Agency
30 would consider essential information to make a determination about a MOA.

31 Two reviewers commented that even though the MOA for 1,4-dioxane is not clear
32 there is substantial evidence that the MOA is non-genotoxic. One of these reviewers also
33 suggested that a nonlinear cancer risk assessment model should be utilized.

34 One reviewer suggested adding more text to the summary statement to fully
35 reflect the available MOA information which should be tied to the conclusion and choice
36 of an extrapolation model.
37

1 **Response:** The Agency agrees with the reviewer not to rule out a toxic metabolite as the
2 toxic moiety. In Section 5.5.1.2 text is included relating that there is not enough
3 information to determine whether the parent compound, its metabolite(s), or a
4 combination is responsible for the observed toxicities following exposure to 1,4-dioxane.

5 It is not feasible to describe the exact data that would be necessary to conclude
6 that a particular MOA was operating to induce the tumors observed following
7 1,4-dioxane exposure. In general, the data would fit the general criteria described in the
8 U.S. EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)). For
9 1,4-dioxane, several MOA hypotheses have been proposed and are explored for the
10 observed liver tumors in Section 4.7.3. This analysis represents the extent to which data
11 could provide support for any particular MOA.

12 One reviewer suggested that the evidence indicating that 1,4-dioxane is not
13 genotoxic supports a nonlinear approach to low-dose extrapolation. In accordance with
14 the U.S. EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)), the
15 absence of evidence for genotoxicity does not invoke the use of nonlinear low-dose
16 extrapolation, nor does it define a MOA. A nonlinear low-dose extrapolation can be
17 utilized when a MOA supporting a nonlinear dose response is identified. For 1,4-dioxane
18 this is not the case; a cancer MOA for any of the tumor types observed in animal models
19 has not been elucidated. Therefore, as concluded in the Toxicological Review, the
20 application of a nonlinear low-dose extrapolation approach was not supported.

21 Additional text has been added to Section 5.4.3.2 to relay the fact that several
22 reviewers recommended that the MOA data support the use of a nonlinear extrapolation
23 approach to estimate human carcinogenic risk associated with exposure to 1,4-dioxane
24 and that such an approach should be presented in the Toxicological Review. Additional
25 text has also been added to the summary statement in Section 6.2.3 stating that the weight
26 of evidence is inadequate to establish a MOA(s) by which 1,4-dioxane induces peritoneal,
27 mammary, or nasal tumors in rats and liver tumors in rats and mice (see Section 4.7.3 for
28 a more detailed discussion of 1,4-dioxane's hypothesized MOAs).

- 29
30 3. A two-year drinking water cancer bioassay ([JBRC, 1998](#)) was selected as the principal study
31 for the development of an oral slope factor (OSF). Please comment on the appropriateness of
32 the selection of the principal study. Has the rationale for this choice been transparently and
33 objectively described?

34 **Comment:**

35 Seven reviewers agreed with the choice of the JBRC ([1998](#)) study as the principal
36 study for the development of an OSF. However, two reviewers that agreed with the
37 choice of JBRC ([1998](#)) also commented on the description and evaluation of the study.

1 One reviewer commented the evaluation of the study should be separated from the
2 evaluation/selection of endpoints within the study. The other reviewer suggested that
3 details on the following aspects should be added to improve transparency of the study:
4 (1) rationale for selection of doses; (2) temporal information on body weight for
5 individual treatment groups; (3) temporal information on mortality rates; and (4) dosing
6 details.

7 One reviewer thought that the complete rationale for selection of the JBRC ([1998](#))
8 study was not provided because there was no indication of whether the study was
9 conducted under GLP conditions, and the study was not peer reviewed or published. This
10 reviewer noted the NCI ([1978](#)) study was not appropriate for use, but that the Kociba et
11 al. ([1974](#)) study may have resulted in a lower POD had they employed both sexes of mice
12 and combined benign and malignant tumors.

13
14 ***Response:*** Since the External Peer Review draft of the *Toxicological Review of*
15 *1,4-Dioxane* was released ([U.S. EPA, 2009b](#)), the cancer portion of the study conducted
16 by the JBRC laboratory was published in the peer-reviewed literature as Kano et al.
17 ([2009](#)). This manuscript was reviewed by EPA. EPA determined that the data published
18 by Kano et al. ([2009](#)) should be included in the assessment of 1,4-dioxane for several
19 reasons: (1) while the JBRC ([1998](#)) was a detailed laboratory report, it was not peer-
20 reviewed; (2) the JBRC improved the diagnosis of pre- and neoplastic lesions in the liver
21 according to the current diagnostic criteria and submitted the manuscript based on this
22 updated data; (3) the Kano et al. ([2009](#)) peer-reviewed manuscript included additional
23 information such as body weight growth curves and means and standard deviations of
24 estimated dose for both rats and mice of both sexes. Thus, the Toxicological Review was
25 updated to reflect the inclusion of the data from Kano et al. ([2009](#)), and Appendix E was
26 added for a clear and transparent display of the data included in the multiple reports.

27 In response to the peer reviewers, dose information was updated throughout the
28 assessment and are also provided in detail in Section 4.2.1.2.6, along with temporal
29 information on body weights and mortality. Text was also added to Section 4.2.1.2.6
30 regarding the choice of high dose selection as included in the Kano et al. ([2009](#))
31 manuscript. Additional discussion regarding the mortality rates was also added to
32 Section 5.4.1 in selection of the critical study for the oral cancer assessment.
33 Documentation that the study was conducted in accordance with Organization for
34 Economic Co-operation and Development (OECD) Principles of Good Laboratory
35 Practice (GLP) is provided in the manuscript ([Kano, et al., 2009](#)) and this was also added
36 to the text in Section 4.2.1.2.6.

37

- 1 4. Combined liver tumors (adenomas and carcinomas) in female Cjr:BDF1 mice from the JBRC
2 ([1998](#)) study were chosen as the most sensitive species and gender for the derivation of the
3 final OSF. Please comment on the appropriateness of the selections of species and gender.
4 Please comment on whether the rationale for these selections is scientifically justified. Has
5 the rationale for these choices been transparently and objectively described?

6 **Comment:** Six reviewers agreed the female Cjr:BDF1 mice should be used for the
7 derivation of the OSF. Five of these reviewers agreed with the rationale for the selection
8 of the female Cjr:BDF1 mouse as the most sensitive gender and species. However, one
9 reviewer suggested that the specific rationale (i.e., that the final OSF is determined by
10 selecting the gender/species that gives the greatest OSF value) be stated clearly in a
11 paragraph separate from the other considerations of study selection.

12 One reviewer was unsure of both the scientific justification for combining benign
13 and malignant liver tumors, as well as the background incidence of the observed liver
14 tumors in historical control Cjr:BDF1 male and female mice.

15 One reviewer commented that the scientific basis for the selection of female
16 Cjr:BDF1 mice was unclear. This reviewer thought that the rationale for the choice of
17 this strain/sex compared to all others was not clearly articulated.

18
19 **Response:** Using the approach described in the *Guidelines for Carcinogen Risk*
20 *Assessment* ([U.S. EPA, 2005a](#)) studies were first evaluated based on their quality and
21 suitability for inclusion in the assessment. Once the studies were found to be of sufficient
22 quality for inclusion in the assessment, the dose-response analysis was performed with
23 the goal of determining the most appropriate endpoint and species for use in the
24 derivation of an OSF. These topics are discussed in detail in Section 4.7 and 5.4.

25 Benign and malignant tumors that arise from the same cell type (e.g.,
26 hepatocellular) may be combined to more clearly identify the weight of evidence for a
27 chemical. This is in accordance with the US EPA's 2005 Guidelines for Carcinogen Risk
28 Assessment as referenced in the Toxicological Review. In the absence of a MOA (MOA
29 analysis described in detail in Section 4.7.) for 1,4-dioxane carcinogenicity, it is not
30 possible to determine which species may more closely resemble humans. Text in Section
31 5.4.4 indicates that the calculation of an OSF for 1,4-dioxane is based upon the dose-
32 response data for the most sensitive species and gender.

- 33
34 5. Has the scientific justification for deriving a quantitative cancer assessment been
35 transparently and objectively described? Regarding liver cancer, a linear low-dose
36 extrapolation approach was utilized to derive the OSF. Please provide detailed comments on
37 whether this approach to dose-response assessment is scientifically sound, appropriately

1 conducted, and objectively and transparently described in the document. Please identify and
2 provide the rationale for any alternative approaches for the determination of the OSF and
3 discuss whether such approaches are preferred to EPA’s approach.

4 **Comment:** Four reviewers agreed with the approach for the dose-response assessment.
5 One reviewer commented that even if a nongenotoxic MOA were identified for
6 1,4-dioxane it may not be best evaluated by threshold modeling. One reviewer
7 commented the use of the female mouse data provided an appropriate health protective
8 and scientifically valid approach.

9 One reviewer commented that the basic adjustments and extrapolation method for
10 derivation of the OSF were clearly and adequately described, but disagreed with the
11 linear low-dose extrapolation. This reviewer suggested that the lack of certainty regarding
12 the MOA was not a sufficient cause to default to a linear extrapolation. Another reviewer
13 commented that the rationale for a linear low-dose extrapolation to derive the OSF was
14 not clear, but may be in accordance with current Agency policy in the absence of a
15 known MOA. This reviewer also commented that 1,4-dioxane appears to be non-
16 genotoxic and nonlinear models should be tested on the available data to determine if
17 they provide a better fit and are more appropriate.

18 One reviewer thought that the justification for a linear extrapolation was not
19 clearly provided and that a disconnect between the MOA summary and the choice of a
20 linear extrapolation model existed. In addition, this reviewer commented that the
21 pharmacokinetic information did not support the use of a linear extrapolation approach,
22 but rather use of animal PBPK models to extrapolate from high to low dose that would
23 result in a mixture of linear and nonlinear extrapolation models was warranted.

24 One reviewer suggested consideration of an integrated assessment of the cancer
25 and noncancer endpoints; however, if linear low-dose extrapolation remains the approach
26 of choice by the Agency, then the effect of choosing BMRs other than 10% was
27 recommended to at least be included in the uncertainty discussion. Using BMRs lower
28 than 10% may allow for the identification of a risk level for which the low-dose slope is
29 ‘best’ estimated.

30
31 **Response:** The EPA conducted a cancer MOA analysis evaluating all of the
32 available data for 1,4-dioxane. Application of the framework in the USEPA’s Guidelines
33 for Carcinogen Risk Assessment ([2005a](#)) demonstrates that the available evidence to
34 support any hypothesized MOA for 1,4-dioxane-induced tumors does not exist. In the
35 absence of a MOA, the USEPA’s Guidelines for Carcinogen Risk Assessment ([2005a](#))
36 indicate that a low dose linear extrapolation should be utilized for dose response analysis
37 (see Section 5.4). Some of the potential uncertainty associated with this conclusion was

1 characterized in Section 5.5. Note that there is no scientific basis to indicate that in the
2 absence of evidence for genotoxicity a nonlinear low-dose extrapolation should be used.
3 As concluded in the Toxicological Review, the application of a nonlinear low-dose
4 extrapolation approach was not supported.

5 With regards to the PBPK model available for 1,4-dioxane, it is clear that there
6 currently exist deficiencies within the model and as such, the model was not utilized for
7 interspecies extrapolation. Given the deficiencies and uncertainty in the 1,4-dioxane
8 model it also does not provide support for a MOA.

9 Lastly, in the absence of a MOA for 1,4-dioxane carcinogenicity it is not possible
10 to harmonize the cancer and noncancer effects to assess the risk of health effects due to
11 exposure. However, the choice of the BMDL₁₀, which was more than 15-fold lower than
12 the response at the lowest dose (66 mg/kg-day), was reconsidered in response to a public
13 comment. BMDs and BMDLs were calculated using a BMR of 30 and 50% extra risk
14 (BMD₃₀, BMDL₃₀, BMD₅₀, and BMDL₅₀). A BMR of 50% was used as it resulted in a
15 BMDL closest to the response level at the lowest dose tested in the bioassay.

A.2. PUBLIC COMMENTS

16 Comments on the *Toxicological Review of 1,4-Dioxane* submitted by the public are summarized
17 below in the following categories: Oral reference dose for 1,4-dioxane, carcinogenicity of
18 1,4-dioxane, PBPK modeling, and other comments.

A.2.1. Oral reference dose (RfD) for 1,4-dioxane

19 **Comment:** An UF for database deficiencies is not necessary because of considerable
20 evidence showing no reproductive or developmental effects from 1,4-dioxane exposure.

21
22 **Response:** Due to the lack of a multigenerational reproductive study for 1,4-dioxane an
23 UF of 3 was retained for database deficiencies. Without clear evidence showing a lack of
24 reproductive or developmental effects in a multigenerational reproductive study, there is
25 still uncertainty in this area.

A.2.2. Carcinogenicity of 1,4-dioxane

27 **Comment:** Using liver tumors as the basis for the oral CSF is more appropriate than
28 nasal tumors (1988 IRIS assessment of 1,4-dioxane); however, the use of mouse liver
29 tumor data is inappropriate because it is inconsistent with other liver models both
30 quantitatively and in the dose-response pattern. High mortality rates in the study are also
31 a limitation. Liver tumor data from rats should be used instead, which represents a better
32 animal model for 1,4-dioxane carcinogenicity assessment.

1
2 **Response:** Even though the dose-response is different for mice and rats, the female mice
3 were considered to be appropriate for the carcinogenicity assessment for several reasons.
4 The female mouse liver tumors from the Kano et al. (2009) report were found to be the
5 most sensitive species and endpoint. Section 4.2.1.2.6 was updated to include additional
6 information on mortality rates. The majority of the animals lived past 52 weeks (only 4
7 females died prior to 52 weeks, 2 in each the mid- and high-dose groups). The cause of
8 death in the female mice that died between 1 and 2 years was attributed to liver tumors.

9
10 **Comment:** The OSF was based on the most sensitive group, Crj:BDF1 mice; however
11 BDF1 mice have a high background rate of liver tumors. The incidence of liver tumors in
12 historical controls for this gender/species should be considered in the assessment.
13 Sensitivity of the test species/gender as well as other criteria should be considered in the
14 selection of the appropriate study, including internal and external validity as outlined in
15 Lewandowski and Rhomberg (2005). The female Crj:BDF1 mice had a low survival rate
16 that should be considered in the selection of the animal model for 1,4-dioxane
17 carcinogenicity.

18
19 **Response:** Katagiri et al. (1998) summarized the incidence of hepatocellular adenomas
20 and carcinomas in control male and female BDF1 mice from ten 2-year bioassays at the
21 JBRC. For female mice, out of 499 control mice, the incidence rates were 4.4% for
22 hepatocellular adenomas and 2.0% for hepatocellular carcinomas. Kano et al. (2009)
23 reported a 10% incidence rate for hepatocellular adenomas and a 0% incidence rate for
24 hepatocellular carcinomas in control female BDF1. These incidence rates are near the
25 historical control values and thus are appropriate for consideration in this assessment.
26 Additional text regarding these historical controls was added to the study description in
27 Section 4.2.1.2.6.

28
29 **Comment:** Low-dose linear extrapolation for the oral CSF is not appropriate nor justified
30 by the data. The weight of evidence supports a threshold (nonlinear) MOA when
31 metabolic pathway is saturated at high doses. Nonlinear extrapolations should be
32 evaluated and presented for 1,4-dioxane. Oral CSFs should be derived and presented
33 using both the $BW^{3/4}$ scaling as well as available PBPK models to extrapolate across
34 species.

35
36 **Response:** The absence of evidence for genotoxicity/mutagenicity does not indicate the
37 use of nonlinear low-dose extrapolation. For 1,4-dioxane, a MOA to explain the

1 induction of tumors does not exist so the nature of the low-dose region of the dose-
2 response is unknown. The oral CSF for 1,4-dioxane was derived using $BW^{3/4}$ scaling for
3 interspecies extrapolation. The PBPK and empirical models available for 1,4-dioxane
4 were evaluated and found not to be adequate for use in this assessment, described in
5 detail in Appendix B.

6
7 **Comment:** The POD for the BDF1 female mouse is 15-fold lower than the lowest dose
8 in the bioassay, thus the POD is far below the lower limit of the data and does not follow
9 the U.S. EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)).

10
11 **Response:** The comment is correct that the animal $BMDL_{10}$ was more than 15-fold
12 lower than the response at the lowest dose (66 mg/kg-day) in the bioassay. BMDs and
13 BMDLs were calculated using a BMR of 30 and 50% extra risk (BMD_{30} , $BMDL_{30}$,
14 BMD_{50} , and $BMDL_{50}$). A BMR of 50% was chosen as it resulted in a BMDL closest to
15 the response level at the lowest dose tested in the bioassay.

16
17 **Comment:** The geometric mean of the oral cancer slope factors (as done with B[a]P &
18 DDT) should have been used instead of relying on the female BDF1 mouse data, since a
19 MOA could not be determined for 1,4-dioxane.

20
21 **Response:** In accordance with the BMD technical guidance document ([U.S. EPA, 2000a](#)),
22 averaging tumor incidence is not a standard or default approach. Averaging the tumor
23 incidence response diminishes the effect seen in the sensitive species/gender.

24
25 **Comment:** EPA should critically reexamine the choice of JBRC ([1998](#)) as the principal
26 study since it has not been published or peer-reviewed. A transcript of e-mail
27 correspondence should be provided.

28
29 **Response:** JBRC ([1998](#)) was published as conference proceedings as Yamazaki et al.
30 ([1994](#)) and recently in the peer-reviewed literature as Kano et al. ([2009](#)). Additional study
31 information was also gathered from the authors ([Yamazaki, 2006](#)) and is available upon
32 request from the IRIS Hotline. The peer-reviewed and published data from Kano et al.
33 ([2009](#)) was incorporated into the final version of the *Toxicological Review of*
34 *1,4-Dioxane*.

35
36 **Comment:** The WOE does not support a cancer descriptor of *likely to be carcinogenic to*
37 *humans* determination, but rather *suggestive human carcinogen at the high dose levels*

1 *used in rodent studies* seems more appropriate for the following reasons: 1) lack of
2 conclusive human epidemiological data; 2) 1,4-dioxane is not mutagenic; and 3) evidence
3 at high doses it would act via cell proliferation MOA.
4

5 ***Response:*** A cancer classification of “*likely*,” based on evidence of liver carcinogenicity
6 in several two-year bioassays conducted in three strains of rats, two strains of mice, and
7 in guinea pigs was chosen. Also, mesotheliomas of the peritoneum, mammary, and nasal
8 tumors have been observed in rats. The Agency agrees that human epidemiological
9 studies are inconclusive. The evidence at any dose is insufficient to determine a MOA.
10

A.2.3. PBPK Modeling

11 ***Comment:*** EPA should have used and considered PBPK models to derive the oral
12 toxicity values (rat to human extrapolation) rather than relying on a default method. The
13 draft did not consider the Sweeney et al. (2008) model. The PBPK model should be used
14 for both noncancer and cancer dose extrapolation.
15

16 ***Response:*** The Agency evaluated the Sweeney et al. (2008) publication and this was
17 included in Appendix B of the document. Text was added to the main document in
18 Section 3.5.2.4 and 3.5.3 regarding the evaluation of Sweeney et al. (2008). This model
19 was determined not to be appropriate for interspecies extrapolation. Additionally, see
20 response to the external peer review panel comment B4.
21

22 ***Comment:*** EPA should use the modified inhalation inputs used in the Reitz et al. (1990)
23 model and the updated input parameters provided in Sweeney et al. (2008) and add a
24 compartment for the kidney
25

26 ***Response:*** See response to previous comment regarding evaluation of Sweeney et al.
27 (2008). Modification of the model to add a kidney compartment is not within the scope
28 of this assessment.

A.2.4. Other Comments

29 ***Comment:*** EPA should consider the Kasai et al. (2009; 2008) studies for inhalation and
30 MOA relevance.
31

32 ***Response:*** The 13 week and 2-year inhalation studies by Kasai et al. (2009; 2008) were
33 published late in the development stage of this assessment. The IRIS Program will

1 evaluate these recently published 1,4-dioxane inhalation data for the potential to derive
2 an RfC in a separate assessment.

3

4 **Comment:** 1,4-Dioxane is not intentionally added to cosmetics and personal care
5 products – correct sentence on page 4.

6

7 **Response:** This oversight was corrected in the document.

APPENDIX B. EVALUATION OF EXISTING PBPK MODELS FOR 1,4-DIOXANE

B.1. BACKGROUND

1 Several pharmacokinetic models have been developed to predict the absorption,
2 distribution, metabolism, and elimination of 1,4-dioxane in rats and humans. Single
3 compartment, empirical models for rats ([Young, et al., 1978a](#); [Young, et al., 1978b](#)) and humans
4 ([Young, et al., 1977](#)) were developed to predict blood levels of 1,4-dioxane and urine levels of
5 the primary metabolite, β -hydroxyethoxy acetic acid (HEAA). Physiologically based
6 pharmacokinetic (PBPK) models that describe the kinetics of 1,4-dioxane using biologically
7 realistic flow rates, tissue volumes and affinities, metabolic processes, and elimination behaviors,
8 were also developed ([Fisher, et al., 1997](#); [Leung & Paustenbach, 1990](#); [Reitz, et al., 1990](#)).

9 In developing updated toxicity values for 1,4-dioxane, the available PBPK models were
10 evaluated for their ability to predict observations made in experimental studies of rat and human
11 exposures to 1,4-dioxane. The model of Reitz et al. ([1990](#)) was identified for further
12 consideration to assist in the derivation of toxicity values. Issues related to the biological
13 plausibility of parameter values in the Reitz et al. ([1990](#)) human model were identified. The
14 model was able to predict the only available human inhalation data set ([Young, et al., 1977](#)) by
15 increasing (i.e., doubling) parameter values for human alveolar ventilation, cardiac output, and
16 the blood:air partition coefficient above the measured values. Furthermore, the measured value
17 for the slowly perfused tissue:air partition coefficient (i.e., muscle) was replaced with the
18 measured liver value to improve the fit. Analysis of the Young et al. ([1977](#)) human data
19 suggested that the apparent volume of distribution (V_d) for 1,4-dioxane was approximately 10-
20 fold higher in rats than humans, presumably due to species differences in tissue partitioning or
21 other process not represented in the model. Subsequent exercising of the model demonstrated
22 that selecting a human slowly perfused tissue:air partition coefficient much lower than the
23 measured rat value resulted in better agreement between model predictions of 1,4-dioxane in
24 blood and experimental observations. Based upon these observations, several model parameters
25 (e.g., metabolism/elimination parameters) were re-calibrated using biologically plausible values
26 for flow rates and tissue:air partition coefficients.

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#).

1 This appendix describes activities conducted in the evaluation of the empirical models
2 ([Young, et al., 1978a](#); [Young, et al., 1978b](#); [Young, et al., 1977](#)), and re-calibration and
3 exercising of the Reitz et al. ([1990](#)) PBPK model, and evaluation of the Sweeney et al. ([2008](#))
4 model to determine the potential utility of the PBPK models for 1,4-dioxane for interspecies and
5 route-to-route extrapolation.

B.2. SCOPE

6 The scope of this effort consisted of implementation of the Young et al. ([1978a](#); [1978b](#);
7 [1977](#)) empirical rat and human models using the acslXtreme simulation software, re-calibration
8 of the Reitz et al. ([1990](#)) human PBPK model, and evaluation of model parameters published by
9 Sweeney et al. ([2008](#)). Using the model descriptions and equations given in Young et al. ([1978a](#);
10 [1978b](#); [1977](#)), model code was developed for the empirical models and executed, simulating the
11 reported experimental conditions. The model output was then compared with the model output
12 reported in Young et al. ([1978a](#); [1978b](#); [1977](#)).

13 The PBPK model of Reitz et al. ([1990](#)) was re-calibrated using measured values for
14 cardiac and alveolar flow rates and tissue:air partition coefficients. The predictions of blood and
15 urine levels of 1,4-dioxane and HEAA, respectively, from the re-calibrated model were
16 compared with the empirical model predictions of the same dosimeters to determine whether the
17 re-calibrated PBPK model could perform similarly to the empirical model. As part of the PBPK
18 model evaluation, EPA performed a sensitivity analysis to identify the model parameters having
19 the greatest influence on the primary dosimeter of interest, the blood level of 1,4-dioxane.
20 Variability data for the experimental measurements of the tissue:air partition coefficients were
21 incorporated to determine a range of model outputs bounded by biologically plausible values for
22 these parameters. Model parameters from Sweeney et al. ([2008](#)) were also tested to evaluate the
23 ability of the PBPK model to predict human data following exposure to 1,4-dioxane.

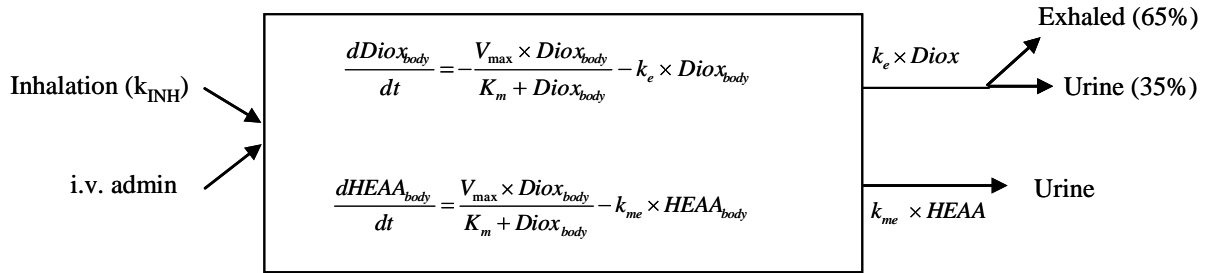
B.3. IMPLEMENTATION OF THE EMPIRICAL MODELS IN acslXtreme

24 The empirical models of Young et al. ([1978a](#); [1978b](#); [1977](#)) for 1,4-dioxane in rats and
25 humans were reproduced using acslXtreme, version 2.3 (Aegis Technologies, Huntsville, AL).
26 Model code files were developed using the equations described in the published papers.
27 Additional files containing experiment-specific information (i.e., BWs, exposure levels, and
28 duration) were also generated.

B.3.1. Model Descriptions

29 The empirical model of Young et al. ([1978a](#); [1978b](#)) for 1,4-dioxane in rats is shown in
30 Figure B-1. This is a single-compartment model that describes the absorption and metabolism
31 kinetics of 1,4-dioxane in blood and urine. No information is reported describing pulmonary

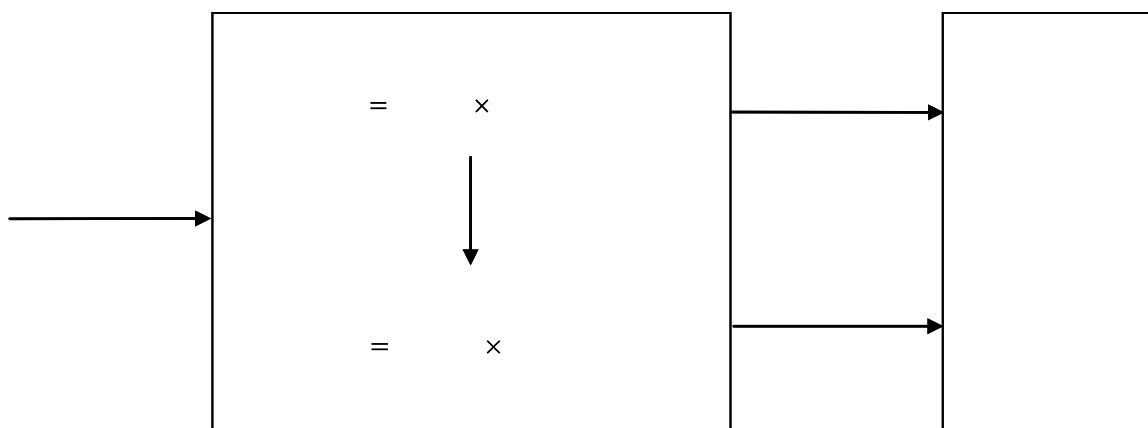
1 absorption or intravenous (i.v.) injection/infusion of 1,4-dioxane. The metabolism of
 2 1,4-dioxane and subsequent appearance of HEAA is described by Michaelis-Menten kinetics
 3 governed by a maximum rate (V_{max} , $\mu\text{g/mL-hour}$) and affinity constant (K_m , $\mu\text{g/mL}$). Both
 4 1,4-dioxane and HEAA are eliminated via the first-order elimination rate constants, k_e and k_{me} ,
 5 respectively (hour^{-1}) by which 35% of 1,4-dioxane and 100% of HEAA appear in the urine,
 6 while 65% of 1,4-dioxane is exhaled. Blood concentration of 1,4-dioxane is determined by
 7 dividing the instantaneous amount of 1,4-dioxane in blood by a V_d of 301 mL/kg BW.



Source: Used with permission from Taylor & Francis, Young et al. (1978a; 1978b).

Figure B-1. Schematic representation of empirical model for 1,4-dioxane in rats.

8 Figure B-2 illustrates the empirical model for 1,4-dioxane in humans as described in
 9 Young et al. (1977). Like the rat model, the human model predicts blood 1,4-dioxane and
 10 urinary 1,4-dioxane and HEAA levels using a single-compartment structure. However, the
 11 metabolism of 1,4-dioxane to HEAA in humans is modeled as a first-order process governed by
 12 a rate constant, K_M (hour^{-1}). Urinary deposition of 1,4-dioxane and HEAA is described using the
 13 first order rate constants, $k_{e(\text{diox})}$ and $k_{me(\text{HEAA})}$, respectively. Pulmonary absorption is described
 14 by a fixed rate of 76.1 mg/hour (k_{INH}). Blood concentrations of 1,4-dioxane and HEAA are
 15 calculated as instantaneous amount (mg) divided by $V_{d(\text{diox})}$ or $V_{d(\text{HEAA})}$, respectively (104 and
 16 480 mL/kg BW, respectively).



Source: Used with permission from Taylor & Francis, Young et al. (1977).

Figure B-2. Schematic representation of empirical model for 1,4-dioxane in humans.

B.3.2. Modifications to the Empirical Models

1 Several modifications were made to the empirical models. The need for the
 2 modifications arose in some cases from incomplete reporting of the Young et al. (1978a; 1978b;
 3 1977) studies and in other cases from the desire to add capabilities to the models to assist in the
 4 derivation of toxicity values.

5 For the rat model, no information was given by Young et al. (1978a; 1978b) regarding
 6 the parameterization of pulmonary absorption (or exhalation) or i.v. administration of
 7 1,4-dioxane. Therefore, additional parameters were added to simulate these processes in the
 8 simplest form. To replicate 1,4-dioxane inhalation, a first-order rate constant, k_{INH} (hour^{-1}), was
 9 introduced. k_{INH} was multiplied by the inhalation concentration and the respiratory minute
 10 volume of 0.238 L/minute (Young, et al., 1978a; 1978b). The value for k_{INH} was estimated by
 11 optimization against the blood time course data of Young et al. (1978a; 1978b). Intravenous
 12 (i.v.) administration was modeled as instantaneous appearance of the full dose at the start of the
 13 simulation. Rat urinary HEAA data were reported by Young et al. (1978a; 1978b) in units of
 14 concentration. To simulate urinary HEAA concentration, an estimate of urine volume was
 15 required. Since observed urinary volumes were not reported by Young et al. (1978a; 1978b), a
 16 standard rat urine production rate of 0.00145 L/hour was used.

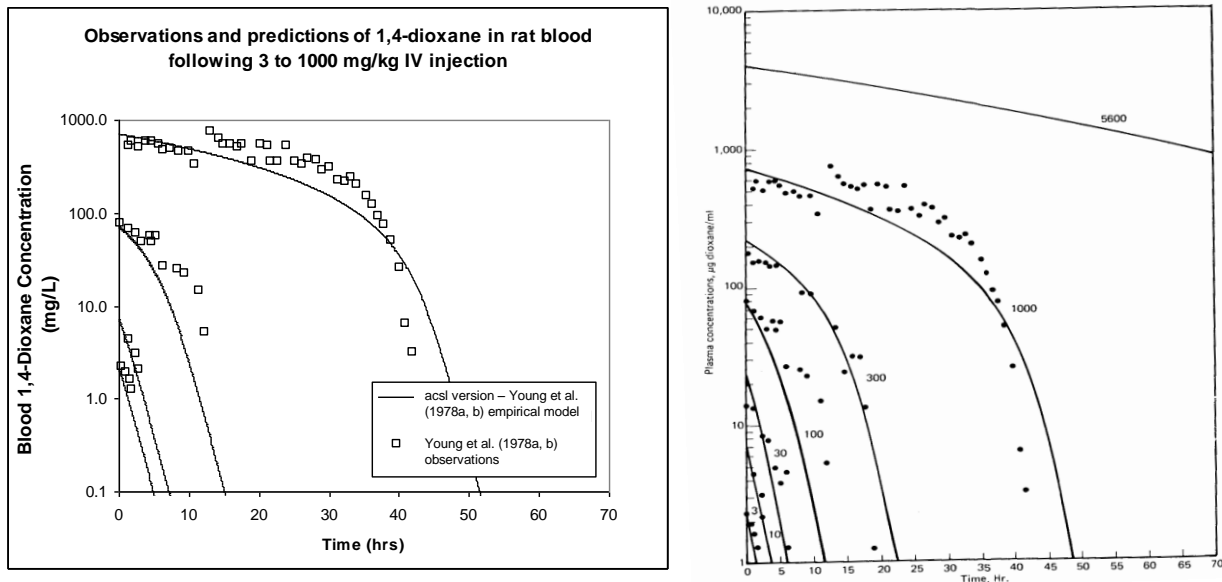
17 For humans, Young et al. (1977) used a fixed 1,4-dioxane inhalation uptake rate of
 18 76.1 mg/hour, which corresponded to observations during a 50 ppm exposure. In order to
 19 facilitate user-specified inhalation concentrations, pulmonary absorption was modeled. The
 20 modeling was performed identically to the rat model, but using a human minute volume of
 21 7 L/minute. Urinary HEAA data were reported by Young et al. (1977) as a cumulative amount
 22 (mg) of HEAA. Cumulative amount of HEAA in the urine is readily calculated from the rate of

1 transfer of HEAA from plasma to urine, so no modification was necessary to simulate this dose
2 metric for humans.

3 Neither empirical model of Young et al. ([1978a](#); [1978b](#); [1977](#)) described oral uptake of
4 1,4-dioxane. Adequate data to estimate oral absorption parameters are not available for either
5 rats or humans; therefore, neither empirical model was modified to include oral uptake.

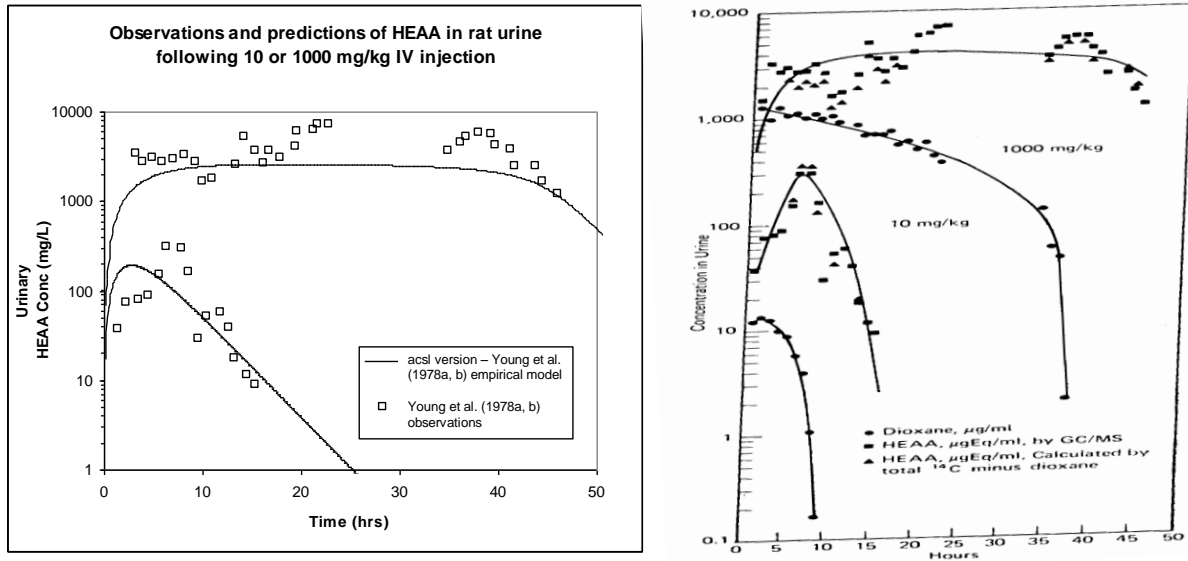
B.3.3. Results

6 The acslXtreme implementation of the Young et al. ([1978a](#); [1978b](#)) rat empirical model
7 simulates the 1,4-dioxane blood levels from the i.v. experiments identically to the model output
8 reported in the published paper (Figure B-3). However, the acslXtreme version predicts urinary
9 HEAA concentrations in rats that are approximately threefold lower and reach a maximum
10 sooner than the predicted levels reported in the paper (Figure B-4). These discrepancies may be
11 due, at least in part, to the reliance in the acslXtreme implementation on a constant, standard,
12 urine volume rather than experimental measurements, which may have been different from the
13 assumed value and may have varied over time. Unreported model parameters (e.g., lag times for
14 appearance of excreted HEAA in bladder urine) may also contribute to the discrepancy.



Source: Used with permission from Taylor & Francis, Young et al. ([1978a](#); [1978b](#)).

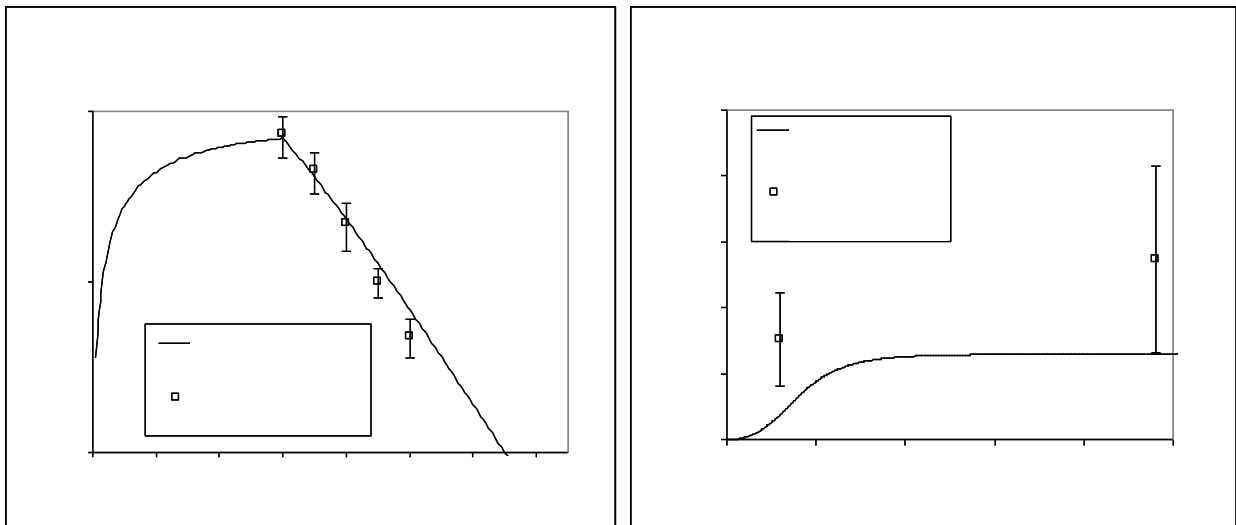
Figure B-3. Output of 1,4-dioxane blood level data from the acslXtreme implementation (left) and published (right) empirical rat model simulations of i.v. administration experiments.



Source: Used with permission from Taylor & Francis, Young et al. ([1978a](#); [1978b](#)).

Figure B-4. Output of HEAA urine level data from acslXtreme implementation (left) and published (right) empirical rat model simulations of i.v. administration experiments.

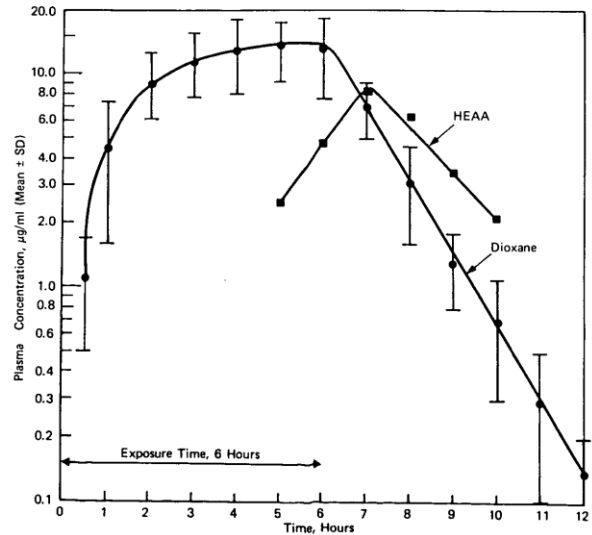
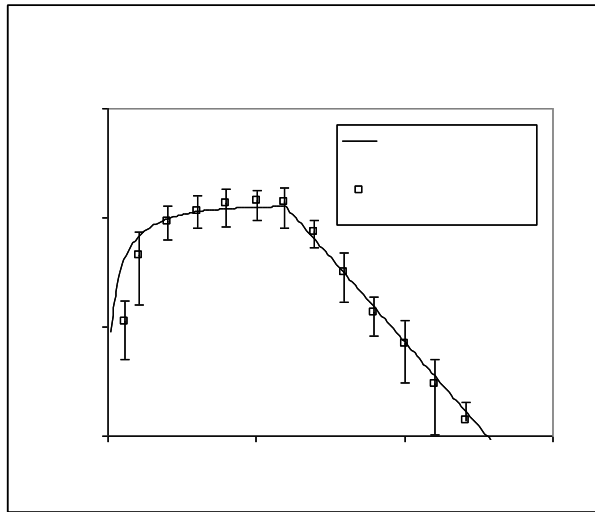
- 1 The Young et al. ([1978a](#); [1978b](#)) report did not provide model predictions for the 50-ppm
- 2 inhalation experiment. However, the acslXtreme implementation produces blood 1,4-dioxane
- 3 predictions that are quite similar to the reported observations (Figure B-5). As with the urine
- 4 data from the i.v. experiment, the acslXtreme-predicted urinary HEAA concentrations are
- 5 approximately threefold lower than the observations, presumably for the same reasons discussed
- 6 above for the i.v. predictions.



Source: Used with permission from Taylor & Francis, Young et al. ([1978a](#); [1978b](#)).

Figure B-5. acslXtreme predictions of blood 1,4-dioxane and urine HEAA levels from the empirical rat model simulations of a 6-hour, 50-ppm inhalation exposure.

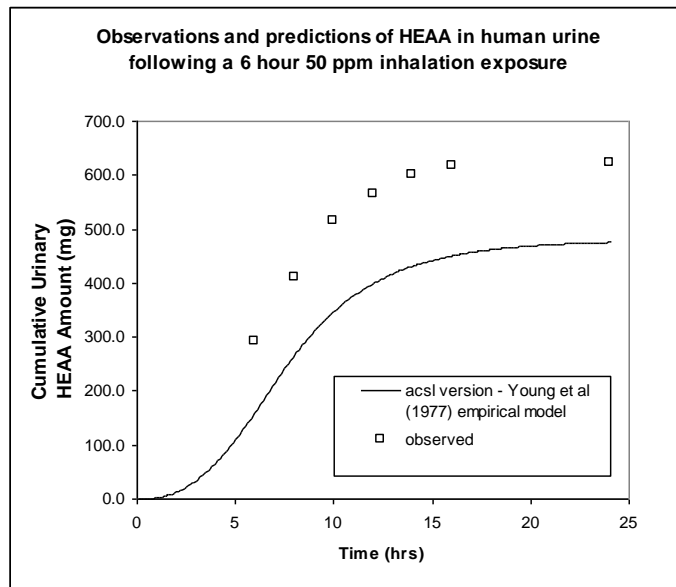
1 Inhalation data for a single exposure level (50 ppm) are available for humans. The
 2 acslXtreme predictions of the blood 1,4-dioxane observations are identical to the predictions
 3 reported in Young et al. ([1977](#)) (Figure B-6). Limited blood HEAA data were reported, and the
 4 specimen analysis was highly problematic (e.g., an analytical interference was sometimes present
 5 from which HEAA could not be separated). For this reason, Young et al. ([1977](#)) did not compare
 6 predictions of the blood HEAA data to observations in their manuscript.



Source: Used with permission from Taylor & Francis, Young et al. (1978a; 1978b).

Figure B-6. Output of 1,4-dioxane blood level data from the acslXtreme implementation (left) and published (right) empirical human model simulations of a 6-hour, 50-ppm inhalation exposure.

1 Data for cumulative urinary HEAA amounts are provided in Young et al. (1977), and no
 2 analytical problems for these data were reported. Nevertheless, model predictions for urinary
 3 HEAA were not presented in the manuscript. The acslXtreme prediction of the HEAA kinetics
 4 profile is similar to the observations, although predicted values are approximately 1.5- to 2-fold
 5 lower than the observed values (Figure B-7). Unlike urinary HEAA observations in the rat,
 6 human observations were reported as cumulative amount produced, negating the need for urine
 7 volume data. Therefore, discrepancies between model predictions and experimental observations
 8 for humans cannot be attributed to uncertainties in urine volumes in the subjects. Further
 9 evaluation of the Young et al. (1977) empirical model was conducted against subchronic
 10 inhalation exposure data reported by Kasai et al. (2008). In the experimental study, male and
 11 female F344 rats were exposed to 0, 100, 200, 400, 800, 1,600, 3,200, or 6,400ppm 1,4-dioxane
 12 in a 13-week inhalation study. The simulations of the Young et al. (1977) model did not provide
 13 an adequate fit (Figure B-8) for the measured plasma levels at each exposure level of 1,4-dioxane
 14 as reported by Kasai et al. (2008).
 15



Source: Used with permission from Taylor & Francis, Young et al. (1977).

Figure B-7. Observations and acslXtreme predictions of cumulative HEAA in human urine following a 6-hour, 50-ppm inhalation exposure.

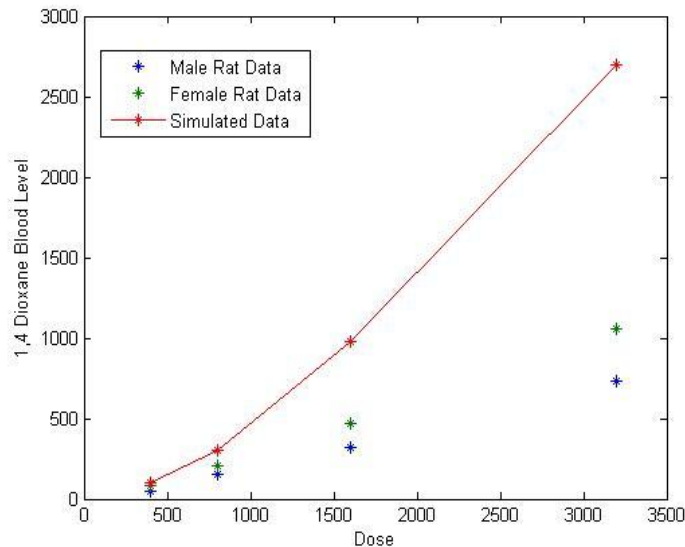


Figure B-8. EPA-modified Young et al. empirical model prediction (line) of plasma 1,4-dioxane levels in rats following exposure to 1,4-dioxane for 13 weeks compared to data from Kasai et al. (2008).

B.3.4. Conclusions for Empirical Model Implementation

- 1 The empirical models described by Young et al. (1978a; 1978b; 1977) for rats and
- 2 humans were implemented using acslXtreme. The models were modified to allow for user-
- 3 defined inhalation levels by addition of a first-order rate constant for pulmonary uptake of

1 1,4-dioxane, fitted to the inhalation data. No modifications were made for oral absorption as
2 adequate data are not available for parameter estimation. The acslXtreme predictions of
3 1,4-dioxane in the blood are identical to the published predictions for simulations of 6-hour, 50-
4 ppm inhalation exposures in rats and humans and 3 to 1,000 mg/kg i.v. doses in rats (Figures B-
5 3, B-5, and B-6). However, the acslXtreme version predicts lower urinary HEAA concentrations
6 in rats appearing earlier than either the Young et al. (1978a; 1978b) model predictions or the
7 experimental observations. The lower predicted urinary HEAA levels in the acslXtreme
8 implementation for rats is likely due to use of default values for urine volume in the absence of
9 measured volumes. The reason for differences in time-to-peak levels is unknown, but may be
10 the result of an unreported adjustment by Young et al. (1978a; 1978b) in model parameter
11 values. Additionally, the modified Young et al. (1978a; 1978b; 1977) model failed to provide
12 adequate fit to blood data reported following subchronic inhalation of 1,4-dioxane in rats (Kasai,
13 et al., 2008). For humans, Young et al. (1977) did not report model predictions of urinary HEAA
14 levels. The urinary HEAA levels predicted by acslXtreme were low relative to the observations.
15 However, unlike the situation in rats, these data are not dependent on unreported urine volumes
16 (observations were reported as cumulative HEAA amount rather than HEAA concentration), but
17 reflect the model parameter values reported by Young et al. (1977). Presently, there is no
18 explanation for the lack of fit of the reported urinary HEAA elimination rate constant to the
19 observations.

B.4. INITIAL RE-CALIBRATION OF THE PBPK MODEL

20 Concern regarding adjustments made to some of the parameter values in Reitz et al.
21 (1990) prompted a re-calibration of the Reitz et al. (1990) human PBPK model using more
22 biologically plausible values for all measured parameter values. Reitz et al. (1990) doubled the
23 measured physiological flows and blood:air partition coefficient and substituted the slowly-
24 perfused tissue:air partition coefficient with the liver:air value in order to attain an adequate fit to
25 the observations. This approach increases uncertainty in these parameter values, and in the
26 utilization of the model for cross-species dose extrapolation. Therefore, the model was re-
27 calibrated using parameter values that are more biologically plausible to determine whether an
28 adequate fit of the model to the available data can be attained.

B.4.1. Sources of Values for Flow Rates

29 The cardiac output of 30 L/hour/kg^{0.74} (Table B-1) reported by Reitz et al. (Reitz, et al.,
30 1990) is approximately double the mean resting value of 14 L/hour/kg^{0.74} reported in the widely
31 accepted compendium of Brown et al. (1997). Resting cardiac output was reported to be 5.2
32 L/minute (or 14 L/hour/kg^{0.74}), while strenuous exercise resulted in a flow of 9.9 L/minute (or 26
33 L/hour/kg^{0.74}) (Brown, et al., 1997). Brown et al. (1997) also cite the ICRP (1975) as having a

1 mean respiratory minute volume of 7.5 L/minute, which results in an alveolar ventilation rate of
 2 5 L/minute (assuming 33% lung dead space), or 13 L/minute/kg^{0.74}. Again, this is roughly half
 3 the value of 30 L/hour/kg^{0.74} employed for this parameter by Reitz et al. (1990). Young et al.
 4 (1977) reported that the human subjects exposed to 50 ppm for 6 hours were resting inside a
 5 walk-in exposure chamber. Thus, use of cardiac output and alveolar ventilation rates of 30
 6 L/hour/kg^{0.74} is not consistent with the experimental conditions being simulated.

Table B-1. Human PBPK model parameter values for 1,4-dioxane

Parameter	Reitz et al. (1990)	Leung and Paustenbach (1990)	Sweeney et al. (2008)	EPA ^c
Physiological Flows				
Cardiac output (QCC) ^a	30	--	--	17.0
Alveolar ventilation (QPC) ^a	30	--	--	17.7
Partition Coefficients (PCs)				
Blood:air (PB)	3,650	1,825 ± 94	1,666 ± 287	1,850
Fat:air (PFA)	851	851 ± 118	--	851
Liver:air (PLA)	1,557	1,557 ± 114	1,862 ± 739 ^b	1,557
Rapidly perfused tissue:air (PRA)	1,557	--	--	1,557
Slowly perfused tissue:air (PSA)	1,557	997 ± 254	1,348 ± 290 ^b	166
Metabolic Constants				
Maximum rate for 1,4-dioxane metabolism (V _{maxC}) ^d	6.35	--	--	5.49
Metabolic affinity constant (K _m) ^e	3.00	--	--	9.8
HEAA urinary elimination rate constant (k _{me}) ^f	0.56	--	--	0.44

^aL/hour/kg BW^{0.74}

^bMeasurement for rat tissue

^cBiologically plausible values utilized by EPA in this assessment

^dmg/hour/kg BW^{0.75}

^emg/L

^fhour⁻¹

7 Examination of the experimental data of Young et al. (1977) yields an estimated alveolar
 8 ventilation to be 7 L/minute (or 16 L/hour/kg^{0.74}) for volunteers having a mean BW of 84 kg.
 9 This rate is based on the Young et al. (1977) estimate of 76.1 mg/hour for 1,4-dioxane uptake.
 10 Based on these findings, the cardiac output and alveolar ventilation rates of 17.0 and 17.7
 11 L/hour/kg^{0.74} were biologically plausible for the experimental subjects. These rate estimates are
 12 based on calculations made using empirical data and are consistent with standard human values
 13 and the experimental conditions (i.e., subject exertion level) reported by Young et al. (1977).
 14 Therefore, these flow values were chosen for the model re-calibration.

B.4.2. Sources of Values for Partition Coefficients

1 Two data sources are available for the tissue:air equilibrium partition coefficients for
2 1,4-dioxane: Leung and Paustenbach (1990) and Sweeney et al. (2008). Both investigators
3 report mean values and standard deviations for human blood:air, rat liver:air, and rat muscle:air
4 (e.g., slowly perfused tissue:air), while Leung and Paustenbach et al. (1990) also reported values
5 for rat fat:air (Table B-1).

B.4.3. Calibration Method

6 The PBPK model was twice re-calibrated using the physiological flow values suggested
7 values (current EPA assessment, see Table B-1) and the partition coefficients of Leung and
8 Paustenbach (1990) and Sweeney et al. (2008) separately. For each calibration, the metabolic
9 parameters $V_{\max C}$ and K_m , were simultaneously fit (using the parameter estimation tool provided
10 in the acsIXtreme software) to the output of 1,4-dioxane blood concentrations generated by the
11 acsIXtreme implementation of the Young et al. (1977) empirical human model for a 6 hour,
12 50 ppm inhalation exposure. Subsequently, the HEAA urinary elimination rate constant, k_{me} ,
13 was fitted to the urine HEAA predictions from the empirical model. The empirical model
14 predictions, rather than experimental observations, were used to provide a more robust data set
15 for model fitting, as the empirical model simulation provided 240 data points (one prediction
16 every 0.1 hour) compared with hourly experimental observations, and to avoid introducing error
17 by calibrating the model to data digitally captured from Young et al. (1977).

B.4.4. Results

18 Results of the model re-calibration are provided in Table B-2. The re-calibrated values
19 for $V_{\max C}$ and k_{me} associated with the Leung and Paustenbach (1990) or Sweeney et al. (2008)
20 tissue:air partition coefficients are very similar. However, the fitted value for K_m using the
21 Sweeney et al. (2008) partition coefficients is far lower (0.0001 mg/L) than that resulting from
22 use of the Leung and Paustenbach (1990) partition coefficients (2.5 mg/L). This appears to be
23 due to the higher slowly perfused tissue:air partition coefficient determined by Sweeney et al.
24 (2008) (1,348 vs. 997), resulting in a higher apparent V_d than if the Leung and Paustenbach
25 (1990) value is used. Thus, the optimization algorithm selects a low K_m , artificially saturating
26 metabolism in an effort to drive predicted blood 1,4-dioxane levels closer to the empirical model
27 output. Saturation of metabolism during a 50 ppm inhalation exposure is inconsistent with the
28 observed kinetics.

Table B-2. PBPK metabolic and elimination parameter values resulting from re-calibration of the human model using alternative values for physiological flow rates^a and tissue:air partition coefficients

Source of Partition Coefficients	Leung and Paustenbach (1990)	Sweeney et al. (2008)
Maximum rate for 1,4-dioxane metabolism (V_{maxC}) ^b	16.9	20.36
Metabolic affinity constant (K_m) ^c	2.5	0.0001
HEAA urinary elimination rate constant (k_{me}) ^d	0.18	0.17

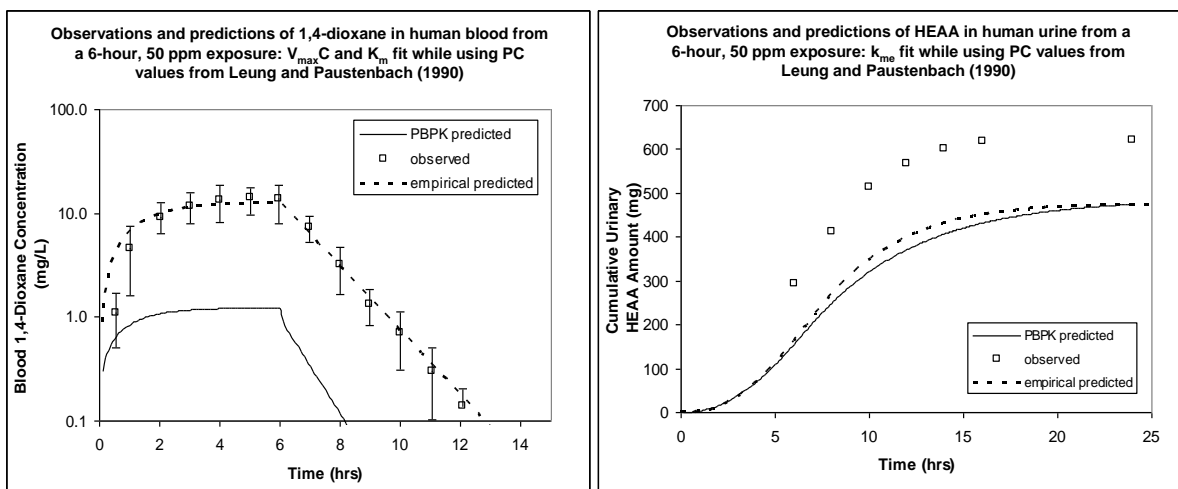
^aCardiac output = 17.0 L/hour/kg BW^{0.74}, alveolar ventilation = 17.7 L/hour/kg BW^{0.74}

^bmg/hour/kg BW^{0.75}

^cmg/L

^dhour⁻¹

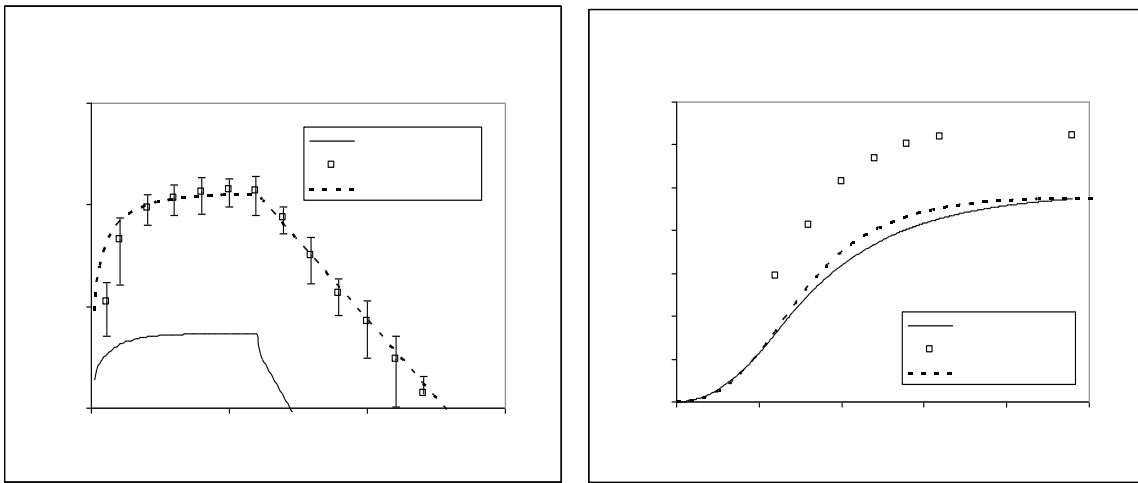
1 Plots of predicted and experimentally observed blood 1,4-dioxane and urinary HEAA
 2 levels are shown in Figure B-9. Neither re-calibration resulted in an adequate fit to the blood
 3 1,4-dioxane data from the empirical model output or the experimental observations. Re-
 4 calibration using either the Leung and Paustenbach (1990) or Sweeney et al. (2008) partition
 5 coefficients resulted in blood 1,4-dioxane predictions that were at least 10-fold lower than
 6 empirical model predictions or observations.



Source: Used with permission from Elsevier, Ltd., Leung and Paustenbach (1990).

Figure B-9. Predicted and observed blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) following re-calibration of the human PBPK model with tissue:air partition coefficient values.

7 The refitted values for k_{me} resulted in HEAA levels in urine that were very similar to the
 8 empirical model output (compare Figures B-7, B-9, and B-10), which was not surprising, given
 9 the fitting of a single parameter to the data.



Source: Used with permission of Oxford Journals, Sweeney et al. (2008).

Figure B-10. Predicted and observed blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) following re-calibration of the human PBPK model with tissue:air partition coefficient values.

1 Outputs of the blood 1,4-dioxane and urinary HEAA levels using the suggested (Table B-
 2 1) parameters are shown in Figure B-11. These outputs rely on a very low value for the slowly
 3 perfused tissue:air partition coefficient (166) that is six- to eightfold lower than the measured
 4 values reported in Leung and Paustenbach (1990) and Sweeney et al. (2008), and 10-fold lower
 5 than the value used by Reitz et al. (1990). While the predicted maximum blood 1,4-dioxane
 6 levels are much closer to the observations, the elimination kinetics are markedly different,
 7 producing higher predicted elimination rates compared to observations during the post-exposure
 8 phase of the experiment.

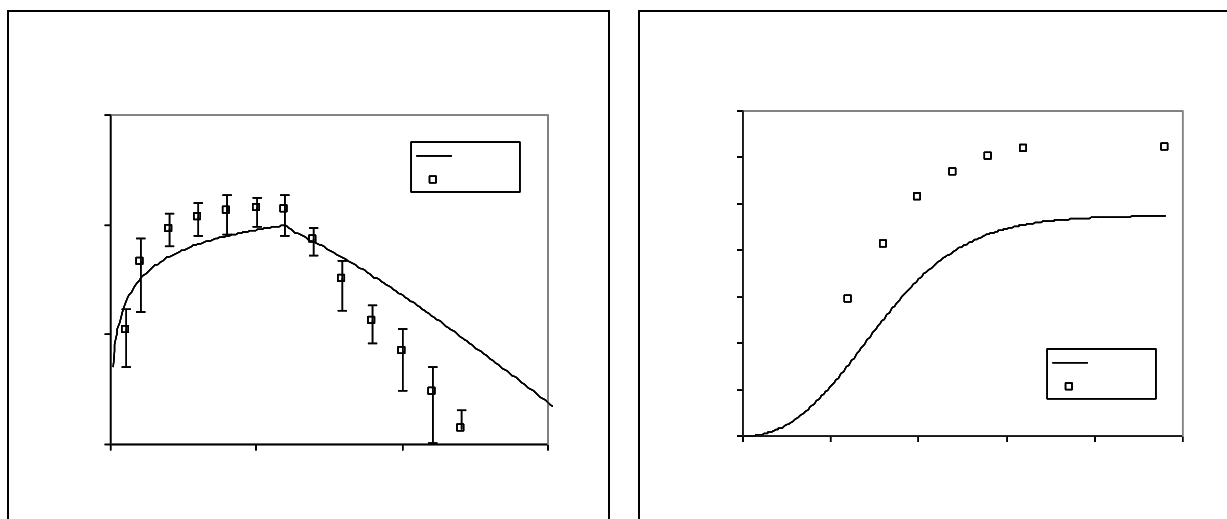


Figure B-11. Predicted and observed blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) using EPA estimated biologically plausible parameters (Table B-1).

B.4.5. Conclusions for PBPK Model Implementation

1 Re-calibration of the human PBPK model was performed using experiment-specific
 2 values for cardiac output and alveolar ventilation ([Young, et al., 1977](#)) and measured mean
 3 tissue:air 1,4-dioxane partition coefficients reported by Leung and Paustenbach ([1990](#)) or
 4 Sweeney et al. ([2008](#)). The resulting predictions of 1,4-dioxane in blood following a 6-hour, 50-
 5 ppm inhalation exposure were 10-fold (or more) lower than either the observations or the
 6 empirical model predictions, while the predictions of urinary HEAA by the PBPK and empirical
 7 models were similar to each other, but lower than observed values (Figures B-9 and B-10).
 8 Output from the model using biologically plausible parameter values (Table B-1), Figure B-11
 9 shows that application of a value for the slowly perfused tissue:air partition coefficient, which is
 10 10-fold lower than the measured value reported by Leung and Paustenbach ([1990](#)), results in
 11 closer agreement of the predictions to observations during the exposure phase, but not during the
 12 elimination phase. Thus, model re-calibration using experiment-specific flow rates and mean
 13 measured partition coefficients does not result in an adequate fit of the PBPK model to the
 14 available data.

15 The Sweeney et al. (2008) PBPK model consisted of compartments for fat, liver, slowly
 16 perfused, and other well perfused tissues. Lung and stomach compartments were used to
 17 describe the route of exposure, and an overall volume of distribution compartment was used for
 18 calculation of urinary excretion levels of 1,4-dioxane and its metabolite, HEAA. Metabolic
 19 constants (VmaxC and Km) for the rat PBPK model were derived by optimization data from an
 20 i.v. exposure of 1,000 mg/kg data (Young, et al., 1978a; Young, et al., 1978b) for induced
 21 metabolism. For uninduced metabolism data generated by i.v. exposures to 3, 10, 30, and 100

1 mg/kg were used (Young, et al., 1978a; Young, et al., 1978b). Data generated from the 300
2 mg/kg i.v. exposure was not used to estimate VmaxC and Km. The best fitting values for VmaxC
3 to estimate the blood data from the Young et al. (1978a; 1978b) study using the Sweeney et al.
4 (2008) model resulted in VmaxC values of 12.7, 10.8, 7.4 mg/kg-hr; suggesting a gradual dose
5 dependent increase in metabolic rate with dose. These estimates were for a range of doses
6 between 3 and 1,000 mg/kg i.v. dose. Although the Sweeney et al. (2008) model utilized two
7 values for VmaxC (induced and uninduced), the PBPK model does not include dose-dependent
8 function description of the change of Vmax for i.v. doses between 100 and 1,000 mg/kg. PBPK
9 model outputs were compared with other data not used in fitting model parameters by visual
10 inspection. The model predictions gave adequate match to the 1,4-dioxane exhalation data after a
11 1,000 mg/kg i.v. dose. 1,4-Dioxane exhalation was overpredicted by a factor of about 3 for the
12 10 mg/kg i.v. dose. Similarly, the simulations of exhaled 1,4-dioxane after oral dosing were
13 adequate at 1,000 mg/kg, and 100 mg/kg (within 50%), but poor at 10 mg/kg (model
14 overpredicted by a factor of five). The fit of the model to the human data (Young, et al., 1977)
15 was also problematic (Sweeney, et al., 2008). Using physiological parameters of Brown et al.
16 (1997) and measured partitioning parameters (Leung & Paustenbach, 1990; Sweeney, et al.,
17 2008) with no metabolism, measured blood 1,4-dioxane concentrations reported by Young et al.
18 (1977) could not be achieved unless the estimated exposure concentration was increased from 53
19 to 100 ppm. Inclusion of any metabolism necessarily decreased predicted blood concentrations.
20 If estimated metabolism rates were used with the reported exposure concentration, urinary
21 metabolite excretion was underpredicted (Sweeney, et al., 2008). Thus, the models were
22 inadequate to use for rat to human extrapolation.

B.4.6. SENSITIVITY ANALYSIS

23 A sensitivity analysis of the Reitz et al. (1990) model was performed to determine which
24 PBPK model parameters exert the greatest influence on the outcome of dosimeters of interest—
25 in this case, the concentration of 1,4-dioxane in blood. Knowledge of model sensitivity is useful
26 for guiding the choice of parameter values to minimize model uncertainty.

B.4.7. Method

27 A univariate sensitivity analysis was performed on all of the model parameters for two
28 endpoints: blood 1,4-dioxane concentrations after 1 and 4 hours of exposure. These time points
29 were chosen to assess sensitivity during periods of rapid uptake (1 hour) and as the model
30 approached steady state (4 hours) for blood 1,4-dioxane. Model parameters were perturbed 1%
31 above and below nominal values and sensitivity coefficients were calculated as follows:

$$f'(x) \approx \frac{f(x + \Delta x) - f(x)}{\Delta x} \cdot \frac{x}{f(x)}$$

1 where x is the model parameter, $f(x)$ is the output variable, Δx is the perturbation of the
2 parameter from the nominal value, and $f'(x)$ is the sensitivity coefficient. The sensitivity
3 coefficients were scaled to the nominal value of x and $f(x)$ to eliminate the potential effect of
4 units of expression. As a result, the sensitivity coefficient is a measure of the proportional
5 change in the blood 1,4-dioxane concentration produced by a proportional change in the
6 parameter value, with a maximum value of 1.

B.4.8. Results

7 The sensitivity coefficients for the seven most influential model parameters at 1 and
8 4 hours of exposure are shown in Figure B-12. The three parameters with the highest sensitivity
9 coefficients in descending order are alveolar ventilation (QPC) (1.0), the blood:air partition
10 coefficient (PB) (0.65), and the slowly perfused tissue:air partition coefficient (PSA) (0.51). Not
11 surprisingly, these were the parameters that were doubled or given surrogate values in the Reitz
12 et al. (1990) model in order to achieve an adequate fit to the data. Because of the large influence
13 of these parameters on the model, it is important to assign values to these parameters in which
14 high confidence is placed, in order to reduce model uncertainty.

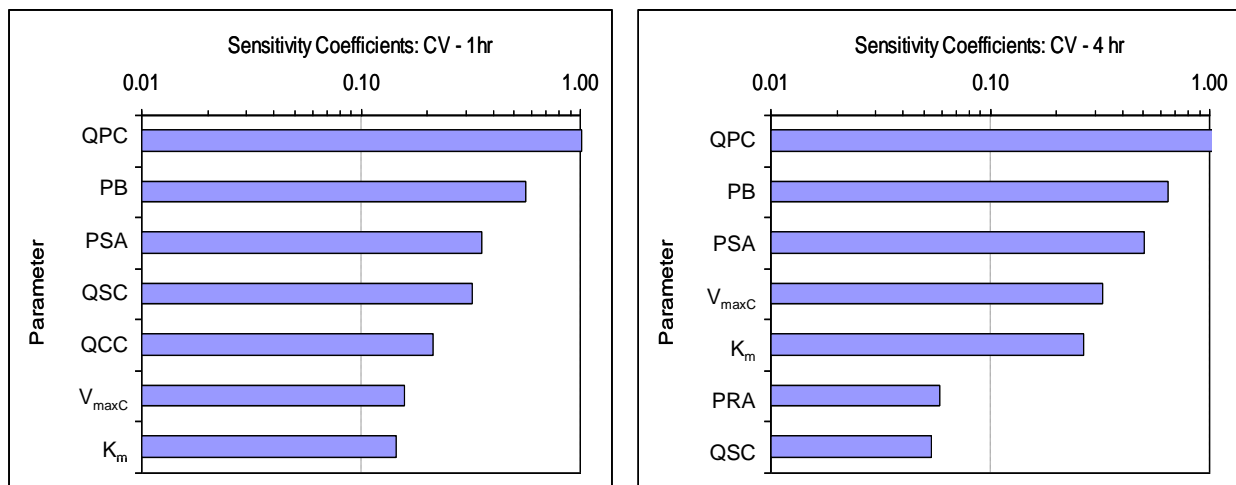


Figure B-12. The highest seven sensitivity coefficients (and associated parameters) for blood 1,4-dioxane concentrations (CV) at 1 (left) and 4 (right) hours of a 50-ppm inhalation exposure.

B.5. PBPK MODEL EXERCISES USING BIOLOGICALLY PLAUSIBLE PARAMETER BOUNDARIES

15 The PBPK model includes numerous physiological parameters whose values are typically
16 taken from experimental observations. In particular, values for the flow rates (cardiac output and
17 alveolar ventilation) and tissue:air partition coefficients (i.e., mean and standard deviations) are
18 available from multiple sources as means and variances. The PBPK model was exercised by

1 varying the partition coefficients over the range of biological plausibility (parameter mean \pm
2 2 standard deviations), re-calibrating the metabolism and elimination parameters, and exploring
3 the resulting range of blood 1,4-dioxane concentration time course predictions. Cardiac output
4 and alveolar ventilation were not varied because the experiment-specific values used did not
5 include any measure of inter-individual variation.

B.5.1. Observations Regarding the Volume of Distribution

6 Young et al. ([1978a](#); [1978b](#)) used experimental observations to estimate a V_d for
7 1,4-dioxane in rats of 301 mL, or 1,204 mL/kg BW. For humans, the V_d was estimated to be
8 104 mL/kg BW ([Young, et al., 1977](#)). It is possible that a very large volume of the slowly
9 perfused tissues in the body of rats and humans may be a significant contributor to the estimated
10 10-fold difference in distribution volumes for the two species. This raises doubt regarding the
11 appropriateness of using the measured rat slowly perfused tissue:air partition coefficient as a
12 surrogate values for humans in the PBPK model.

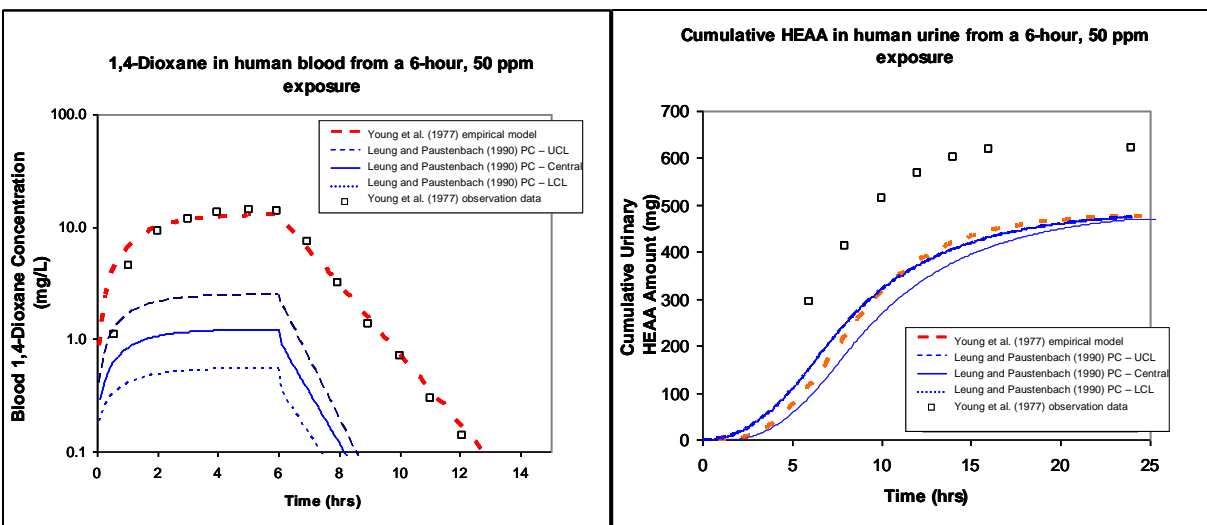
B.5.2. Defining Boundaries for Parameter Values

13 Given the possible 10-fold species differences in the apparent V_d for 1,4-dioxane in rats
14 and humans, boundary values for the partition coefficients were chosen to exercise the PBPK
15 model across its performance range to either minimize or maximize the simulated V_d . This was
16 accomplished by defining biologically plausible values for the partition coefficients as the
17 mean \pm 2 standard deviations of the measured values. Thus, to minimize the simulated V_d for
18 1,4-dioxane, the selected blood:air partition coefficient was chosen to be the mean + 2 standard
19 deviations, while all of the other tissue:air partition coefficients were chosen to be the mean – 2
20 standard deviations. This created conditions that would sequester 1,4-dioxane in the blood, away
21 from other tissues. To maximize the simulated 1,4-dioxane V_d , the opposite selections were
22 made: blood and other tissue:air partition coefficients were chosen as the mean – 2 standard
23 deviations and mean + 2 standard deviations, respectively. Subsequently, V_{maxC} , K_m , and k_{me}
24 were optimized to the empirical model output data as described in Section B.4.3. This procedure
25 was performed for both the Leung and Paustenbach ([1990](#)) and Sweeney et al. ([2008](#)) partition
26 coefficients (Table B-1). The two predicted time courses resulting from the re-calibrated model
27 with partition coefficients chosen to minimize or maximize the 1,4-dioxane V_d represent the
28 range of model performance as bounded by biologically plausible parameter values.

B.5.3. Results

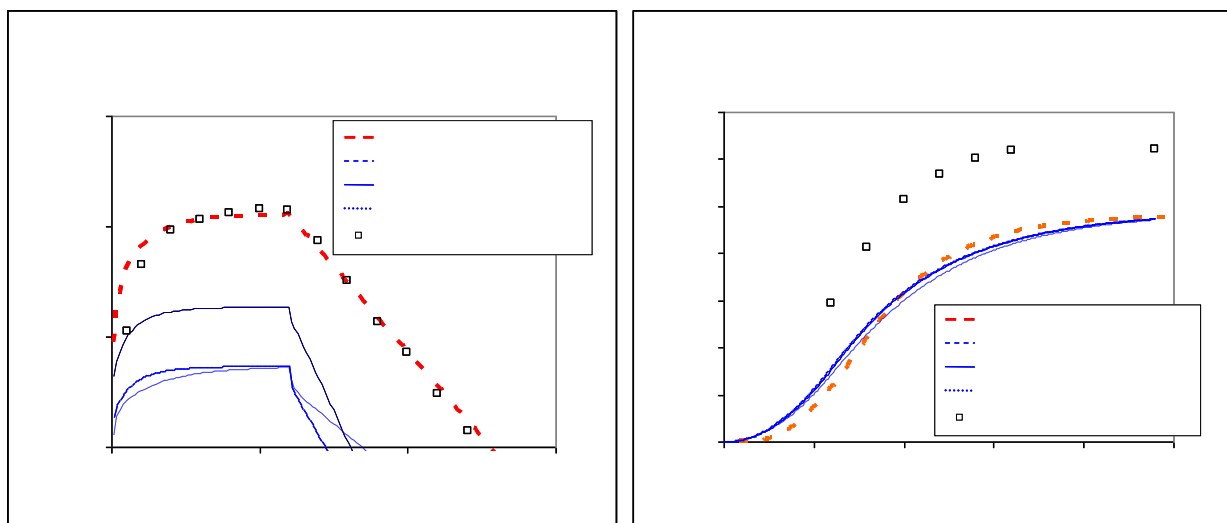
29 The predicted time courses for a 6-hour, 50-ppm inhalation exposure for the re-calibrated
30 human PBPK model with mean (central tendency) and \pm 2 standard deviations from the mean
31 values for partition coefficients are shown in Figure B-13 for the Leung and Paustenbach ([1990](#))
32 values and Figure B-14 for the Sweeney et al. ([2008](#)) values. The resulting fitted values for

1 V_{maxC} , K_m , and k_{me} , are given in Table B-3. By bounding the tissue:air partition coefficients with
2 upper and lower limits on biologically plausible values from Leung and Paustenbach (1990) or
3 Sweeney et al. (2008), the model predictions are still at least six- to sevenfold lower than either
4 the empirical model output or the experimental observations. The range of possible urinary
5 HEAA predictions brackets the prediction of the empirical model, but this agreement is not
6 surprising, as the cumulative rate of excretion depends only on the rate of metabolism of
7 1,4-dioxane, and not on the apparent V_d for 1,4-dioxane. These data show that the PBPK model
8 cannot adequately reproduce the predictions of blood 1,4-dioxane concentrations of the Young
9 et al. (1977) human empirical model or the experimental observations when constrained by
10 biologically plausible values for physiological flow rates and tissue:air partition coefficients.



Source: Used with permission of Elsevier, Ltd., Leung and Paustenbach (1990)

Figure B-13. Comparisons of the range of PBPK model predictions from upper and lower boundaries on partition coefficients with empirical model predictions and experimental observations for blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) from a 6-hour, 50-ppm inhalation exposure.



Source: Used with permission of Oxford Journals, Sweeney et al. (2008); Used with permission of Taylor & Francis, Young et al. (1977).

Figure B-14. Comparisons of the range of PBPK model predictions from upper and lower boundaries on partition coefficients with empirical model predictions and experimental observations for blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) from a 6-hour, 50-ppm inhalation exposure.

Table B-3. PBPK metabolic and elimination parameter values resulting from recalibration of the human model using biologically plausible values for physiological flow rates^a and selected upper and lower boundary values for tissue:air partition coefficients

Source of partition coefficients	Leung and Paustenbach (1990)		Sweeney et al. (2008)	
	For maximal V_d	For minimal V_d	For maximal V_d	For minimal V_d
Maximum rate for 1,4-dioxane metabolism (V_{maxc}) ^b	14.95	18.24	17.37	21.75
Metabolic dissociation constant (K_m) ^c	5.97	0.0001	4.88	0.0001
HEAA urinary elimination rate constant (k_{me}) ^d	0.18	0.17	0.26	0.19

^aCardiac output = 17.0 L/hour/kg BW^{0.74}, alveolar ventilation = 17.7 L/hour/kg BW^{0.74}

^bmg/hour/kg BW^{0.75}

^cmg/L

^dhour⁻¹

B.5.4. Alternative Model Parameterization

- 1 Since the PBPK model does not predict the experimental observations of Young et al.
- 2 (1977) when parameterized by biologically plausible values, an exercise was performed to
- 3 explore alternative parameters and values capable of producing an adequate fit of the data. Since

1 the metabolism of 1,4-dioxane appears to be linear in humans for a 50-ppm exposure ([Young, et](#)
2 [al., 1977](#)), the parameters V_{maxC} and K_m were replaced by a zero-order, non-saturable metabolism
3 rate constant, k_{LC} . This rate constant was fitted to the experimental blood 1,4-dioxane data using
4 partition coefficient values of Sweeney et al. ([2008](#)) to minimize the V_d (i.e., maximize the blood
5 1,4-dioxane levels). The resulting model predictions are shown in Figure B-15. As before, the
6 maximum blood 1,4-dioxane levels were approximately sevenfold lower than the observed
7 values.

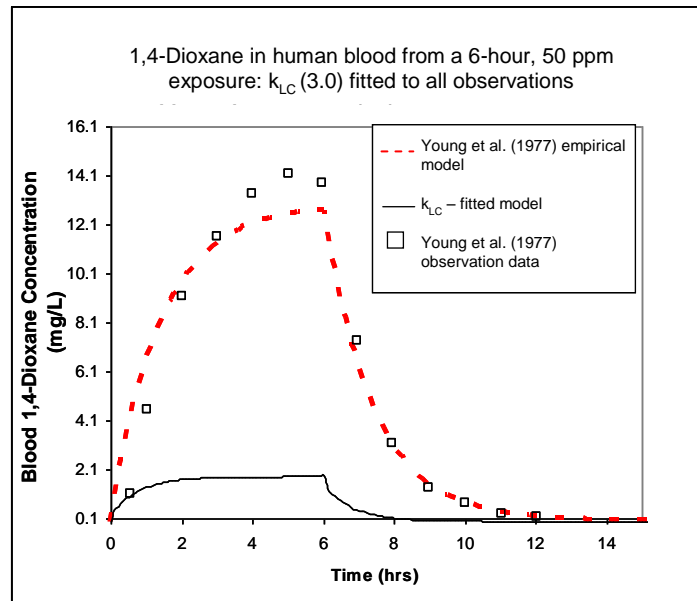


Figure B-15. Predictions of blood 1,4-dioxane concentration following calibration of a zero-order metabolism rate constant, k_{LC} , to the experimental data.

8 A re-calibration was performed using only the data from the exposure phase of the
9 experiment, such that the elimination data did not influence the initial metabolism and tissue
10 distribution. The model predictions from this exercise are shown in Figure B-16. These
11 predictions are more similar to the observations made during the exposure phase of the
12 experiment; however, this is achieved at greatly reduced elimination rate (compare Figures B-11
13 and B-16).

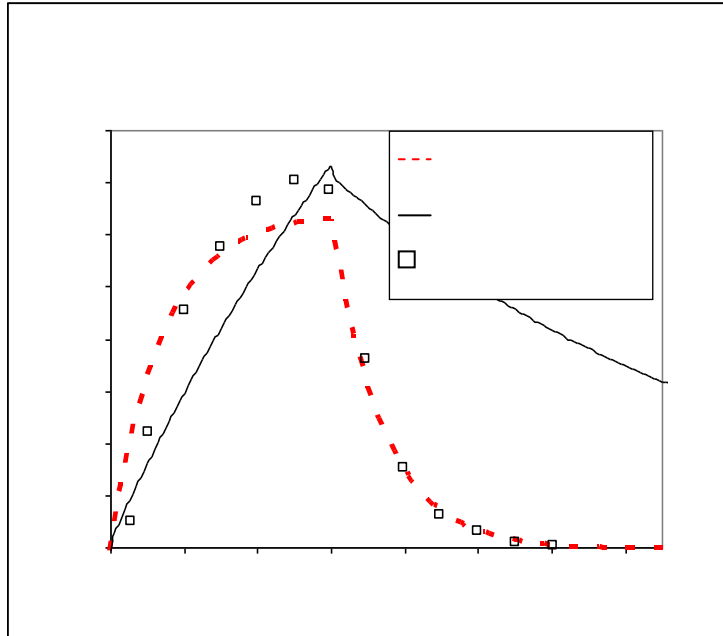


Figure B-16. Predictions of blood 1,4-dioxane concentration following calibration of a zero-order metabolism rate constant, k_{LC} , to only the exposure phase of the experimental data.

1 Finally, the model was re-calibrated by simultaneously fitting k_{LC} and the slowly
 2 perfused tissue:air partition coefficient to the experimental data with no bounds on possible
 3 values (except that they be non-zero). The fitted slowly perfused tissue:air partition coefficient
 4 was an extremely low (and biologically unlikely) value of 0.0001. The resulting model
 5 predictions, however, were closer to the observations than even the empirical model predictions
 6 (Figure B-17). These exercises show that better fits to the observed blood 1,4-dioxane kinetics
 7 are achieved only when parameter values are adjusted in a way that corresponds to a substantial
 8 decrease in apparent V_d of 1,4-dioxane in the human, relative to the rat (e.g., decreasing the
 9 slowly perfused tissue:air partition coefficient to extremely low values, relative to observations).
 10 Downward adjustment of the elimination parameters (e.g., decreasing k_{LC}) increases the
 11 predicted blood concentrations of 1,4-dioxane, achieving better agreement with observations
 12 during the exposure phase of the experiment; however, it results in unacceptably slow
 13 elimination kinetics, relative to observations following cessation of exposure. These
 14 observations suggest that some other process not captured in the present PBPK model structure is
 15 responsible for the species differences in 1,4-dioxane V_d and the inability to reproduce the
 16 human experimental inhalation data with biologically plausible parameter values.

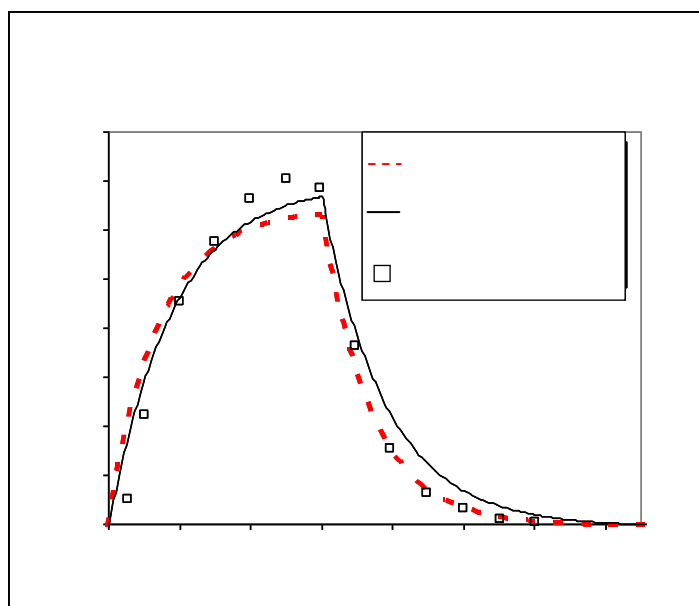


Figure B-17. Predictions of blood 1,4-dioxane concentration following simultaneous calibration of a zero-order metabolism rate constant, k_{LC} , and slowly perfused tissue:air partition coefficient to the experimental data.

B.6. CONCLUSIONS

1 The rat and human empirical models of Young et al. ([1978a](#); [1978b](#); [1977](#)) were
 2 successfully implemented in acslXtreme and perform identically to the models reported in the
 3 published papers (Figures 3-3 through 3-6), with the exception of the lower predicted HEAA
 4 concentrations and early appearance of the peak HEAA levels in rat urine. The early appearance
 5 of peak HEAA levels cannot presently be explained, but may result from manipulations of k_{me} or
 6 other parameters by Young et al. ([1978a](#); [1978b](#)) that were not reported. The lower predictions
 7 of HEAA levels are likely due to reliance on a standard urine volume production rate in the
 8 absence of measured (but unreported) urine volumes. While the human urinary HEAA
 9 predictions were lower than observations, this is due to parameter fitting of Young et al. ([1977](#)).
 10 No model output was published in Young et al. ([1977](#)) for comparison. The empirical models
 11 were modified to allow for user-defined inhalation exposure levels. However, no modifications
 12 were made to model oral exposures because adequate data to parameterize such modifications do
 13 not exist for rats or humans. The inhalation Young et al. (1977) model failed to provide adequate
 14 fits to the subchronic exposure plasma levels of 1,4-dioxane in rats using the data from the Kasai
 15 et al. (2008) study.

16 Several procedures were applied to the human PBPK model to determine if an adequate
 17 fit of the model to the empirical model output or experimental observations could be attained
 18 using biologically plausible values for the model parameters. The re-calibrated model
 19 predictions for blood 1,4-dioxane levels do not come within 10-fold of the experimental values

1 using measured tissue:air partition coefficients from Leung and Paustenbach ([1990](#)) or Sweeney
2 et al. ([2008](#)) (Figures B-[9](#) and B-[10](#)). Use of a slowly perfused tissue:air partition coefficient 10-
3 fold lower than measured values produces exposure-phase predictions that are much closer to
4 observations, but does not replicate the elimination kinetics (Figure B-[11](#)). Re-calibration of the
5 model with upper bounds on the tissue:air partition coefficients results in predictions that are still
6 six- to sevenfold lower than empirical model prediction or observations (Figures B-[13](#) and B-[14](#)).
7 Exploration of the model space using an assumption of first-order metabolism (valid for the 50-
8 ppm inhalation exposure) showed that an adequate fit to the exposure and elimination data can
9 be achieved only when unrealistically low values are assumed for the slowly perfused tissue:air
10 partition coefficient (Figure B-[17](#)). Artificially low values for the other tissue:air partition
11 coefficients are not expected to improve the model fit, because the sensitivity analysis to exert
12 less influence on blood 1,4-dioxane than V_{maxC} and K_m . This suggests that the model structure is
13 insufficient to capture the apparent 10-fold species difference in the blood 1,4-dioxane V_d
14 between rats and humans. In the absence of actual measurements for the human slowly perfused
15 tissue:air partition coefficient, high uncertainty exists for this model parameter value.
16 Differences in the ability of rat and human blood to bind 1,4-dioxane may contribute to the
17 difference in V_d . However, this is expected to be evident in very different values for rat and
18 human blood:air partition coefficients, which is not the case (Table B-1). Therefore, some other,
19 as yet unknown, modification to model structure may be necessary. [Sweeney et al. \(2008\) PBPK](#)
20 [model provided an overall improvement on previous models; however, the Sweeney et al. \(2008\)](#)
21 [inhalation model predictions of animal and human data were problematic.](#)

B.7. acsIXtreme CODE FOR THE YOUNG ET AL. EMPIRCAL MODEL FOR 1,4-DIOXANE IN RATS

```
1 PROGRAM: Young (1978a) rat.csl
2 !-----
3 ! Created by Michael Lumpkin, Syracuse Research Corporation, 08/06
4 ! This program implements the 1-compartment empirical model for 1,4-dioxane
5 ! in rats, developed by Young et al. 1978a, b. Program was modified to run
6 ! in ACSL Xtreme and to include user-defined i.v. and inhalation concentrations
7 !(MLumpkin, 08/06)
8 !-----
9
10 INITIAL
11
12 !*****Timing and Integration Commands*****
13 ALGORITHM IALG=2      !Gear integration algorithm for stiff systems
14 !MERROR %%%=0.01     !Relative error for lead in plasma
15 NSTEPS NSTP=1000 !Number of integration steps per communication interval
16 CINTERVAL CINT=0.1   !Communication interval
17 CONSTANT TSTART=0.   !Start of simulation (hr)
18 CONSTANT TSTOP=70.   !End of simulation (hr)
19
20 !*****MODEL PARAMETERS*****
21 CONSTANT BW=0.215    !Body weight (kg)
22 CONSTANT MINVOL=0.238 !respiratory minute volume (L/min) estimated from Young et al.
23 (1978)
24 CONSTANT IVDOSE = 0. !IV dose (mg/kg)!
25 CONSTANT CONC = 0.  !inhalation concentration (ppm)
26
27 CONSTANT MOLWT=88.105 !mol weight of 1,4-dioxane
28 CONSTANT TCHNG=6.0    !Exposure pulse 1 width (hr)
29 CONSTANT TDUR=24.0   !Exposure duration (hr)
30 CONSTANT TCHNG2=120.0 !Exposure pulse 2 width (hr)
31 CONSTANT TDUR2=168.0 !Exposure duration 2 (hr)
32
33 CONSTANT Vmax=4.008  !(mcg/mL/hr)
34 CONSTANT Km=6.308   !(mcg/mL)
35 CONSTANT Kinh=0.43  !pulmonary absorption constant (/hr)
36 CONSTANT Ke=0.0149  !(/hr)
37 CONSTANT Kme=0.2593 !(/hr)
38 CONSTANT Vd=0.3014  !(L)
39
40 IV = IVDOSE*BW
41 AmDIOXi=IV
42
43 END          !Of Initial Section
44
45 DYNAMIC
```



```

1  DERIVATIVE
2
3  !*** Dioxane inhalation concentration ***
4  CIZONE=PULSE(0.0, TDUR, TCHNG) * PULSE(0.0, TDUR2, TCHNG2)
5      !First pulse is hours/day, second pulse is hours/week
6  CI=CONC*CIZONE*MOLWT/24450.      !Convert to mg/L
7
8  !*** Dioxane metabolism/1st order elimination ***
9  dAmDIOX=(Kinh*CI*(MINVOL*60))-((Vmax*(AmDIOX))/(Km+(AmDIOX)))-
10 (Ke*(AmDIOX))
11 AmDIOX=INTEG(dAmDIOX,AmDIOXi)
12 ConcDIOX=AmDIOX/Vd      !plasma dioxane concentration (mcg/mL)
13 AUCDIOX=INTEG(ConcDIOX,0) !plasma dioxane AUC
14
15 !*** HEAA production and 1st order metabolism ***
16 dAmHEAA=((Vmax*(AmDIOX))/(Km+(AmDIOX)))-(Kme*(AmHEAA))
17 AmHEAA=INTEG(dAmHEAA,0.)
18 ConcHEAA=AmHEAA/Vd !plasma HEAA concentration
19
20 !*** 1st order dioxane elimination to urine ***
21 dAmDIOXu=(Ke*(AmDIOX))*0.35
22 AmDIOXu=INTEG(dAmDIOXu,0.)
23 ConcDIOXu=Ke*AmDIOX*0.35/1.45e-3 !urine production approx 1.45e-3 L/hr in SD rats
24
25 !*** 1st order dioxane exhaled ***
26 dAmDIOXex=(Ke*(AmDIOX))*0.65
27 AmDIOXex=INTEG(dAmDIOXex,0.)
28
29 !*** 1st order HEAA elimination to urine ***
30 dAmHEAAu=(Kme*(AmHEAA))
31 AmHEAAu=INTEG(dAmHEAAu,0.)
32 ConcHEAAu=Kme*AmHEAA/1.45e-3 !urine production approx 1.45e-3 L/hr in SD rats
33
34 END !of Derivative Section
35
36 DISCRETE
37
38 END !of Discrete Section
39
40 TERMT (T .GT. TSTOP)
41
42 END !of Dynamic Section
43
44 TERMINAL
45
46 END !of Terminal Section
47
48 END !of Program

```

B.8. acslXtreme CODE FOR THE YOUNG ET AL. EMPIRICAL MODEL FOR 1,4-DIOXANE IN HUMANS

```
1 PROGRAM: Young (1977) human.csl
2 !-----
3 ! Created by Michael Lumpkin, Syracuse Research Corporation, 01/06
4 ! This program implements the 1-compartment model for 1,4-dioxane in humans,
5 ! developed by Young et al., 1977. Program was modified to run
6 ! in acslXtreme (MLumpkin, 08/06)
7 !-----
8
9 INITIAL
10
11 !*****Timing and Integration Commands*****
12 ALGORITHM IALG=2      !Gear integration algorithm for stiff systems
13 !MERROR %%%=0.01     !Relative error for lead in plasma
14 NSTEPS NSTP=1000 !Number of integration steps per communication interval
15 CINTERVAL CINT=0.1   !Communication interval
16 CONSTANT TSTART=0.   !Start of simulation (hr)
17 CONSTANT TSTOP=120.  !End of simulation (hr)
18
19 !*****MODEL PARAMETERS*****
20 !CONSTANT DATA=1     !Optimization dataset
21 CONSTANT MOLWT=88.105 !mol weight for 1,4-dioxane
22 CONSTANT DOSE=0.      !Dose (mg/kg)
23 CONSTANT CONC=0.      !Inhalation concentration (ppm)
24 CONSTANT BW=84.1      !Body weight (kg)
25 CONSTANT MINVOL=7.0   !pulmonary minute volume (L/min)
26 CONSTANT F=1.0        !Fraction of dose absorbed
27 CONSTANT kinh=1.06    !Rate constant for inhalation (mg/hr); optimized by MHL
28 CONSTANT ke=0.0033    !Rate constant for dioxane elim to urine (hr-1)
29 CONSTANT km=0.7096    !Rate constant for metab of dioxane to HEAA (hr-1)
30 CONSTANT kme=0.2593   !Rate constant for transfer from rapid to blood (hr-1)
31 CONSTANT VdDkg=0.104  !Volume of distribution for dioxane (L/kg BW)
32
33 CONSTANT VdMkg=0.480  !Volume of distribution for HEAA (L/kg BW)
34 CONSTANT OStart=0.    !Time of first oral dose (hr)
35 CONSTANT OPeriod=120. !Oral Dose pulse period (hr)
36 CONSTANT OWidth=1.    !Width (gavage/drink time) of oral dose (hr)
37
38 CONSTANT IStart=0.     !Time of inhalation onset (hr)
39 CONSTANT IPeriod=120.  !Inhalation pulse period (hr)
40 CONSTANT IWidth=6.     !Width (duration) of inhalation exposure (hr)
41
42 END                    !Of Initial Section
43
44 DYNAMIC
45
46 DERIVATIVE
```

```

1  !****VARIABLES and DEFINED VALUES****
2  VdD=BW*VdDkg    !Volume of distribution for dioxane
3  VdM=BW*VdMkg    !Volume of distribution for HEAA
4
5  InhalePulse=PULSE(IStart,IPeriod,IWidth)
6  Inhale=CONC*InhalePulse*MOLWT/24450.    !Convert to mg/L
7
8  !*****DIFFERENTIAL EQUATIONS FOR COMPARTMENTS****
9
10 !*** Dioxane in the body (plasma) ***
11 dAMTbD=(Kinh*Inhale*(MINVOL*60))-(AMTbD*km)-(AMTbD*ke)
12 AMTbD=INTEG(dAMTbD,0.)
13 CbD=AMTbD/VdD
14 AUCbD=INTEG(CbD,0)
15
16 !*** HEAA in the body (plasma)***
17 dAMTbM=AMTbD*km-AMTbM*kme
18 AMTbM=INTEG(dAMTbM,0.)
19 CbM=AMTbM/VdM
20
21 !*** Cumulative Dioxane in the urine ***
22 dAMTuD=(AMTbD*ke)
23 AMTuD=INTEG(dAMTuD,0.)
24
25 !*** Cumulative HEAA in the urine ***
26 dAMTuM=(AMTbM*kme)
27 AMTuM=INTEG(dAMTuM,0.)
28
29 END                !Of Derivative Section
30
31 DISCRETE
32
33 END                !of Discrete Section
34
35 TERMT (T .GT. TSTOP)
36
37 END                !Of Dynamic Section
38
39 TERMINAL
40
41 END                !of Terminal Section
42
43 END                !of Program

```

B.9. acsIXtreme CODE FOR THE REITZ ET AL. PBPK MODEL FOR 1,4- DIOXANE

```
1  (Reitz, et al., 1990)
2  PROGRAM: DIOXANE.CSL (Used in Risk Estimation Procedures)
3  !Added a venous blood compartment and 1st order elim of metab.'
4  !Mass Balance Checked OK for Inhal, IV, Oral, and Water RHR'
5  !Defined Dose Surrogates for Risk Assessment 01/04/89'
6  !Modified the Inhal Route to use PULSE for exposure conditions'
7  !Modifications by GLDiamond, Aug2004, marked as !**
8  !
9  !Metabolism of dioxane modified by MLumpkin, Oct2006, to include 1st order
10 !or saturable kinetics. For 1st order, set VmaxC=0; for M-Menten, set K1C=0.
11 !
12 INITIAL
13
14  INTEGER I
15  I=1
16  ! ARRAY TDATA(20) ! CONSTANT TDATA=999, 19*1.0E-6 !**
17  CONSTANT  BW = 0.40  !'Body weight (kg)'
18  CONSTANT  QPC = 15.  !'Alveolar ventilation rate (l/hr)'
19  CONSTANT  QCC = 15.  !'Cardiac output (l/hr)'
20
21  !Flows to Tissue Compartments'
22  CONSTANT  QLC = 0.25  !'Fractional blood flow to liver'
23  CONSTANT  QFC = 0.05  !'Fractional blood flow to fat'
24  CONSTANT  QSC = 0.18  !'Fractional blood flow to slow'
25  QRC = 1.0 - (QFC + QSC + QLC)
26  CONSTANT  SPDC = 1.0  ! diffusion constant for slowly perfused tissues
27
28  !Volumes of Tissue/Blood Compartments'
29  CONSTANT  VLC = 0.04  !'Fraction liver tissue'
30  CONSTANT  VFC = 0.07  !'Fraction fat tissue'
31  CONSTANT  VRC = 0.05  !'Fraction Rapidly Perf tissue'
32  CONSTANT  VBC = 0.05  !'Fraction as Blood'
33  VSC = 0.91 - (VLC + VFC + VRC + VBC)
34
35  !Partition Coefficients'
36  CONSTANT  PLA = 1557.  !'Liver/air partition coefficient'
37  CONSTANT  PFA = 851.  !'Fat/air partition coefficient'
38  CONSTANT  PSA = 2065.  !'Muscle/air (Slow Perf) partition'
39  CONSTANT  PRA = 1557.  !'Richly perfused tissue/air partition'
40  CONSTANT  PB = 1850.  !'Blood/air partition coefficient'
41
42  !Other Compound Specific Parameters'
43  CONSTANT  MW = 88.1  !'Molecular weight (g/mol)'
44  CONSTANT  KLC = 12.0  ! temp zero-order metab constant
45  CONSTANT  VMAXC = 13.8  !'Maximum Velocity of Metabol.'
46  CONSTANT  KM = 29.4  !'Michaelis Menten Constant'
47  CONSTANT  ORAL = 0.0  !'Oral Bolus Dose (mg/kg)'
```

```

1  CONSTANT  KA = 5.0  !'Oral uptake rate (/hr)'
2  CONSTANT WATER = 0.0  !'Conc in Water (mg/liter, ppm)'
3  CONSTANT WDOSE=0.0  !'Water dose (mg/kg/day) **
4  CONSTANT  IV = 0.0  !'IV dose (mg/kg)'
5  CONSTANT CONC = 0.0  !'Inhaled concentration (ppm)'
6  CONSTANT  KME = 0.276  !'Urinary Elim constant for met (hr-1)'
7
8  !Timing commands'
9  CONSTANT  TSTOP = 50  !'Length of experiment (hrs)'
10 CONSTANT  TCHNG = 6  !'Length of inhalation exposure (hrs)'
11 CINTERVAL CINT=0.1
12 CONSTANT WIDD=24.  !***
13 CONSTANT PERD=24.  !***
14 CONSTANT PERW=168. !***
15 CONSTANT WIDW=168. !***
16 CONSTANT DAT=0.017  !***
17
18 !Scaled parameters calculated in this section of Program'
19  QC=QCC*BW**0.74
20  QP=QPC*BW**0.74
21  QL=QLC*QC
22  QF=QFC*QC
23  QS=QSC*QC
24  QR=QRC*QC
25  VL=VLC*BW
26  VF=VFC*BW
27  VS=VSC*BW
28  VR=VRC*BW
29  VB=VBC*BW
30  PL=PLA/PB
31  PR=PRA/PB
32  PS=PSA/PB
33  PF=PFA/PB
34  KL = KLC*bw**0.7 ! Zero-order metab constant
35  VMAX = VMAXC*BW**0.7
36  DOSE = ORAL*BW  !'Initial Amount in Stomach'
37  AB0 = IV*BW  !'Initial Amount in Blood'
38  !DRINK = 0.102*BW**0.7*WATER/24 !'Input from water (mg/hr)' !***
39  !DRINKA = 0.102*BW**0.7*WATER/DAT !'Input from water (mg/hr)' !***
40  DRINKA=WDOSE*BW/DAT
41  CV = AB0/VB  !'Initialize CV'
42
43 END  !'End of INITIAL'
44
45 DYNAMIC
46
47  ALGORITHM IALG = 2  !'Gear method for stiff systems'
48  TERMT(T .GE. TSTOP )

```

```

1      CR = AR/VR
2      CS = AS/VS
3      CF = AF/VF
4      BODY = AL + AR + AS + AF + AB + TUMMY
5      BURDEN = AM + BODY
6      TMASS = BURDEN + AX + AMEX
7
8      !Calculate the Interval Excretion Data here:'
9      !      DAX = AMEX-AMEX2
10     !      IF(DOSE .LE. 0.0 .AND. IV .LE. 0.0 ) GO TO SKIP1
11     !      PCTAX = 100*(AX - AX2)/(DOSE + IV*BW)
12     !      PCTMX = 100*(AMEX - AMEX2)/(DOSE + IV*BW)
13     !      SKIP1.. CONTINUE
14     !      IF(T .LT. TDATA(I) .OR. I .GE. 20 ) GO TO SKIP
15     !      AX2=AX
16     !      AMEX2=AMEX
17     !      I=I+1
18     !      SKIP.. CONTINUE
19
20     !DISCRETE EXPOSE
21     ! CIZONE = 1.0 ! CALL LOGD(.TRUE.) Turns on inhalation exposure?
22     !END
23     !DISCRETE CLEAR
24     ! CIZONE = 0.0 ! CALL LOGD(.TRUE.)
25     !END
26
27     DERIVATIVE
28
29     !Use Zero-Crossing Form of DISCRETE Function Here'
30     ! SCHEDULE command must be in DERIVATIVE section'
31     ! DAILY = PULSE (0.0, PER1, TCHNG )
32     ! WEEKLY = PULSE (0.0, PER2, LEN2 )
33     ! SWITCHY = DAILY * WEEKLY
34     !SCHEDULE EXPOSE .XP. SWITCHY - 0.995
35     !SCHEDULE CLEAR .XN. SWITCHY - 0.005
36
37     DAILY=PULSE(0.0,PERD,WIDD)
38     WEEKLY=PULSE(0.0,PERW,WIDW)
39     SWITCHY = DAILY * WEEKLY
40
41     !*****Modified Here for Wong*****'
42     CI = CONC * MW / 24451.0 * SWITCHY!**
43
44     !CA = Concentration in arterial blood (mg/l)'
45     CA = (QC*CV+QP*CI)/(QC+(QP/PB))
46     CX = CA/PB
47
48     DRINK=DRINKA*SWITCHY      !**

```

```

1
2 !TUMMY = Amount in stomach'
3 RTUMMY = -KA*TUMMY
4 TUMMY = INTEG(RTUMMY,DOSE)
5 !RAX = Rate of Elimination in Exhaled air'
6 RAX = QP*CX
7 AX = INTEG(RAX, 0.0)
8
9 !AS = Amount in slowly perfused tissues (mg)'
10 RAS = SPDC*(CA-CVS) !now governed by diffusion-limited constant, SPDC, instead of QS
11 AS = INTEG(RAS,0.)
12 CVS = AS/(VS*PS)
13
14 !AR = Amount in rapidly perfused tissues (mg)'
15 RAR = QR*(CA-CVR)
16 AR = INTEG(RAR,0.)
17 CVR = AR/(VR*PR)
18
19 !AF = Amount in fat tissue (mg)'
20 RAF = QF*(CA-CVF)
21 AF = INTEG(RAF,0.)
22 CVF = AF/(VF*PF)
23
24 !AL = Amount in liver tissue (mg)'
25 RAL = QL*(CA-CVL) - KL*CVL - VMAX*CVL/(KM+CVL) + KA*TUMMY + DRINK
26 AL = INTEG(RAL,0.)
27 CVL = AL/(VL*PL)
28
29 !Metabolism comments updated by EDM on 2/1/10
30 !AM = Amount metabolized (mg)'
31 RMEX = (KL*CVL)+(VMAX*CVL/(KM+CVL)) !Rate of 1,4-dioxane metabolism
32 RAM = (KL*CVL)+(VMAX*CVL)/(KM+CVL) - KME*AM !Rate of change of metabolite
33 in body
34
35 AM = INTEG(RAM, 0.0) !'Amt Metabolite in body
36 CAM = AM/BW !'Conc Metabolite in body'
37 AMEX = INTEG(KME*AM, 0.0) !'Amt Metabolite Excreted via urine'
38
39 !AB = Amount in Venous Blood'
40 RAB = QF*CVF + QL*CVL + QS*CVS + QR*CVR - QC*CV
41 AB = INTEG(RAB, AB0)
42 CV = AB/VB
43 AUCV = INTEG(CV, 0.0)
44
45 !Possible Dose Surrogates for Risk Assessment Defined Here'
46
47 CEX = 0.667*CX + 0.333*CI !'Conc in Exhal Air'
48 AVECON = PLA * (CEX+CI)/2 !'Ave Conc in Nose Tissue'

```

```

1  AUCCON = INTEG(AVECON, 0.0)      !'Area under Curve (Nose)
2
3  AUCMET = INTEG(CAM, 0.0)         !'Area under Curve (Metab)'
4
5  CL = AL/VL                       !'Conc Liver Tissue'
6  AUCL = INTEG(CL, 0.0)            !'Area under Curve (Liver)'
7  AAUCL=AUCL/TIME
8
9  ! Dose Surrogates are Average Area under Time/Conc Curve per 24 hrs'
10 IF (T .GT. 0) TIME=T
11   dayS = TIME/24.0
12   NOSE = AUCCON/DAYS              !'Nasal Turbinates'
13   LIVER = AUCL/DAYS               !'Liver Tissues'
14   METAB = AUCMET/DAYS             !'Stable Metabolite'
15
16 END    !'End of dynamic'
17
18 END ! End of TERMINAL
19
20 END    !'End of PROGRAM

```


APPENDIX C. DETAILS OF BMD ANALYSIS FOR ORAL RfD FOR 1,4-DIOXANE

C.1. CORTICAL TUBULE DEGENERATION

1 All available dichotomous models in the Benchmark Dose Software (version 2.1.1) were
2 fit to the incidence data shown in Table C-1, for cortical tubule degeneration in male and female
3 Osborne-Mendel rats exposed to 1,4-dioxane in the drinking water ([NCI, 1978](#)). Doses
4 associated with a BMR of a 10% extra risk were calculated.

Table C-1. Incidence of cortical tubule degeneration in Osborne-Mendel rats exposed to 1,4-dioxane in drinking water for 2 years

Males (mg/kg-day)			Females (mg/kg-day)		
0	240	530	0	350	640
0/31 ^a	20/31 ^b (65%)	27/33 ^b (82%)	0/31 ^a	0/34	10/32 ^b (31%)

^aStatistically significant trend for increased incidence by Cochran-Armitage test ($p < 0.05$) performed for this review.

^bIncidence significantly elevated compared to control by Fisher's exact test ($p < 0.05$) performed for this review.

Source: NCI ([1978](#)).

5 As assessed by the χ^2 goodness-of-fit test, several models in the software provided
6 adequate fits to the data for the incidence of cortical tubule degeneration in male and female rats
7 ($\chi^2 p \geq 0.1$) (Table C-2). Comparing across models, a better fit is indicated by a lower AIC
8 value ([U.S. EPA, 2000a](#)). As assessed by Akaike's Information Criterion (AIC), the log-probit
9 model provided the best fit to the cortical tubule degeneration incidence data for male rats
10 (Table C-2, Figure C-1) and could be used to derive a POD of 38.5 mg/kg-day for this endpoint.
11 The Weibull model provided the best fit to the data for female rats (Table C-2, Figure C-5) and
12 could be used to derive a POD of 452.4 mg/kg-day for this endpoint. For those models that
13 exhibit adequate fit, models with the lower AIC values are preferred. Differences in AIC values
14 of less than 1 are generally not considered important. BMDS modeling results for all
15 dichotomous models are shown in Table C-2.

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#)

Table C-2. Goodness-of-fit statistics and BMD₁₀ and BMDL₁₀ values from models fit to incidence data for cortical tubule degeneration in male and female Osborne-Mendel rats (NCI, 1978) exposed to 1,4-dioxane in drinking water

1

Model	AIC	<i>p</i> -value ^a	Scaled Residual of Interest	BMD ₁₀ (mg/kg-day)	BMDL ₁₀ (mg/kg-day)
Male					
Gamma ^b	74.458	0.6514	0	28.80	22.27
Logistic	89.0147	0.0011	-1.902	88.48	65.84
Log-logistic ^c	75.6174	1	0	20.85	8.59
Log-probit ^c	74.168	0.7532	0	51.41	38.53
Multistage (2 degree) ^d	74.458	0.6514	0	28.80	22.27
Probit	88.782	0.0011	-1.784	87.10	66.32
Weibull ^b	74.458	0.6514	0	28.80	22.27
Quantal-Linear	74.458	0.6514	0	28.80	22.27
Female					
Gamma ^b	41.9712	0.945	0.064	524.73	437.08
Logistic	43.7495	0.9996	0	617.44	471.92
Log-logistic ^c	41.7501	0.9999	0	591.82	447.21
Log-probit ^c	43.7495	0.9997	0	584.22	436.19
Multistage (2 degree) ^d	48.1969	0.1443	-1.693	399.29	297.86
Probit	43.7495	0.9997	0	596.02	456.42
Weibull ^b	41.75	0.9999	0	596.45	452.36
Quantal-Linear	52.3035	0.03	-2.086	306.21	189.49

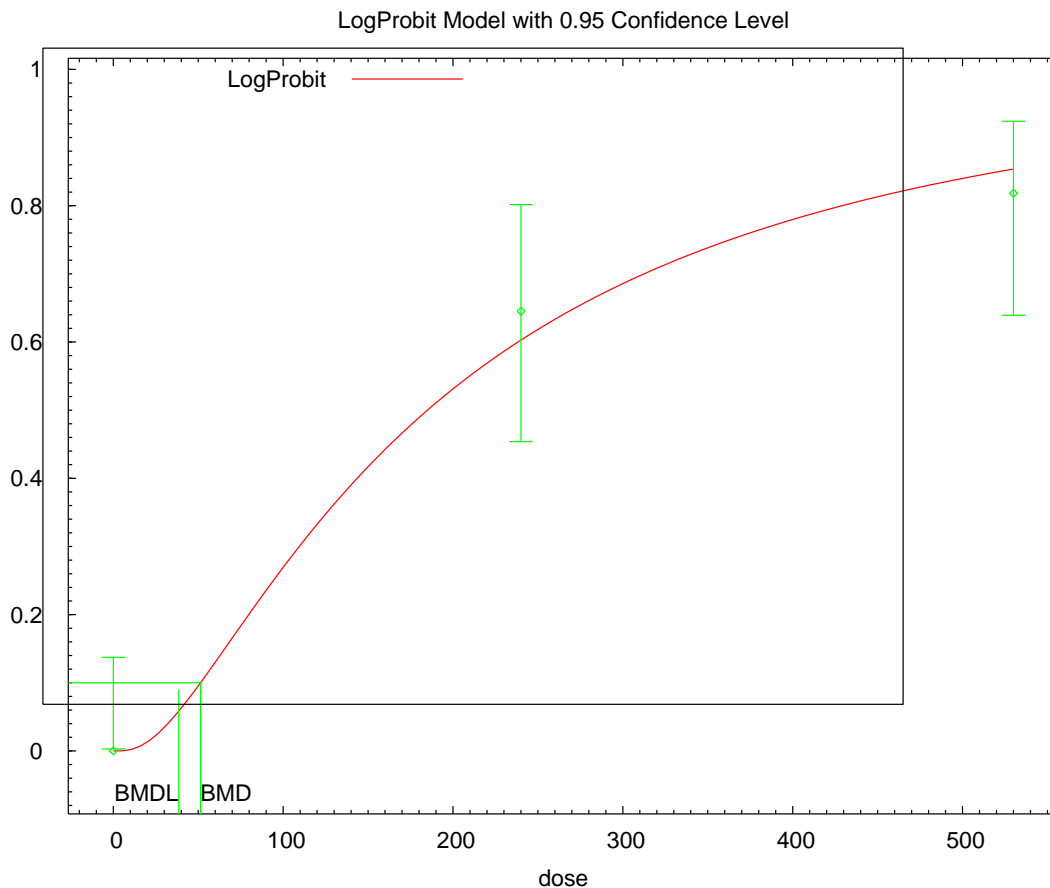
^a *p*-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^bPower restricted to ≥ 1 .

^cSlope restricted to ≥ 1 .

^dBetas restricted to ≥ 0 .

Source: NCI (1978).



14:49 02/01 2010

Source: NCI (1978).

Figure C-1. BMD Log-probit model of cortical tubule degeneration incidence data for male rats exposed to 1,4-dioxane in drinking water for 2 years to support the results in Table C-2.

```

1 =====
2 Probit Model. (Version: 3.1; Date: 05/16/2008)
3 Input Data File: C:\14DBMDS\lnp_nci_mrat_cortdeg_Lnp-BMR10-restrict.(d)
4 Gnuplot Plotting File: C:\14DBMDS\lnp_nci_mrat_cortdeg_Lnp-BMR10-restrict.plt
5                               Mon Feb 01 14:49:17 2010
6 =====
7 BMD5 Model Run
8 ~~~~~
9 The form of the probability function is:
10
11     P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),
12
13     where CumNorm(.) is the cumulative normal distribution function
14
15     Dependent variable = Effect
16     Independent variable = Dose
17     Slope parameter is restricted as slope >= 1
18
19     Total number of observations = 3
20     Total number of records with missing values = 0
21     Maximum number of iterations = 250
22     Relative Function Convergence has been set to: 1e-008
23     Parameter Convergence has been set to: 1e-008

```

1 User has chosen the log transformed model

2
3
4 Default Initial (and Specified) Parameter Values

5 background = 0
6 intercept = -5.14038
7 slope = 1
8
9

10 Asymptotic Correlation Matrix of Parameter Estimates

11 (***) The model parameter(s) -background -slope have been estimated at a boundary
12 point, or have been specified by the user, and do not appear in the correlation
13 matrix)

14
15 intercept
16 intercept 1
17
18

19 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	-5.22131	0.172682	-5.55976	-4.88286
slope	1	NA		

27 NA - Indicates that this parameter has hit a bound implied by some inequality
28 constraint and thus has no standard error.
29
30
31

32 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-35.8087	3			
Fitted model	-36.084	1	0.550629	2	0.7593
Reduced model	-65.8437	1	60.07	2	<.0001

39 AIC: 74.168
40
41

42 Goodness of Fit

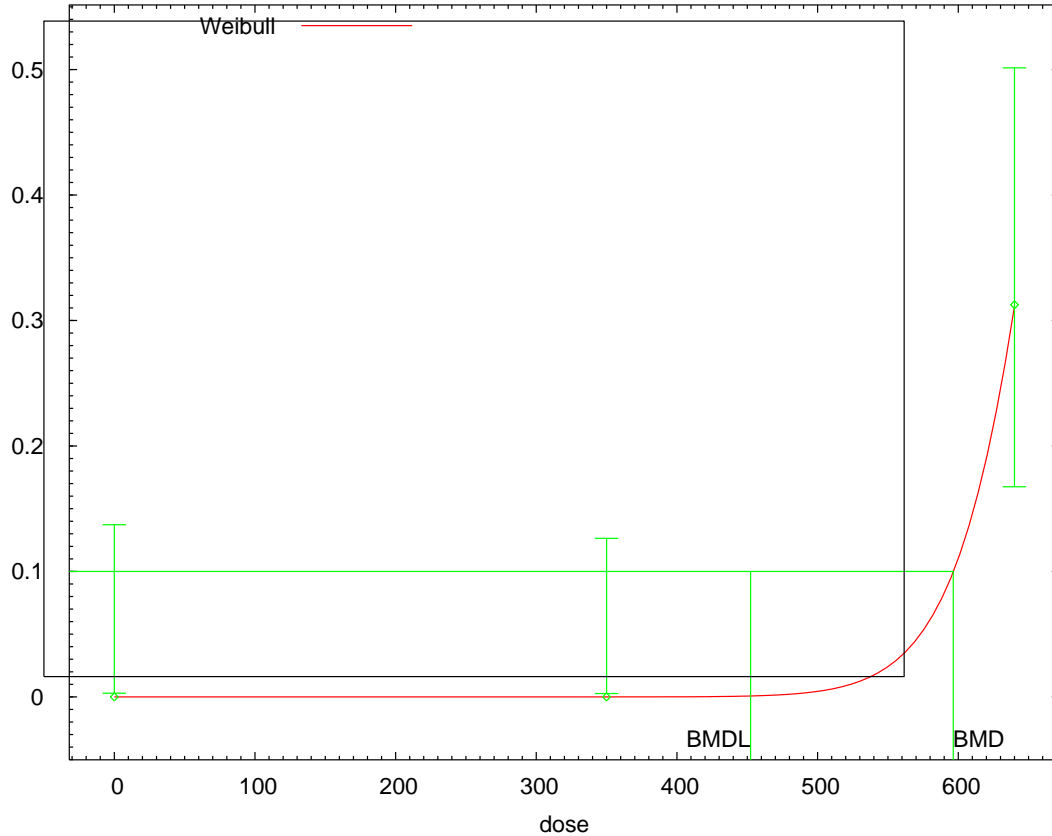
Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	31	0.000
240.0000	0.6023	18.672	20.000	31	0.487
530.0000	0.8535	28.166	27.000	33	-0.574

49
50 Chi^2 = 0.57 d.f. = 2 P-value = 0.7532
51
52

53 Benchmark Dose Computation

54 Specified effect = 0.1
55 Risk Type = Extra risk
56 Confidence level = 0.95
57 BMD = 51.4062
58 BMDL = 38.5284

Weibull Model with 0.95 Confidence Level



14:20 12/04 2009

Source: NCI (1978).

Figure C-2. BMD Weibull model of cortical tubule degeneration incidence data for female rats exposed to 1,4-dioxane in drinking water for 2 years to support the results in Table C-2.

```
1 =====
2 Weibull Model using Weibull Model (Version: 2.12; Date: 05/16/2008)
3 Input Data File: Z:\14Dioxane\BMSD\wei_nci_frat_cortdeg_Wei-BMR10-Restrict.(d)
4 Gnuplot Plotting File: Z:\14Dioxane\BMSD\wei_nci_frat_cortdeg_Wei-BMR10-Restrict.plt
5                               Fri Dec 04 14:20:41 2009
6 =====
7 BMSD Model Run
8 ~~~~~
9 The form of the probability function is:
10
11 P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]
12
13 Dependent variable = Effect
14 Independent variable = Dose
15 Power parameter is restricted as power >=1
16
17 Total number of observations = 3
18 Total number of records with missing values = 0
19 Maximum number of iterations = 250
20 Relative Function Convergence has been set to: 1e-008
21 Parameter Convergence has been set to: 1e-008
22
23
24
```

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46
47
48
49
50
51

Default Initial (and Specified) Parameter Values

Background = 0.015625
Slope = 1.55776e-010
Power = 3.33993

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Power have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

Slope Slope
Slope -1.5

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	1.15454e-051	1.#QNAN	1.#QNAN	1.#QNAN
Power	18	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-19.8748	3			
Fitted model	-19.875	1	0.000487728	2	0.9998
Reduced model	-32.1871	1	24.6247	2	<.0001

AIC: 41.75

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	31	0.000
350.0000	0.0000	0.000	0.000	34	-0.016
640.0000	0.3125	9.999	10.000	32	0.000

Chi^2 = 0.00 d.f. = 2 P-value = 0.9999

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 596.445
BMDL = 452.359

C.2. LIVER HYPERPLASIA

1 All available dichotomous models in the Benchmark Dose Software (version 2.1.1) were
2 fit to the incidence data shown in Table C-3, for liver hyperplasia in male and female
3 F344/DuCrj rats exposed to 1,4-dioxane in the drinking water ([JBRC, 1998](#); [Kano, et al., 2009](#)).
4 Benchmark doses associated with a BMR of a 10% extra risk were calculated.

Table C-3. Incidence of liver hyperplasia in F344/DuCrj rats exposed to 1,4-dioxane in drinking water^a

Males (mg/kg-day)				Females (mg/kg-day)			
0	11	55	274	0	18	83	429
3/40	2/45	9/35 ^a	12/22 ^c	2/38 ^b	2/37	9/38	24/24 ^c

^aDose information from Kano et al. (2009) and incidence data from sacrificed animals from JBRC (1998).

^bIncidence significantly elevated compared to control by χ^2 test ($p < 0.05$).

^cIncidence significantly elevated compared to control by χ^2 test ($p < 0.01$).

Sources: Kano et al. (2009); JBRC (1998).

5 For incidence of liver hyperplasia in F344 male rats, the logistic, probit, and
6 dichotomous-Hill models all exhibited a statistically significant lack of fit (i.e., χ^2 p -value < 0.1 ;
7 see Table C-4), and thus should not be considered further for identification of a POD. All of the
8 remaining models exhibited adequate fit, but the AIC values for the gamma, multistage, quantal-
9 linear, and Weibull models were lower than the AIC values for the log-logistic and log-probit
10 models. Finally, the AIC values for gamma, multistage, quantal-linear, and Weibull models in
11 Table C-4 are equivalent and, in this case, essentially represent the same model. Therefore,
12 consistent with the external review draft Benchmark Dose Technical Guidance ([U.S. EPA,](#)
13 [2000a](#)), any of them with equal AIC values (gamma, multistage, quantal-linear, or Weibull)
14 could be used to identify a POD for this endpoint of 23.8 mg/kg-day.

15 For liver hyperplasias in F344 female rats exposed to 1,4-dioxane, the quantal-linear and
16 dichotomous-Hill models did not result in a good fit (i.e., χ^2 p -value < 0.1 ; See Table C-4). The
17 multistage (3-degree) model had the lowest AIC value and was selected as the best-fitting model.
18 Therefore, consistent with the BMD technical guidance document ([U.S. EPA, 2000a](#)), the
19 BMDL from the multistage (3-degree) model was selected to yield a POD for this endpoint of
20 27.1 mg/kg-day.

Table C-4. Benchmark dose modeling results based on the incidence of liver hyperplasias in male and female F344 rats exposed to 1,4-dioxane in drinking water for 2 years

Model	AIC	<i>p</i> -value ^a	Scaled Residual of Interest	BMD ₁₀ (mg/kg-day)	BMDL ₁₀ (mg/kg-day)
Male					
Gamma ^b	114.172	0.3421	0.886	35.90	23.81
Logistic	117.047	0.0706	1.869	83.56	63.29
Log-logistic ^c	115.772	0.1848	0.681	33.39	16.96
Log-probit ^c	115.57	0.1431	1.472	54.91	37.05
Multistage ^d (2 degree)	114.172	0.3421	0.886	35.90	23.81
Probit	116.668	0.0859	1.804	76.69	58.57
Weibull ^b	114.172	0.3421	0.886	35.90	23.81
Quantal-Linear	114.172	0.3421	0.886	35.90	23.81
Dichotomous-Hill	117.185	NC ^e	-0.2398	32.01	14.84
Female					
Gamma ^b	78.8357	0.9783	0	70.78	40.51
Logistic	77.0274	0.9174	-0.016	54.66	41.11
Log-logistic ^c	78.8357	0.9781	0	77.72	51.21
Log-probit ^c	78.8357	0.9781	0	74.64	50.97
Multistage ^d (2 degree)	76.9718	0.9563	-0.107	56.06	31.17
Multistage ^d (3 degree)	76.8351	0.9999	0	65.28	27.08
Probit	77.0308	0.9095	0.017	52.53	38.44
Weibull ^b	78.8349	0.9995	0	66.47	36.14
Quantal-Linear	87.3833	0.0245	-1.116	21.52	15.61
Dichotomous-Hill	2972.99	NC ^e	0	NC ^e	NC ^e

^a*p*-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^bPower restricted to ≥ 1 .

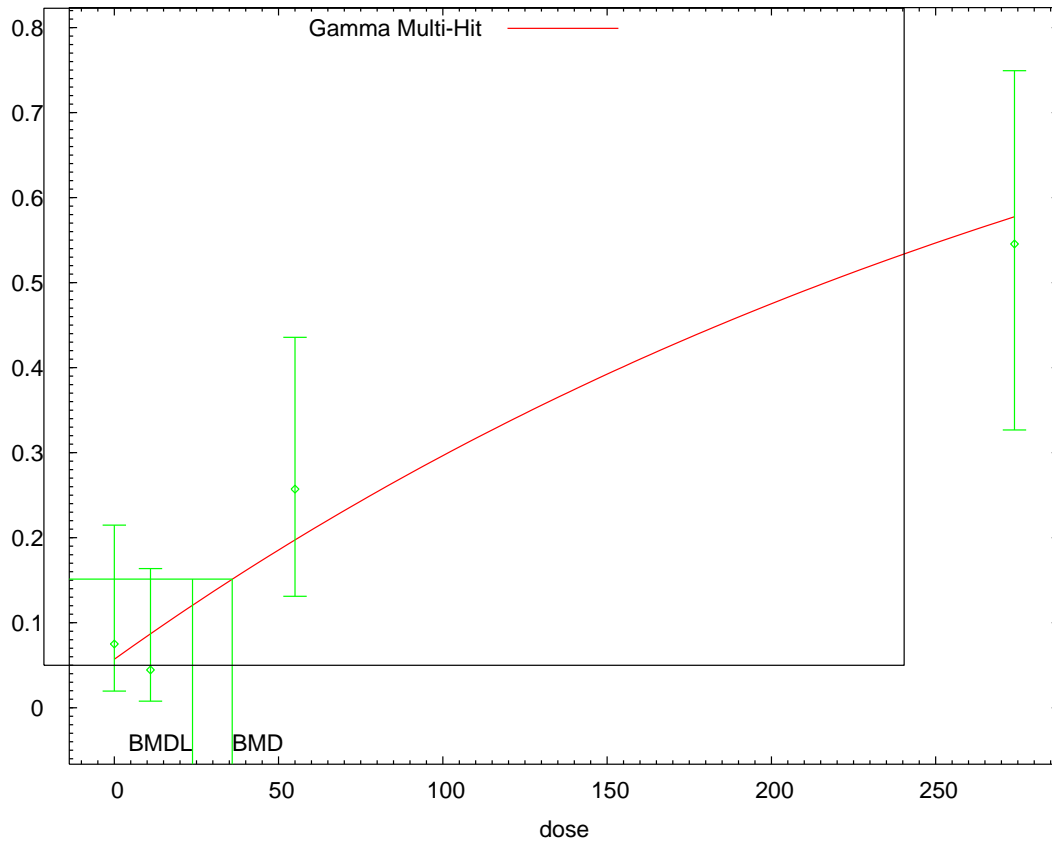
^cSlope restricted to ≥ 1 .

^dBetas restricted to ≥ 0 .

^eNC=Not calculated.

Sources: Kano et al. (2009); JBRC (1998).

Gamma Multi-Hit Model with 0.95 Confidence Level



14:35 12/04 2009

Figure C-3. BMD gamma model of liver hyperplasia incidence data for F344 male rats exposed to 1,4-dioxane in drinking water for 2 years to support results Table C-4.

```
1 =====
2 Gamma Model. (Version: 2.13; Date: 05/16/2008)
3 Input Data File: Z:\14Dioxane\BMDS\gam_jbrcl1998_mrat_liver_hyper_Gam-BMR10-
4 Restrict.(d)
5 Gnuplot Plotting File: Z:\14Dioxane\BMDS\gam_jbrcl1998_mrat_liver_hyper_Gam-BMR10-
6 Restrict.plt
7
8                               Fri Dec 04 14:35:02 2009
9 =====
10  BMDS Model Run
11 ~~~~~
12  The form of the probability function is:
13
14  P[response]= background+(1-background)*CumGamma[slope*dose,power],
15  where CumGamma(.) is the cumulative Gamma distribution function
16
17  Dependent variable = Effect
18  Independent variable = Dose
19  Power parameter is restricted as power >=1
20
21  Total number of observations = 4
22  Total number of records with missing values = 0
23  Maximum number of iterations = 250
24  Relative Function Convergence has been set to: 1e-008
```

1 Parameter Convergence has been set to: 1e-008

2
3
4 Default Initial (and Specified) Parameter Values

5 Background = 0.0853659
6 Slope = 0.00479329
7 Power = 1.3
8
9

10 Asymptotic Correlation Matrix of Parameter Estimates

11 (***) The model parameter(s) -Power have been estimated at a boundary point, or have
12 been specified by the user, and do not appear in the correlation matrix)

13
14 Background Slope
15 Background 1 -0.36
16 Slope -0.36 1
17

18 Parameter Estimates

19 95.0% Wald Confidence Interval
20 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
21 Background 0.0569658 0.0278487 0.00238329 0.111548
22 Slope 0.00293446 0.000814441 0.00133818 0.00453073
23 Power 1 NA
24

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

27
28 Analysis of Deviance Table

29
30 Model Log(likelihood) # Param's Deviance Test d.f. P-value
31 Full model -53.9471 4
32 Fitted model -55.0858 2 2.27725 2 0.3203
33 Reduced model -67.6005 1 27.3066 3 <.0001
34
35 AIC: 114.172
36
37

38 Goodness of Fit

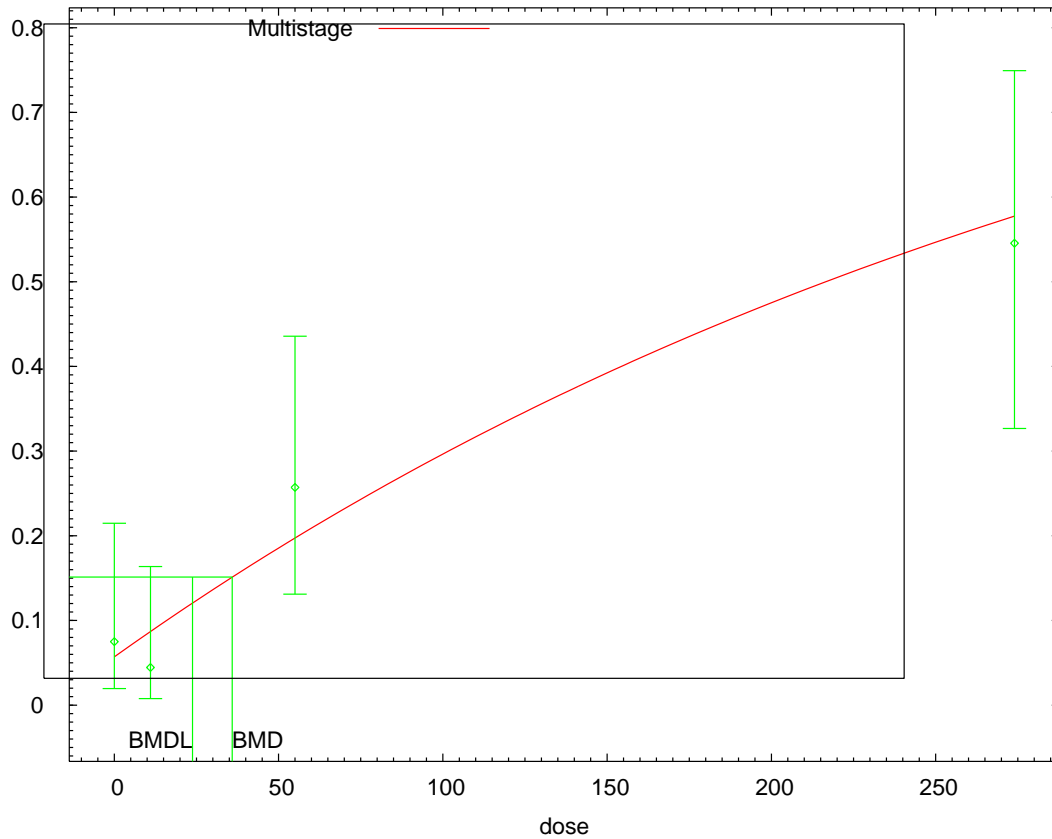
39
40 Dose Est. Prob. Expected Observed Size Scaled Residual
41 -----
42 0.0000 0.0570 2.279 3.000 40 0.492
43 11.0000 0.0869 3.911 2.000 45 -1.011
44 55.0000 0.1975 6.913 9.000 35 0.886
45 274.0000 0.5780 12.715 12.000 22 -0.309
46

47 Chi^2 = 2.15 d.f. = 2 P-value = 0.3421
48
49

50 Benchmark Dose Computation

51 Specified effect = 0.1
52 Risk Type = Extra risk
53 Confidence level = 0.95
54 BMD = 35.9046
55 BMDL = 23.8065

Multistage Model with 0.95 Confidence Level



14:35 12/04 2009

Figure C-4. BMD multistage (2 degree) model of liver hyperplasia incidence data for F344 male rats exposed to 1,4-dioxane in drinking water for 2 years to support results Table C-4.

```

1  =====
2  Multistage Model. (Version: 3.0; Date: 05/16/2008)
3  Input Data File: Z:\14Dioxane\BMDS\mst_jbrcl1998_mrnat_liver_hyper_Mst-BMR10-
4  restrict.(d)
5  Gnuplot Plotting File: Z:\14Dioxane\BMDS\mst_jbrcl1998_mrnat_liver_hyper_Mst-BMR10-
6  Restrict.plt
7
8  Fri Dec 04 14:35:06 2009
9  =====
10 BMDS Model Run
11 ~~~~~
12 The form of the probability function is:
13
14 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2)]
15
16 The parameter betas are restricted to be positive
17
18 Dependent variable = Effect
19 Independent variable = Dose
20
21 Total number of observations = 4
22 Total number of records with missing values = 0
23 Total number of parameters in model = 3
24 Total number of specified parameters = 0
25 Degree of polynomial = 2
26

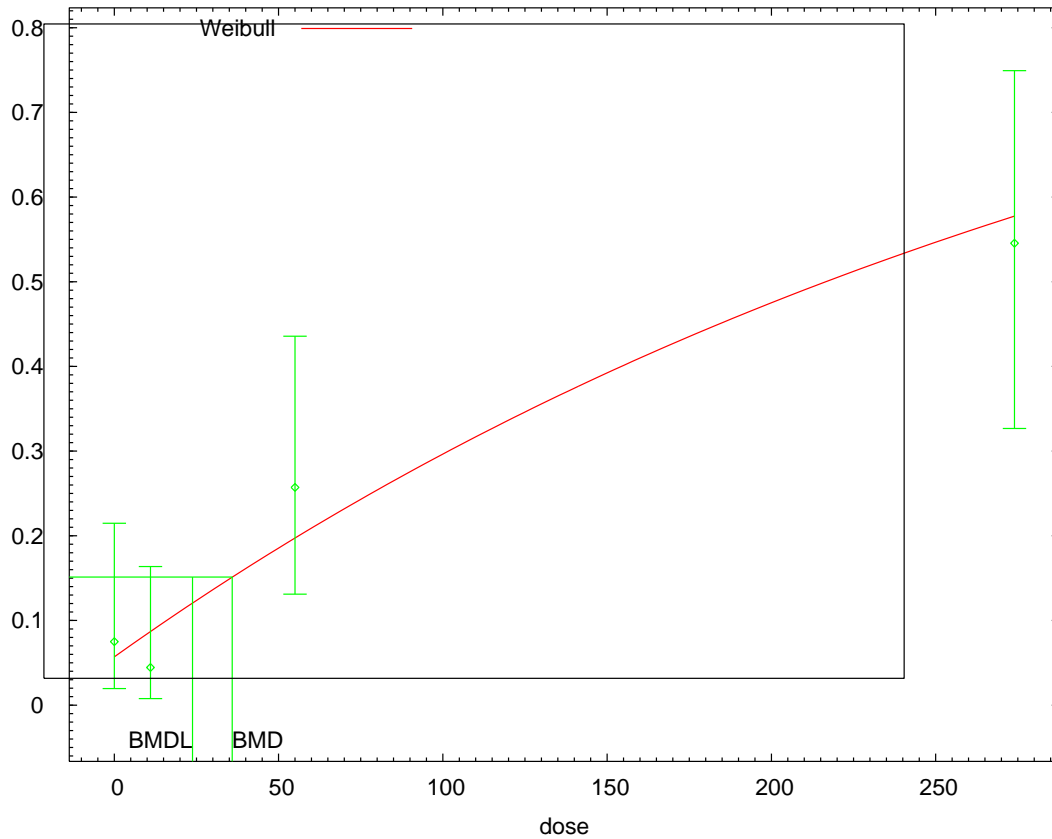
```

```

1 Maximum number of iterations = 250
2 Relative Function Convergence has been set to: 1e-008
3 Parameter Convergence has been set to: 1e-008
4
5
6
7           Default Initial Parameter Values
8           Background =      0.0750872
9             Beta(1) =      0.00263797
10            Beta(2) =              0
11
12
13           Asymptotic Correlation Matrix of Parameter Estimates
14 (***) The model parameter(s) -Beta(2) have been estimated at a boundary point, or have
15 been specified by the user, and do not appear in the correlation matrix)
16
17           Background      Beta(1)
18 Background      1      -0.49
19 Beta(1)      -0.49      1
20
21
22           Parameter Estimates
23           95.0% Wald Confidence Interval
24 Variable      Estimate      Std. Err.      Lower Conf. Limit      Upper Conf. Limit
25 Background      0.0569658      *      *      *
26 Beta(1)      0.00293446      *      *      *
27 Beta(2)      0      *      *      *
28
29 * - Indicates that this value is not calculated.
30
31
32
33           Analysis of Deviance Table
34
35 Model      Log(likelihood) # Param's Deviance Test d.f. P-value
36 Full model      -53.9471      4
37 Fitted model      -55.0858      2      2.27725      2      0.3203
38 Reduced model      -67.6005      1      27.3066      3      <.0001
39
40 AIC:      114.172
41
42
43           Goodness of Fit
44
45 Dose      Est._Prob.      Expected      Observed      Size      Scaled Residual
46 -----
47 0.0000      0.0570      2.279      3.000      40      0.492
48 11.0000      0.0869      3.911      2.000      45      -1.011
49 55.0000      0.1975      6.913      9.000      35      0.886
50 274.0000      0.5780      12.715      12.000      22      -0.309
51
52 Chi^2 = 2.15      d.f. = 2      P-value = 0.3421
53
54
55           Benchmark Dose Computation
56 Specified effect =      0.1
57 Risk Type =      Extra risk
58 Confidence level =      0.95
59 BMD =      35.9046
60 BMDL =      23.8065
61 BMDU =      82.1206
62
63 Taken together, (23.8065, 82.1206) is a 90% two-sided confidence interval for the BMD

```

Weibull Model with 0.95 Confidence Level



14:35 12/04 2009

Figure C-5. BMD Weibull model of liver hyperplasia incidence data for F344 male rats exposed to 1,4-dioxane in drinking water for 2 years to support the results in Table C-4.

```
1 =====
2 Weibull Model using Weibull Model (Version: 2.12; Date: 05/16/2008)
3 Input Data File: Z:\14Dioxane\BMDS\wei_jbrcl1998_mrat_liver_hyper_Wei-BMR10-
4 Restrict.(d)
5 Gnuplot Plotting File: Z:\14Dioxane\BMDS\wei_jbrcl1998_mrat_liver_hyper_Wei-BMR10-
6 Restrict.plt
7                               Fri Dec 04 14:35:08 2009
8 =====
9  BMDS Model Run
10 ~~~~~
11  The form of the probability function is:
12
13  P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]
14
15  Dependent variable = Effect
16  Independent variable = Dose
17  Power parameter is restricted as power >=1
18
19  Total number of observations = 4
20  Total number of records with missing values = 0
21  Maximum number of iterations = 250
22  Relative Function Convergence has been set to: 1e-008
23  Parameter Convergence has been set to: 1e-008
24
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26
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Default Initial (and Specified) Parameter Values

Background = 0.0853659
Slope = 0.00253609
Power = 1

Asymptotic Correlation Matrix of Parameter Estimates

(** The model parameter(s) -Power have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Background	Slope
Background	1	-0.36
Slope	-0.36	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0569661	0.0278498	0.00238155	0.111551
Slope	0.00293445	0.000814445	0.00133816	0.00453073
Power	1	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-53.9471	4			
Fitted model	-55.0858	2	2.27725	2	0.3203
Reduced model	-67.6005	1	27.3066	3	<.0001
AIC:	114.172				

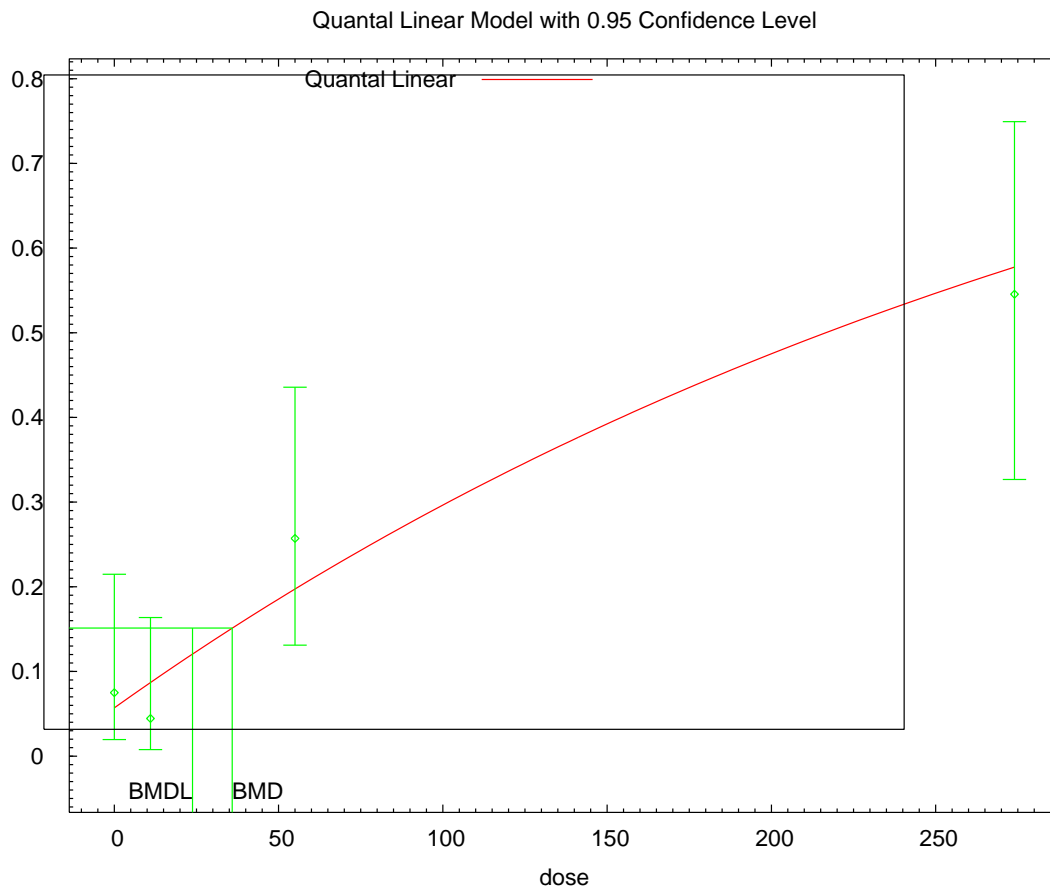
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0570	2.279	3.000	40	0.492
11.0000	0.0869	3.911	2.000	45	-1.011
55.0000	0.1975	6.913	9.000	35	0.886
274.0000	0.5780	12.715	12.000	22	-0.309

Chi^2 = 2.15 d.f. = 2 P-value = 0.3421

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 35.9047
BMDL = 23.8065



14:35 12/04 2009

Figure C-6. BMD quantal-linear model of liver hyperplasia incidence data for F344 male rats exposed to 1,4-dioxane in drinking water for 2 years to support the results in Table C-4.

```

1  =====
2  Quantal Linear Model using Weibull Model (Version: 2.12; Date: 05/16/2008)
3  Input Data File: Z:\14Dioxane\BMSD\qln_jbrcl1998_mrat_liver_hyper_Qln-BMR10.(d)
4  Gnuplot Plotting File: Z:\14Dioxane\BMSD\qln_jbrcl1998_mrat_liver_hyper_Qln-BMR10.plt
5  Fri Dec 04 14:35:09 2009
6  =====
7  BMSD Model Run
8  ~~~~~
9  The form of the probability function is:
10
11  P[response] = background + (1-background)*[1-EXP(-slope*dose)]
12
13
14  Dependent variable = Effect
15  Independent variable = Dose
16
17  Total number of observations = 4
18  Total number of records with missing values = 0
19  Maximum number of iterations = 250
20  Relative Function Convergence has been set to: 1e-008
21  Parameter Convergence has been set to: 1e-008
22
23  Default Initial (and Specified) Parameter Values
24  Background = 0.0853659
25  Slope = 0.00253609
26  Power = 1 Specified

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

	Background	Slope
Background	1	-0.36
Slope	-0.36	1

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval		
			Lower Conf. Limit	Upper Conf. Limit	
Background	0.0569665	0.02785	0.00238157	0.111551	
Slope	0.00293447	0.000814452	0.00133818	0.00453077	

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-53.9471	4			
Fitted model	-55.0858	2	2.27725	2	0.3203
Reduced model	-67.6005	1	27.3066	3	<.0001
AIC:	114.172				

Goodness of Fit

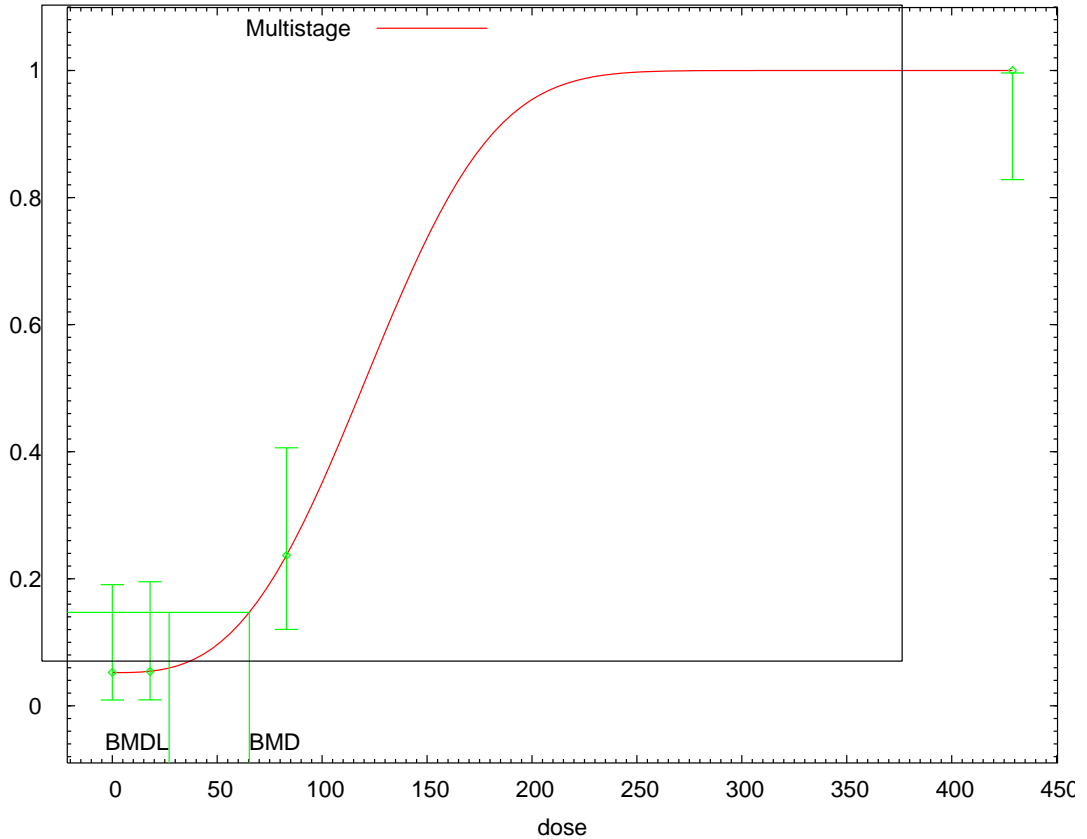
Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0570	2.279	3.000	40	0.492
11.0000	0.0869	3.911	2.000	45	-1.011
55.0000	0.1975	6.913	9.000	35	0.886
274.0000	0.5780	12.716	12.000	22	-0.309

Chi^2 = 2.15 d.f. = 2 P-value = 0.3421

Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	35.9044
BMDL =	23.8065

Multistage Model with 0.95 Confidence Level



10:30 05/21 2010

Source: JBRC (1998).

Figure C-7. BMD log-probit model of liver hyperplasia incidence data for F344 female rats exposed to 1,4-dioxane in drinking water for 2 years to support the results in Table C-4.

```

1  =====
2  Multistage Model. (Version: 3.0; Date: 05/16/2008)
3  Input Data File: H:\14Dioxane\BMDS\mst_jbrcl1998_frat_liver_hyper_Mst-BMR10-Restrict-
4  3deg. (d)
5  Gnuplot Plotting File: H:\14Dioxane\BMDS\mst_jbrcl1998_frat_liver_hyper_Mst-BMR10-
6  Restrict-3deg.plt
7
8  =====
9  BMD Model Run
10 ~~~~~
11 The form of the probability function is:
12
13 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2-
14 beta3*dose^3)]
15
16 The parameter betas are restricted to be positive
17
18   Dependent variable = Effect
19   Independent variable = Dose
20
21 Total number of observations = 4
22 Total number of records with missing values = 0
23 Total number of parameters in model = 4

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Total number of specified parameters = 0
Degree of polynomial = 3
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0
Beta(1) = 0
Beta(2) = 0
Beta(3) = 1.2696e+012

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1), -Beta(2) have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Background	Beta(3)
Background	1	-0.55
Beta(3)	-0.55	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0523101	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	3.78712e-007	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-36.4175	4			
Fitted model	-36.4175	2	0.00016582	2	0.9999
Reduced model	-79.9164	1	86.9979	3	<.0001
AIC:	76.8351				

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0523	1.988	2.000	38	0.009
18.0000	0.0544	2.013	2.000	37	-0.009
83.0000	0.2368	8.999	9.000	38	0.000
429.0000	1.0000	24.000	24.000	24	0.000

Chi^2 = 0.00 d.f. = 2 P-value = 0.9999

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 65.2814
BMDL = 27.0766
BMDU = 91.3457

Taken together, (27.0766, 91.3457) is a 90% two-sided confidence interval for the BMD

APPENDIX D. DETAILS OF BMD ANALYSIS FOR ORAL CSF FOR 1,4-DIOXANE

1 Dichotomous models available in the Benchmark Dose Software (BMDS) (version 2.1.1)
2 were fit to the incidence data for hepatocellular carcinoma and/or adenoma for mice and rats, as
3 well as nasal cavity tumors, peritoneal mesotheliomas, and mammary gland adenomas in rats
4 exposed to 1,4-dioxane in the drinking water. Doses associated with a benchmark response
5 (BMR) of a 10% extra risk were calculated. BMD₁₀ and BMDL₁₀ values from the best fitting
6 model, determined by adequate global- fit ($\chi^2 p \geq 0.1$) and AIC values, are reported for each
7 endpoint ([U.S. EPA, 2000a](#)). If the multistage cancer model is not the best fitting model for a
8 particular endpoint, the best-fitting multistage cancer model for that endpoint is also presented as
9 a point of comparison.

10 A summary of the model predictions for the Kano et al. ([2009](#)) study are shown in
11 Table D-1. The data and BMD modeling results are presented separately for each dataset as
12 follows:

- 13 • Hepatic adenomas and carcinomas in female F344 rats (Tables D-2 and D-3; Figure D-1)
- 14 • Hepatic adenomas and carcinomas in male F344 rats (Tables D-4 and D-5; Figures D-2
15 and D-3)
- 16 • Significant tumor incidence data at sites other than the liver (i.e., nasal cavity, mammary
17 gland, and peritoneal) in male and female F344 rats (Table D-6)
 - 18 ○ Nasal cavity tumors in female F344 rats (Table D-7; Figure D-4)
 - 19 ○ Nasal cavity tumors in male F344 rats (Table D-8; Figure D-5)
 - 20 ○ Mammary gland adenomas in female F344 rats (Table D-9; Figures D-6 and D-7)
 - 21 ○ Peritoneal mesotheliomas in male F344 rats (Table D-10; Figures D-8 and D-9)
- 22 • Hepatic adenomas and carcinomas in female BDF1 mice (Tables D-11, D-12, and D-13;
23 Figures D-10, D-11, D-12, and D-13)
- 24 • Hepatic adenomas and carcinomas in male BDF1 mice (Tables D-14 and D-15; Figures
25 D-14 and D-15)

26 Data and BMD modeling results from the additional chronic bioassays ([Kociba, et al., 1974](#);
27 [NCI, 1978](#)) were evaluated for comparison with the data from Kano et al. ([2009](#)). These results
28 are presented as follows:

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#)

- 1 • Summary of BMDS dose-response modeling estimates associated with liver and nasal
2 tumor incidence data resulting from chronic oral exposure to 1,4-dioxane in rats and mice
3 (Table D-16)
- 4 • Incidence of hepatocellular carcinoma and nasal squamous cell carcinoma in male and
5 female Sherman rats (combined) ([Kociba, et al., 1974](#)) treated with 1,4-dioxane in the
6 drinking water for 2 years (Table D-17)
 - 7 ○ BMDS dose-response modeling results for incidence of hepatocellular carcinoma in
8 male and female Sherman rats (combined) ([Kociba, et al., 1974](#)) exposed to
9 1,4-dioxane in drinking water for 2 years (Table D-18; Figures D-16 and D-17)
 - 10 ○ BMDS dose-response modeling results for incidence of nasal squamous cell
11 carcinoma in male and female Sherman rats (combined) ([Kociba, et al., 1974](#))
12 exposed to 1,4-dioxane in the drinking water for 2 years (Table D-19; Figure D-18)
- 13 • Incidence of nasal cavity squamous cell carcinoma and hepatocellular adenoma in
14 Osborne-Mendel rats ([NCI, 1978](#)) exposed to 1,4-dioxane in the drinking water (Table D-
15 20)
 - 16 ○ BMDS dose-response modeling results for incidence of hepatocellular adenoma in
17 female Osborne-Mendel rats ([NCI, 1978](#)) exposed to 1,4-dioxane in the drinking
18 water for 2 years (Table D-21; Figures D-19 and D-20)
 - 19 ○ BMDS dose-response modeling results for incidence of nasal cavity squamous cell
20 carcinoma in female Osborne-Mendel rats ([NCI, 1978](#)) exposed to 1,4-dioxane in the
21 drinking water for 2 years (Table D-22; Figures D-21 and D-22)
 - 22 ○ BMDS dose-response modeling results for incidence of nasal cavity squamous cell
23 carcinoma in male Osborne-Mendel rats ([NCI, 1978](#)) exposed to 1,4-dioxane in the
24 drinking water for 2 years (Table D-23; Figures D-23 and D-24)
- 25 • Incidence of hepatocellular adenoma or carcinoma in male and female B6C3F₁ mice
26 ([NCI, 1978](#)) exposed to 1,4-dioxane in drinking water (Table D-24)
 - 27 ○ BMDS dose-response modeling results for the combined incidence of hepatocellular
28 adenoma or carcinoma in female B6C3F₁ mice ([NCI, 1978](#)) exposed to 1,4-dioxane in
29 the drinking water for 2 years (Table D-25; Figure D-25)
 - 30 ○ BMDS dose-response modeling results for incidence of combined hepatocellular
31 adenoma or carcinoma in male B6C3F₁ mice ([NCI, 1978](#)) exposed to 1,4-dioxane in
32 the drinking water for 2 years (Table D-26; Figures D-26 and D-27).

D.1. GENERAL ISSUES AND APPROACHES TO BMDS MODELING

D.1.1. Combining Data on Adenomas and Carcinomas

33 The incidence of adenomas and the incidence of carcinomas within a dose group at a site
34 or tissue in rodents are sometimes combined. [This practice is based upon the hypothesis that](#)

1 [adenomas may develop into carcinomas if exposure at the same dose was continued \(McConnell,](#)
2 [et al., 1986; U.S. EPA, 2005a\)](#). The incidence at high doses of both tumors in rat and mouse
3 liver is high in the key study ([Kano, et al., 2009](#)). The incidence of hepatic adenomas and
4 carcinomas was summed without double-counting them so as to calculate the combined
5 incidence of either a hepatic carcinoma or a hepatic adenoma in rodents.

6 The variable N is used to denote the total number of animals tested in the dose group.
7 The variable Y is used here to denote the number of rodents within a dose group that have
8 characteristic X, and the notation Y(X) is used to identify the number with a specific
9 characteristic X. Modeling was performed on the adenomas and carcinomas separately and the
10 following combinations of tumor types:

- 11 • Y(adenomas) = number of animals with adenomas, whether or not carcinomas are
12 present;
- 13 • Y(carcinomas) = number of animals with carcinomas, whether or not adenomas are also
14 present;
- 15 • Y(either adenomas or carcinomas) = number of animals with adenomas or carcinomas,
16 not both = Y(adenomas) + Y(carcinomas) – Y(both adenomas and carcinomas);
- 17 • Y(neither adenomas nor carcinomas) = number of animals with no adenomas and no
18 carcinomas = N - Y(either adenomas or carcinomas).

D.1.2. Model Selection Criteria

19 Multiple models were fit to each dataset. The model selection criteria used in the BMD
20 technical guidance document ([U.S. EPA, 2000a](#)) were applied as follows:

- 21 • *p*-value for goodness-of-fit > 0.10
- 22 • AIC smaller than other acceptable models
- 23 • χ^2 residuals as small as possible
- 24 • No systematic patterns of deviation of model from data

25 Additional criteria were applied to eliminate implausible dose-response functions:

- 26 • Monotonic dose-response functions, e.g. no negative coefficients of polynomials in MS
27 models
- 28 • No infinitely steep dose-response functions near 0 (control dose), achieved by requiring
29 the estimated parameters “power” in the Weibull and Gamma models and “slope” in the
30 log-logistic model to have values ≥ 1 .

31 Because no single set of criteria covers all contingencies, an extended list of preferred models are
32 presented below in Table D-1.

D.1.3. Summary

1 The BMDS models recommended to calculate rodent BMD and BMDL values and
 2 corresponding human BMD_{HED} and BMDL_{HED} values are summarized in Table D-1.

Table D-1. Recommended models for rodents exposed to 1,4-dioxane in drinking water ([Kano, et al., 2009](#))

Endpoint	Model selection criterion	Model Type	AIC	p-value	BMD ^a mg/kg-day	BMDL ^a mg/kg-day	BMD _{HED} ^a mg/kg-day	BMDL _D ^a mg/kg-day
Female F344 Rat								
Hepatic Tumors	Lowest AIC	Multistage (2 degree)	91.5898	0.4516	79.83	58.09	19.84	14.43
Mammary Gland Tumors	Lowest AIC	LogLogistic	194.151	0.8874	161.01	81.91	40.01	20.35
Nasal Cavity Tumors	Lowest AIC	Multistage (3 degree)	42.6063	0.9966	381.65	282.61	94.84	70.23
Male R344 Rat								
Hepatic Tumors	Lowest AIC	Probit	147.787	0.9867	62.20	51.12	17.43	14.33
Peritoneal Mesothelioma	Lowest AIC	Probit	138.869	0.9148	93.06	76.32	26.09	21.39
Nasal Cavity Tumors	Lowest AIC	Multistage (3 degree)	24.747	0.9989	328.11	245.63	91.97	68.85
Female BDF1 Mouse								
Hepatic Tumors	Lowest AIC	LogLogistic	176.214	0.1421	5.54	3.66	0.83	0.55
	BMR 50%	LogLogistic	176.214	0.1421	49.88 ^b	32.93 ^b	7.51 ^b	4.95 ^b
Male BDF1 Mouse								
Hepatic Tumors	Lowest AIC	Log-Logistic	248.839	0.3461	34.78	16.60	5.63	2.68

3
 4 ^aValues for BMR 10% unless otherwise noted.
 5 ^bBMR 50%.

D.2. FEMALE F344 RATS: HEPATIC CARCINOMAS AND ADENOMAS

6 The incidence data for hepatic carcinomas and adenomas in female F344 rats ([Kano, et](#)
 7 [al., 2009](#)) are shown in Table D-2.

Table D-2. Data for hepatic adenomas and carcinomas in female F344 rats (Kano, et al., 2009)

Tumor type	Dose (mg/kg-day)			
	0	18	83	429
Hepatocellular adenomas	3	1	6	48
Hepatocellular carcinomas	0	0	0	10
Either adenomas or carcinomas	3	1	6	48
Neither adenomas nor carcinomas	47	49	44	2
Total number per group	50	50	50	50

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

1 Note that the incidence of rats with adenomas, with carcinomas, and with either
 2 adenomas or carcinomas are monotone non-decreasing functions of dose except for 3 female rats
 3 in the control group. These data therefore appear to be appropriate for dose-response modeling
 4 using BMDS.

5 The results of the BMDS modeling for the entire suite of models are presented in
 6 Table D-3.

Table D-3. BMDS dose-response modeling results for the combined incidence of hepatic adenomas and carcinomas in female F344 rats (Kano, et al., 2009)

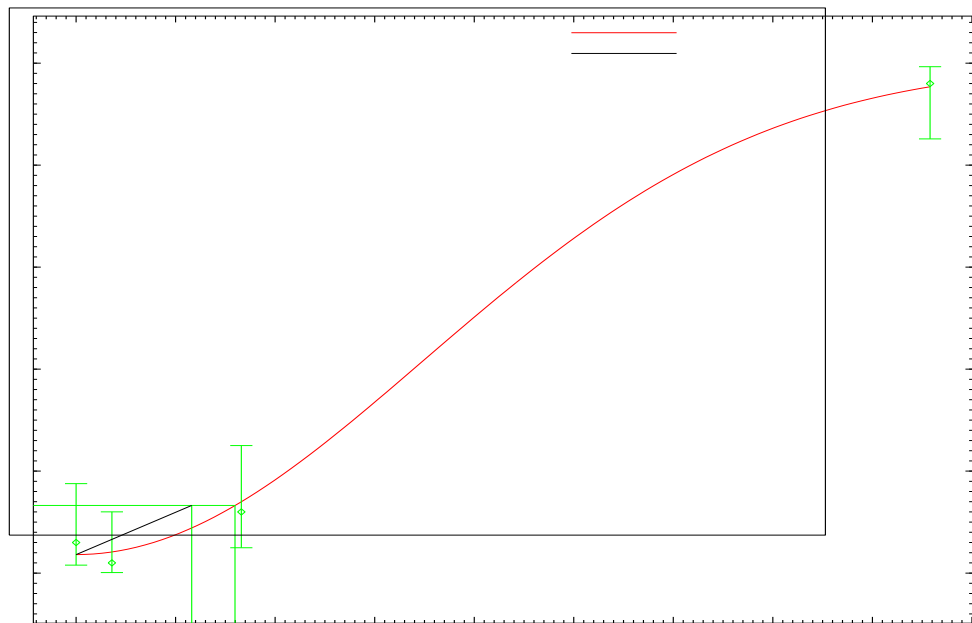
Model	AIC	p-value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	93.1067	0.3024	89.46	62.09	0.027	22.23	15.43
Logistic	91.7017	0.4459	93.02	71.60	0.077	23.12	17.79
LogLogistic	93.102	0.3028	88.34	65.52	0.016	21.95	16.28
LogProbit ^b	93.0762	0.3074	87.57	66.19	0.001	21.76	16.45
Multistage-Cancer (1 degree)	114.094	0.0001	25.58	19.92	-1.827	6.36	4.95
Multistage-Cancer (2 degree) ^c	91.5898	0.4516	79.83	58.09	-0.408	19.84	14.43
Multistage-Cancer (3 degree)	93.2682	0.2747	92.81	59.31	0.077	23.06	14.74
Probit	91.8786	0.3839	85.46	67.84	-0.116	21.24	16.86
Weibull	93.2255	0.2825	92.67	59.89	0.088	23.03	14.88
Quantal-Linear	114.094	0.0001	25.58	19.92	-1.827	6.36	4.95
Dichotomous-Hill	4458.37	NC ^d	NC ^d	NC ^d	0	0	0

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bSlope restricted ≥ 1 .

^cBest-fitting model.

^dValue unable to be calculated (NC: not calculated) by BMDS.



Source: Used with permission of Elsevier, Ltd., Kano et al. (2009).

Figure D-1. Multistage BMD model (2 degree) for the combined incidence of hepatic adenomas and carcinomas in female F344 rats.

```

1  =====
2  Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_hepato_adcar_Msc-
4  BMR10-2poly.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_hepato_adcar_Msc-BMR10-2poly.plt
7  Mon Oct 26 08:20:52 2009
8  =====
9  BMDS Model Run
10 ~~~~~
11
12 The form of the probability function is:
13 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2)]
14
15 The parameter betas are restricted to be positive
16
17 Dependent variable = Effect
18 Independent variable = Dose
19
20 Total number of observations = 4
21 Total number of records with missing values = 0
22 Total number of parameters in model = 3
23 Total number of specified parameters = 0
24 Degree of polynomial = 2
25
26 Maximum number of iterations = 250
27 Relative Function Convergence has been set to: 1e-008
28 Parameter Convergence has been set to: 1e-008
29
30 Default Initial Parameter Values
31 Background = 0.0281572

```


1 Beta(1) = 0
 2 Beta(2) = 1.73306e-005
 3
 4 Asymptotic Correlation Matrix of Parameter Estimates (***) The model parameter(s) -
 5 Beta(1) have been estimated at a boundary point, or have been specified by the user,
 6 and do not appear in the correlation matrix)
 7

	Background	Beta(2)
Background	1	-0.2
Beta(2)	-0.2	1

11
 12 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0362773	*	*	*
Beta(1)	0	*	*	*
Beta(2)	1.65328e-005	*	*	*

18
 19 * - Indicates that this value is not calculated.
 20

21
 22 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-42.9938	4			
Fitted model	-43.7949	2	1.60218	2	0.4488
Reduced model	-120.43	1	154.873	3	<.0001

29 AIC: 91.5898

30
 31 Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0363	1.814	3.000	50	0.897
18.0000	0.0414	2.071	1.000	50	-0.760
83.0000	0.1400	7.001	6.000	50	-0.408
429.0000	0.9540	47.701	48.000	50	0.202

39
 40 Chi^2 = 1.59 d.f. = 2 P-value = 0.4516
 41

42 Benchmark Dose Computation

43
 44 Specified effect = 0.1
 45 Risk Type = Extra risk
 46 Confidence level = 0.95
 47 BMD = 79.8299
 48 BMDL = 58.085
 49 BMDU = 94.0205
 50

51 Taken together, (58.085 , 94.0205) is a 90% two-sided confidence interval for the BMD

52
 53 Multistage Cancer Slope Factor = 0.00172161

D.3. MALE F344 RATS: HEPATIC CARCINOMAS AND ADENOMAS

1 The data for hepatic adenomas and carcinomas in male F344 rats ([Kano, et al., 2009](#)) are
2 shown in Table D-4.

**Table D-4. Data for hepatic adenomas and carcinomas in male F344 rats
([Kano, et al., 2009](#))**

Tumor type	Dose (mg/kg-day)			
	0	11	55	274
Hepatocellular adenomas	3	4	7	32
Hepatocellular carcinomas	0	0	0	14
Either adenomas or carcinomas	3	4	7	39
Neither adenomas nor carcinomas	47	46	43	11
Total number per group	50	50	50	50

Source: Used with permission from Elsevier, Ltd., Kano et al. ([2009](#)).

3
4 Note that the incidence of rats with hepatic adenomas, carcinomas, and with either
5 adenomas or carcinomas are monotone non-decreasing functions of dose. These data therefore
6 appear to be appropriate for dose-response modeling using BMDS.

7 The results of the BMDS modeling for the entire suite of models tested using the data for
8 hepatic adenomas and carcinomas for male F344 rats are presented in Table D-5.

Table D-5. BMDS dose-response modeling results for the combined incidence of adenomas and carcinomas in livers of male F344 rats (Kano, et al., 2009)

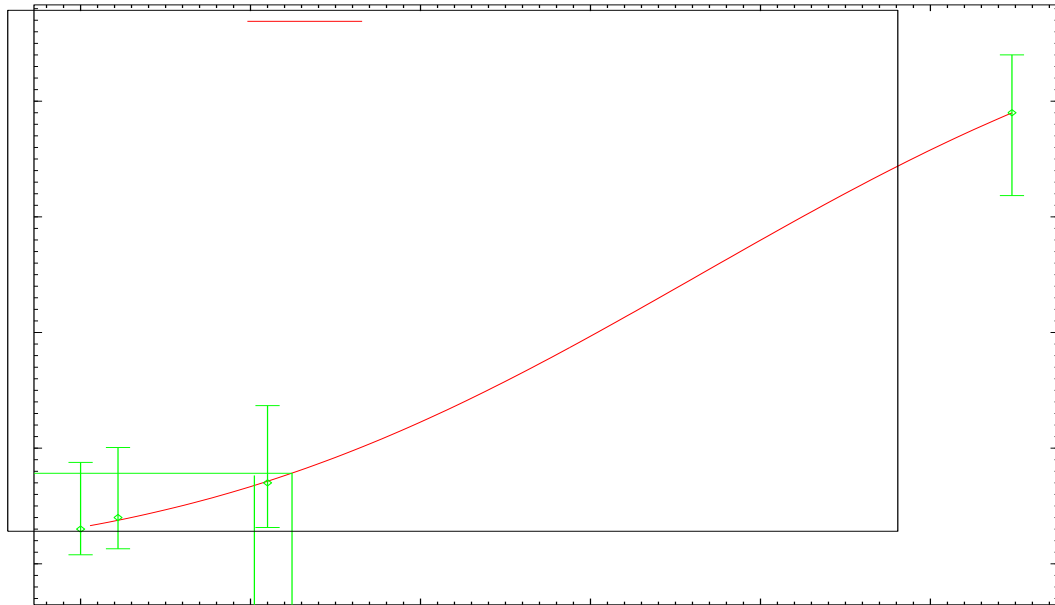
Model	AIC	p-value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	149.884	0.7257	62.41	30.79	-0.03	17.49	8.63
Logistic	147.813	0.9749	68.74	55.39	0.097	19.27	15.53
LogLogistic	149.886	0.7235	62.10	34.61	-0.021	17.41	9.70
LogProbit ^b	149.913	0.6972	61.70	37.49	-0.003	17.29	10.51
Multistage-Cancer (1 degree)	152.836	0.0978	23.82	18.34	-0.186	6.68	5.14
Multistage-Cancer (2 degree)	149.814	0.8161	61.68	28.26	-0.063	17.29	7.92
Multistage-Cancer (3 degree)	149.772	0.9171	63.62	27.49	-0.024	17.83	7.71
Probit ^c	147.787	0.9867	62.20	51.12	-0.05	17.43	14.33
Weibull	149.856	0.7576	62.63	30.11	-0.039	17.56	8.44
Quantal-Linear	152.836	0.0978	23.82	18.34	-0.186	6.68	5.14
Dichotomous-Hill	4441.71	NC ^d	NC ^d	NC ^d	0	0	0

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bSlope restricted ≥ 1 .

^cBest-fitting model.

^dValue unable to be calculated (NC: not calculated) by BMDS.



Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-2. Probit BMD model for the combined incidence of hepatic adenomas and carcinomas in male F344 rats.

```

1  =====
2  Probit Model. (Version: 3.1; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kano2009_mratt_hepato_adcar_Pr-
4  BMR10.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kano2009_mratt_hepato_adcar_Pr-BMR10.plt
7  Mon Oct 26 08:32:08 2009
8  =====
9  BMDS Model Run
10 ~~~~~
11
12 The form of the probability function is:
13 P[response] = CumNorm(Intercept+Slope*Dose),
14 where CumNorm(.) is the cumulative normal distribution function
15
16 Dependent variable = Effect
17 Independent variable = Dose
18 Slope parameter is not restricted
19
20 Total number of observations = 4
21 Total number of records with missing values = 0
22 Maximum number of iterations = 250
23 Relative Function Convergence has been set to: 1e-008
24 Parameter Convergence has been set to: 1e-008
25
26
27 Default Initial (and Specified) Parameter Values
28 background = 0 Specified
29 intercept = -1.51718
30 slope = 0.00831843
31

```

1 Asymptotic Correlation Matrix of Parameter Estimates
 2 (** The model parameter(s) -background have been estimated at a boundary point, or
 3 have been specified by the user, and do not appear in the correlation matrix)
 4

	intercept	slope
intercept	1	-0.69
slope	-0.69	1

10 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
intercept	1.53138	0.160195	-1.84535	-1.2174
slope	0.00840347	0.000976752	0.00648907	0.0103179

17 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-71.8804	4			
Fitted model	-71.8937	2	0.0265818	2	0.9868
Reduced model	-115.644	1	87.528	3	<.0001
AIC:	147.787				

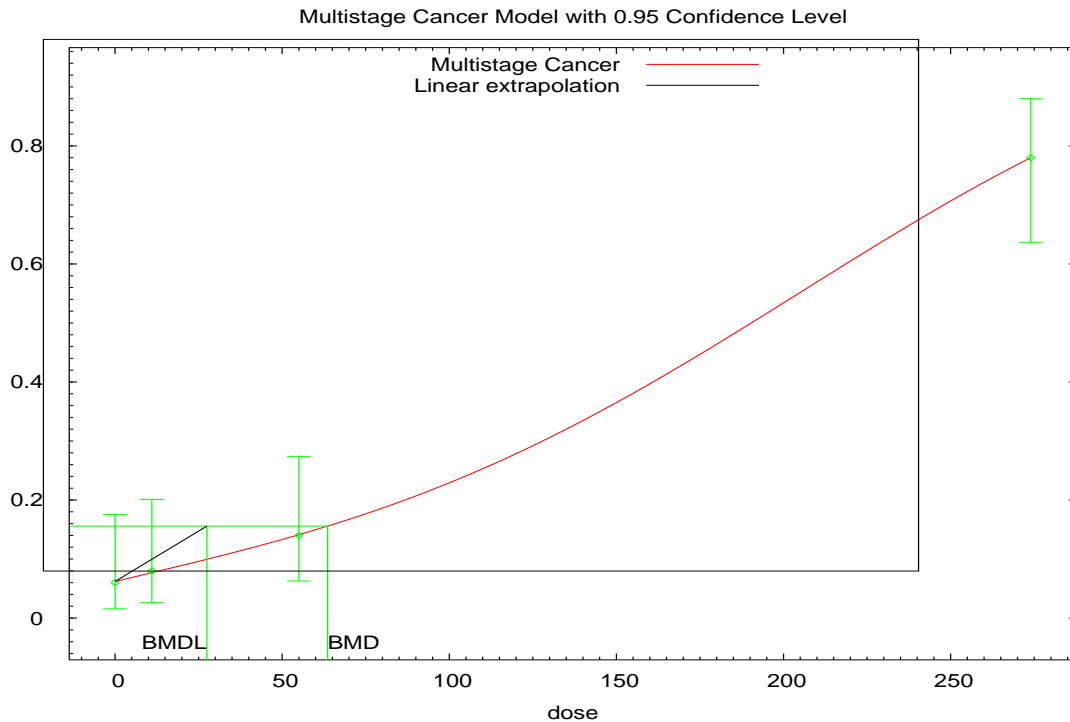
27 Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0628	3.142	3.000	50	-0.083
11.0000	0.0751	3.754	4.000	50	0.132
55.0000	0.1425	7.125	7.000	50	-0.050
274.0000	0.7797	38.985	39.000	50	0.005

36 Chi^2 = 0.03 d.f. = 2 P-value = 0.9867

38 Benchmark Dose Computation

40 Specified effect = 0.1
 41 Risk Type = Extra risk
 42 Confidence level = 0.95
 43 BMD = 62.1952
 44 BMDL = 51.1158



07:32 10/26 2009

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-3. Multistage BMD model (3 degree) for the combined incidence of hepatic adenomas and carcinomas in male F344 rats.

```

1  =====
2  Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrato_hepato_adcar_Msc-
4  BMR10-3poly.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrato_hepato_adcar_Msc-BMR10-3poly.plt
7  Mon Oct 26 08:32:08 2009
8  =====
9
10  BMDS Model Run
11  ~~~~~
12
13  The form of the probability function is: P[response] = background + (1-background)*[1-
14  EXP(-beta1*dose^1-beta2*dose^2-beta3*dose^3)]
15
16  The parameter betas are restricted to be positive
17
18  Dependent variable = Effect
19  Independent variable = Dose
20
21  Total number of observations = 4
22  Total number of records with missing values = 0
23  Total number of parameters in model = 4
24  Total number of specified parameters = 0
25  Degree of polynomial = 3
26
27  Maximum number of iterations = 250
28  Relative Function Convergence has been set to: 1e-008
29  Parameter Convergence has been set to: 1e-008
30
31  Default Initial Parameter Values

```

1 Background = 0.0623822
 2 Beta(1) = 0.00142752
 3 Beta(2) = 0
 4 Beta(3) = 5.14597e-008
 5 Asymptotic Correlation Matrix of Parameter Estimates
 6 (***) The model parameter(s) -Beta(2) have been estimated at a boundary point, or have
 7 been specified by the user, and do not appear in the correlation matrix)
 8

	Background	Beta(1)	Beta(3)
Background	1	-0.67	0.58
Beta(1)	-0.67	1	-0.95
Beta(3)	0.58	-0.95	1

15 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0619918	*	*	*
Beta(1)	0.001449	*	*	*
Beta(2)	0	*	*	*
Beta(3)	5.11829e-008	*	*	*

24 * - Indicates that this value is not calculated.

28 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-71.8804	4			
Fitted model	-71.8858	3	0.0107754	1	0.9173
Reduced model	-115.644	1	87.528	3	<.0001
AIC:	149.772				

38 Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0620	3.100	3.000	50	-0.058
11.0000	0.0769	3.844	4.000	50	0.083
55.0000	0.1412	7.059	7.000	50	-0.024
274.0000	0.7799	38.997	39.000	50	0.001

47 Chi^2 = 0.01 d.f. = 1 P-value = 0.9171

50 Benchmark Dose Computation

52 Specified effect = 0.1
 53 Risk Type = Extra risk
 54 Confidence level = 0.95
 55 BMD = 63.6179
 56 BMDL = 27.4913
 57 BMDU = 123.443

59 Taken together, (27.4913, 123.443) is a 90% two-sided confidence interval for the BMD

61 Multistage Cancer Slope Factor = 0.00363752

D.4. F344 RATS: TUMORS AT OTHER SITES

1 The data for tumors at sites other than the liver in male and female F344 rats ([Kano, et](#)
2 [al., 2009](#)) are shown in Table D-6. Note that the incidence of rats with these endpoints are
3 monotone non-decreasing functions (except female peritoneal mesotheliomas). These data
4 therefore appear to be appropriate for dose-response modeling using BMDS.

Table D-6. Data for significant tumors at other sites in male and female F344 rats ([Kano, et al., 2009](#))

Tumor site and type	Dose (mg/kg-day)							
	Female				Male			
	0	18	83	429	0	11	55	274
Nasal cavity squamous cell carcinoma	0	0	0	7	0	0	0	3
Peritoneal mesothelioma	1	0	0	0	2	2	5	28
Mammary gland adenoma	6	7	10	16	0	1	2	2
Total number per group	50	50	50	50	50	50	50	50

Source: Used with permission from Elsevier, Ltd., Kano et al., ([2009](#)).

5 The results of the BMDS modeling for the entire suite of models are presented in Tables
6 D-7 through Table D-10 for tumors in the nasal cavity, mammary gland, and peritoneal cavity.

Table D-7. BMDS dose-response modeling results for the incidence of nasal cavity tumors in female F344 rats^a (Kano, et al., 2009)

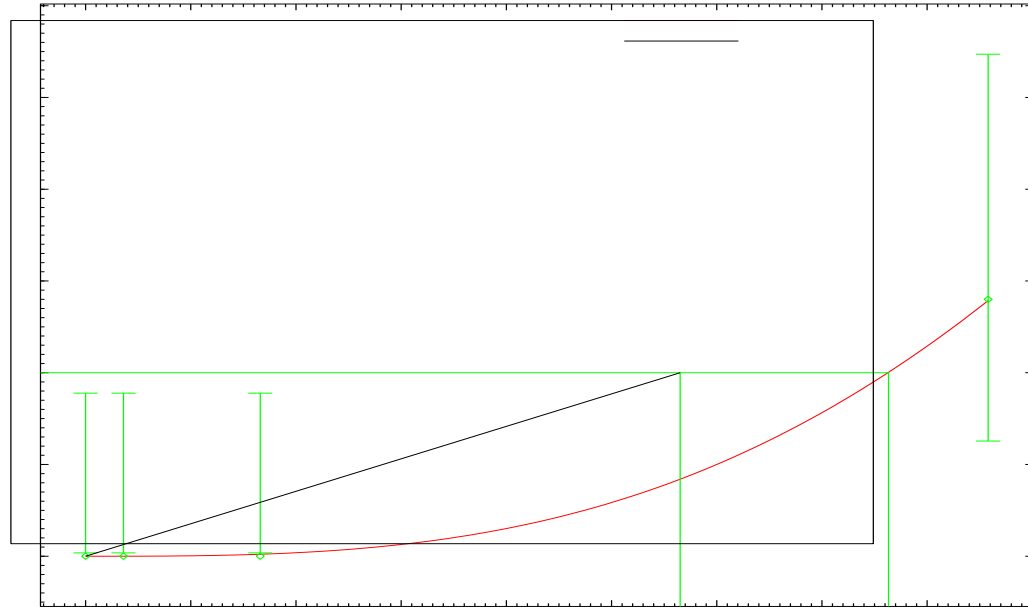
Model	AIC	p-value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^b	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	44.4964	1	403.82	269.03	0	100.35	66.85
Logistic	44.4963	1	421.54	351.74	0	104.75	87.41
LogLogistic	44.4963	1	413.69	268.85	0	102.80	66.81
LogProbit ^c	44.4963	1	400.06	260.38	0	99.42	64.71
Multistage-Cancer (1 degree)	45.6604	0.6184	375.81	213.84	0.595	93.39	53.14
Multistage-Cancer (2 degree)	43.0753	0.9607	366.07	274.63	0.109	90.97	68.24
Multistage-Cancer (3 degree) ^d	42.6063	0.9966	381.65	282.61	0.021	94.84	70.23
Probit	44.4963	1	414.11	333.31	0	102.91	82.83
Weibull	44.4963	1	414.86	273.73	0	103.09	68.02
Quantal-Linear	45.6604	0.6184	375.81	213.84	0.595	93.39	53.14
Dichotomous-Hill	46.4963	0.9997	413.96	372.57	1.64×10 ⁻⁸	102.87	92.58

^aNasal cavity tumors in female F344 rats include squamous cell carcinoma and esthesioneuro-epithelioma.

^bMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^cSlope restricted ≥ 1 .

^dBest-fitting model.



Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-4. Multistage BMD model (3 degree) for nasal cavity tumors in female F344 rats.

```

1  =====
2  Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_nasal_car_Msc-
4  BMR10-3poly.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_nasal_car_Msc-BMR10-3poly.plt
7  Mon Oct 26 08:28:58 2009
8  =====
9  BMDS Model Run
10 ~~~~~
11 The form of the probability function is: P[response] = background + (1-
12 background)*[1-EXP(-beta1*dose^1-beta2*dose^2-beta3*dose^3)]
13
14 The parameter betas are restricted to be positive
15
16 Dependent variable = Effect
17 Independent variable = Dose
18 Total number of observations = 4
19 Total number of records with missing values = 0
20 Total number of parameters in model = 4
21 Total number of specified parameters = 0
22 Degree of polynomial = 3
23
24 Maximum number of iterations = 250
25 Relative Function Convergence has been set to: 1e-008
26 Parameter Convergence has been set to: 1e-008
27
28 Default Initial Parameter Values
29 Background = 0
30 Beta(1) = 0
31 Beta(2) = 0
32 Beta(3) = 1.91485e-009

```

1 Asymptotic Correlation Matrix of Parameter Estimates
2 (***) The model parameter(s) -Background -Beta(1) -Beta(2)
3 have been estimated at a boundary point, or have been specified by the user,
4 and do not appear in the correlation matrix)
5
6 Beta(3) Beta(3)
7 Beta(3) 1
8
9 Parameter Estimates
10
11 95.0% Wald Confidence Interval
12 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
13 Background 0 * * *
14 Beta(1) 0 * * *
15 Beta(2) 0 * * *
16 Beta(3) 1.89531e-009 * * *
17
18 * - Indicates that this value is not calculated.
19
20
21 Analysis of Deviance Table
22
23 Model Log(likelihood) # Param's Deviance Test d.f. P-value
24 Full model -20.2482 4
25 Fitted model -20.3031 1 0.109908 3 0.9906
26 Reduced model -30.3429 1 20.1894 3 0.0001551
27
28 AIC: 42.6063
29
30
31 Goodness of Fit
32
33 Dose Est._Prob. Expected Observed Size Scaled Residual
34 -----
35 0.0000 0.0000 0.000 0.000 50 0.000
36 18.0000 0.0000 0.001 0.000 50 -0.024
37 83.0000 0.0011 0.054 0.000 50 -0.233
38 429.0000 0.1390 6.949 7.000 50 0.021
39
40 Chi^2 = 0.06 d.f. = 3 P-value = 0.9966
41
42
43 Benchmark Dose Computation
44
45 Specified effect = 0.1
46 Risk Type = Extra risk
47 Confidence level = 0.95
48 BMD = 381.651
49 BMDL = 282.609
50 BMDU = 500.178
51
52 Taken together, (282.609, 500.178) is a 90% two-sided confidence interval for the BMD
53
54 Multistage Cancer Slope Factor = 0.000353846

Table D-8. BMDS dose-response modeling results for the incidence of nasal cavity tumors in male F344 rats^a (Kano, et al., 2009)

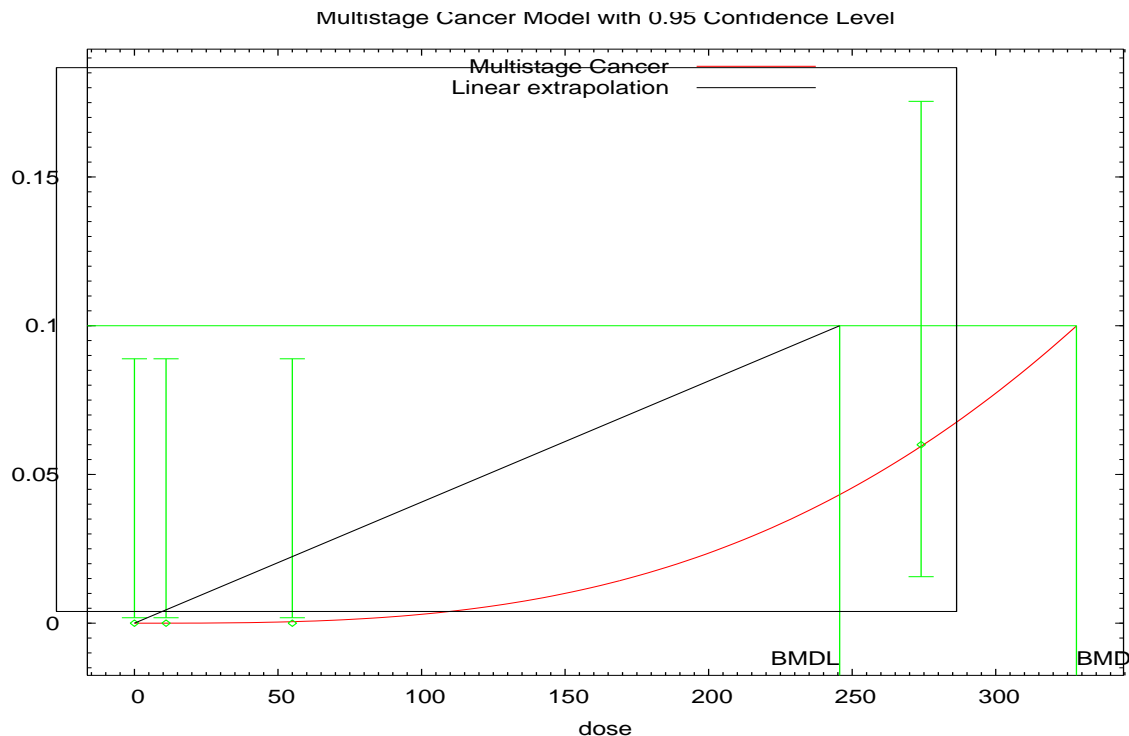
Model	AIC	<i>p</i> -value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^b	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	26.6968	1	299.29	244.10	0	83.89	68.42
Logistic	26.6968	1	281.06	261.29	0	78.78	73.24
LogLogistic	26.6968	1	288.31	245.29	0	80.81	68.75
LogProbit ^c	26.6968	1	303.06	238.86	0	84.94	66.95
Multistage-Cancer (1 degree)	26.0279	0.8621	582.49	256.43	0.384	163.28	71.88
Multistage-Cancer (2 degree)	24.9506	0.988	365.19	242.30	0.073	102.37	67.92
Multistage-Cancer (3 degree) ^d	24.747	0.9989	328.11	245.63	0.015	91.97	68.85
Probit	26.6968	1	287.96	257.01	0	80.72	72.04
Weibull	26.6968	1	288.00	246.36	0	80.73	69.06
Quantal-Linear	26.0279	0.8621	582.49	256.43	0.384	163.28	71.88
Dichotomous-Hill	28.6968	0.9994	290.52	261.47	6.25×10 ⁻⁵	81.44	73.29

^aNasal cavity tumors in male F344 rats include squamous cell carcinoma, Sarcoma: NOS, rhabdomyosarcoma, and esthesioneuro-epithelioma.

^bMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^cSlope restricted ≥ 1 .

^dBest-fitting model.



07:34 10/26 2009

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-5. Multistage BMD model (3 degree) for nasal cavity tumors in male F344 rats.

```

1 =====
2 Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3 Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_nasal_car_Msc-
4 BMR10-3poly.(d)
5 Gnuplot Plotting File:
6 L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_nasal_car_Msc-BMR10-3poly.plt
7 Mon Oct 26 08:34:20 2009
8 =====
9 BMDS Model Run
10 ~~~~~
11 The form of the probability function is: P[response] = background + (1-background)*[1-
12 EXP(-beta1*dose^1-beta2*dose^2-beta3*dose^3) ]
13
14 The parameter betas are restricted to be positive
15
16 Dependent variable = Effect
17 Independent variable = Dose
18 Total number of observations = 4
19 Total number of records with missing values = 0
20 Total number of parameters in model = 4
21 Total number of specified parameters = 0
22 Degree of polynomial = 3
23
24 Maximum number of iterations = 250
25 Relative Function Convergence has been set to: 1e-008
26 Parameter Convergence has been set to: 1e-008
27
28 Default Initial Parameter Values
29 Background = 0

```

1 Beta(1) = 0
 2 Beta(2) = 0
 3 Beta(3) = 3.01594e-009
 4
 5

6 Asymptotic Correlation Matrix of Parameter Estimates

7
 8 (***) The model parameter(s) -Background -Beta(1) -Beta(2)
 9 have been estimated at a boundary point, or have been specified by the user,
 10 and do not appear in the correlation matrix)
 11

12 Beta(3)
 13 Beta(3) 1
 14

15
 16 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	2.98283e-009	*	*	*

24
 25 * - Indicates that this value is not calculated.
 26
 27

28
 29 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-11.3484	4			
Fitted model	-11.3735	1	0.0502337	3	0.9971
Reduced model	-15.5765	1	8.45625	3	0.03747
AIC:	24.747				

38
 39 Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50	0.000
11.0000	0.0000	0.000	0.000	50	-0.014
55.0000	0.0005	0.025	0.000	50	-0.158
274.0000	0.0595	2.976	3.000	50	0.015

48 Chi^2 = 0.03 d.f. = 3 P-value = 0.9989
 49
 50

51 Benchmark Dose Computation

52
 53 Specified effect = 0.1
 54 Risk Type = Extra risk
 55 Confidence level = 0.95
 56 BMD = 328.108
 57 BMDL = 245.634
 58 BMDU = 1268.48
 59

60 Taken together, (245.634, 1268.48) is a 90% two-sided confidence interval for the BMD

61
 62 Multistage Cancer Slope Factor = 0.00040711

Table D-9. BMDS dose-response modeling results for the incidence of mammary gland adenomas in female F344 rats ([Kano, et al., 2009](#))

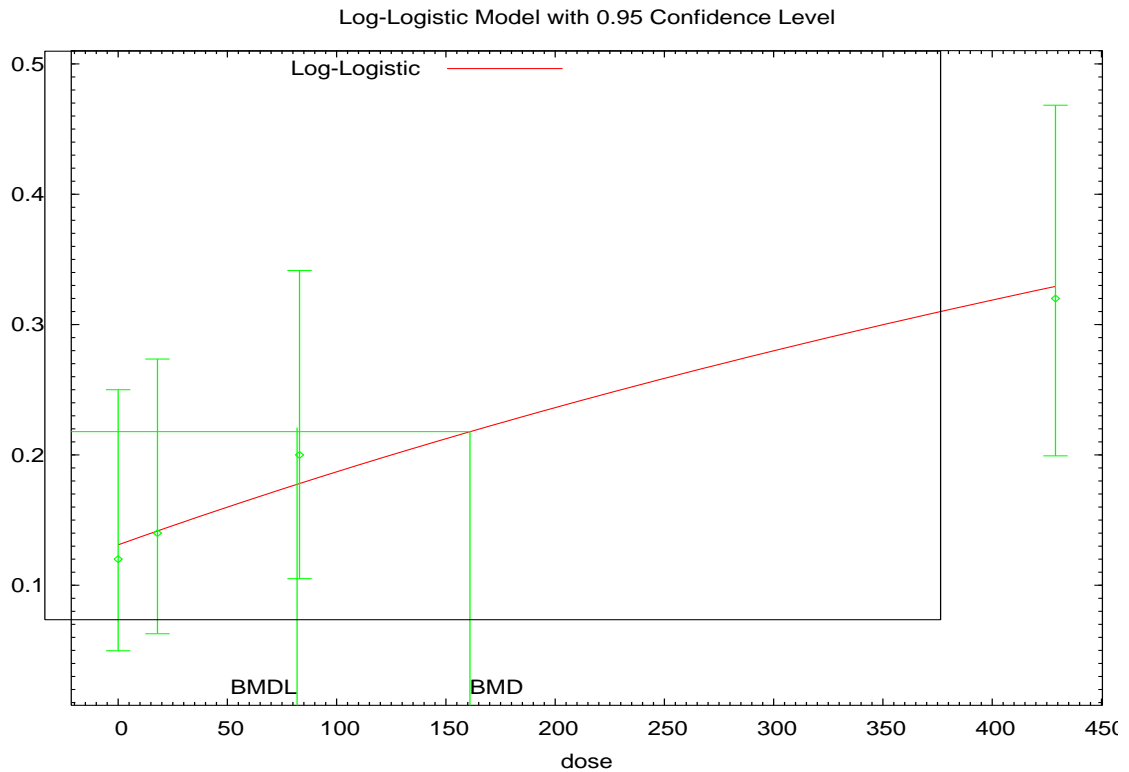
Model	AIC	p-value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	194.222	0.8559	176.66	99.13	0.465	43.90	24.63
Logistic	194.475	0.7526	230.35	159.73	0.612	57.24	39.69
LogLogistic ^b	194.151	0.8874	161.01	81.91	0.406	40.01	20.35
LogProbit ^c	195.028	0.5659	270.74	174.66	-0.075	67.28	43.41
Multistage-Cancer (1 degree)	194.222	0.8559	176.66	99.13	0.465	43.90	24.63
Multistage-Cancer (2 degree)	194.222	0.8559	176.66	99.13	0.465	43.90	24.63
Multistage-Cancer (3 degree)	194.222	0.8559	176.66	99.13	0.465	43.90	24.63
Probit	194.441	0.7656	223.04	151.60	0.596	55.43	37.67
Weibull	194.222	0.8559	176.65	99.13	0.465	43.90	24.63
Quantal-Linear	194.222	0.8559	176.65	99.13	0.465	43.90	24.63
Dichotomous-Hill	197.916	NC ^d	94.06	14.02	3.49×10 ⁻⁵	23.37	3.48

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bBest-fitting model.

^cSlope restricted ≥ 1 .

^dValue unable to be calculated (NC: not calculated) by BMDS.



11:31 02/01 2010

Source: Use with permission from Elsevier, Ltd., Kano et al. ([2009](#)).

Figure D-6. LogLogistic BMD model for mammary gland adenomas in female F344 rats.

```

1  =====
2  Logistic Model. (Version: 2.12; Date: 05/16/2008)
3  Input Data File: C:\14DBMDS\lnl_kano2009_frat_mamm_ad_Lnl-BMR10-Restrict.(d)
4  Gnuplot Plotting File: C:\14DBMDS\lnl_kano2009_frat_mamm_ad_Lnl-BMR10-Restrict.plt
5  Mon Feb 01 11:31:31 2010
6  =====
7  BMDs Model Run
8  ~~~~~
9  The form of the probability function is:
10
11  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
12
13  Dependent variable = Effect
14  Independent variable = Dose
15  Slope parameter is restricted as slope >= 1
16
17  Total number of observations = 4
18  Total number of records with missing values = 0
19  Maximum number of iterations = 250
20  Relative Function Convergence has been set to: 1e-008
21  Parameter Convergence has been set to: 1e-008
22
23  User has chosen the log transformed model
24
25  Default Initial Parameter Values
26  background = 0.12
27  intercept = -7.06982
28  slope = 1
29  Asymptotic Correlation Matrix of Parameter Estimates

```


(*** The model parameter(s) -slope have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	background	intercept
background	1	-0.53
intercept	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0.130936	*	*	*
intercept	-7.2787	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-94.958	4			
Fitted model	-95.0757	2	0.235347	2	0.889
Reduced model	-98.6785	1	7.4409	3	0.0591
AIC:	194.151				

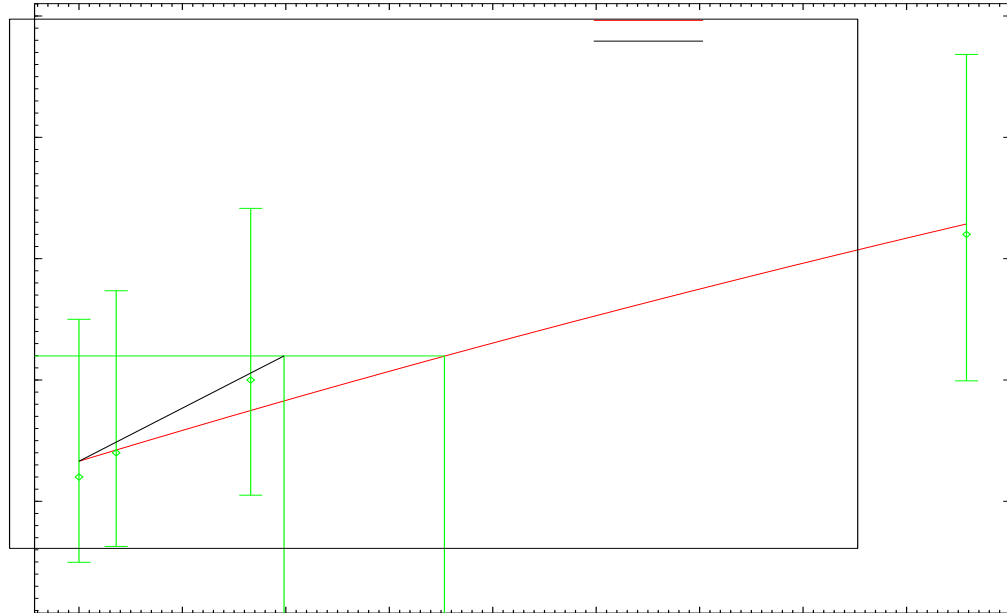
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1309	6.547	6.000	50	-0.229
18.0000	0.1416	7.080	7.000	50	-0.032
83.0000	0.1780	8.901	10.000	50	0.406
429.0000	0.3294	16.472	16.000	50	-0.142

Chi^2 = 0.24 d.f. = 2 P-value = 0.8874

Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	161.012
BMDL =	81.9107



Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-7. Multistage BMD model (1 degree) for mammary gland adenomas in female F344 rats.

```

1  =====
2  Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMSD\msc_kano2009_frat_mamm_ad_Msc-BMR10-
4  lpoly.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMSD\msc_kano2009_frat_mamm_ad_Msc-BMR10-lpoly.plt
7  Mon Oct 26 08:27:02 2009
8  =====
9  BMSD Model Run
10 ~~~~~
11 The form of the probability function is:
12
13  $P[\text{response}] = \text{background} + (1-\text{background}) * [1-\text{EXP}(-\text{beta}1 * \text{dose}^1)]$ 
14
15 The parameter betas are restricted to be positive
16
17 Dependent variable = Effect
18 Independent variable = Dose
19
20 Total number of observations = 4
21 Total number of records with missing values = 0
22 Total number of parameters in model = 2
23 Total number of specified parameters = 0
24 Degree of polynomial = 1
25
26 Maximum number of iterations = 250
27 Relative Function Convergence has been set to: 1e-008
28 Parameter Convergence has been set to: 1e-008
29
30 Default Initial Parameter Values
31 Background = 0.136033
32 Beta(1) = 0.000570906

```

1 Asymptotic Correlation Matrix of Parameter Estimates

2
3

	Background	Beta(1)
4 Background	1	-0.58
5 Beta(1)	-0.58	1

7
8 Parameter Estimates

9
10

11 Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
12 Background	.133161	*	*	*
13 Beta(1)	0.000596394	*	*	*

14
15 * - Indicates that this value is not calculated.

16
17
18
19 Analysis of Deviance Table

20

21 Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
22 Full model	-94.958	4			
23 Fitted model	-95.111	2	0.305898	2	0.8582
24 Reduced model	-98.6785	1	7.4409	3	0.0591
25 AIC:	194.222				

26
27
28
29 Goodness of Fit

30

31 Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
32 -----	-----	-----	-----	-----	-----
33 0.0000	0.1332	6.658	6.000	50	-0.274
34 18.0000	0.1424	7.121	7.000	50	-0.049
35 83.0000	0.1750	8.751	10.000	50	0.465
36 429.0000	0.3288	16.442	16.000	50	-0.133

37
38 Chi^2 = 0.31 d.f. = 2 P-value = 0.8559

39
40
41 Benchmark Dose Computation

42
43 Specified effect = 0.1
44 Risk Type = Extra risk
45 Confidence level = 0.95
46 BMD = 176.663
47 BMDL = 99.1337
48 BMDU = 501.523

49
50 Taken together, (99.1337, 501.523) is a 90% two-sided confidence interval for the BMD

51
52 Multistage Cancer Slope Factor = 0.00100874

Table D-10. BMDS dose-response modeling results for the incidence of peritoneal mesotheliomas in male F344 rats ([Kano, et al., 2009](#))

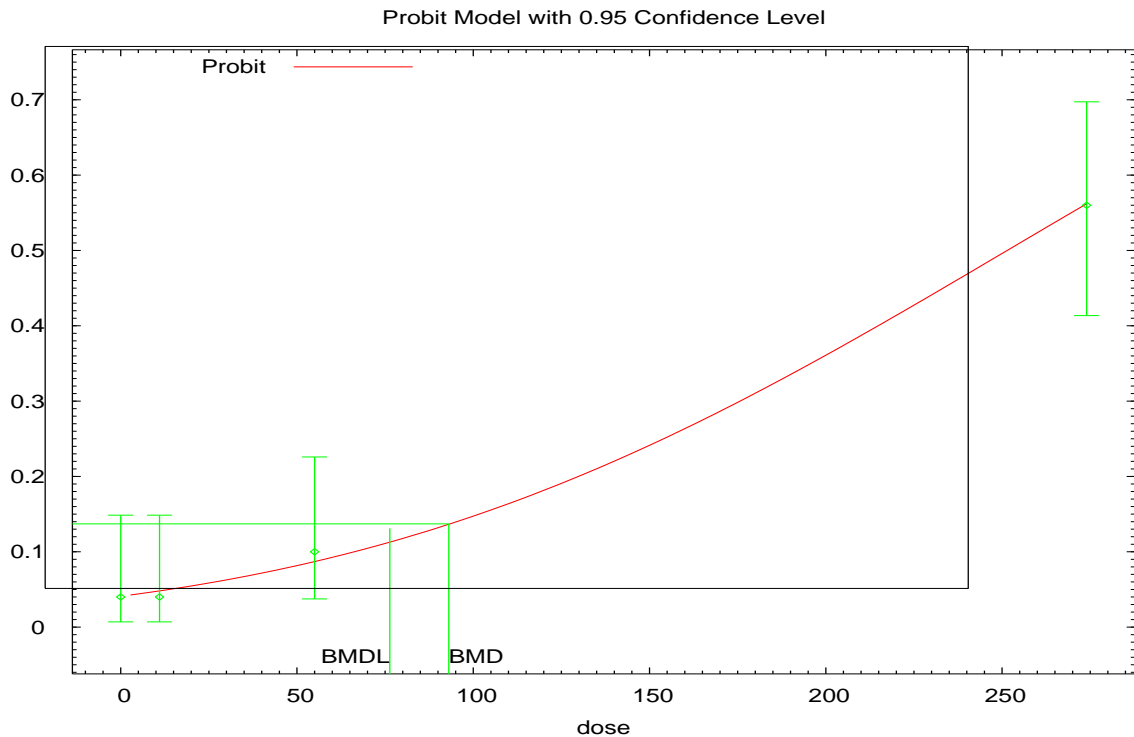
Model	AIC	<i>p</i> -value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	140.701	0.9189	73.52	35.62	0.018	20.61	9.98
Logistic	139.016	0.8484	103.52	84.35	0.446	29.02	23.65
LogLogistic	140.699	0.9242	72.56	36.37	0.014	20.34	10.19
LogProbit ^b	140.69	0.9852	70.29	52.59	0.001	19.70	14.74
Multistage-Cancer (1 degree)	140.826	0.3617	41.04	30.51	-1.066	11.50	8.55
Multistage-Cancer (2 degree)	140.747	0.8135	77.73	35.43	0.067	21.79	9.93
Multistage-Cancer (3 degree)	140.747	0.8135	77.73	35.43	0.067	21.79	9.93
Probit ^c	138.869	0.9148	93.06	76.32	0.315	26.09	21.39
Weibull	140.709	0.8915	74.77	35.59	0.027	20.96	9.97
Quantal-Linear	140.826	0.3617	41.04	30.51	-1.066	11.50	8.55
Dichotomous-Hill	2992	NC ^d	NC ^d	NC ^d	0	0	0

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bSlope restricted ≥ 1 .

^cBest-fitting model.

^dValue unable to be calculated (NC: not calculated) by BMDS.



07:41 10/26 2009

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-8. Probit BMD model for peritoneal mesotheliomas in male F344 rats.

```

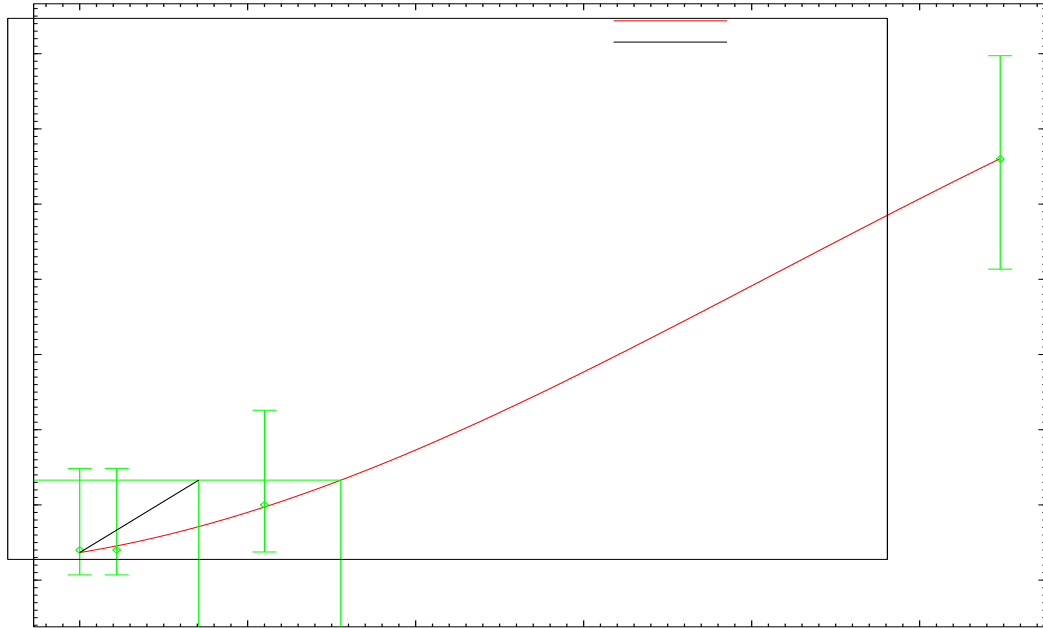
1  =====
2  Probit Model. (Version: 3.1; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kano2009_mrat_peri_meso_Pr-
4  BMR10.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kano2009_mrat_peri_meso_Pr-
7  BMR10.plt
8  Mon Oct 26 08:41:29 2009
9  =====
10 BMDS Model Run
11 ~~~~~
12 The form of the probability function is: P[response] = CumNorm(Intercept+Slope*Dose),
13 where CumNorm(.) is the cumulative normal distribution function
14
15 Dependent variable = Effect
16 Independent variable = Dose
17 Slope parameter is not restricted
18
19 Total number of observations = 4
20 Total number of records with missing values = 0
21 Maximum number of iterations = 250
22 Relative Function Convergence has been set to: 1e-008
23 Parameter Convergence has been set to: 1e-008
24
25 Default Initial (and Specified) Parameter Values
26 background = 0 Specified
27 intercept = -1.73485
28 slope = 0.00692801
29
30 Asymptotic Correlation Matrix of Parameter Estimates

```

```

1 (***) The model parameter(s) -background have been estimated at a boundary point, or
2 have been specified by the user, and do not appear in the correlation matrix )
3
4      intercept      slope
5  intercept          1      -0.75
6  slope             -0.75      1
7
8                      Parameter Estimates
9                      95.0% Wald Confidence Interval
10 Variable           Estimate      Std. Err.      Lower Conf. Limit      Upper Conf. Limit
11 intercept          -1.73734      0.18348      -2.09695      -1.37772
12 slope              0.00691646     0.000974372    0.00500672      0.00882619
13
14                      Analysis of Deviance Table
15      Model           Log(likelihood) # Param's  Deviance  Test d.f.  P-value
16      Full model      -67.3451      4
17      Fitted model    -67.4344      2      0.178619      2      0.9146
18      Reduced model   -95.7782      1      56.8663      3      <.0001
19      AIC:            138.869
20
21                      Goodness of Fit
22
23      Dose      Est._Prob.      Expected      Observed      Size      Scaled
24      -----
25      0.0000      0.0412      2.058      2.000      50      -0.041
26      11.0000      0.0483      2.417      2.000      50      -0.275
27      55.0000      0.0874      4.370      5.000      50      0.315
28      274.0000      0.5627      28.134      28.000      50      -0.038
29
30      Chi^2 = 0.18      d.f. = 2      P-value = 0.9148
31      Benchmark Dose Computation
32      Specified effect =      0.1
33      Risk Type =      Extra risk
34      Confidence level =      0.95
35      BMD =      93.0615
36      BMDL =      76.3242
37
38

```



Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-9. Multistage BMD (2 degree) model for peritoneal mesotheliomas in male F344 rats.

```

1  =====
2  Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMSD\msc_kano2009_mrat_peri_meso_Msc-
4  BMR10-2poly.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMSD\msc_kano2009_mrat_peri_meso_Msc-BMR10-2poly.plt
7  Mon Oct 26 08:41:28 2009
8  =====
9  BMSD Model Run
10 ~~~~~
11
12 The form of the probability function is:
13
14 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2)]
15
16 The parameter betas are restricted to be positive
17
18
19 Dependent variable = Effect
20 Independent variable = Dose
21
22 Total number of observations = 4
23 Total number of records with missing values = 0
24 Total number of parameters in model = 3
25 Total number of specified parameters = 0
26 Degree of polynomial = 2
27
28 Maximum number of iterations = 250
29 Relative Function Convergence has been set to: 1e-008
30 Parameter Convergence has been set to: 1e-008
31

```

1 Default Initial Parameter Values
 2 Background = 0.0358706
 3 Beta(1) = 0.000816174
 4 Beta(2) = 7.47062e-006
 5
 6

7 Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)	Beta(2)
Background	1	-0.67	0.59
Beta(1)	-0.67	1	-0.98
Beta(2)	0.59	-0.98	1

13 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0366063	*	*	*
Beta(1)	0.000757836	*	*	*
Beta(2)	7.6893e-006	*	*	*

21 * - Indicates that this value is not calculated.

22 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-67.3451	4			
Fitted model	-67.3733	3	0.056567	1	0.812
Reduced model	-95.7782	1	56.8663	3	<.0001
AIC:	140.747				

33 Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0366	1.830	2.000	50	0.128
11.0000	0.0455	2.275	2.000	50	-0.186
55.0000	0.0972	4.859	5.000	50	0.067
274.0000	0.5605	28.027	28.000	50	-0.008

42 Chi^2 = 0.06 d.f. = 1 P-value = 0.8135

45 Benchmark Dose Computation

47 Specified effect = 0.1
 48 Risk Type = Extra risk
 49 Confidence level = 0.95
 50 BMD = 77.7277
 51 BMDL = 35.4296
 52 BMDU = 118.349

54 Taken together, (35.4296, 118.349) is a 90% two-sided confidence interval for the BMD

56 Multistage Cancer Slope Factor = 0.0028225

D.5. FEMALE BDF1 MICE: HEPATIC CARCINOMAS AND ADENOMAS

1 Data for female BDF1 mouse hepatic carcinomas and adenomas are shown in Table D-
2 11. Note that the incidence of carcinomas and the incidence of either adenomas or carcinomas
3 are monotone non-decreasing functions of dose. These data therefore appear to be appropriate
4 for dose-response modeling using BMDS. However, the incidence of adenomas clearly reaches
5 a peak value at 66 mg/kg-day and then decreases sharply with increasing dose. This cannot be
6 modeled by a multistage model using only non-negative coefficients. To some extent the
7 incidence of “either adenomas or carcinomas” retains some of the inverted-U shaped dose-
8 response of the adenomas, which dominate based on their high incidence at the lowest dose
9 groups (66 and 278 mg/kg-day), thus is not well characterized by any multistage model.

Table D-11. Data for hepatic adenomas and carcinomas in female BDF1 mice
([Kano, et al., 2009](#))

Tumor type	Dose (mg/kg-day)			
	0	66	278	964
Hepatocellular adenomas	5	31	20	3
Hepatocellular carcinomas	0	6	30	45
Either adenomas or carcinomas	5	35	41	46
Neither adenomas nor carcinomas	45	15	9	4
Total number per group	50	50	50	50

Source: Used with permission from Elsevier, Ltd., Kano et al. ([2009](#)).

10 The results of the BMDS modeling for the entire suite of models for hepatic adenomas
11 and carcinomas in female BDF1 mice are presented in Table D-12. The multistage models did
12 not provide reasonable fits to the incidence data for hepatocellular adenoma or carcinoma in
13 female BDF1 mice. The log-logistic model provided the best-fit to the data as indicated by the
14 AIC and p -value as was chosen as the best-fitting model to carry forward in the analysis;
15 however, this model resulted in a $BMDL_{10}$ much lower than the response level at the lowest dose
16 in the study ([Kano, et al., 2009](#)). Thus, the log-logistic model was run for BMRs of 30 and 50%.
17 The output from these models are shown in Figures D-11 and D-12. A summary of the BMD
18 results for BMRs of 10, 30, and 50% are shown in Table D-13. Using a higher BMR resulted in
19 BMDLs closer to the lowest observed response data, and a BMR of 50% was chosen to carry
20 forward in the analysis.

21 The graphical output from fitting these models suggested that a simpler model obtained
22 by dropping the data point for the highest dose (964 mg/kg-day) might also be adequate. This
23 was tested and the results did not affect the choice of the model, nor significantly affect the
24 resulting BMDs and BMDLs.

Table D-12. BMDS dose-response modeling results for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice (Kano, et al., 2009)

1

Model	AIC	<i>p</i> -value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	203.331	0	26.43	19.50	-2.654	3.98	2.94
Logistic	214.951	0	58.05	44.44	3.201	8.74	6.69
LogLogistic ^b	176.214	0.1421	5.54	3.66	-0.121	0.83	0.55
LogProbit ^c	198.354	0	26.37	19.57	-1.166	3.97	2.95
Multistage-Cancer (1 degree)	203.331	0	26.43	19.50	-2.654	3.98	2.94
Multistage-Cancer (2 degree)	203.331	0	26.43	19.50	-2.654	3.98	2.94
Multistage-Cancer (3 degree)	203.331	0	26.43	19.50	-2.654	3.98	2.94
Probit	217.671	0	69.89	56.22	3.114	10.5	8.46
Weibull	203.331	0	26.43	19.50	-2.654	3.98	2.94
Quantal-Linear	203.331	0	26.43	19.50	-2.654	3.98	2.94
Dichotomous-Hill	7300.48	NC ^d	NC ^d	NC ^d	0	0	0

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bBest-fitting model, lowest AIC value.

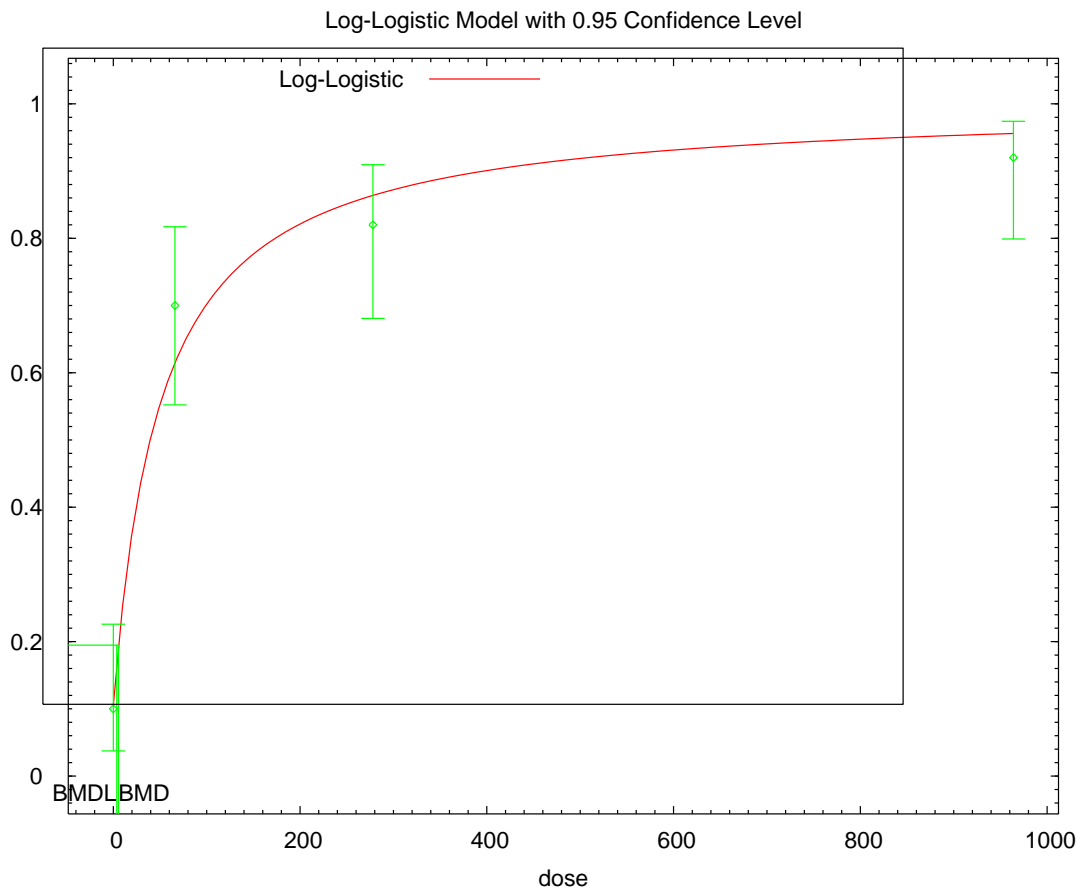
^cSlope restricted ≥ 1 .

^dValue unable to be calculated (NC: not calculated) by BMDS.

Table D-13. BMDS LogLogistic dose-response modeling results using BMRs of 10, 30, and 50% for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice (Kano, et al., 2009).

BMR	AIC	<i>p</i> -value	BMD mg/kg-day	BMDL mg/kg-day	χ^2 ^a	BMD _{HED} mg/kg-day	BMDL _{HED} mg/kg-day
10%	176.214	0.1421	5.54	3.66	-0.121	0.83	0.55
30%	176.214	0.1421	21.38	14.11	-0.121	3.22	2.12
50%	176.214	0.1421	49.88	32.93	0	7.51	4.95

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.



11:26 05/12 2010

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-10. LogLogistic BMD model for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice with a BMR of 10%.

```

1  =====
2  Logistic Model. (Version: 2.12; Date: 05/16/2008)
3  Input Data File:
4  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR10-
5  Restrict.(d)
6  Gnuplot Plotting File:
7  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR10-
8  Restrict.plt
9
10  Wed May 12 11:26:35 2010
11  =====
12  BMDS Model Run
13  ~~~~~
14  The form of the probability function is:
15  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
16
17  Dependent variable = Effect
18  Independent variable = Dose
19  Slope parameter is restricted as slope >= 1
20
21  Total number of observations = 4
22  Total number of records with missing values = 0
23  Maximum number of iterations = 250

```

1
2
3
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11
12
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Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.1
intercept = -4.33618
slope = 1

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

	background	intercept
background	1	-0.32
intercept	-0.32	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0.105265	*	*	*
intercept	-3.90961	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-84.3055	4			
Fitted model	-86.107	2	3.6029	2	0.1651
Reduced model	-131.248	1	93.8853	3	<.0001
AIC:	176.214				

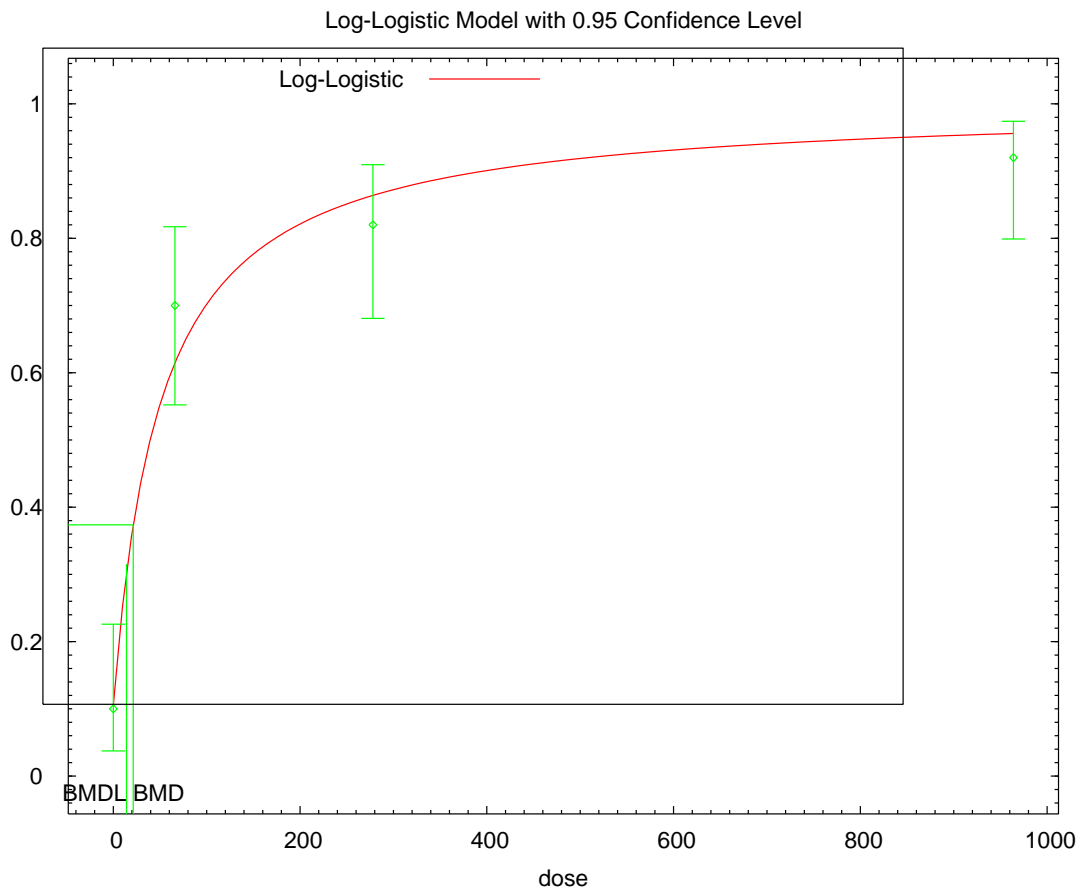
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1053	5.263	5.000	50	-0.121
66.0000	0.6149	30.743	35.000	50	1.237
278.0000	0.8639	43.194	41.000	50	-0.905
964.0000	0.9560	47.799	46.000	50	-1.240

Chi^2 = 3.90 d.f. = 2 P-value = 0.1421

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 5.54218
BMDL = 3.65848



11:26 05/12 2010

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-11. LogLogistic BMD model for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice with a BMR of 30%.

```

1  =====
2  Logistic Model. (Version: 2.12; Date: 05/16/2008)
3  Input Data File:
4  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR30-
5  Restrict.(d)
6  Gnuplot Plotting File:
7  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR30-
8  Restrict.plt
9
10                                     Wed May 12 11:26:36 2010
11  =====
12  BMDS Model Run
13  ~~~~~
14  The form of the probability function is:
15  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
16
17  Dependent variable = Effect
18  Independent variable = Dose
19  Slope parameter is restricted as slope >= 1
20
21  Total number of observations = 4
22  Total number of records with missing values = 0
23  Maximum number of iterations = 250
24  Relative Function Convergence has been set to: 1e-008
    Parameter Convergence has been set to: 1e-008
  
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User has chosen the log transformed model

Default Initial Parameter Values

background = 0.1
intercept = -4.33618
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	background	intercept
background	1	-0.32
intercept	-0.32	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0.105265	*	*	*
intercept	-3.90961	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-84.3055	4			
Fitted model	-86.107	2	3.6029	2	0.1651
Reduced model	-131.248	1	93.8853	3	<.0001
AIC:	176.214				

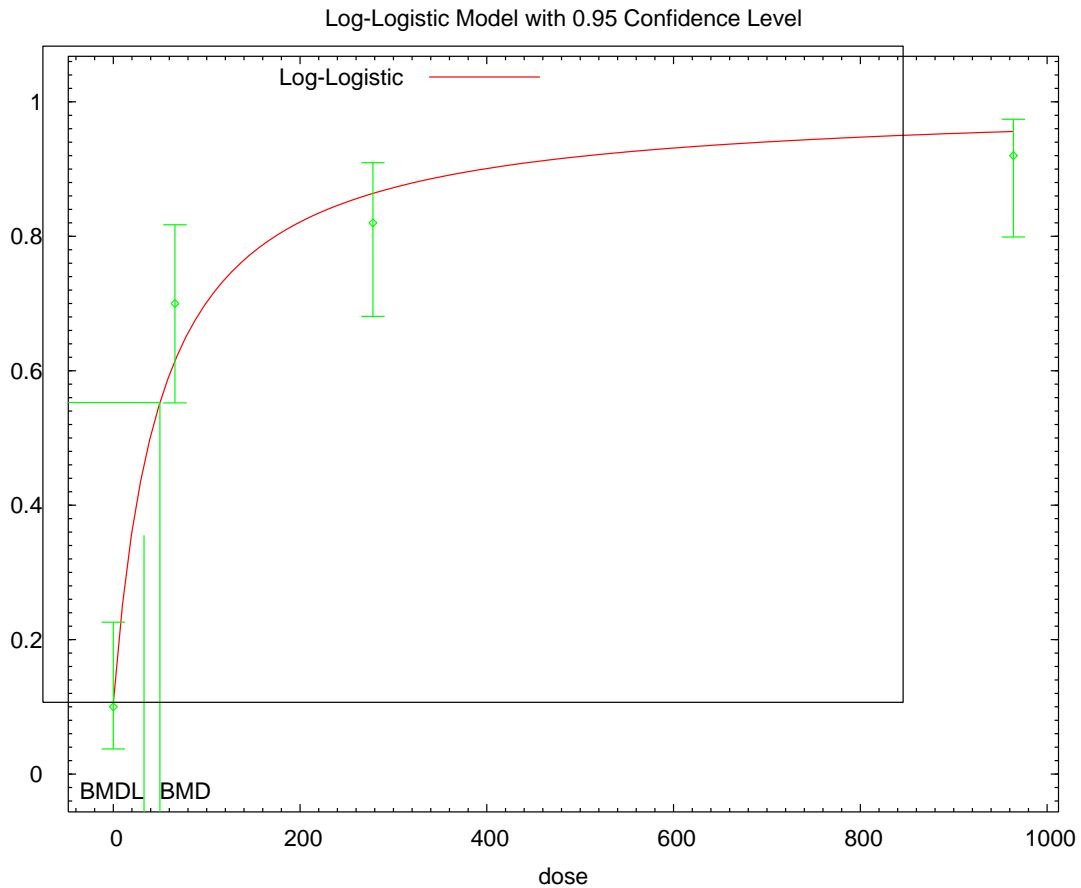
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1053	5.263	5.000	50	-0.121
66.0000	0.6149	30.743	35.000	50	1.237
278.0000	0.8639	43.194	41.000	50	-0.905
964.0000	0.9560	47.799	46.000	50	-1.240

Chi^2 = 3.90 d.f. = 2 P-value = 0.1421

Benchmark Dose Computation

Specified effect = 0.3
Risk Type = Extra risk
Confidence level = 0.95
BMD = 21.377
BMDL = 14.1113



11:26 05/12 2010

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-12. LogLogistic BMD model for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice with a BMR of 50%.

```

1  =====
2  Logistic Model. (Version: 2.12; Date: 05/16/2008)
3  Input Data File:
4  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR50-
5  Restrict.(d)
6  Gnuplot Plotting File:
7  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR50-
8  Restrict.plt
9
10                                     Wed May 12 11:26:36 2010
11  =====
12  BMDS Model Run
13  ~~~~~
14  The form of the probability function is:
15  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
16
17  Dependent variable = Effect
18  Independent variable = Dose
19  Slope parameter is restricted as slope >= 1
20
21  Total number of observations = 4
22  Total number of records with missing values = 0
23  Maximum number of iterations = 250

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Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.1
intercept = -4.33618
slope = 1

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

	background	intercept
background	1	-0.32
intercept	-0.32	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0.105265	*	*	*
intercept	-3.90961	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-84.3055	4			
Fitted model	-86.107	2	3.6029	2	0.1651
Reduced model	-131.248	1	93.8853	3	<.0001
AIC:	176.214				

Goodness of Fit

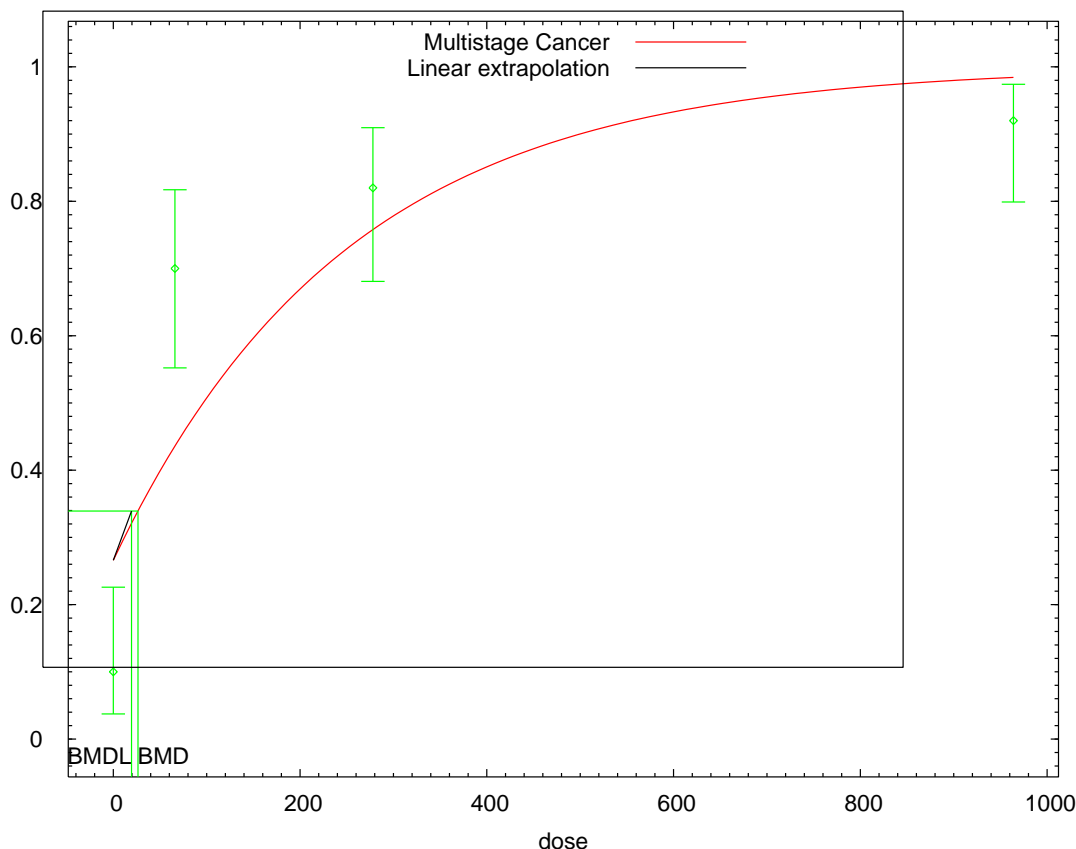
Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1053	5.263	5.000	50	-0.121
66.0000	0.6149	30.743	35.000	50	1.237
278.0000	0.8639	43.194	41.000	50	-0.905
964.0000	0.9560	47.799	46.000	50	-1.240

Chi^2 = 3.90 d.f. = 2 P-value = 0.1421

Benchmark Dose Computation

Specified effect = 0.5
Risk Type = Extra risk
Confidence level = 0.95
BMD = 49.8797
BMDL = 32.9263

Multistage Cancer Model with 0.95 Confidence Level



11:26 05/12 2010

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-13. Multistage BMD model (1 degree) for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice.

```

1 =====
2 Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3 Input Data File:
4 L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_fmouse_hepato_adcar_Msc-BMR10-1poly.(d)
5 Gnuplot Plotting File:
6 L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_fmouse_hepato_adcar_Msc-BMR10-1poly.plt
7                                     Wed May 12 11:26:31 2010
8 =====
9 BMSD Model Run
10 ~~~~~
11 The form of the probability function is:
12 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
13
14 The parameter betas are restricted to be positive
15
16 Dependent variable = Effect
17 Independent variable = Dose
18
19 Total number of observations = 4
20 Total number of records with missing values = 0
21 Total number of parameters in model = 2
22 Total number of specified parameters = 0
23 Degree of polynomial = 1
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Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

Background = 0.51713
Beta(1) = 0.00201669

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.65
Beta(1)	-0.65	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.265826	*	*	*
Beta(1)	0.00398627	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-84.3055	4			
Fitted model	-99.6653	2	30.7195	2	2.1346928e-007
Reduced model	-131.248	1	93.8853	3	<.0001

AIC: 203.331

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2658	13.291	5.000	50	-2.654
66.0000	0.4357	21.783	35.000	50	3.770
278.0000	0.7576	37.880	41.000	50	1.030
964.0000	0.9843	49.213	46.000	50	-3.651

Chi^2 = 35.65 d.f. = 2 P-value = 0.0000

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 26.4309
BMDL = 19.5045
BMDU = 37.5583

Taken together, (19.5045, 37.5583) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00512702

D.6. MALE BDF1 MICE: HEPATIC CARCINOMAS AND ADENOMAS

1 Data for hepatic carcinomas and adenomas in male BDF1 mice ([Kano, et al., 2009](#)) are
2 shown in Table D-14. Note that the incidence of carcinomas and the incidence of either
3 adenomas or carcinomas are monotone non-decreasing functions of dose. These data therefore
4 appear to be appropriate for dose-response modeling using BMDS. However, the incidence of
5 adenomas clearly reaches a peak value at 191 mg/kg-day and then decreases sharply with
6 increasing dose. This cannot be modeled by a multistage model using only non-negative
7 coefficients. To some extent the incidence of “either adenomas or carcinomas or both” retains
8 some of the inverted-U shaped dose-response of the adenomas, which dominate based on their
9 high incidence at the lowest dose groups (49 and 191 mg/kg-day), thus is not well characterized
10 by any multistage model.

Table D-14. Data for hepatic adenomas and carcinomas in male BDF1 mice
([Kano, et al., 2009](#))

Tumor type	Dose (mg/kg-day)			
	0	49	191	677
Hepatocellular adenomas	9	17	23	11
Hepatocellular carcinomas	15	20	23	36
Either adenomas or carcinomas	23	31	37	40
Neither adenomas nor carcinomas	27	19	13	10
Total number per group	50	50	50	50

Source: Used with permission from Elsevier, Ltd., Kano et al. ([2009](#)).

11 The results of the BMDS modeling for the entire suite of models for hepatic adenomas
12 and carcinomas in male BDF1 mice are presented in Table D-15.

Table D-15. BMDS dose-response modeling results for the combined incidence of hepatic adenomas and carcinomas in male BDF1 mice ([Kano, et al., 2009](#))

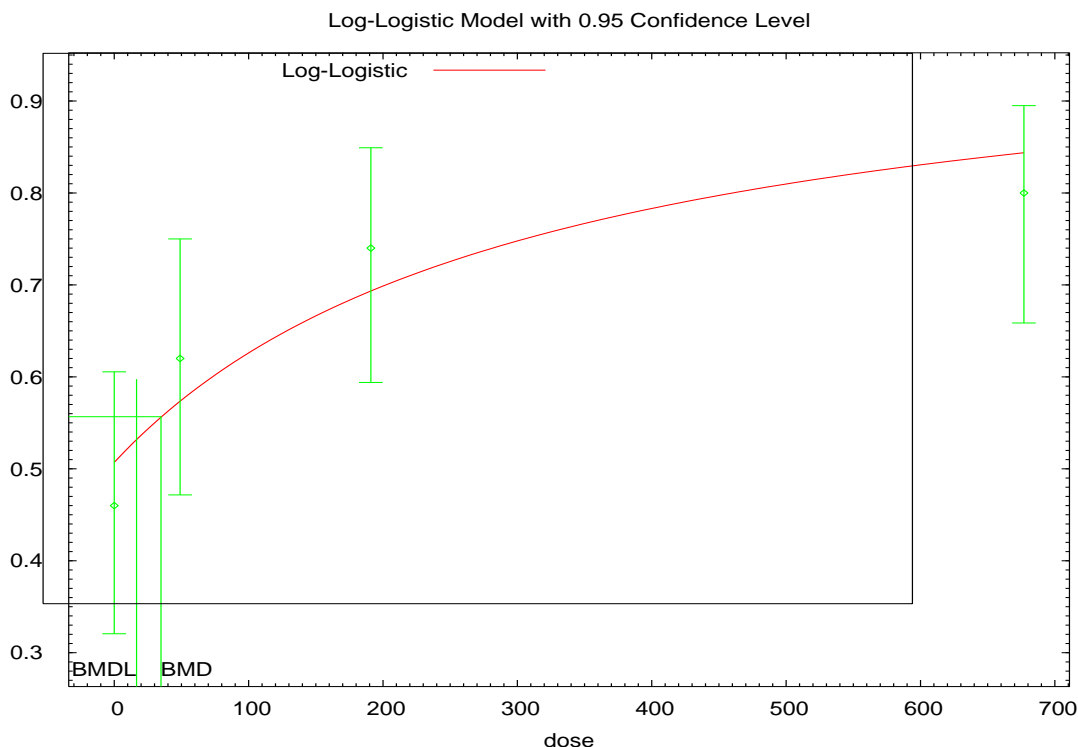
Model	AIC	<i>p</i> -value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	250.551	0.1527	70.99	44.00	0.605	11.48	7.12
Logistic	251.187	0.112	91.89	61.98	0.529	14.86	10.02
LogLogistic ^b	248.839	0.3461	34.78	16.60	0.656	5.63	2.68
LogProbit ^c	252.244	0.0655	133.53	78.18	0.016	21.60	12.64
Multistage-Cancer (1 degree)	250.551	0.1527	70.99	44.00	0.605	11.48	7.12
Multistage-Cancer (2 degree)	250.551	0.1527	70.99	44.00	0.605	11.48	7.12
Multistage-Cancer (3 degree)	250.551	0.1527	70.99	44.00	0.605	11.48	7.12
Probit	251.326	0.1048	97.01	67.36	0.518	15.69	10.90
Weibull	250.551	0.1527	70.99	44.00	0.605	11.48	7.12
Quantal-Linear	250.551	0.1527	70.99	44.00	0.605	11.48	7.12
Dichotomous-Hill	250.747	NC ^d	11.60	1.63	-1.25×10 ⁻⁵	1.88	0.26

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bBest-fitting model.

^cSlope restricted ≥ 1 .

^dValue unable to be calculated (NC: not calculated) by BMDS.



07:30 10/26 2009

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-14. LogLogistic BMD model for the combined incidence of hepatic adenomas and carcinomas in male BDF1 mice.

```

1  =====
2  Logistic Model. (Version: 2.12; Date: 05/16/2008)
3  Input Data File:
4  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_mmouse_hepato_adcar_Lnl-BMR10-
5  Restrict.(d)
6  Gnuplot Plotting File:
7  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_mmouse_hepato_adcar_Lnl-BMR10-
8  Restrict.plt
9  Thu Nov 12 09:09:36 2009
10 =====
11  BMDS Model Run
12  ~~~~~
13  The form of the probability function is:
14  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
15
16  Dependent variable = Effect
17  Independent variable = Dose
18  Slope parameter is restricted as slope >= 1
19
20  Total number of observations = 4
21  Total number of records with missing values = 0
22  Maximum number of iterations = 250
23  Relative Function Convergence has been set to: 1e-008
24  Parameter Convergence has been set to: 1e-008
25
26  User has chosen the log transformed model
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Default Initial Parameter Values

background = 0.46
intercept = -5.58909
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	background	intercept
background	1	-0.69
intercept	-0.69	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0.507468	*	*	*
intercept	-5.74623	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-121.373	4			
Fitted model	-122.419	2	2.09225	2	0.3513
Reduced model	-128.859	1	14.9718	3	0.001841

AIC: 248.839

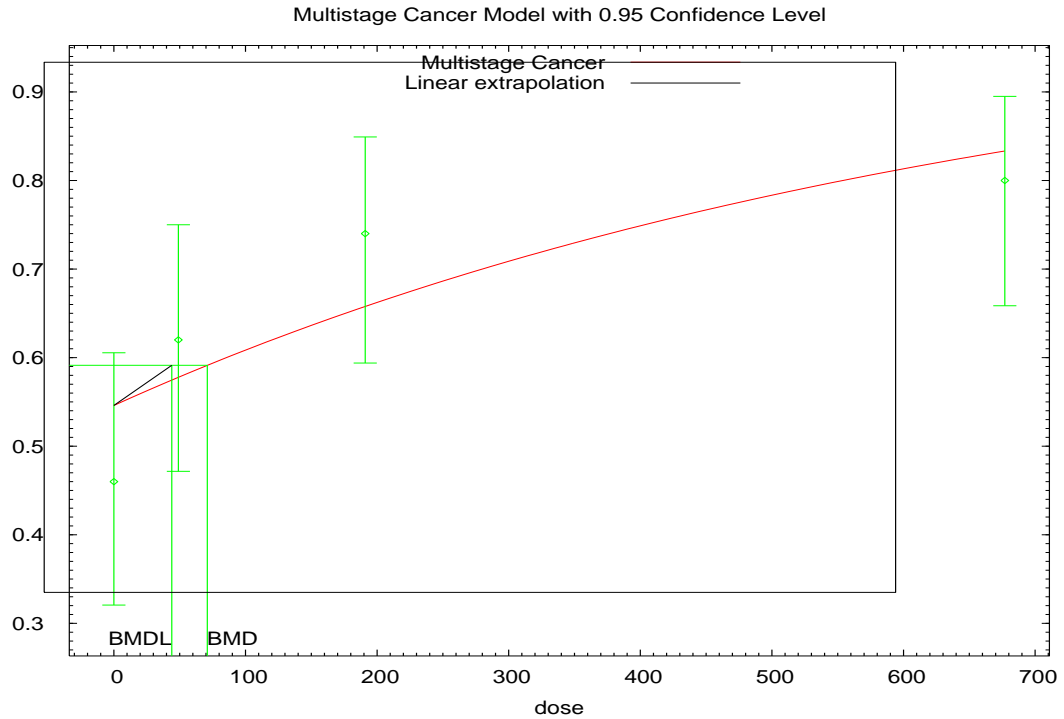
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.5075	25.373	23.000	50	-0.671
49.0000	0.5741	28.707	31.000	50	0.656
191.0000	0.6941	34.706	37.000	50	0.704
677.0000	0.8443	42.214	40.000	50	-0.863

Chi^2 = 2.12 d.f. = 2 P-value = 0.3461

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 34.7787
BMDL = 16.5976



07:30 10/26 2009

Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-15. Multistage BMD model (1 degree) for the combined incidence of hepatic adenomas and carcinomas in male BDF1 mice.

```

1 =====
2 Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3 Input Data File:
4 L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mmouse_hepato_adcar_Msc-BMR10-1poly.(d)
5 Gnuplot Plotting File:
6 L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mmouse_hepato_adcar_Msc-BMR10-1poly.plt
7 Mon Oct 26 08:30:50 2009
8 =====
9 BMDS Model Run
10 ~~~~~
11
12 The form of the probability function is:
13 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
14
15 The parameter betas are restricted to be positive
16
17 Dependent variable = Effect
18 Independent variable = Dose
19
20 Total number of observations = 4
21 Total number of records with missing values = 0
22 Total number of parameters in model = 2
23 Total number of specified parameters = 0
24 Degree of polynomial = 1
25
26 Maximum number of iterations = 250
27 Relative Function Convergence has been set to: 1e-008
28 Parameter Convergence has been set to: 1e-008
29

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Default Initial Parameter Values

Background = 0.573756
Beta(1) = 0.00123152

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.58
Beta(1)	-0.58	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.545889	*	*	*
Beta(1)	0.00148414	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-121.373	4			
Fitted model	-123.275	2	3.80413	2	0.1493
Reduced model	-128.859	1	14.9718	3	0.001841
AIC:	250.551				

Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.5459	27.294	23.000	50	-1.220
49.0000	0.5777	28.887	31.000	50	0.605
191.0000	0.6580	32.899	37.000	50	1.223
677.0000	0.8337	41.687	40.000	50	-0.641

Chi^2 = 3.76 d.f. = 2 P-value = 0.1527

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 70.9911
BMDL = 44.0047
BMDU = 150.117

Taken together, (44.0047, 150.117) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00227248

D.7. BMD MODELING RESULTS FROM ADDITIONAL CHRONIC BIOASSAYS

1 Data and BMDS modeling results for the additional chronic bioassays ([Kociba, et al.,](#)
 2 [1974](#); [NCI, 1978](#)) were evaluated for comparison with the Kano et al. ([2009](#)) study. These
 3 results are presented in the following sections.

4 The BMDS dose-response modeling estimates and HEDs that resulted are presented in
 5 detail in the following sections and a summary is provided in Table D-16.

Table D-16. Summary of BMDS dose-response modeling estimates associated with liver and nasal tumor incidence data resulting from chronic oral exposure to 1,4-dioxane in rats and mice

Endpoint	Model selection criterion	Model Type	AIC	p-value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Kociba et al., (1974) Male and Female (combined) Sherman Rats								
Hepatic Tumors ^a	Lowest AIC	Probit	84.3126	0.606	1113.94	920.62	290.78	240.31
Nasal Cavity Tumors ^b	Lowest AIC	Multistage (3 degree)	26.4156	0.9999	1717.16	1306.29	448.24	340.99
NCI, (1978) Female Osborne-Mendel Rats								
Hepatic Tumors ^c	Lowest AIC	LogLogistic	84.2821	0.7333	111.46	72.41	28.75	18.68
Nasal Cavity Tumors ^b	Lowest AIC	LogLogistic	84.2235	0.2486	155.32	100.08	40.07	25.82
NCI, (1978) Male Osborne-Mendel Rats								
Nasal Cavity Tumors ^b	Lowest AIC	LogLogistic	92.7669	0.7809	56.26	37.26	16.10	10.66
NCI, (1978) Female B6C3F ₁ Mice								
Hepatic Tumors ^d	Lowest AIC, Multistage model	Multistage (2 degree)	85.3511	1	160.68	67.76	23.12	9.75
NCI, (1978) Male B6C3F ₁ Mice								
Hepatic Tumors ^d	Lowest AIC	Gamma	177.539	0.7571	601.69	243.92	87.98	35.67

^aIncidence of hepatocellular carcinoma.

^bIncidence of nasal squamous cell carcinoma.

^cIncidence of hepatocellular adenoma.

^dIncidence of hepatocellular adenoma or carcinoma.

D.7.1. Hepatocellular Carcinoma and Nasal Squamous Cell Carcinoma ([Kociba, et al., 1974](#))

1 The incidence data for hepatocellular carcinoma and nasal squamous cell carcinoma are
2 presented in Table D-17. The predicted BMD_{10 HED} and BMDL_{10 HED} values are also presented in
3 Tables D-18 and D-19 for hepatocellular carcinomas and nasal squamous cell carcinomas,
4 respectively.

Table D-17. Incidence of hepatocellular carcinoma and nasal squamous cell carcinoma in male and female Sherman rats (combined) ([Kociba, et al., 1974](#)) treated with 1,4-dioxane in the drinking water for 2 years

Animal Dose (mg/kg-day) (average of male and female dose)	Incidence of hepatocellular carcinoma ^a	Incidence of nasal squamous cell carcinoma ^a
0	1/106 ^b	0/106 ^c
14	0/110	0/110
121	1/106	0/106
1307	10/66 ^d	3/66 ^d

^aRats surviving until 12 months on study.

^b $p < 0.001$; positive dose-related trend (Cochran-Armitage test).

^c $p < 0.01$; positive dose-related trend (Cochran-Armitage test).

^d $p < 0.001$; Fisher's Exact test.

Source: Used with permission from Elsevier, Ltd., Kociba et al. ([1974](#)).

Table D-18. BMDS dose-response modeling results for the incidence of hepatocellular carcinoma in male and female Sherman rats (combined) ([Kociba, et al., 1974](#)) exposed to 1,4-dioxane in the drinking water for 2 years

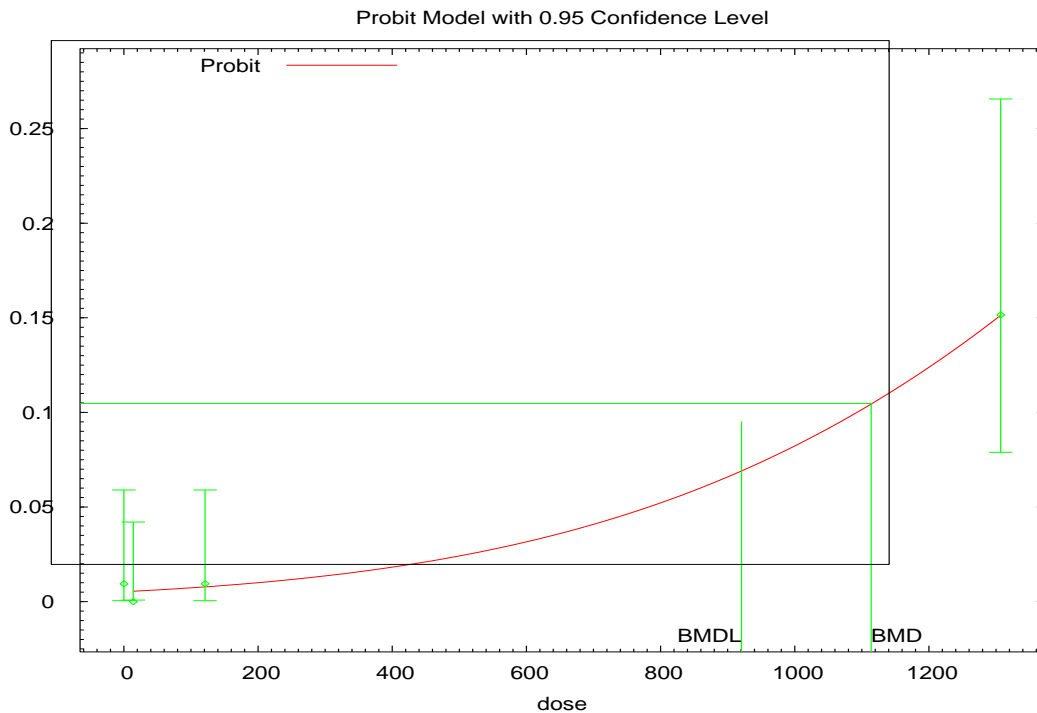
Model	AIC	<i>p</i> -value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	86.2403	0.3105	985.13	628.48	-0.005	257.15	164.05
Logistic	84.3292	0.6086	1148.65	980.95	-0.004	299.84	256.06
LogLogistic	86.2422	0.3103	985.62	611.14	-0.005	257.28	159.53
LogProbit ^b	84.4246	0.5977	1036.97	760.29	-0.011	270.68	198.46
Multistage-Cancer (1 degree)	85.1187	0.3838	940.12	583.58	0.279	245.40	152.33
Multistage-Cancer (2 degree)	86.2868	0.3109	1041.72	628.56	-0.006	271.92	164.07
Multistage-Cancer (3 degree)	86.2868	0.3109	1041.72	628.56	-0.006	271.92	164.08
Probit ^c	84.3126	0.606	1113.94	920.62	-0.005	290.78	240.31
Weibull	86.2443	0.3104	998.33	629.93	-0.005	260.60	164.43
Quantal-Linear	85.1187	0.3838	940.12	583.58	0.279	245.40	152.33
Dichotomous-Hill	1503.63	NC ^d	NC ^d	NC ^d	0	0	0

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bSlope restricted ≥ 1 .

^cBest-fitting model.

^dValue unable to be calculated (NC: not calculated) by BMDS.



11:54 10/27 2009

Source: Used with permission from Elsevier, Ltd., Kociba et al. (1974).

Figure D-16. Probit BMD model for the incidence of hepatocellular carcinoma in male and female Sherman rats exposed to 1,4-dioxane in drinking water.

```

1 =====
2 Probit Model. (Version: 3.1; Date: 05/16/2008)
3 Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kociba_mf_rat_hepato_car_Pr-
4 BMR10.(d)
5 Gnuplot Plotting File:
6 L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kociba_mf_rat_hepato_car_Pr-
7 BMR10.plt
8 Tue Oct 27 12:54:14 2009
9 =====
10 BMDS Model Run
11 ~~~~~
12 The form of the probability function is:
13 P[response] = CumNorm(Intercept+Slope*Dose),where CumNorm(.) is the cumulative normal
14 distribution function
15
16 Dependent variable = Effect
17 Independent variable = Dose
18 Slope parameter is not restricted
19
20 Total number of observations = 4
21 Total number of records with missing values = 0
22 Maximum number of iterations = 250
23 Relative Function Convergence has been set to: 1e-008
24 Parameter Convergence has been set to: 1e-008
25
26 Initial (and Specified) Parameter Values
27 background = 0 Specified
28 intercept = -2.62034

```

1 slope = 0.0012323
 2 Asymptotic Correlation Matrix of Parameter Estimates
 3 (***) The model parameter(s) -background have been estimated at a boundary point, or
 4 have been specified by the user, and do not appear in the correlation matrix)
 5

	intercept	slope
intercept	1	-0.82
slope	-0.82	1

10 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
intercept	-2.55961	0.261184	-3.07152	-2.0477
slope	0.00117105	0.000249508	0.000682022	0.00166008

19 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-39.3891	4			
Fitted model	-40.1563	2	1.53445	2	0.4643
Reduced model	-53.5257	1	28.2732	3	<.0001

26 AIC: 84.3126

29 Goodness of Fit

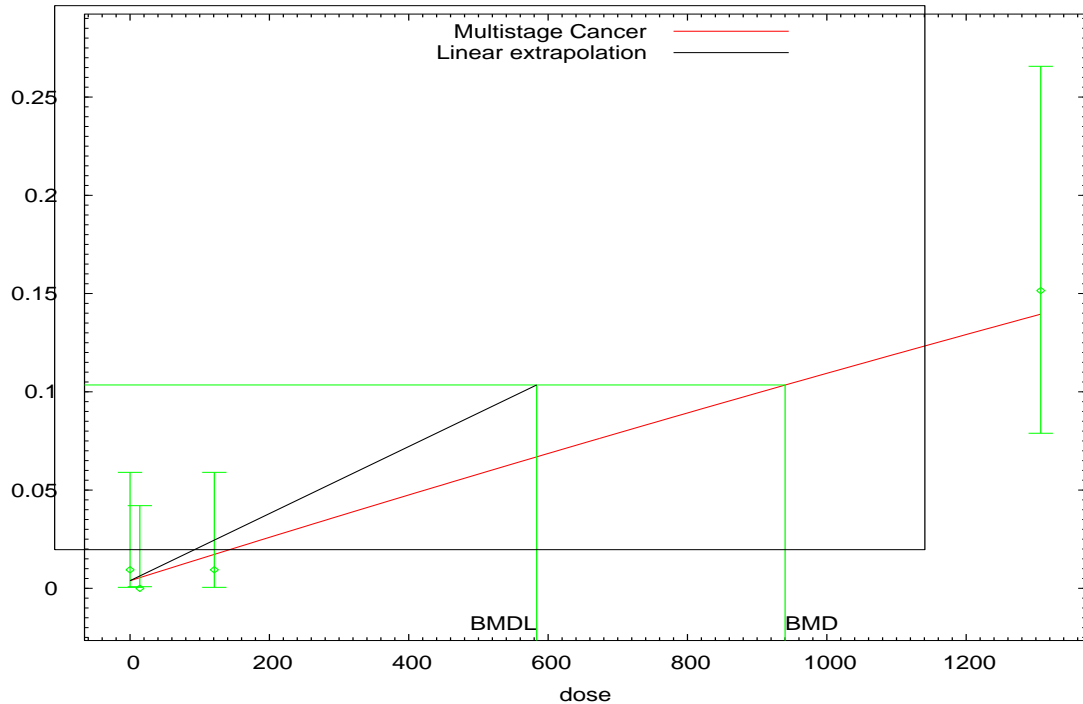
Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0052	0.555	1.000	106	0.598
14.0000	0.0055	0.604	0.000	110	-0.779
121.0000	0.0078	0.827	1.000	106	0.191
1307.0000	0.1517	10.014	10.000	66	-0.005

38 Chi^2 = 1.00 d.f. = 2 P-value = 0.6060

41 Benchmark Dose Computation

43 Specified effect = 0.1
 44 Risk Type = Extra risk
 45 Confidence level = 0.95
 46 BMD = 1113.94
 47 BMDL = 920.616

Multistage Cancer Model with 0.95 Confidence Level



11:54 10/27 2009

Source: Used with permission from Elsevier, Ltd., Kociba et al. (1974).

Figure D-17. Multistage BMD model (1 degree) for the incidence of hepatocellular carcinoma in male and female Sherman rats exposed to 1,4-dioxane in drinking water.

```

1 =====
2 Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3 Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kociba_mf_rat_hepato_car_Msc-
4 BMR10-1poly.(d)
5 Gnuplot Plotting File:
6 L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kociba_mf_rat_hepato_car_Msc-BMR10-1poly.plt
7 Tue Oct 27 12:54:10 2009
8 =====
9 BMDS Model Run
10 ~~~~~
11
12 The form of the probability function is:
13
14 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
15
16 The parameter betas are restricted to be positive
17
18 Dependent variable = Effect
19 Independent variable = Dose
20
21 Total number of observations = 4
22 total number of records with missing values = 0
23 Total number of parameters in model = 2
24 Total number of specified parameters = 0
25 Degree of polynomial = 1
26
27 Maximum number of iterations = 250
28 Relative Function Convergence has been set to: 1e-008
29 Parameter Convergence has been set to: 1e-008

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Default Initial Parameter Values

Background = 0.000925988
Beta(1) = 0.000124518

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.44
Beta(1)	-0.44	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0038683	*	*	*
Beta(1)	0.000112071	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-39.3891	4			
Fitted model	-40.5594	2	2.34056	2	0.3103
Reduced model	-53.5257	1	28.2732	3	<.0001
AIC:	85.1187				

Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0039	0.410	1.000	106	0.923
14.0000	0.0054	0.597	0.000	110	-0.775
121.0000	0.0173	1.832	1.000	106	-0.620
1307.0000	0.1396	9.213	10.000	66	0.279

Chi^2 = 1.92 d.f. = 2 P-value = 0.3838

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 940.124
BMDL = 583.576
BMDU = 1685.88

Taken together, (583.576, 1685.88) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000171357

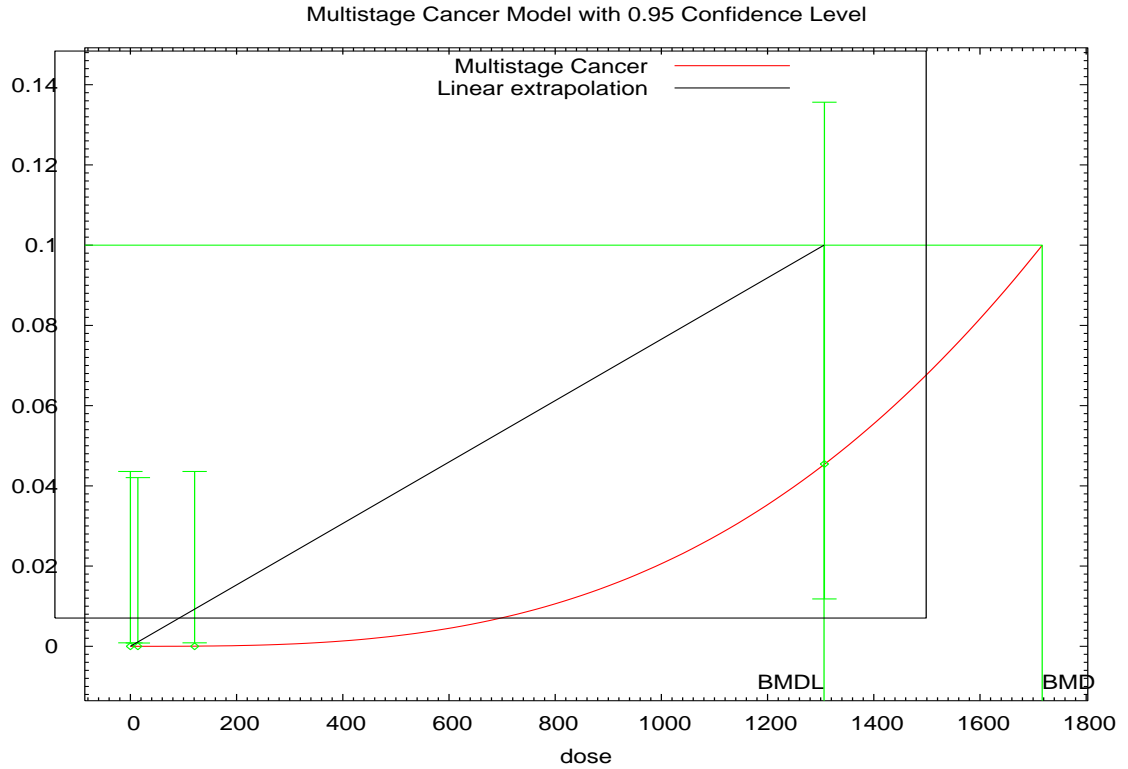
Table D-19. BMD5 dose-response modeling results for the incidence of nasal squamous cell carcinoma in male and female Sherman rats (combined) ([Kociba, et al., 1974](#)) exposed to 1,4-dioxane in the drinking water for 2 years

Model	AIC	<i>p</i> -value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	28.4078	1	1572.09	1305.86	0	410.37	340.87
Logistic	28.4078	1	1363.46	1306.67	0	355.91	341.09
LogLogistic	28.4078	1	1464.77	1306.06	0	382.35	340.93
LogProbit ^b	28.4078	1	1644.38	1305.49	0	429.24	340.78
Multistage-Cancer (1 degree)	27.3521	0.9163	3464.76	1525.36	0.272	904.42	398.17
Multistage-Cancer (2 degree)	26.4929	0.9977	1980.96	1314.37	0.025	517.10	343.10
Multistage-Cancer (3 degree) ^c	26.4156	0.9999	1717.16	1306.29	0.002	448.24	340.99
Probit	28.4078	1	1419.14	1306.44	0	370.44	341.03
Weibull	28.4078	1	1461.48	1306.11	0	381.50	340.94
Quantal-Linear	27.3521	0.9163	3464.76	1525.35	0.272	904.42	398.17
Dichotomous-Hill	30.4078	0.9997	1465.77	1319.19	5.53×10 ⁻⁷	382.62	344.35

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bSlope restricted ≥ 1 .

^cBest-fitting model.



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Figure D-18. Multistage BMD model (3 degree) for the incidence of nasal squamous cell carcinoma in male and female Sherman rats exposed to 1,4-dioxane in drinking water.

```

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3 =====
4 Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
5 Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kociba_mf_rat_nasal_car_Msc-
6 BMR10-3poly. (d)
7 Gnuplot Plotting File:
8 L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kociba_mf_rat_nasal_car_Msc-BMR10-3poly.plt
9 Tue Oct 27 07:25:02 2009
10 =====
11 BMDS Model Run
12 ~~~~~
13 The form of the probability function is:
14
15 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2-
16 beta3*dose^3)]
17
18 The parameter betas are restricted to be positive
19
20 Dependent variable = Effect
21 Independent variable = Dose
22
23 Total number of observations = 4
24 Total number of records with missing values = 0
25 Total number of parameters in model = 4
26 Total number of specified parameters = 0
27 Degree of polynomial = 3
28

```

```

1 Maximum number of iterations = 250
2 Relative Function Convergence has been set to: 1e-008
3 Parameter Convergence has been set to: 1e-008
4 Default Initial Parameter Values
5 Background = 0
6 Beta(1) = 0
7 Beta(2) = 0
8 Beta(3) = 2.08414e-011
9
10
11 Asymptotic Correlation Matrix of Parameter Estimates
12
13 (***) The model parameter(s) -Background -Beta(1) -Beta(2)
14 have been estimated at a boundary point, or have been specified by the user,
15 and do not appear in the correlation matrix )
16
17 Beta(3) Beta(3)
18 Beta(3) 1
19
20
21 Parameter Estimates
22
23 95.0% Wald Confidence Interval
24 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
25 Background 0 * * *
26 Beta(1) 0 * * *
27 Beta(2) 0 * * *
28 Beta(3) 2.08088e-011 * * *
29
30 * - Indicates that this value is not calculated.
31
32
33
34 Analysis of Deviance Table
35
36 Model Log(likelihood) # Param's Deviance Test d.f. P-value
37 Full model -12.2039 4
38 Fitted model -12.2078 1 0.00783284 3 0.9998
39 Reduced model -17.5756 1 10.7433 3 0.0132
40
41 AIC: 26.4156
42
43
44 Goodness of Fit
45
46 Dose Est._Prob. Expected Observed Size Scaled Residual
47 -----
48 0.0000 0.0000 0.000 0.000 106 0.000
49 14.0000 0.0000 0.000 0.000 110 -0.003
50 121.0000 0.0000 0.004 0.000 106 -0.063
51 1307.0000 0.0454 2.996 3.000 66 0.002
52
53 Chi^2 = 0.00 d.f. = 3 P-value = 0.9999
54
55
56 Benchmark Dose Computation
57
58 Specified effect = 0.1
59 Risk Type = Extra risk
60 Confidence level = 0.95
61 BMD = 1717.16
62 BMDL = 1306.29
63 BMDU = 8354.46
64
65 Taken together, (1306.29, 8354.46) is a 90% two-sided confidence interval for the BMD
66

```

D.7.2. Nasal Cavity Squamous Cell Carcinoma and Liver Hepatocellular Adenoma in Osborne-Mendel Rats (NCI, 1978)

2 The incidence data for hepatocellular adenoma (female rats) and nasal squamous cell
 3 carcinoma (male and female rats) are presented in Table D-20. The log-logistic model
 4 adequately fit both the male and female rat nasal squamous cell carcinoma data, as well as
 5 female hepatocellular adenoma incidence data. For all endpoints and genders evaluated in this
 6 section, compared to the multistage models, the log-logistic model had a higher *p*-value, as well
 7 as both a lower AIC and lower BMDL. The results of the BMDS modeling for the entire suite of
 8 models are presented in Tables D-21 through D-23.

Table D-20. Incidence of nasal cavity squamous cell carcinoma and hepatocellular adenoma in Osborne-Mendel rats (NCI, 1978) exposed to 1,4-dioxane in the drinking water

Male rat Animal Dose (mg/kg-day) ^a			
	0	240 ^b	530
Nasal cavity squamous cell carcinoma	0/33 ^c	12/26 ^d	16/33 ^d
Female rat Animal Dose (mg/kg-day) ^a			
	0	350	640
Nasal cavity squamous cell carcinoma	0/34 ^c	10/30 ^d	8/29 ^d
Hepatocellular adenoma	0/31 ^c	10/30 ^d	11/29 ^d

^aTumor incidence values were adjusted for mortality (NCI, 1978).

^bGroup not included in statistical analysis by NCI (1978) because the dose group was started a year earlier without appropriate controls.

^c*p* ≤ 0.001; positive dose-related trend (Cochran-Armitage test).

^d*p* ≤ 0.001; Fisher’s Exact test.

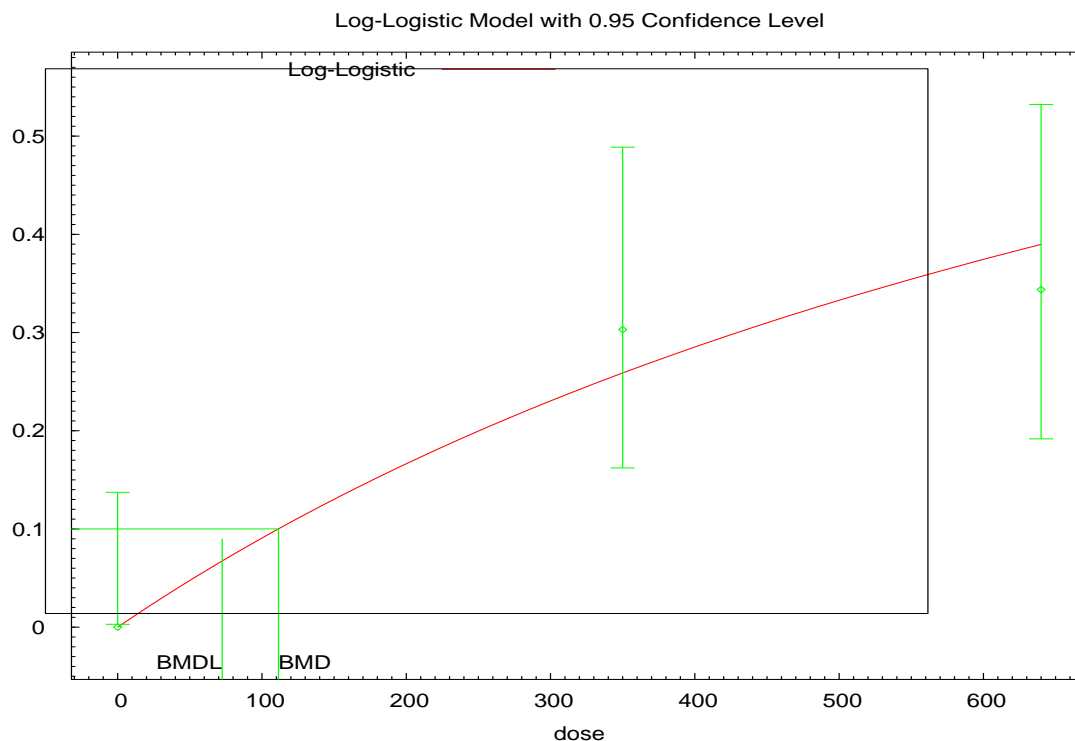
Source: NCI (1978).

Table D-21. BMDS dose-response modeling results for the incidence of hepatocellular adenoma in female Osborne-Mendel rats (NCL, 1978) exposed to 1,4-dioxane in the drinking water for 2 years

Model	AIC	<i>p</i> -value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	84.6972	0.5908	132.36	94.06	0	34.144	24.26
Logistic	92.477	0.02	284.09	220.46	1.727	73.29	56.87
LogLogistic ^b	84.2821	0.7333	111.46	72.41	0	28.75	18.68
LogProbit	85.957	0.3076	209.47	160.66	1.133	54.04	41.45
Multistage-Cancer (1 degree)	84.6972	0.5908	132.36	94.06	0	34.14	24.26
Multistage-Cancer (2 degree)	84.6972	0.5908	132.36	94.06	0	34.14	24.26
Probit	91.7318	0.0251	267.02	207.18	1.7	68.88	53.44
Weibull	84.6972	0.5908	132.36	94.06	0	34.14	24.26
Quantal-Linear	84.6972	0.5908	132.36	94.06	0	34.14	24.26

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bBest-fitting model.



06:32 10/27 2009

Source: NCI (1978).

Figure D-19. LogLogistic BMD model for the incidence of hepatocellular adenoma in female Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```

1  =====
2  Logistic Model. (Version: 2.12; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMSD\lnl_nci_frat_hepato_ad_Lnl-BMR10-
4  Restrict.(d)
5  Gnuplot Plotting File: L:\Priv\NCEA_HPAG\14Dioxane\BMSD\lnl_nci_frat_hepato_ad_Lnl-
6  BMR10-Restrict.plt
7  Tue Oct 27 07:32:13 2009
8  =====
9  BMSD Model Run
10 ~~~~~
11 The form of the probability function is:
12 P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
13
14 Dependent variable = Effect
15 Independent variable = Dose
16 Slope parameter is restricted as slope >= 1
17
18 Total number of observations = 3
19 Total number of records with missing values = 0
20 Maximum number of iterations = 250
21 Relative Function Convergence has been set to: 1e-008
22 Parameter Convergence has been set to: 1e-008
23
24 User has chosen the log transformed model
25
26 Default Initial Parameter Values
27 background = 0
28 intercept = -6.62889
29 slope = 1

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Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background -slope have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	intercept
intercept	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-6.91086	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-40.8343	3			
Fitted model	-41.141	1	0.613564	2	0.7358
Reduced model	-50.4308	1	19.1932	2	<.0001

AIC: 84.2821

Goodness of Fit

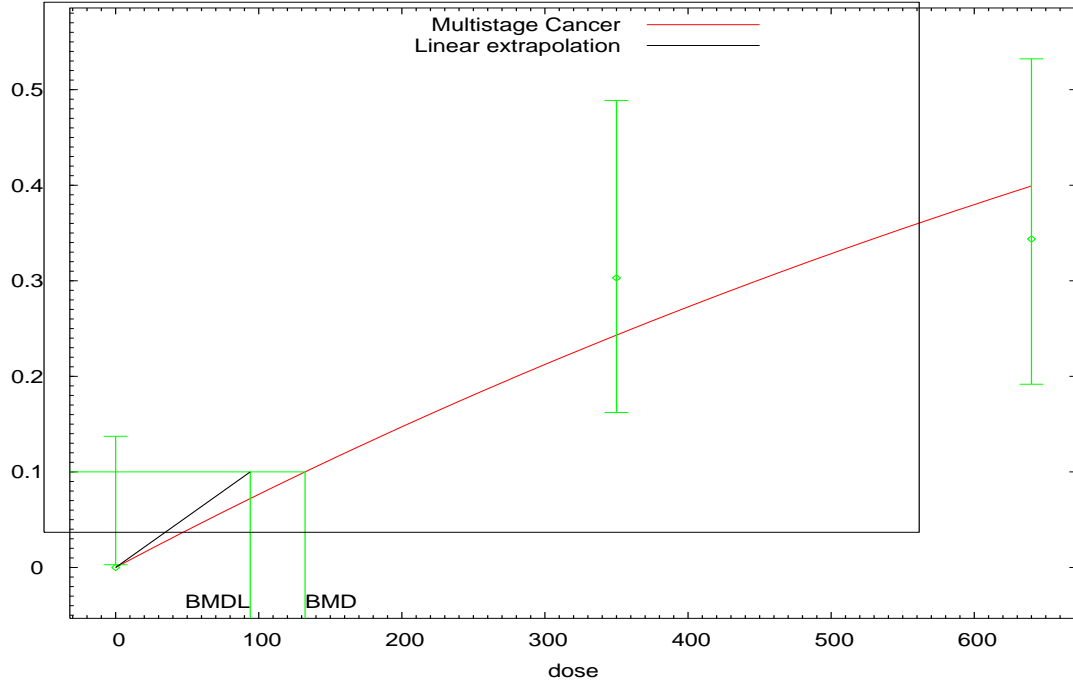
Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	31	0.000
350.0000	0.2587	8.536	10.000	33	0.582
640.0000	0.3895	12.464	11.000	32	-0.531

Chi^2 = 0.62 d.f. = 2 P-value = 0.7333

Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	111.457
BMDL =	72.4092

Multistage Cancer Model with 0.95 Confidence Level



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Source: NCI (1978).

Figure D-20. Multistage BMD model (1 degree) for the incidence of hepatocellular adenoma in female Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```

1  =====
2  Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_hepato_ad_Msc-BMR10-
4  lpoly.(d)
5  Gnuplot Plotting File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_hepato_ad_Msc-
6  BMR10-lpoly.plt
7  Tue Oct 27 07:32:16 2009
8  =====
9  BMDS Model Run
10 ~~~~~
11
12 The form of the probability function is:
13
14 P[response] = background + (1-background)*[1-EXP(-betal*dose^1)]
15
16 The parameter betas are restricted to be positive
17
18 Dependent variable = Effect
19 Independent variable = Dose
20
21 Total number of observations = 3
22 Total number of records with missing values = 0
23 Total number of parameters in model = 2
24 Total number of specified parameters = 0
25 Degree of polynomial = 1
26
27 Maximum number of iterations = 250
28 Relative Function Convergence has been set to: 1e-008
29 Parameter Convergence has been set to: 1e-008
30

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Default Initial Parameter Values

Background = 0.0385912

Beta(1) = 0.000670869

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00079602	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-40.8343	3			
Fitted model	-41.3486	1	1.02868	2	0.5979
Reduced model	-50.4308	1	19.1932	2	<.0001

AIC: 84.6972

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	31	0.000
350.0000	0.2432	8.024	10.000	33	0.802
640.0000	0.3992	12.774	11.000	32	-0.640

Chi^2 = 1.05 d.f. = 2 P-value = 0.5908

Benchmark Dose Computation

Specified effect = 0.1
 Risk Type = Extra risk
 Confidence level = 0.95
 BMD = 132.359
 BMDL = 94.0591
 BMDU = 194.33

Taken together, (94.0591, 194.33) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00106316

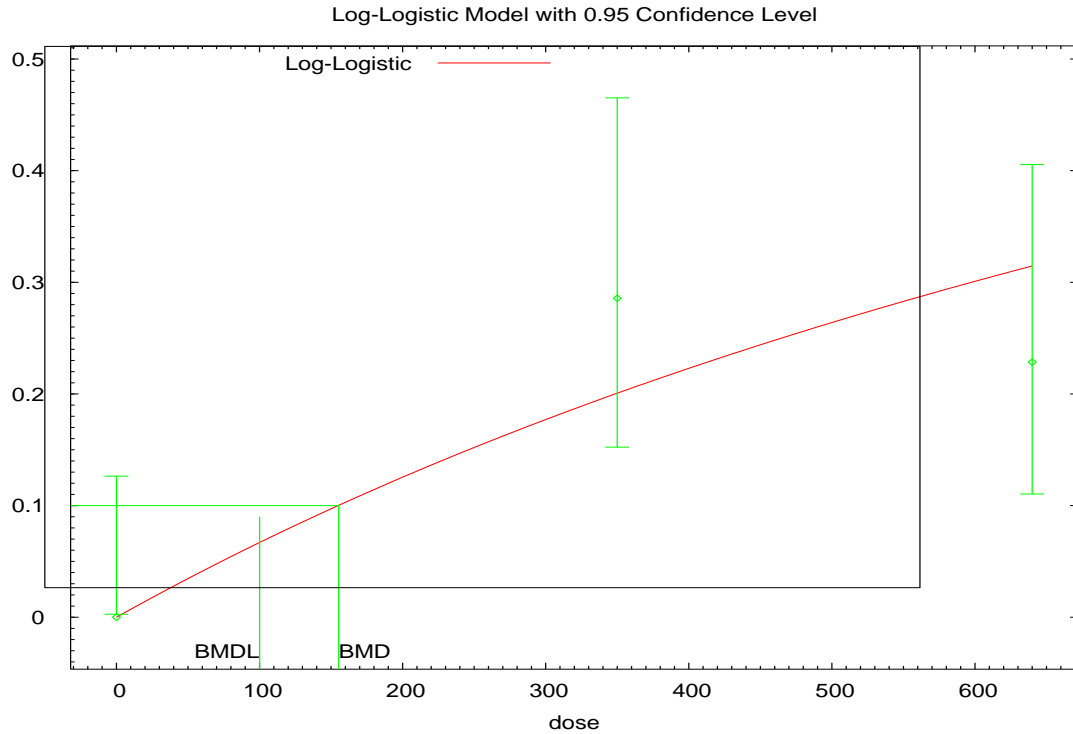
Table D-22. BMDS dose-response modeling results for the incidence of nasal cavity squamous cell carcinoma in female Osborne-Mendel rats ([NCI, 1978](#)) exposed to 1,4-dioxane in the drinking water for 2 years

Model	AIC	<i>p</i>-value	BMD₁₀ mg/kg-day	BMDL₁₀ mg/kg-day	χ^2^a	BMD_{10 HED} mg/kg-day	BMDL_{10 HED} mg/kg-day
Gamma	84.7996	0.1795	176.28	122.27	1.466	45.47	31.54
Logistic	92.569	0.0056	351.51	268.75	2.148	90.68	69.33
LogLogistic ^b	84.2235	0.2486	155.32	100.08	0	40.07	25.82
LogProbit ^c	87.3162	0.0473	254.73	195.76	1.871	65.71	50.50
Multistage-Cancer (1 degree)	84.7996	0.1795	176.28	122.27	1.466	45.47	31.54
Multistage-Cancer (2 degree)	84.7996	0.1795	176.28	122.27	1.466	45.47	31.54
Probit	91.9909	0.0064	328.46	251.31	2.136	84.73	64.83
Weibull	84.7996	0.1795	176.28	122.27	1.466	45.47	31.54
Quantal-Linear	84.7996	0.1795	176.28	122.27	1.466	45.47	31.54

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bBest-fitting model.

^cSlope restricted ≥ 1 .



06:30 10/27 2009

Source: NCI (1978).

Figure D-21. LogLogistic BMD model for the incidence of nasal cavity squamous cell carcinoma in female Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```

1  =====
2  Logistic Model. (Version: 2.12; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMSD\lnl_nci_frat_nasal_car_Lnl-BMR10-
4  Restrict.(d)
5  Gnuplot Plotting File: L:\Priv\NCEA_HPAG\14Dioxane\BMSD\lnl_nci_frat_nasal_car_Lnl-
6  BMR10-Restrict.plt
7  Tue Oct 27 07:30:09 2009
8  =====
9  BMSD Model Run
10 ~~~~~
11
12 The form of the probability function is:
13
14 P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
15
16
17 Dependent variable = Effect
18 Independent variable = Dose
19 Slope parameter is restricted as slope >= 1
20
21 Total number of observations = 3
22 Total number of records with missing values = 0
23 Maximum number of iterations = 250
24 Relative Function Convergence has been set to: 1e-008
25 Parameter Convergence has been set to: 1e-008
26
27

```

```

1 User has chosen the log transformed model
2
3
4 Default Initial Parameter Values
5 background = 0
6 intercept = -6.64005
7 slope = 1
8
9
10 Asymptotic Correlation Matrix of Parameter Estimates
11 (***) The model parameter(s) -background -slope have been estimated at a boundary
12 point, or have been specified by the user, and do not appear in the correlation
13 matrix)
14
15         intercept
16 intercept      1
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
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55

```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-7.24274	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-39.7535	3			
Fitted model	-41.1117	1	2.71651	2	0.2571
Reduced model	-47.9161	1	16.3252	2	0.0002851
AIC:	84.2235				

Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	34	0.000
350.0000	0.2002	7.008	10.000	35	1.264
640.0000	0.3140	10.992	8.000	35	-1.090

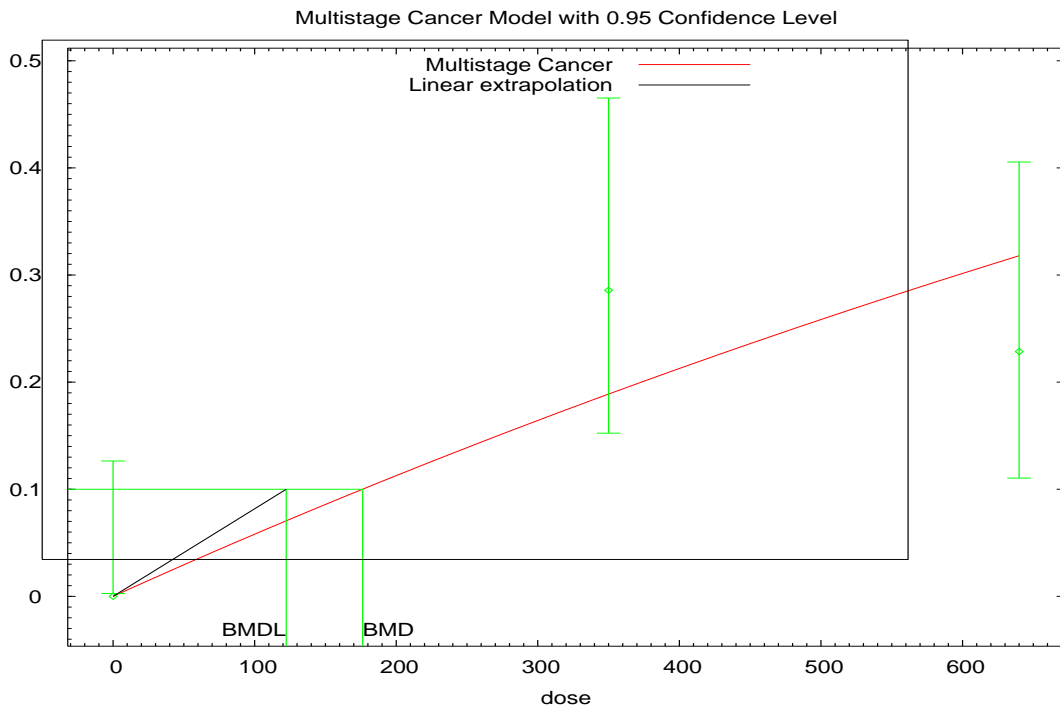
Chi^2 = 2.78 d.f. = 2 P-value = 0.2486

Benchmark Dose Computation

```

51 Specified effect = 0.1
52 Risk Type = Extra risk
53 Confidence level = 0.95
54 BMD = 155.324
55 BMDL = 100.081

```



06:30 10/27 2009

Source: NCI (1978).

Figure D-22. Multistage BMD model (1 degree) for the incidence of nasal cavity squamous cell carcinoma in female Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```

1 =====
2 Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3 Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_nasal_car_Msc-BMR10-
4 lpoly.(d)
5 Gnuplot Plotting File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_nasal_car_Msc-
6 BMR10-lpoly.plt
7 Tue Oct 27 07:30:12 2009
8 =====
9 BMDS Model Run
10 ~~~~~
11 The form of the probability function is:
12 P[response] = background + (1-background)*[1-EXP(-betal*dose^1)]
13
14 The parameter betas are restricted to be positive
15
16 Dependent variable = Effect
17 Independent variable = Dose
18
19 Total number of observations = 3
20 Total number of records with missing values = 0
21 Total number of parameters in model = 2
22 Total number of specified parameters = 0
23 Degree of polynomial = 1
24
25 Maximum number of iterations = 250
26 Relative Function Convergence has been set to: 1e-008
27 Parameter Convergence has been set to: 1e-008
28

```

```

1  Default Initial Parameter Values
2  Background =      0.0569154
3  Beta(1) =        0.00042443
4
5  Asymptotic Correlation Matrix of Parameter Estimates
6  (** The model parameter(s) -Background have been estimated at a boundary point, or
7  have been specified by the user, and do not appear in the correlation matrix)
8
9
10     Beta(1)
11     Beta(1)      1
12
13     Parameter Estimates
14
15     Variable      Estimate      Std. Err.      95.0% Wald Confidence Interval
16     Background      0          *          Lower Conf. Limit      Upper Conf. Limit
17     Beta(1)        0.000597685      *          *          *
18
19 * - Indicates that this value is not calculated.
20
21     Analysis of Deviance Table
22
23     Model      Log(likelihood)  # Param's  Deviance  Test d.f.  P-value
24     Full model      -39.7535      3
25     Fitted model    -41.3998      1      3.29259      2      0.1928
26     Reduced model   -47.9161      1      16.3252      2      0.0002851
27
28     AIC:      84.7996
29
30     Goodness of Fit
31
32     Dose      Est._Prob.      Expected      Observed      Size      Scaled
33     -----      -----      -----      -----      -----      -----
34     0.0000      0.0000      0.000      0.000      34      0.000
35     350.0000      0.1888      6.607      10.000      35      1.466
36     640.0000      0.3179      11.125      8.000      35      -1.134
37
38     Chi^2 = 3.44      d.f. = 2      P-value = 0.1795
39
40     Benchmark Dose Computation
41     Specified effect =      0.1
42     Risk Type =      Extra risk
43     Confidence level =      0.95
44     BMD =      176.281
45     BMDL =      122.274
46     BMDU =      271.474
47
48     Taken together, (122.274, 271.474) is a 90% two-sided confidence interval for the BMD
49
50     Multistage Cancer Slope Factor =      0.000817837

```

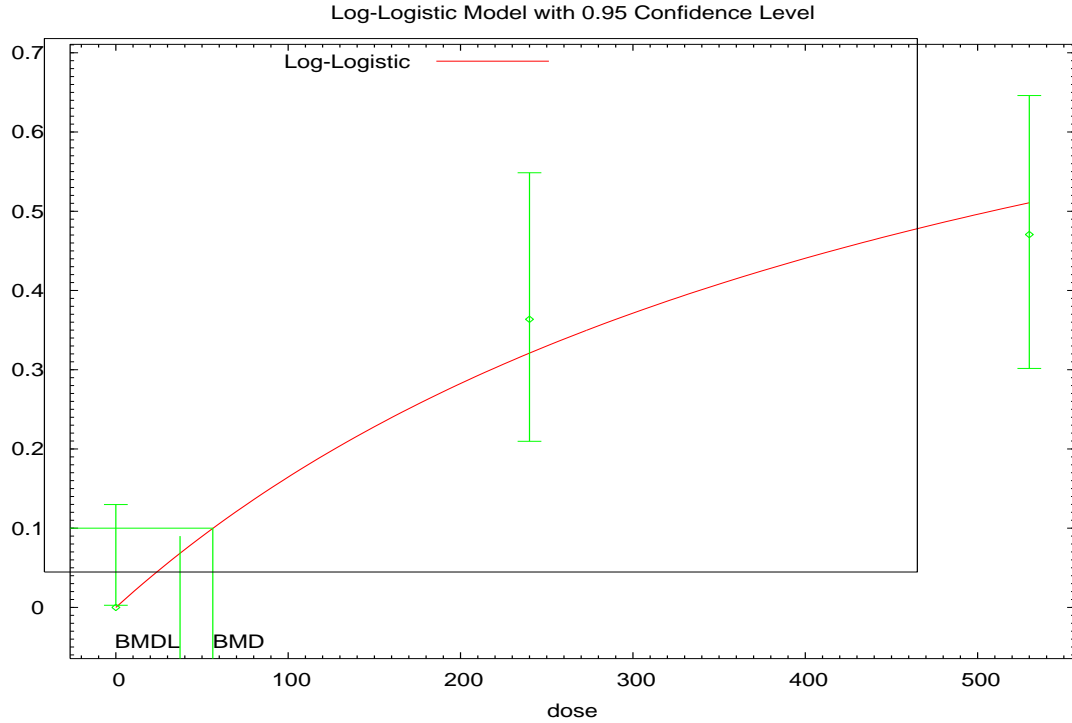
Table D-23. BMDS dose-response modeling results for the incidence of nasal cavity squamous cell carcinoma in male Osborne-Mendel rats ([NCL, 1978](#)) exposed to 1,4-dioxane in the drinking water for 2 years

Model	AIC	<i>p</i> -value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	93.6005	0.5063	73.94	54.724	0	21.17	15.66
Logistic	103.928	0.0061	179.05	139.26	2.024	51.25	39.86
LogLogistic ^b	92.7669	0.7809	56.26	37.26	0	16.10	10.66
LogProbit ^c	95.0436	0.2373	123.87	95.82	1.246	35.46	27.43
Multistage-Cancer (1 degree)	93.6005	0.5063	73.94	54.72	0	21.16	15.66
Multistage-Cancer (2 degree)	93.6005	0.5063	73.94	54.72	0	21.16	15.66
Probit	103.061	0.0078	168.03	131.61	2.024	48.10	37.67
Weibull	93.6005	0.5063	73.94	54.72	0	21.17	15.66
Quantal-Linear	93.6005	0.5063	73.94	54.72	0	21.17	15.66

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bBest-fitting model.

^cSlope restricted ≥ 1 .



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Source: NCI (1978).

Figure D-23. LogLogistic BMD model for the incidence of nasal cavity squamous cell carcinoma in male Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```

1  =====
2  Logistic Model. (Version: 2.12; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_mrat_nasal_car_Lnl-BMR10-
4  Restrict.(d)
5  Gnuplot Plotting File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_mrat_nasal_car_Lnl-
6  BMR10-Restrict.plt
7  Tue Oct 27 07:27:57 2009
8  =====
9  BMDS Model Run
10 ~~~~~
11
12 The form of the probability function is:
13 P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
14
15 Dependent variable = Effect
16 Independent variable = Dose
17 Slope parameter is restricted as slope >= 1
18
19 Total number of observations = 3
20 Total number of records with missing values = 0
21 Maximum number of iterations = 250
22 Relative Function Convergence has been set to: 1e-008
23 Parameter Convergence has been set to: 1e-008
24
25 User has chosen the log transformed model
26

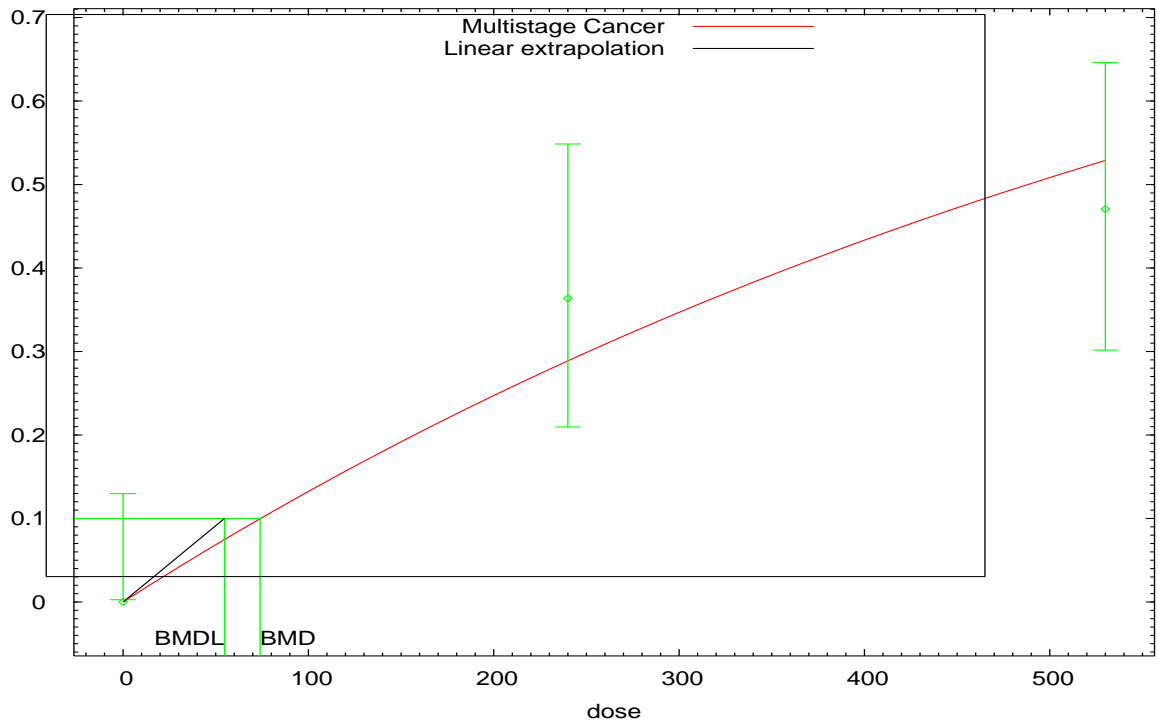
```

```

1  Default Initial Parameter Values
2  background =      0
3  intercept =     -6.08408
4  slope =          1
5
6  Asymptotic Correlation Matrix of Parameter Estimates
7  (***) The model parameter(s) -background -slope have been estimated at a boundary
8  point, or have been specified by the user, and do not appear in the correlation
9  matrix)
10
11      intercept
12  intercept      1
13
14      Parameter Estimates
15
16      95.0% Wald Confidence Interval
17  Variable      Estimate      Std. Err.      Lower Conf. Limit      Upper Conf. Limit
18  background      0          *          *          *
19  intercept     -6.2272      *          *          *
20  slope          1          *          *          *
21
22  * - Indicates that this value is not calculated.
23
24      Analysis of Deviance Table
25
26      Model      Log(likelihood)  # Param's  Deviance  Test d.f.  P-value
27      Full model      -45.139      3
28      Fitted model    -45.3835      1      0.488858      2      0.7832
29      Reduced model   -59.2953      1      28.3126      2      <.0001
30
31      AIC:          92.7669
32
33      Goodness of Fit
34
35      Dose      Est._Prob.      Expected      Observed      Size      Scaled
36      -----
37      0.0000      0.0000      0.000      0.000      33      0.000
38      240.0000      0.3216      10.612      12.000      33      0.517
39      530.0000      0.5114      17.388      16.000      34      -0.476
40
41      Chi^2 = 0.49      d.f. = 2      P-value = 0.7809
42      Benchmark Dose Computation
43
44      Specified effect =      0.1
45      Risk Type =      Extra risk
46      Confidence level =      0.95
47      BMD =      56.2596
48      BMDL =      37.256

```


Multistage Cancer Model with 0.95 Confidence Level



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Source: NCI (1978).

Figure D-24. Multistage BMD model (1 degree) for the incidence of nasal cavity squamous cell carcinoma in male Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```

1  =====
2  Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_mrat_nasal_car_Msc-BMR10-
4  lpoly.(d)
5  Gnuplot Plotting File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_mrat_nasal_car_Msc-
6  BMR10-lpoly.plt
7
8  Tue Oct 27 07:28:00 2009
9  =====
10 BMS Model Run
11 ~~~~~
12 The form of the probability function is:
13 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
14
15 The parameter betas are restricted to be positive
16
17 Dependent variable = Effect
18 Independent variable = Dose
19
20 Total number of observations = 3
21 Total number of records with missing values = 0
22 Total number of parameters in model = 2
23 Total number of specified parameters = 0
24 Degree of polynomial = 1
25
26 Maximum number of iterations = 250
27 Relative Function Convergence has been set to: 1e-008
28 Parameter Convergence has been set to: 1e-008
29 Default Initial Parameter Values

```

```

1 Background = 0.0578996
2 Beta(1) = 0.00118058
3
4 Asymptotic Correlation Matrix of Parameter Estimates
5 (** The model parameter(s) -Background have been estimated at a boundary point, or
6 have been specified by the user, and do not appear in the correlation matrix)
7
8
9
10
11
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41
42
43
44
45
46
47
48
49

```

Background = 0.0578996
Beta(1) = 0.00118058

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

Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00142499	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-45.139	3			
Fitted model	-45.8002	1	1.32238	2	0.5162
Reduced model	-59.2953	1	28.3126	2	<.0001

AIC: 93.6005

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	33	-0.000
240.0000	0.2896	9.558	12.000	33	0.937
530.0000	0.5301	18.024	16.000	34	-0.695

Chi^2 = 1.36 d.f. = 2 P-value = 0.5063

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 73.9379
BMDL = 54.7238
BMDU = 103.07

Taken together, (54.7238, 103.07) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00182736

D.7.3. Hepatocellular Adenoma or Carcinoma in B6C3F₁ Mice (NCI, 1978)

1 The incidence data for hepatocellular adenoma or carcinoma in male and female
2 mice are presented in Table D-24. The 2-degree polynomial model (betas restricted ≥ 0)
3 was the lowest degree polynomial that provided an adequate fit to the female mouse data
4 (Figure D-25), while the gamma model provided the best fit to the male mouse data
5 (Figure D-26). The results of the BMDS modeling for the entire suite of models are
6 presented in Tables D-25 and D-26 for the female and male data, respectively.

Table D-24. Incidence of hepatocellular adenoma or carcinoma in male and female B6C3F₁ mice (NCI, 1978) exposed to 1,4-dioxane in drinking water

Male mouse Animal Dose (mg/kg-day) ^a			Female mouse Animal Dose (mg/kg-day) ^a		
0	720	830	0	380	860
8/49 ^b	19/50 ^d	28/47 ^c	0/50 ^b	21/48 ^c	35/37 ^c

^aTumor incidence values were not adjusted for mortality.

^b $p < 0.001$, positive dose-related trend (Cochran-Armitage test).

^c $p < 0.001$ by Fisher's Exact test pair-wise comparison with controls.

^d $p = 0.014$.

Source: NCI (1978).

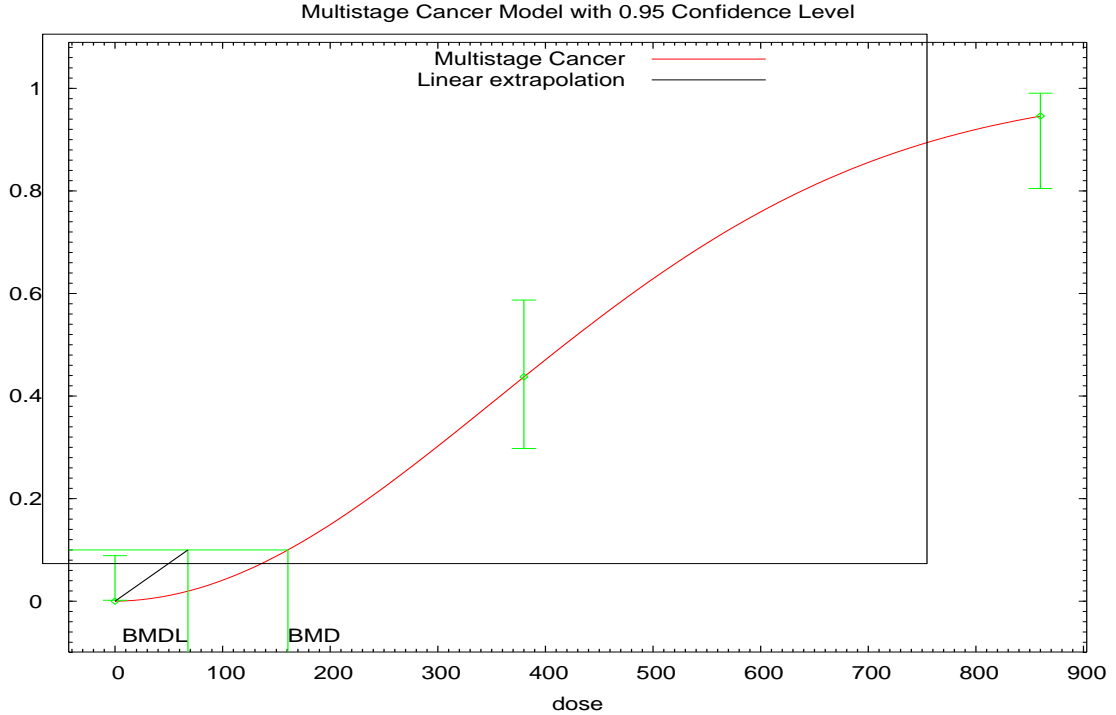
Table D-25. BMDS dose-response modeling results for the combined incidence of hepatocellular adenoma or carcinoma in female B6C3F₁ mice (NCL, 1978) exposed to 1,4-dioxane in the drinking water for 2 years

Model	AIC	<i>p</i> -value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma	85.3511	1	195.69	105.54	0	28.16	15.19
Logistic	89.1965	0.0935	199.63	151.35	0.675	28.72	21.78
LogLogistic	85.3511	1	228.08	151.16	0	32.82	21.75
LogProbit ^b	85.3511	1	225.8	150.91	0	32.49	21.71
Multistage-Cancer (1 degree)	89.986	0.0548	49.10	38.80	0	7.06	5.58
Multistage-Cancer (2 degree) ^c	85.3511	1	160.68	67.76	0	23.12	9.75
Probit	88.718	0.1165	188.24	141.49	-1.031	27.08	20.36
Weibull	85.3511	1	161.77	89.27	0	23.28	12.84
Quantal-Linear	89.986	0.0548	49.10	38.80	0	7.065	5.58

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bSlope restricted ≥ 1 .

^cBest-fitting model.



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Source: NCI (1978).

Figure D-25. Multistage BMD model (2 degree) for the incidence of hepatocellular adenoma or carcinoma in female B6C3F₁ mice exposed to 1,4-dioxane in drinking water.

```

1  =====
2  Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_fmouse_hepato_adcar_Msc-
4  BMR10-2poly.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_fmouse_hepato_adcar_Msc-BMR10-2poly.plt
7  Tue Oct 27 07:36:26 2009
8  =====
9  BMD5 Model Run
10 ~~~~~
11
12 The form of the probability function is:
13 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2)]
14
15 The parameter betas are restricted to be positive
16
17 Dependent variable = Effect
18 Independent variable = Dose
19
20 Total number of observations = 3
21 Total number of records with missing values = 0
22 Total number of parameters in model = 3
23 Total number of specified parameters = 0
24 Degree of polynomial = 2
25
26
27 Maximum number of iterations = 250
28 Relative Function Convergence has been set to: 1e-008

```

```

1 Parameter Convergence has been set to: 1e-008
2
3 Default Initial Parameter Values
4 Background = 0
5 Beta(1) = 2.68591e-005
6 Beta(2) = 3.91383e-006
7
8
9 Asymptotic Correlation Matrix of Parameter Estimates
10 (** The model parameter(s) -Background have been estimated at a boundary point, or
11 have been specified by the user, and do not appear in the correlation matrix)
12
13 Beta(1) Beta(2)
14 Beta(1) 1 -0.92
15 Beta(2) -0.92 1
16
17
18 Parameter Estimates
19
20 95.0% Wald Confidence Interval
21 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
22 Background 0 * * *
23 Beta(1) 2.686e-005 * * *
24 Beta(2) 3.91382e-006 * * *
25
26 * - Indicates that this value is not calculated.
27
28
29 Analysis of Deviance Table
30
31 Model Log(likelihood) # Param's Deviance Test d.f. P-value
32 Full model -40.6756 3
33 Fitted model -40.6756 2 3.20014e-010 1 1
34 Reduced model -91.606 1 101.861 2 <.0001
35
36 AIC: 85.3511
37
38 Goodness of Fit
39
40 Dose Est. Prob. Expected Observed Size Scaled Residual
41 -----
42 0.0000 0.0000 0.000 0.000 50 0.000
43 380.0000 0.4375 21.000 21.000 48 0.000
44 860.0000 0.9459 35.000 35.000 37 0.000
45
46 Chi^2 = 0.00 d.f. = 1 P-value = 1.0000
47
48
49 Benchmark Dose Computation
50 Specified effect = 0.1
51 Risk Type = Extra risk
52 Confidence level = 0.95
53 BMD = 160.678
54 BMDL = 67.7635
55 BMDU = 186.587
56
57 Taken together, (67.7635, 186.587) is a 90% two-sided confidence interval for the BMD
58
59 Multistage Cancer Slope Factor = 0.00147572

```

Table D-26. BMDS dose-response modeling results for the combined incidence of hepatocellular adenoma or carcinoma in male B6C3F₁ mice (NCI, 1978) exposed to 1,4-dioxane in drinking water

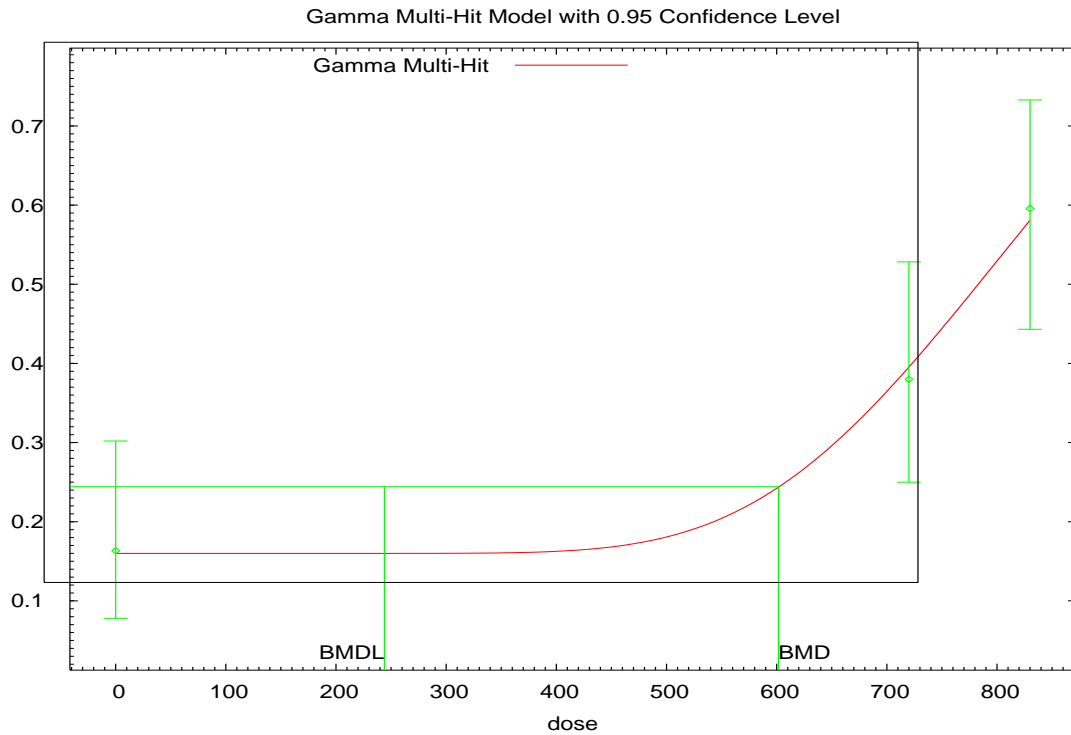
Model	AIC	<i>p</i> -value	BMD ₁₀ mg/kg-day	BMDL ₁₀ mg/kg-day	χ^2 ^a	BMD _{10 HED} mg/kg-day	BMDL _{10 HED} mg/kg-day
Gamma ^b	177.539	0.7571	601.69	243.92	-0.233	87.98	35.67
Logistic	179.9	0.1189	252.66	207.15	0.214	36.94	30.29
LogLogistic	179.443	NC ^c	622.39	283.04	0	91.01	41.39
LogProbit ^d	179.443	NC ^c	631.51	305.44	0	92.34	44.66
Multistage-Cancer (1 degree)	180.618	0.0762	164.29	117.37	0.079	24.02	17.16
Multistage-Cancer (2 degree)	179.483	0.1554	354.41	126.24	0.124	51.82	18.46
Probit	179.984	0.1128	239.93	196.90	0.191	35.08	28.79
Weibull	179.443	NC ^c	608.81	249.71	0	89.02	36.51
Quantal-Linear	180.618	0.0762	164.29	117.37	0.079	24.02	17.16

^aMaximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

^bBest-fitting model.

^cValue unable to be calculated (NC: not calculated) by BMDS.

^dSlope restricted ≥ 1 .



06:34 10/27 2009

Source: NCI (1978).

Figure D-26. Gamma BMD model for the incidence of hepatocellular adenoma or carcinoma in male B6C3F₁ mice exposed to 1,4-dioxane in drinking water.

```

1  =====
2  Gamma Model. (Version: 2.13; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\gam_nci_mmouse_hepato_adcar_Gam-
4  BMR10-Restrict.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\gam_nci_mmouse_hepato_adcar_Gam-BMR10-Restrict.plt
7  Tue Oct 27 07:34:35 2009
8  =====
9  BMDS Model Run
10 ~~~~~
11
12 The form of the probability function is:
13 P[response]= background+(1-background)*CumGamma[slope*dose,power],
14 where CumGamma(.) is the cummulative Gamma distribution function
15
16 Dependent variable = Effect
17 Independent variable = Dose
18 Power parameter is restricted as power >=1
19
20 Total number of observations = 3
21 Total number of records with missing values = 0
22 Maximum number of iterations = 250
23 Relative Function Convergence has been set to: 1e-008
24 Parameter Convergence has been set to: 1e-008
25
26 Default Initial (and Specified) Parameter Values
27 Background = 0.17
28 Slope = 0.000671886
29 Power = 1.3
30

```


1 Asymptotic Correlation Matrix of Parameter Estimates
 2 (***) The model parameter(s) -Power have been estimated at a boundary point, or have
 3 been specified by the user, and do not appear in the correlation matrix)
 4

	Background	Slope
Background	1	-0.52
Slope	-0.52	1

9 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.160326	0.0510618	0.060247	0.260405
Slope	0.0213093	0.000971596	0.019405	0.0232136
Power	18	NA		

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

19 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-86.7213	3			
Fitted model	-86.7693	2	0.096042	1	0.7566
Reduced model	-96.715	1	19.9875	2	<.0001
AIC:	177.539				

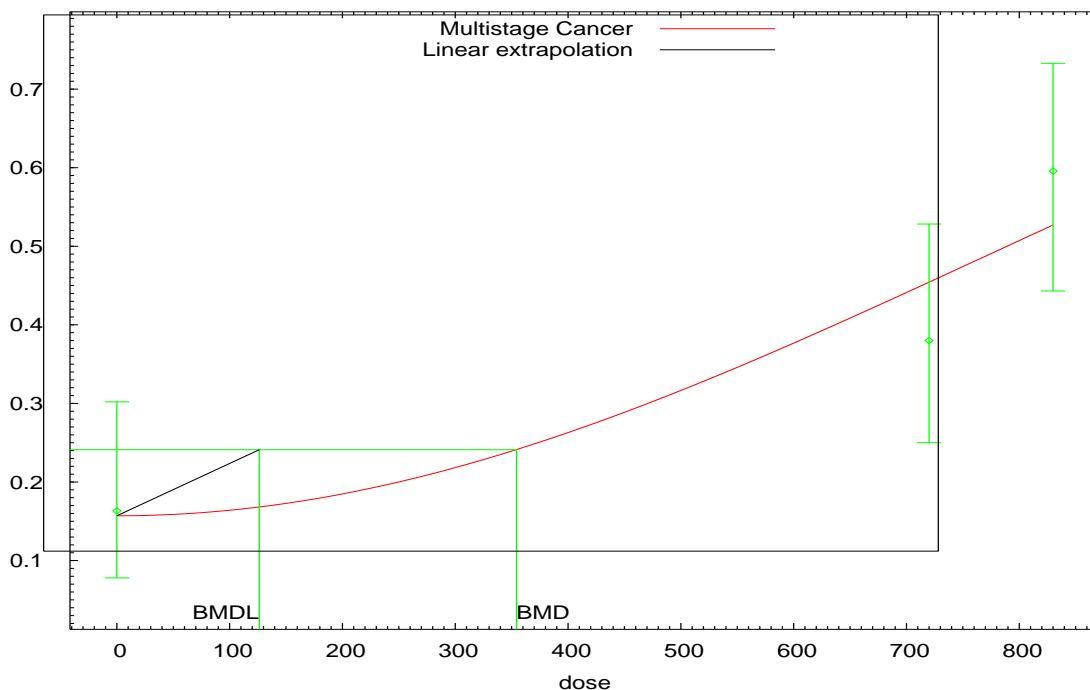
28 Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1603	7.856	8.000	49	0.056
720.0000	0.3961	19.806	19.000	50	-0.233
830.0000	0.5817	27.339	28.000	47	0.196

36 Chi^2 = 0.10 d.f. = 1 P-value = 0.7571

37 Benchmark Dose Computation
 38 Specified effect = 0.1
 39 Risk Type = Extra risk
 40 Confidence level = 0.95
 41 BMD = 601.692
 42 BMDL = 243.917

Multistage Cancer Model with 0.95 Confidence Level



06:34 10/27 2009

Source: NCI (1978).

Figure D-27. Multistage BMD model (2 degree) for the incidence of hepatocellular adenoma or carcinoma in male B6C3F₁ mice exposed to 1,4-dioxane in drinking water.

```

1  =====
2  Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3  Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_mmouse_hepato_adcar_Msc-
4  BMR10-2poly.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_mmouse_hepato_adcar_Msc-BMR10-2poly.plt
7  Tue Oct 27 07:34:42 2009
8  =====
9  BMDS Model Run
10 ~~~~~
11
12 The form of the probability function is: P[response] = background + (1-background)*[1-
13 EXP(-beta1*dose^1-beta2*dose^2)]
14
15 The parameter betas are restricted to be positive
16
17 Dependent variable = Effect
18 Independent variable = Dose
19
20 Total number of observations = 3
21 Total number of records with missing values = 0
22 Total number of parameters in model = 3
23 Total number of specified parameters = 0
24 Degree of polynomial = 2
25 Maximum number of iterations = 250
26 Relative Function Convergence has been set to: 1e-008
27 Parameter Convergence has been set to: 1e-008

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Default Initial Parameter Values

Background = 0.131156
Beta(1) = 0
Beta(2) = 9.44437e-007

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1) have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.72
Beta(2)	-0.72	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.1568	*	*	*
Beta(1)	0	*	*	*
Beta(2)	8.38821e-007	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-86.7213	3			
Fitted model	-87.7413	2	2.04001	1	0.1532
Reduced model	-96.715	1	19.9875	2	<.0001
AIC:	179.483				

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1568	7.683	8.000	49	0.124
720.0000	0.4541	22.707	19.000	50	-1.053
830.0000	0.5269	24.764	28.000	47	0.946

Chi^2 = 2.02 d.f. = 1 P-value = 0.1554

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 354.409
BMDL = 126.241
BMDU = 447.476

Taken together, (126.241, 447.476) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000792138

APPENDIX E. COMPARISON OF SEVERAL DATA REPORTS FOR THE JBRC 2-YEAR 1,4-DIOXANE DRINKING WATER STUDY

1 As described in detail in Section 4.2.1.2.6 of this *Toxicological Review of 1,4-Dioxane*,
2 the JBRC conducted a 2-year drinking water study on the effects of 1,4-dioxane in both sexes of
3 rats and mice. The results from this study have been reported three times, once as conference
4 proceedings ([Yamazaki, et al., 1994](#)), once as a detailed laboratory report ([JBRC, 1998](#)), and
5 once as a published manuscript ([Kano, et al., 2009](#)). After the External Peer Review draft of the
6 *Toxicological Review of 1,4-Dioxane* ([U.S. EPA, 2009b](#)) had been released, the Kano et al.
7 ([2009](#)) manuscript was published; thus, minor changes to the *Toxicological Review of*
8 *1,4-Dioxane* occurred.

9 The purpose of this appendix is to provide a clear and transparent comparison of the
10 reporting of this 2-year 1,4-dioxane drinking water study. The variations included: (1) the level
11 of detail on dose information reported; (2) categories for incidence data reported (e.g., all
12 animals or sacrificed animals); and (3) analysis of non- and neoplastic lesions. Even though the
13 data contained in the reports varied, the differences were minor and did not significantly
14 affect the qualitative or quantitative cancer assessment.

15 Tables contained within this appendix provide a comparison of the variations in the
16 reported data ([JBRC, 1998](#); [Kano, et al., 2009](#); [Yamazaki, et al., 1994](#)). Tables E-1 and E-2
17 show the histological nonneoplastic findings provided for male and female F344 rats,
18 respectively. Tables E-3 and E-4 show the histological neoplastic findings provided for male
19 and female F344 rats, respectively. Tables E-5 and E-6 show the histological nonneoplastic
20 findings provided for male and female F344 rats, respectively. Tables E-7 and E-8 show the
21 histological neoplastic findings provided for male and female Crj:BDF1 mice, respectively.

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#)

Table E-1. Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male F344 rats

		Yamazaki et al. (1994) ^a				JBRC (1998)				Kano et al. (2009)			
		Drinking water concentration (ppm)											
		0	200	1,000	5,000	0	200	1,000	5,000	0	200	1,000	5,000
		Calculated Dose (Intake [mg/kg-day]) ^{b,c}											
		Not reported				Control (0)	8-24 (16)	41-121 (81)	209-586 (398)	0	11±1	55±3	274±18
Nasal respiratory epithelium; nuclear enlargement	All animals	Not reported				0/50	0/50	0/50	26/50	0/50	0/50	0/50	26/50 ^e
	Sacrificed animals	Not reported				0/40	0/45	0/35	12/22 ^e	Not reported			
Nasal respiratory epithelium; squamous cell metaplasia	All animals	0/50	0/50	0/50	31/50	0/50	0/50	0/50	31/50	0/50	0/50	0/50	31/50 ^e
	Sacrificed animals	Not reported				0/40	0/45	0/35	15/22 ^e	Not reported			
Nasal respiratory epithelium; squamous cell hyperplasia	All animals	0/50	0/50	0/50	2/50	0/50	0/50	0/50	2/50	0/50	0/50	0/50	2/50
	Sacrificed animals	Not reported				0/40	0/45	0/35	1/22	Not reported			
Nasal gland; proliferation	All animals	0/50	0/50	0/50	5/50	Not reported				Not reported			
	Sacrificed animals	Not reported				Not reported				Not reported			
Nasal olfactory epithelium; nuclear enlargement	All animals	Not reported				0/50	0/50	5/50	38/50	0/50	0/50	5/50	38/50 ^e
	Sacrificed animals	Not reported				0/40	0/45	4/35	20/22 ^e	Not reported			
Nasal olfactory epithelium; respiratory metaplasia	All animals	Not reported				12/50	11/50	20/50	43/50	Not reported			
	Sacrificed animals	Not reported				10/40	11/45	17/35	22/22 ^e	Not reported			
Nasal olfactory epithelium; atrophy	All animals	Not reported				0/50	0/50	0/50	36/50	Not reported			
	Sacrificed animals	Not reported				0/40	0/45	0/35	17/22 ^e	Not reported			
Lamina propria; hydropic change	All animals	Not reported				0/50	0/50	0/50	46/50	Not reported			
	Sacrificed animals	Not reported				0/40	0/45	0/35	20/22 ^e	Not reported			
Lamina propria; sclerosis	All animals	Not reported				0/50	0/50	1/50	44/50	Not reported			
	Sacrificed animals	Not reported				0/40	0/45	1/35	20/22 ^e	Not reported			
Nasal cavity; adhesion	All animals	Not reported				0/50	0/50	0/50	48/50	Not reported			
	Sacrificed animals	Not reported				0/40	0/45	0/35	21/22 ^e	Not reported			
Nasal cavity; inflammation	All animals	Not reported				0/50	0/50	0/50	13/50	Not reported			
	Sacrificed animals	Not reported				0/40	0/45	0/35	7/22 ^e	Not reported			
Hyperplasia; liver	All animals	3/50	2/10	10/50	24/50	3/50	2/50	10/50	24/50	Not reported			
	Sacrificed animals	Not reported				3/40	2/45	9/35 ^f	12/22 ^e	Not reported			

		Yamazaki et al. (1994) ^a	JBRC (1998)				Kano et al. (2009)			
Spongiosis hepatitis; liver	All animals	12/50 20/50 25/50 40/50	12/50	20/50	25/50	40/50	Not reported			
	Sacrificed animals	Not reported	12/40	20/45	21/35 ^f	21/22 ^e	Not reported			
Clear cell foci; liver	All animals	Not reported	3/50	3/50	9/50	8/50	3/50	3/50	9/50	8/50
	Sacrificed animals	Not reported	3/40	3/45	9/35 ^f	7/22 ^e	Not reported			
Acidophilic cell foci; liver	All animals	Not reported	Not reported				12/50	8/50	7/50	5/50
	Sacrificed animals	Not reported	Not reported				Not reported			
Basophilic cell foci; liver	All animals	Not reported	7/50	11/50	6/50	16/50	7/50	11/50	8/50	16/50 ^f
	Sacrificed animals	Not reported	7/40	11/45	6/35	8/22 ^f	Not reported			
Mixed-cell foci; liver	All animals	Not reported	2/50	8/50	14/50	13/50	2/50	8/50	14/50 ^e	13/50 ^e
	Sacrificed animals	Not reported	2/40	8/45	14/35 ^e	22/22 ^e	Not reported			
Nuclear enlargement; kidney proximal tubule	All animals	Not reported	0/50	0/50	0/50	50/50	Not reported			
	Sacrificed animals	Not reported	0/40	0/45	0/35	22/22 ^e	Not reported			

^aDose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.

^bJBRC (1998) reported an estimated chemical intake range (of doses) for the animals; and the midpoint of the range (shown in parentheses) was used in the external peer review draft of this document (U.S. EPA, 2009b).

^cKano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010).

^dJBRC did not report statistical significance for the “All animals” comparison.

^ep ≤ 0.01 by χ^2 test.

^fp ≤ 0.05 by χ^2 test.

Table E-2. Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female F344 rats

		Yamazaki et al. (1994) ^a	JBRC (1998)				Kano et al. (2009)						
		Drinking water concentration (ppm)											
		0	200	1,000	5,000	0	200	1,000	5,000	0	200	1,000	5,000
		Calculated Dose (Intake [mg/kg-day]) ^{b,c}											
		Not reported				Control (0)	12-29 (21)	56-149 (103)	307-720 (514)	0	18±3	83±14	429±69
Nasal respiratory epithelium; nuclear enlargement	All animals	Not reported				0/50	0/50	0/50	13/50	0/50	0/50	0/50	13/50 ^e
	Sacrificed animals	Not reported				0/38	0/37	0/38	7/24 ^e	Not reported			
Nasal respiratory epithelium; squamous cell metaplasia	All animals	0/50	0/50	0/50	35/50	0/50	0/50	0/50	35/50	0/50	0/50	0/50	35/50 ^e
	Sacrificed animals	Not reported				0/38	0/37	0/38	18/24 ^e	Not reported			
Nasal respiratory epithelium; squamous cell hyperplasia	All animals	0/50	0/50	0/50	5/50	0/50	0/50	0/50	5/50	0/50	0/50	0/50	5/50
	Sacrificed animals	Not reported				0/38	0/37	0/38	4/24 ^f	Not reported			
Nasal gland;	All animals	0/50	0/50	0/50	11/50	0/50	0/50	0/50	11/50	Not reported			

		Yamazaki et al. (1994) ^a	JBRC (1998)				Kano et al. (2009)
proliferation	Sacrificed animals	Not reported	0/38	0/37	0/38	8/24 ^e	Not reported
	All animals	Not reported	0/50	0/50	28/50	39/50	0/50 0/50 28/50 ^e 39/50 ^e
Nasal olfactory epithelium; nuclear enlargement	Sacrificed animals	Not reported	0/38	0/37	24/38 ^e	22/24 ^e	Not reported
	All animals	Not reported	2/50	0/50	2/50	42/50	Not reported
Nasal olfactory epithelium; respiratory metaplasia	Sacrificed animals	Not reported	1/38	0/37	1/38	24/24 ^e	Not reported
	All animals	Not reported	0/50	0/50	1/50	40/50	Not reported
Nasal olfactory epithelium; atrophy	Sacrificed animals	Not reported	0/38	0/37	1/38	22/24 ^e	Not reported
	All animals	Not reported	0/50	0/50	0/50	46/50	Not reported
Lamina propria; hydropic change	Sacrificed animals	Not reported	0/38	0/37	0/38	23/24 ^e	Not reported
	All animals	Not reported	0/50	0/50	0/50	48/50	Not reported
Lamina propria; sclerosis	Sacrificed animals	Not reported	0/38	0/37	0/38	23/24 ^e	Not reported
	All animals	Not reported	0/50	0/50	0/50	46/50	Not reported
Nasal cavity; adhesion	Sacrificed animals	Not reported	0/38	0/37	0/38	24/24 ^e	Not reported
	All animals	Not reported	0/50	0/50	1/50	15/50	Not reported
Nasal cavity; inflammation	Sacrificed animals	Not reported	0/38	0/37	1/38	7/24 ^e	Not reported
	All animals	3/50 2/50 11/50 47/50	3/50	2/50	11/50	47/50	Not reported
Liver; hyperplasia	Sacrificed animals	Not reported	2/38	2/37	9/38	24/24 ^e	Not reported
	All animals	0/50 0/50 1/50 20/50	0/50	0/50	1/50	20/50	Not reported
Liver; spongiosis hepatitis	Sacrificed animals	Not reported	0/38	0/37	1/38	14/24 ^e	Not reported
	All animals	Not reported	0/50	1/50	1/50	8/50	Not reported
Liver; cyst formation	Sacrificed animals	Not reported	0/38	1/37	0/38	5/24 ^f	Not reported
	All animals	Not reported	Not reported	Not reported	Not reported	1/50 1/50 5/50 4/50	
Liver; clear cell foci	Sacrificed animals	Not reported	Not reported	Not reported	Not reported	Not reported	
	All animals	Not reported	Not reported	Not reported	Not reported	1/50 1/50 1/50 1/50	
Liver; acidophilic cell foci	Sacrificed animals	Not reported	Not reported	Not reported	Not reported	Not reported	
	All animals	Not reported	Not reported	Not reported	Not reported	23/50 27/50 31/50 8/50 ^e	
Liver; basophilic cell foci	Sacrificed animals	Not reported	Not reported	Not reported	Not reported	Not reported	
	All animals	Not reported	1/50	1/50	3/50	11/50	1/50 1/50 3/50 11/50 ^f
Liver; mixed-cell foci	Sacrificed animals	Not reported	1/38	1/37	3/38	7/24 ^f	Not reported
	All animals	Not reported	0/50	0/50	6/50	39/50	Not reported
Kidney proximal tubule; nuclear enlargement	Sacrificed animals	Not reported	0/38	0/37	6/38	22/24 ^e	Not reported

	Yamazaki et al. (1994) ^a	JBRC (1998)	Kano et al. (2009)
--	-------------------------------------	-------------	--------------------

^aDose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.

^bJBRC (1998) reported an estimated chemical intake range (of doses) for the animals; and the midpoint of the range (shown in parentheses) was used in the external peer review draft of this document (U.S. EPA, 2009b).

^cKano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010).

^dJBRC did not report statistical significance for the “All animals” comparison.

^ep ≤ 0.01 by χ^2 test.

^fp ≤ 0.05 by χ^2 test.

Table E-3. Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male F344 rats

		Yamazaki et al. (1994) ^a				JBRC (1998)				Kano et al. (2009)			
		Drinking water concentration (ppm)											
		0	200	1,000	5,000	0	200	1,000	5,000	0	200	1,000	5,000
		Calculated Dose (Intake [mg/kg-day]) ^{b,c}											
		Not reported				Control (0)	8-24 (16)	41-121 (81)	209-586 (398)	0	11±1	55±3	274±18
Nasal cavity													
Squamous cell carcinoma	All animals	0/50	0/50	0/50	3/50	0/50	0/50	0/50	3/50 ^e	0/50	0/50	0/50	3/50 ^e
	Sacrificed animals	Not reported				Not reported				Not reported			
Sarcoma NOS	All animals	0/50	0/50	0/50	2/50	0/50	0/50	0/50	2/50	0/50	0/50	0/50	2/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Rabdomyosarcoma	All animals	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Esthesioneuroepithelioma	All animals	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Liver													
Hepatocellular adenoma	All animals	0/50	2/50	4/50	24/50	0/50	2/50	4/49	24/50 ^{d,e}	3/50	4/50	7/50	32/50 ^{d,e}
	Sacrificed animals	Not reported				Not reported				Not reported			
Hepatocellular carcinoma	All animals	0/50	0/50	0/50	14/50	0/50	0/50	0/49	14/50 ^{d,e}	0/50	0/50	0/50	14/50 ^{d,e}
	Sacrificed animals	Not reported				Not reported				Not reported			

		Yamazaki et al. (1994) ^a	JBRC (1998)				Kano et al. (2009)						
Hepatocellular adenoma or carcinoma	All animals	Not reported	0/50	2/50	4/49	33/50 ^{d,e}	3/50	4/50	7/50	39/50 ^{d,e}			
	Sacrificed animals	Not reported	Not reported				Not reported						
Tumors at other sites													
Peritoneum mesothelioma	All animals	2/50	2/50	5/50	28/50	2/50	2/50	5/50	28/50 ^{d,e}	2/50	2/50	5/50	28/50 ^{d,e}
	Sacrificed animals	Not reported				Not reported				Not reported			
Subcutis fibroma	All animals	5/50	3/50	5/50	12/50	5/50	3/50	5/50	12/50 ^e	5/50	3/50	5/50	12/50 ^e
	Sacrificed animals	Not reported				Not reported				Not reported			
Mammary gland fibroadenoma	All animals	1/50	1/50	0/50	4/50	1/50	1/50	0/50	4/50 ^e	1/50	1/50	0/50	4/50 ^e
	Sacrificed animals	Not reported				Not reported				Not reported			
Mammary gland adenoma	All animals	0/50	0/50	0/50	0/50	Not reported				0/50	1/50	2/50	2/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Mammary gland fibroadenoma or adenoma	All animals	Not reported				Not reported				1/50	2/50	2/50	6/50 ^e
	Sacrificed animals	Not reported				Not reported				Not reported			

^aDose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.

^bJBRC (1998) reported an estimated chemical intake range (of doses) for the animals; and the midpoint of the range (shown in parentheses) was used in the external peer review draft of this document (U.S. EPA, 2009b).

^cKano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010).

^dp ≤ 0.01 by Fisher's Exact test.

^eSignificantly increased by Peto test for trend p < 0.01.

Table E-4. Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female F344 rats

		Yamazaki et al. (1994) ^a				JBRC (1998)				Kano et al. (2009)			
		Drinking water concentration (ppm)											
		0	200	1,000	5,000	0	200	1,000	5,000	0	200	1,000	5,000
		Calculated Dose (Intake [mg/kg-day]) ^{b,c}											
		Not Reported				Control (0)	12-29 (21)	56-149 (103)	307-720 (514)	0	18±3	83±14	429±69
Nasal cavity													
Squamous cell carcinoma	All animals	0/50	0/50	0/50	7/50	0/50	0/50	0/50	7/50 ^{d,f}	0/50	0/50	0/50	7/50 ^{e,f}
	Sacrificed animals	Not reported				Not reported				Not reported			

		Yamazaki et al. (1994) ^a				JBRC (1998)				Kano et al. (2009)			
Sarcoma NO _s	All animals	0/50	0/50	0/50	0/50	Not reported				0/50 0/50 0/50 0/50			
	Sacrificed animals	Not reported				Not reported				Not reported			
Rabdomyosarcoma	All animals	0/50	0/50	0/50	0/50	Not reported				0/50 0/50 0/50 0/50			
	Sacrificed animals	Not reported				Not reported				Not reported			
Esthesioneuroepithelioma	All animals	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Liver													
Hepatocellular adenoma	All animals	1/50	0/50	5/50	38/50	1/50	0/50	5/50	38/50 ^{e,f}	3/50	1/50	6/50	48/50 ^{e,f}
	Sacrificed animals	Not reported				Not reported				Not reported			
Hepatocellular carcinoma	All animals	0/50	0/50	0/50	10/50	1/50	0/50	0/50	10/50 ^{e,f}	0/50	0/50	0/50	10/50 ^{e,f}
	Sacrificed animals	Not reported				Not reported				Not reported			
Hepatocellular adenoma or carcinoma	All animals	Not reported				1/50	0/50	5/50	40/50 ^{e,f}	3/50	1/50	6/50	48/50 ^{e,f}
	Sacrificed animals	Not reported				Not reported				Not reported			
Tumors at other sites													
Peritoneum mesothelioma	All animals	1/50	0/50	0/50	0/50	Not reported				1/50 0/50 0/50 0/50			
	Sacrificed animals	Not reported				Not reported				Not reported			
Subcutis fibroma	All animals	0/50	2/50	1/50	0/50	Not reported				0/50 2/50 1/50 0/50			
	Sacrificed animals	Not reported				Not reported				Not reported			
Mammary gland fibroadenoma	All animals	3/50	2/50	1/50	3/50	Not reported				3/50 2/50 1/50 3/50			
	Sacrificed animals	Not reported				Not reported				Not reported			
Mammary gland adenoma	All animals	6/50	7/50	10/50	16/50	6/50	7/50	10/50	16/50 ^{d,f}	6/50	7/50	10/50	16/50 ^{d,f}
	Sacrificed animals	Not reported				Not reported				Not reported			
Mammary gland fibroadenoma or adenoma	All animals	Not reported				Not reported				8/50 8/50 11/50 18/50 ^{d,f}			
	Sacrificed animals	Not reported				Not reported				Not reported			

^aDose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.

^bJBRC (1998) reported an estimated chemical intake range (of doses) for the animals; and the midpoint of the range (shown in parentheses) was used in the external peer review draft of this document (U.S. EPA, 2009b).

^cKano et al. (2009) reported a mean intake dose for each group \pm standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010).

^d $p \leq 0.05$ by Fisher's Exact test.

^e $p \leq 0.01$ by Fisher's Exact test.

^fSignificantly increased by Peto test for trend $p < 0.01$.

Table E-5. Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male Crj:BDF1 mice

		Yamazaki et al. (1994)	JBRC (1998) ^d				Kano et al. (2009)						
		Drinking water concentration (ppm)											
		0	500	2,000	8,000	0	500	2,000	8,000	0	500	2,000	8,000
		Calculated Dose (Intake [mg/kg-day]) ^{b,c}											
		Not reported	Control	37-94	144-358	451-1086							
			0	(66)	(251)	(768)	0	49±5	191±21	677±74			
Nasal respiratory epithelium; nuclear enlargement	All animals	Not reported	0/50	0/50	0/50	31/50	0/50	0/50	0/50	31/50 ^e			
	Sacrificed animals	Not reported	0/31	0/33	0/25	19/26 ^e	Not reported						
Nasal olfactory epithelium; nuclear enlargement	All animals	Not reported	0/50	0/50	9/50	49/50	0/50	0/50	9/50 ^e	49/50 ^e			
	Sacrificed animals	Not reported	0/31	0/33	7/25 ^e	26/26 ^e	Not reported						
Nasal olfactory epithelium; atrophy	All animals	Not reported	0/50	0/50	1/50	48/50	Not reported						
	Sacrificed animals	Not reported	0/31	0/33	0/25	26/26 ^e	Not reported						
Nasal cavity; inflammation	All animals	Not reported	1/50	2/50	1/50	25/50	Not reported						
	Sacrificed animals	Not reported	1/31	1/33	1/25	15/26 ^e	Not reported						
Tracheal epithelium; atrophy	All animals	Not reported	0/50	0/50	0/50	42/50	Not reported						
	Sacrificed animals	Not reported	0/31	0/33	0/25	24/26 ^e	Not reported						
Tracheal epithelium; nuclear enlargement	All animals	Not reported	0/50	0/50	0/50	17/50	Not reported						
	Sacrificed animals	Not reported	0/31	0/33	0/25	12/26 ^e	Not reported						
Bronchial epithelium; nuclear enlargement	All animals	Not reported	0/50	0/50	0/50	41/50	Not reported						
	Sacrificed animals	Not reported	0/31	0/33	0/25	24/26 ^e	Not reported						
Bronchial epithelium; atrophy	All animals	Not reported	0/50	0/50	0/50	43/50	Not reported						
	Sacrificed animals	Not reported	0/31	0/33	0/25	26/26 ^e	Not reported						
Lung/bronchial; accumulation of foamy cells	All animals	Not reported	1/50	0/50	0/50	27/50	Not reported						
	Sacrificed animals	Not reported	1/31	0/33	0/25	22/26 ^e	Not reported						
Liver; angiectasis	All animals	Not reported	2/50	3/50	4/50	16/50	Not reported						
	Sacrificed animals	Not reported	2/31	2/33	3/25	8/26 ^f	Not reported						

		Yamazaki et al. (1994)	JBRC (1998) ^d				Kano et al. (2009)
Kidney proximal tubule; nuclear enlargement	All animals	Not reported	0/50	0/50	0/50	39/50	Not reported
	Sacrificed animals	Not reported	0/31	0/33	0/25	22/26 ^e	Not reported
Testis; mineralization	All animals	Not reported	40/50	42/50	38/50	34/50	Not reported
	Sacrificed animals	Not reported	28/31	30/33	24/25 ^f	21/26 ^f	Not reported

^aDose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.

^bJBRC (1998) reported an estimated chemical intake range (of doses) for the animals; and the midpoint of the range (shown in parentheses) was used in the external peer review draft of this document (U.S. EPA, 2009b).

^cKano et al. (2009) reported a mean intake dose for each group \pm standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010).

^dJBRC did not report statistical significance for the “All animals” comparison.

^e $p \leq 0.01$ by χ^2 test.

^f $p \leq 0.05$ by χ^2 test.

Table E-6. Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female Crj:BDF1 mice

		Yamazaki et al. (1994) ^a				JBRC (1998) ^b				Kano et al. (2009)			
		Drinking water concentration (ppm)											
		0	500	2,000	8,000	0	500	2,000	8,000	0	500	2,000	8,000
		Calculated Dose (Intake [mg/kg-day]) ^{b,c}											
		Not reported				Control 0	45- 109 (77)	192- 454 (323)	759- 1374 (1066)	0	66 ± 10	278 ± 40	964 ± 88
Nasal respiratory epithelium; Nuclear enlargement	All animals	Not reported				0/50	0/50	0/50	41/50	0/50	0/50	0/50	41/50 ^e
	Sacrificed animals	Not reported				0/29	0/29	0/17	5/5 ^e	Not reported			
Nasal olfactory epithelium; Nuclear enlargement	All animals	Not reported				0/50	0/50	41/50	33/50	0/50	0/50	41/50 ^e	33/50 ^e
	Sacrificed animals	Not reported				0/29	0/29	17/17 ^e	1/5	Not reported			
Nasal respiratory epithelium; Atrophy	All animals	Not reported				0/50	0/50	0/50	26/50	Not reported			
	Sacrificed animals	Not reported				0/29	0/29	0/17	1/5	Not reported			
Nasal olfactory epithelium; Atrophy	All animals	Not reported				0/50	0/50	1/50	42/50	Not reported			
	Sacrificed animals	Not reported				0/29	0/29	0/17	5/5 ^e	Not reported			
Nasal cavity; Inflammation	All animals	Not reported				2/50	0/50	7/50	42/50	Not reported			
	Sacrificed animals	Not reported				0/29	0/29	5/17 ^e	5/5 ^e	Not reported			
Tracheal epithelium; Atrophy	All animals	Not reported				0/50	0/50	2/50	49/50	Not reported			
	Sacrificed animals	Not reported				0/29	0/29	1/17	5/5 ^e	Not reported			
Bronchial epithelium; Nuclear enlargement	All animals	Not reported				0/50	1/50	22/50	48/50	Not reported			
	Sacrificed animals	Not reported				0/29	1/29	13/17 ^e	5/5 ^e	Not reported			
Bronchial epithelium; Atrophy	All animals	Not reported				0/50	0/50	7/50	50/50	Not reported			
	Sacrificed animals	Not reported				0/29	0/29	3/17	5/5 ^e	Not reported			
Lung/bronchial; Accumulation of foamy cells	All animals	Not reported				0/50	1/50	4/50	45/50	Not reported			
	Sacrificed animals	Not reported				0/29	1/29	3/17	5/5 ^e	Not reported			
Kidney proximal tubule; Nuclear enlargement	All animals	Not reported				0/50	0/50	0/50	8/50	Not reported			
	Sacrificed animals	Not reported				0/29	0/29	0/17	0/5	Not reported			

^aDose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations (ppm) of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.

^bStatistical analysis was not performed for data on 'All animals' in the JBRC (1998) report.

^cJBRC (1998) reported an estimated chemical intake range (of doses) for the animals; and the midpoint of the range (shown in parentheses) was used in the external peer review draft of this document (U.S. EPA, 2009b).

^dKano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010).

^ep ≤ 0.01 by chi-square test.

Table E-7. Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male Crj:BDF1 mice

		Yamazaki et al. (1994) ^a				JBRC (1998)				Kano et al. (2009)			
		Drinking water concentration (ppm)											
		0	500	2,000	8,000	0	500	2,000	8,000	0	500	2,000	8,000
		Calculated Dose (Intake [mg/kg-day]) ^{b,c}											
		Not reported				Control 0	37-94 (66)	144- 358 (251)	451- 1086 (768)	0 49±5 191±21 677±74			
Nasal cavity													
Esthesioneuroepithelioma	All Animals	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Adenocarcinoma	All Animals	0/50	0/50	0/50	0/50	Not reported				0/50	0/50	0/50	0/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Liver													
Hepatocellular adenomas	All Animals	7/50	16/50	22/50	8/50	7/50	16/50	22/50 ^e	8/50	9/50	17/50	23/50 ^e	11/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Hepatocellular carcinomas	All Animals	15/50	20/50	23/50	36/50	15/50	20/50	23/50	36/50 ^{d,e}	15/50	20/50	23/50	36/50 ^{e,f}
	Sacrificed animals	Not reported				Not reported				Not reported			
Either adenoma or carcinoma	All Animals	Not reported				21/50	31/50	37/50	39/50 ^{d,e}	23/50	31/50	37/50 ^d	40/50 ^{e,f}
	Sacrificed animals	Not reported				Not reported				Not reported			

^aDose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.

^bJBRC (1998) reported an estimated chemical intake range (of doses) for the animals; and the midpoint of the range (shown in parentheses) was used in the external peer review draft of this document (U.S. EPA, 2009b).

^cKano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010).

^dp ≤ 0.05 by Fisher's Exact test.

^eSignificantly increased by Peto test for trend p < 0.01.

^fp ≤ 0.01 by Fisher's Exact test.

Table E-8. Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female Crj:BDF1 mice

		Yamazaki et al. (1994) ^a				JBRC (1998)				Kano et al. (2009)					
		Drinking water concentration (ppm)													
		0	500	2,000	8,000	0	500	2,000	8,000	0	500	2,000	8,000		
		Calculated Dose (Intake [mg/kg-day]) ^{b,c}													
Not reported		Control 0				45- 109 (77)	192- 454 (323)	759- 1374 (1066)	0				66 ± 10	278 ± 40	964 ± 88
Nasal Cavity															
Esthesioneruoepithelioma	All animals	0/50	0/50	0/50	0/50	Not reported				0/50	0/50	0/50	0/50		
	Sacrificed animals	Not reported				Not reported				Not reported					
Adenocarcinoma	All animals	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50		
	Sacrificed animals	Not reported				Not reported				Not reported					
Liver															
Hepatocellular adenomas	All animals	4/50	30/50	20/50	2/50	4/50	30/50 ^d	20/50 ^d	2/50 ^e	5/50	31/50 ^d	20/50 ^d	3/50		
	Sacrificed animals	Not reported				Not reported				Not reported					
Hepatocellular carcinomas	All animals	0/50	6/50	30/50	45/50	0/50	6/50 ^f	30/50 ^d	45/50 ^{d,g}	0/50	6/50 ^f	30/50 ^d	45/50 ^{d,g}		
	Sacrificed animals	Not reported				Not reported				Not reported					
Either adenoma or carcinoma	All animals	Not reported				4/50	34/50 ^d	41/50 ^d	46/50 ^{d,g}	5/50	35/50 ^d	41/50 ^d	46/50 ^{d,g}		
	Sacrificed animals	Not reported				Not reported				Not reported					

^aDose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations (ppm) of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.

^bJBRC (1998) reported an estimated chemical intake range (of doses) for the animals; and the midpoint of the range (shown in parentheses) was used in the external peer review draft of this document (U.S. EPA, 2009b).

^cKano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010).

^dp ≤ 0.01 by Fisher's Exact test.

^eSignificantly decreased by Cochran-Armitage test for trend p < 0.05

^fp ≤ 0.05 by Fisher's Exact test.

^gSignificantly increased by Peto test for trend p < 0.01

APPENDIX F. DETAILS OF BMD ANALYSIS FOR INHALATION RfC FOR 1,4-DIOXANE

F.1. CENTRILOBULAR NECROSIS OF THE LIVER

1 All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were
2 fit to the incidence data shown in Table F-1, for centrilobular necrosis of the liver in male
3 F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years (Kasai, et al., 2009). Doses
4 associated with a BMR of a 10% extra risk were calculated.

Table F-1. Incidence of centrilobular necrosis of the liver in F344/DuCrj rats exposed to 1,4-dioxane via inhalation for 2 years.

<u>1,4-dioxane vapor concentration (ppm)</u>			
<u>0</u>	<u>50</u>	<u>250</u>	<u>1,250</u>
<u>1/50</u> <u>(2%)</u>	<u>3/50</u> <u>(6%)</u>	<u>6/50</u> <u>(12%)</u>	<u>12/50^a</u> <u>(24%)</u>

^ap< 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

5
6 As assessed by the χ^2 goodness-of-fit test, several models in the software provided
7 adequate fits to the incidence data of centrilobular necrosis of the liver in male rats ($\chi^2 p \geq 0.1$)
8 (Table F-2). Comparing across adequately fitting models, the BMDL estimates were not within
9 threefold difference of each other. Therefore, in accordance with EPA BMD technical guidance
10 (U.S. EPA, 2000a), the adequately fitting model that resulted in the lowest BMDL was selected
11 as appropriate for deriving a POD which was the Dichotomous-Hill model. BMDS modeling
12 results for all dichotomous models are shown in Table F-2 and the model plot (Figure F-1) and
13 output for the selected Dichotomous-Hill model I are included immediately after the table.

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#)

Table F-2. Goodness-of-fit statistics and BMD₁₀ and BMDL₁₀ values from models fit to incidence data for centrilobular necrosis of the liver in male F344/DuCrj rats exposed to 1,4-dioxane vapors (Kasai, et al., 2009).

Model	AIC	p-value^a	Scaled Residual of Interest	BMD₁₀ (ppm)	BMDL₁₀ (ppm)
Male					
<u>Gamma^b</u>	<u>129.692</u>	<u>0.5099</u>	<u>0.786</u>	<u>502.444</u>	<u>308.113</u>
<u>Logistic</u>	<u>131.043</u>	<u>0.2794</u>	<u>-0.142</u>	<u>794.87</u>	<u>609.269</u>
<u>Log-logistic^c</u>	<u>129.465</u>	<u>0.568</u>	<u>0.676</u>	<u>453.169</u>	<u>258.687</u>
<u>Log-probit^c</u>	<u>132.067</u>	<u>0.1645</u>	<u>-0.175</u>	<u>801.17</u>	<u>539.489</u>
<u>Multistage (2 degree)^d</u>	<u>129.692</u>	<u>0.5099</u>	<u>0.786</u>	<u>502.445</u>	<u>308.112</u>
<u>Probit</u>	<u>130.889</u>	<u>0.2992</u>	<u>-0.167</u>	<u>756.192</u>	<u>567.169</u>
<u>Weibull^b</u>	<u>129.692</u>	<u>0.5099</u>	<u>0.786</u>	<u>502.461</u>	<u>308.113</u>
<u>Quantal-Linear</u>	<u>129.692</u>	<u>0.5099</u>	<u>0.786</u>	<u>502.461</u>	<u>308.113</u>
<u>Dichotomous-Hill^{c, e}</u>	<u>130.404</u>	<u>0.7459</u>	<u>-0.179</u>	<u>219.51</u>	<u>59.5598</u>

^a p-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^b Power restricted to ≥ 1 .

^c Slope restricted to ≥ 1 .

^d Betas restricted to ≥ 0 .

^e Bold indicates best-fit model based on lowest BMDL.

Source: Kasai et al. (2009).

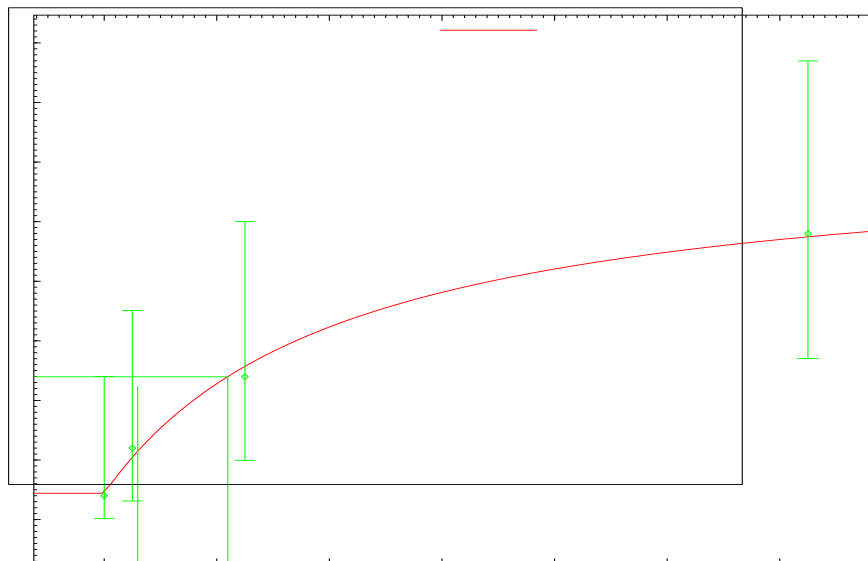


Figure F-1. BMD Dichotomous Hill model of centrilobular necrosis incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-2.

```

1  =====
2  Dichotomous Hill Model. (Version: 1.2; Date: 12/11/2009)
3  Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
4  files/dhl Centr necrosis liver Dhl-BMR10-Restrict.(d)
5  Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
6  files/dhl Centr necrosis liver Dhl-BMR10-Restrict.plt
7  Wed Jan 12 16:34:41 2011
8  =====
9  BMDS Model Run
10 ~~~~~
11 The form of the probability function is:
12
13  $P[\text{response}] = v * g + (v - v * g) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))]$ 
14 where:  $0 \leq g < 1$ ,  $0 < v \leq 1$ 
15 v is the maximum probability of response predicted by the model,
16 and v*g is the background estimate of that probability.
17
18 Dependent variable = Effect
19 Independent variable = Dose
20 Slope parameter is restricted as slope >= 1
21
22 Total number of observations = 4
23 Total number of records with missing values = 0
24 Maximum number of iterations = 250
25 Relative Function Convergence has been set to: 1e-008
26 Parameter Convergence has been set to: 1e-008
27
28 Default Initial Parameter Values
29 v = -9999
30 g = -9999
31 intercept = -8.08245
32 slope = 1
33
34

```

1 Asymptotic Correlation Matrix of Parameter Estimates
 2 (***) The model parameter(s) -slope have been estimated at a boundary point, or have
 3 been specified by the user, and do not appear in the correlation matrix)
 4

	v	g	intercept
v	1	-0.25	-0.89
g	-0.25	1	0.016
intercept	-0.89	0.016	1

10 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
v	0.311077	0.156196	0.00493876	0.617216
g	0.0709966	0.0662298	-0.0588115	0.200805
intercept	-6.06188	1.34538	-8.69878	-3.42498
slope	1	NA		

19 NA - Indicates that this parameter has hit a bound implied by some inequality
 20 constraint and thus has no standard error.
 21

23 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-62.1506	4			
Fitted model	-62.2022	3	0.103279	1	0.7479
Reduced model	-69.3031	1	14.305	3	0.002518

30 AIC: 130.404

32 Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0221	1.104	1.000	50	-0.100
50.0000	0.0522	2.612	3.000	50	0.247
250.0000	0.1285	6.423	6.000	50	-0.179
1250.0000	0.2372	11.861	12.000	50	0.046

41 Chi^2 = 0.10 d.f. = 1 P-value = 0.7459

44 Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	219.51
BMDL =	59.5598

45 **F.2. SPONGIOSIS HEPATIS**

51 All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were
 52 fit to the incidence data shown in Table F-3, for spongiosis hepatitis of the liver in male
 53 F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years (Kasai, et al., 2009). Doses
 54 associated with a BMR of a 10% extra risk were calculated.

Table F-3. Incidence of spongiosis hepatitis of the liver in F344/DuCrj rats exposed to 1,4-dioxane via inhalation for 2 years.

1,4-dioxane vapor concentration (ppm)			
0	50	250	1,250
<u>7/50</u> (14%)	<u>6/50</u> (12%)	<u>13/50</u> (26%)	<u>19/50^a</u> (38%)

^ap < 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

1 As assessed by the χ^2 goodness-of-fit test, several models in the software provided
2 adequate fits to the incidence data of spongiosis of the liver in male rats ($\chi^2 p \geq 0.1$) (Table F-4).
3 BMDL estimates for all adequately fitting models were not within threefold difference of each other
4 (U.S. EPA, 2000a). Therefore, in accordance with EPA BMD technical guidance (U.S. EPA,
5 2000a), the adequately fitting model that resulted in the lowest BMDL was selected as
6 appropriate for deriving a POD which was the Dichotomous-Hill model. However, the
7 Dichotomous-Hill model, warned that the BMDL estimate was “imprecise at best” (see Figure F-
8 2 and subsequent textual model output). Comparing across all models (excluding the
9 dichotomous-hill model), a better fit is indicated by a lower AIC value since the BMDL estimates
10 for all appropriately fitting models were within threefold difference of each other (U.S. EPA,
11 2000a). As assessed by the AIC, the log-logistic model provided the best fit to the spongiosis
12 incidence data for male rats (Table F-4, Figure F-3 and subsequent textual model output) and
13 could be used to derive a POD for this endpoint.

Table F-4. Goodness-of-fit statistics and BMD₁₀ and BMDL₁₀ values from models fit to incidence data for spongiosis hepatitis of the liver in male F344/DuCrj rats (NCI, 1978) exposed to 1,4-dioxane vapors.

Model	AIC	p-value^a	Scaled Residual of Interest	BMD₁₀ (ppm)	BMDL₁₀ (ppm)
Male					
<u>Gamma^b</u>	<u>206.472</u>	<u>0.4482</u>	<u>1.031</u>	<u>369.422</u>	<u>224.993</u>
<u>Logistic</u>	<u>207.141</u>	<u>0.3159</u>	<u>1.242</u>	<u>537.295</u>	<u>392.318</u>
<u>Log-logistic^{c, f}</u>	<u>206.229</u>	<u>0.5102</u>	<u>0.912</u>	<u>314.34</u>	<u>172.092</u>
<u>Log-probit^c</u>	<u>208.147</u>	<u>0.1825</u>	<u>1.536</u>	<u>633.557</u>	<u>414.718</u>
<u>Multistage (2 degree)^d</u>	<u>206.472</u>	<u>0.4482</u>	<u>1.031</u>	<u>369.422</u>	<u>224.993</u>
<u>Probit</u>	<u>207.06</u>	<u>0.3292</u>	<u>1.223</u>	<u>515.483</u>	<u>371.644</u>
<u>Weibull^b</u>	<u>206.472</u>	<u>0.4482</u>	<u>1.031</u>	<u>369.422</u>	<u>224.993</u>
<u>Quantal-Linear</u>	<u>206.472</u>	<u>0.4482</u>	<u>1.031</u>	<u>369.422</u>	<u>224.993</u>
<u>Dichotomous-Hill^{c, e}</u>	<u>206.364</u>	<u>0.4671</u>	<u>1.031</u>	<u>289.919</u>	<u>59.69</u>

^a*p*-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^bPower restricted to ≥ 1 .

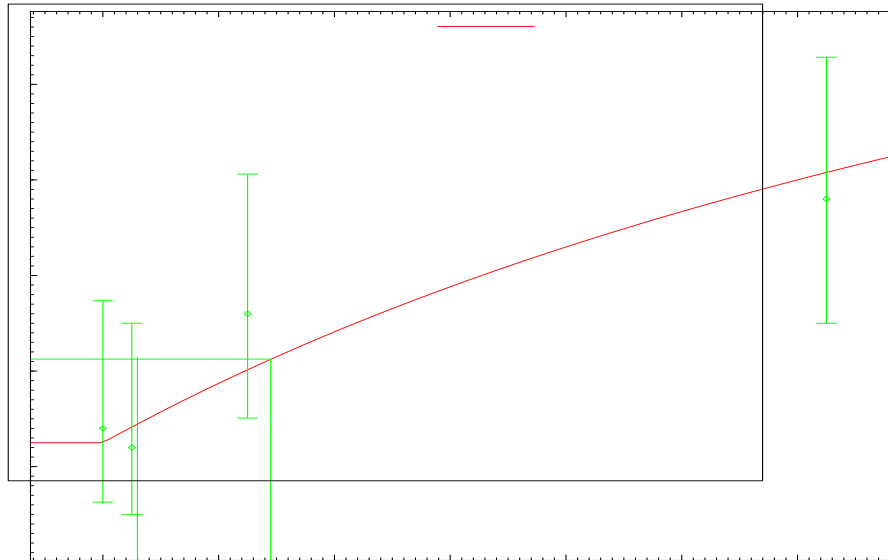
^cSlope restricted to ≥ 1 .

^dBetas restricted to ≥ 0 .

^eModel output warned that the BMDL estimate was “imprecise at best”.

^fBold indicates best-fit model based on lowest AIC.

Source: Kasai et al. (2009).



1

Figure F-2. BMD Dichotomous-Hill model of spongiosis hepatitis incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-4.

```
2 =====
3 Dichotomous Hill Model. (Version: 1.2; Date: 12/11/2009)
4 Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
5 files/dhl spong hepa liver Dhl-BMR10-Restrict.(d)
6 Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
7 files/dhl spong hepa liver Dhl-BMR10-Restrict.plt
8 Wed Jan 12 16:52:46 2011
9 =====
10 BMDS Model Run
11 ~~~~~
12 The form of the probability function is:
13
14  $P[\text{response}] = v * g + (v - v * g) / [1 + \text{EXP}(-\text{intercept} - \text{slope} * \text{Log}(\text{dose}))]$ 
15 where:  $0 \leq g < 1, 0 < v \leq 1$ 
16 v is the maximum probability of response predicted by the model,
17 and  $v * g$  is the background estimate of that probability.
18
19 Dependent variable = Effect
20 Independent variable = Dose
21 Slope parameter is restricted as slope  $\geq 1$ 
22
23 Total number of observations = 4
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Total number of records with missing values = 0
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
 v = -9999
 g = -9999
 intercept = -8.74962
 slope = 1.13892

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

	g	intercept
g	1	-0.53
intercept	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
v	1	NA		
g	0.125	0.0332679	0.0597961	0.190204
intercept	-7.86683	0.396424	-8.6438	-7.08985
slope	1	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-100.45	4			
Fitted model	-101.182	2	1.46273	2	0.4813
Reduced model	-106.633	1	12.3646	3	0.006233

AIC: 206.364

Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1250	6.250	7.000	50	0.321
50.0000	0.1415	7.073	6.000	50	-0.435
250.0000	0.2015	10.075	13.000	50	1.031
1250.0000	0.4084	20.420	19.000	50	-0.409

Chi^2 = 1.52 d.f. = 2 P-value = 0.4671

Benchmark Dose Computation
 Specified effect = 0.1
 Risk Type = Extra risk
 Confidence level = 0.95
 BMD = 289.919

Warning: BMDL computation is at best imprecise for these data
 BMDL = 59.69

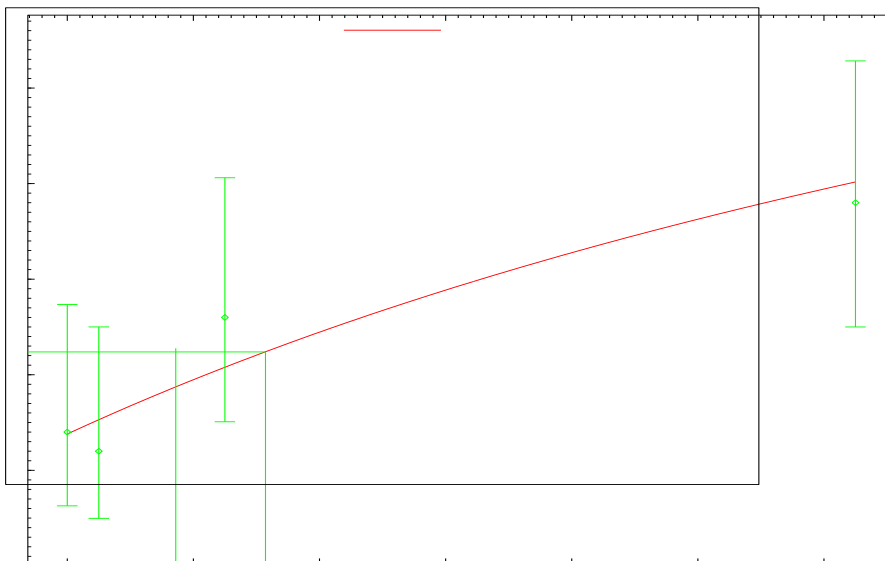


Figure F-3. BMD Log-Logistic model of spongiosis hepatitis incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-4.

```

1  =====
2  Logistic Model. (Version: 2.13; Date: 10/28/2009)
3  Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
4  files/lnl spong hepa liver Lnl-BMR10-Restrict.(d)
5  Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
6  files/lnl spong hepa liver Lnl-BMR10-Restrict.plt
7  Wed Jan 12 16:52:44 2011
8  =====
9  BMD5 Model Run
10 ~~~~~
11 The form of the probability function is:
12
13 P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
14
15 Dependent variable = Effect
16 Independent variable = Dose
17 Slope parameter is restricted as slope >= 1
18
19 Total number of observations = 4
20 Total number of records with missing values = 0
21 Maximum number of iterations = 250
22 Relative Function Convergence has been set to: 1e-008
23 Parameter Convergence has been set to: 1e-008
24
25 User has chosen the log transformed model
26
27 Default Initial Parameter Values
28 background = 0.14
29 intercept = -8.74962
30 slope = 1.13892
31

```

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

	background	intercept
background	1	-0.54
intercept	-0.54	1

Parameter Estimates			95.0% Wald Confidence Interval		
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit	
background	0.13769	*	*	*	*
intercept	-7.9477	*	*	*	*
slope	1	*	*	*	*

16 * - Indicates that this value is not calculated.

19 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-100.45	4			
Fitted model	-101.115	2	1.3283	2	0.5147
Reduced model	-106.633	1	12.3646	3	0.006233

26 AIC: 206.229

29 Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1377	6.885	7.000	50	0.047
50.0000	0.1527	7.633	6.000	50	-0.642
250.0000	0.2077	10.385	13.000	50	0.912
1250.0000	0.4019	20.097	19.000	50	-0.316

38 Chi^2 = 1.35 d.f. = 2 P-value = 0.5102

41 Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	314.34
BMDL =	172.092

47 **F.3. SQUAMOUS CELL METAPLASIA**

47 All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were
 48 fit to the incidence data shown in Table F-5, for squamous cell metaplasia of the respiratory
 49 epithelium in male F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years (NCI, 1978).
 50 Doses associated with a BMR of a 10% extra risk were calculated.

Table F-5. Incidence of squamous cell metaplasia of the respiratory epithelium in F344/DuCrj rats exposed to 1,4-dioxane via inhalation for 2 years.

<u>1,4-dioxane vapor concentration (ppm)</u>			
<u>0</u>	<u>50</u>	<u>250</u>	<u>1,250</u>
<u>0/50</u>	<u>0/50</u>	<u>7/50^b</u> <u>(14%)</u>	<u>44/50^a</u> <u>(88%)</u>

^ap< 0.01 by Fisher's exact test.

^bp< 0.05 by Fisher's exact test.

Source: Kasai et al. (2009).

1 For incidence of squamous cell metaplasia in F344/DuCrj male rats, the logistic and
 2 probit models all exhibited a statistically significant lack of fit (i.e., χ^2 p-value < 0.1; see
 3 Table F-6), and thus should not be considered further for identification of a POD. All of the
 4 remaining models exhibited adequate fit. The BMDL estimates for all appropriately fitting models
 5 were within threefold difference of each other, indicating that BMDL selection should be made based
 6 on model fit (U.S. EPA, 2000a). As assessed by the AIC, the Log-probit model provided the best
 7 fit to the squamous cell metaplasia data for male rats (Table F-6, Figure F-4), and could be used
 8 to derive a POD for this endpoint.

Table F-6. Goodness-of-fit statistics and BMD₁₀ and BMDL₁₀ values from models fit to incidence data for squamous cell metaplasia of the respiratory epithelium in male F344/DuCrj rats exposed to 1,4-dioxane vapors (Kasai, et al., 2009).

<u>Model</u>	<u>AIC</u>	<u>p-value^a</u>	<u>Scaled Residual of Interest</u>	<u>BMD₁₀ (ppm)</u>	<u>BMDL₁₀ (ppm)</u>
<u>Male</u>					
<u>Gamma^b</u>	<u>81.687</u>	<u>0.8682</u>	<u>0.24</u>	<u>218.38</u>	<u>150.329</u>
<u>Logistic</u>	<u>89.4148</u>	<u>0.0464</u>	<u>1.806</u>	<u>370.443</u>	<u>288.535</u>
<u>Log-logistic^c</u>	<u>81.5252</u>	<u>0.9142</u>	<u>0.131</u>	<u>218.218</u>	<u>158.293</u>
<u>Log-probit^{c, e}</u>	<u>81.23</u>	<u>0.9894</u>	<u>0.032</u>	<u>217.79</u>	<u>159.619</u>
<u>Multistage (2 degree)^d</u>	<u>82.6875</u>	<u>0.6188</u>	<u>0.605</u>	<u>231.294</u>	<u>141.025</u>
<u>Probit</u>	<u>87.9361</u>	<u>0.0779</u>	<u>1.681</u>	<u>337.732</u>	<u>268.424</u>
<u>Weibull^b</u>	<u>82.1236</u>	<u>0.7679</u>	<u>0.33</u>	<u>218.435</u>	<u>145.383</u>
<u>Quantal-Linear</u>	<u>92.9215</u>	<u>0.0198</u>	<u>-1.76</u>	<u>87.682</u>	<u>68.8015</u>
<u>Dichotomous-Hill^c</u>	<u>83.1888</u>	<u>0.9995</u>	<u>0</u>	<u>240.867</u>	<u>161.945</u>

^a*p*-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^bPower restricted to ≥ 1 .

^cSlope restricted to ≥ 1 .

^dBetas restricted to ≥ 0 .

^e**Bold indicates best-fit model based on lowest AIC.**

Source: Kasai et al. (2009).

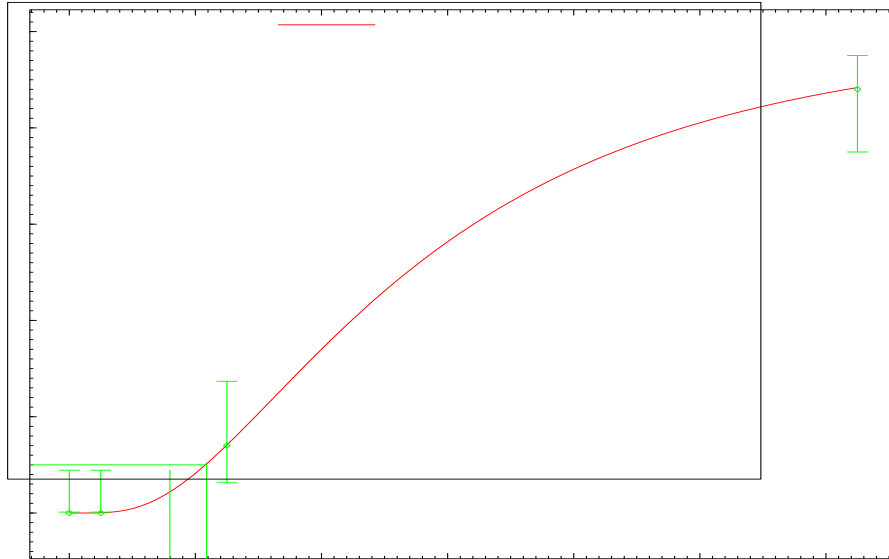


Figure F-4. BMD Log-probit model of squamous cell metaplasia of the respiratory epithelium incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-6.

```
1 =====
2 Probit Model. (Version: 3.2; Date: 10/28/2009)
3 Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
4 files/lnp_squ_cell_meta_re_Lnp-BMR10-Restrict.(d)
5 Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
6 files/lnp_squ_cell_meta_re_Lnp-BMR10-Restrict.plt
7 Thu Jan 13 13:11:09 2011
8 =====
9 BMD5 Model Run
10 ~~~~~
11 The form of the probability function is:
12
13 P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),
14 where CumNorm(.) is the cumulative normal distribution function
15
16 Dependent variable = Effect
17 Independent variable = Dose
18 Slope parameter is restricted as slope  $\geq 1$ 
19
20 Total number of observations = 4
21 Total number of records with missing values = 0
```

1 Maximum number of iterations = 250
 2 Relative Function Convergence has been set to: 1e-008
 3 Parameter Convergence has been set to: 1e-008

4
 5 User has chosen the log transformed model

6
 7 Default Initial (and Specified) Parameter Values
 8 background = 0
 9 intercept = -6.76507
 10 slope = 1.09006

11
 12 Asymptotic Correlation Matrix of Parameter Estimates
 13 (*** The model parameter(s) -background have been estimated at a boundary point, or
 14 have been specified by the user, and do not appear in the correlation matrix)

15
 16 intercept slope
 17 intercept 1 -0.99
 18 slope -0.99 1

19
 20 Parameter Estimates

21
 22 95.0% Wald Confidence Interval
 23 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
 24 background 0 NA
 25 intercept -8.86173 1.2226 -11.258 -6.46548
 26 slope 1.40803 0.193057 1.02965 1.78642

27
 28 NA - Indicates that this parameter has hit a bound implied by some inequality
 29 constraint and thus has no standard error.

30
 31 Analysis of Deviance Table

32
 33 Model Log(likelihood) # Param's Deviance Test d.f. P-value
 34 Full model -38.5944 4
 35 Fitted model -38.615 2 0.041197 2 0.9796
 36 Reduced model -113.552 1 149.916 3 <.0001

37
 38 AIC: 81.23

39
 40 Goodness of Fit

41
 42 Dose Est. Prob. Expected Observed Size Scaled Residual
 43 -----
 44 0.0000 0.0000 0.000 0.000 50 0.000
 45 50.0000 0.0004 0.020 0.000 50 -0.141
 46 250.0000 0.1384 6.922 7.000 50 0.032
 47 1250.0000 0.8808 44.038 44.000 50 -0.017

48
 49 Chi^2 = 0.02 d.f. = 2 P-value = 0.9894

50
 51
 52 Benchmark Dose Computation

53 Specified effect = 0.1
 54 Risk Type = Extra risk
 55 Confidence level = 0.95
 56 BMD = 217.79
 57 BMDL = 159.619

F.4. SQUAMOUS CELL HYPERPLASIA

58 All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were
 59 fit to the incidence data shown in Table F-7, for squamous cell hyperplasia of the respiratory

- 1 [epithelium in male F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years \(NCI, 1978\).](#)
 2 [Doses associated with a BMR of a 10% extra risk were calculated.](#)

Table F-7. Incidence of squamous cell hyperplasia of the respiratory epithelium in F344/DuCrj rats exposed to 1,4-dioxane via inhalation for 2 years.

<u>1,4-dioxane vapor concentration (ppm)</u>			
<u>0</u>	<u>50</u>	<u>250</u>	<u>1,250</u>
<u>0/50</u>	<u>0/50</u>	<u>1/50</u> <u>(2%)</u>	<u>10/50^a</u> <u>(20%)</u>

^ap < 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

- 3 [For incidence of squamous cell hyperplasia in F344/DuCrj male rats, the logistic, probit,](#)
 4 [and quantal-linear models all exhibited a statistically significant lack of fit \(i.e., \$\chi^2\$ p-value < 0.1;](#)
 5 [see Table F-8\), and thus should not be considered further for identification of a POD. All of the](#)
 6 [remaining models exhibited adequate fit. The BMDL estimates for all appropriately fitting models](#)
 7 [were within threefold difference of each other, indicating that BMDL selection should be made based](#)
 8 [on model fit \(U.S. EPA, 2000a\). As assessed by the AIC, the Log-probit model provided the best](#)
 9 [fit to the squamous cell hyperplasia data for male rats \(Table F-8, Figure F-5 and subsequent](#)
 10 [textual model output\), and could be used to derive a POD for this endpoint.](#)

Table F-8. Goodness-of-fit statistics and BMD₁₀ and BMDL₁₀ values from models fit to incidence data for squamous cell hyperplasia of the respiratory epithelium in male F344/DuCrj rats exposed to 1,4-dioxane vapors (Kasai, et al., 2009).

<u>Model</u>	<u>AIC</u>	<u>p-value^a</u>	<u>Scaled Residual of Interest</u>	<u>BMD₁₀ (ppm)</u>	<u>BMDL₁₀ (ppm)</u>
<u>Male</u>					
<u>Gamma^b</u>	<u>81.687</u>	<u>0.8682</u>	<u>0.24</u>	<u>218.38</u>	<u>150.329</u>
<u>Logistic</u>	<u>89.4148</u>	<u>0.0464</u>	<u>1.806</u>	<u>370.443</u>	<u>288.535</u>
<u>Log-logistic^c</u>	<u>81.5252</u>	<u>0.9142</u>	<u>0.131</u>	<u>218.218</u>	<u>158.293</u>
<u>Log-probit^{c, e}</u>	<u>81.23</u>	<u>0.9894</u>	<u>0.032</u>	<u>217.79</u>	<u>159.619</u>
<u>Multistage (2 degree)^d</u>	<u>82.6875</u>	<u>0.6188</u>	<u>0.605</u>	<u>231.294</u>	<u>141.025</u>
<u>Probit</u>	<u>87.9361</u>	<u>0.0779</u>	<u>1.681</u>	<u>337.732</u>	<u>268.424</u>
<u>Weibull^b</u>	<u>82.1236</u>	<u>0.7679</u>	<u>0.33</u>	<u>218.435</u>	<u>145.383</u>
<u>Quantal-Linear</u>	<u>92.9215</u>	<u>0.0198</u>	<u>-1.76</u>	<u>87.682</u>	<u>68.8015</u>
<u>Dichotomous-Hill^c</u>	<u>83.1888</u>	<u>0.9995</u>	<u>0</u>	<u>240.867</u>	<u>161.945</u>

^a*p*-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^bPower restricted to ≥ 1 .

^cSlope restricted to ≥ 1 .

^dBetas restricted to ≥ 0 .

^e**Bold indicates best-fit model based on lowest AIC.**

Source: Kasai et al. (2009).

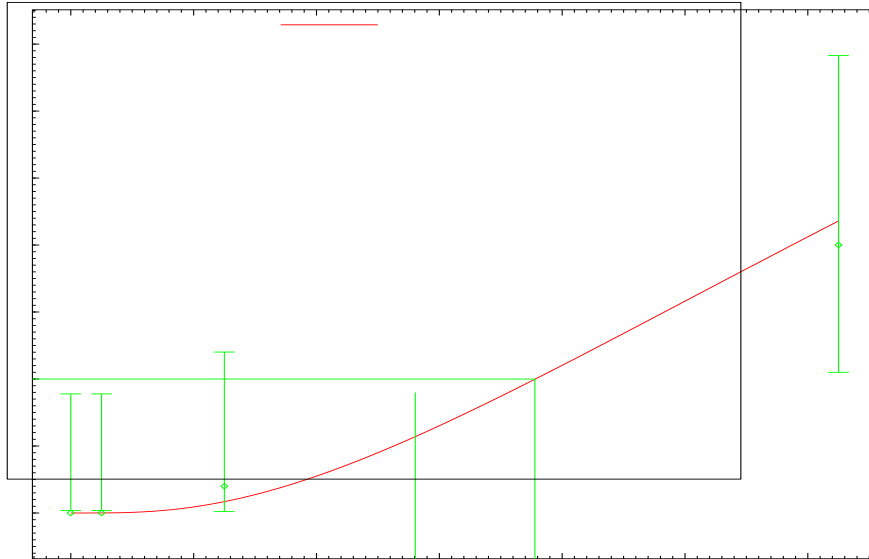


Figure F-5. BMD Log-probit model of squamous cell hyperplasia of the respiratory epithelium incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-8.

```
1 =====
2 Probit Model. (Version: 3.2; Date: 10/28/2009)
3 Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
4 files/lnp_squ_cell_hyper_re_Lnp-BMR10-Restrict.(d)
5 Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
6 files/lnp_squ_cell_hyper_re_Lnp-BMR10-Restrict.plt
7 Thu Jan 13 13:25:05 2011
8 =====
9 BMD5 Model Run
10 ~~~~~
11 The form of the probability function is:
12
13 P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),
14 where CumNorm(.) is the cumulative normal distribution function
15
16 Dependent variable = Effect
17 Independent variable = Dose
18 Slope parameter is restricted as slope >= 1
19
20 Total number of observations = 4
21 Total number of records with missing values = 0
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Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values

background =	0
intercept =	-7.75604
slope =	1

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

intercept	
intercept	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	-7.90911	0.186242	-8.27414	-7.54408
slope	1	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-29.9221	4			
Fitted model	-30.2589	1	0.673572	3	0.8794
Reduced model	-42.5964	1	25.3487	3	<.0001

AIC: 62.5177

Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50	0.000
50.0000	0.0000	0.002	0.000	50	-0.040
250.0000	0.0085	0.424	1.000	50	0.889
1250.0000	0.2182	10.911	10.000	50	-0.312

Chi^2 = 0.89 d.f. = 3 P-value = 0.8282

Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	755.635
BMDL =	560.86

F.5. RESPIRATORY METAPLASIA

58 All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were
 59 fit to the incidence data shown in Table F-9, for respiratory metaplasia of the olfactory

- 1 epithelium in male F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years (NCI, 1978).
 2 Doses associated with a BMR of a 10% extra risk were calculated.

Table F-9. Incidence of respiratory metaplasia of the olfactory epithelium in F344/DuCrj rats exposed to 1,4-dioxane via inhalation for 2 years.

<u>1,4-dioxane vapor concentration (ppm)</u>			
<u>0</u>	<u>50</u>	<u>250</u>	<u>1,250</u>
<u>11/50</u> <u>(22%)</u>	<u>34/50</u> <u>(68%)</u>	<u>49/50^a</u> <u>(98%)</u>	<u>48/50^a</u> <u>(96%)</u>

^ap ≤ 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

3 As assessed by the χ^2 goodness-of-fit test, no models in the software provided adequate
 4 fits to the data for the incidence of respiratory metaplasia of the olfactory epithelium in male rats
 5 ($\chi^2 p \geq 0.1$) (Table F-10). However, given that first non-control dose had a response level
 6 substantially above the desired BMR (i.e. 10%), the use of BMD methods included substantial
 7 model uncertainty. The model uncertainty associated with this dataset is related to low-dose
 8 extrapolation and consistent with BMD technical guidance document (USEPA, 2000), all
 9 available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the
 10 incidence data shown in Table F-9 with the highest dose group omitted. As assessed by the χ^2
 11 goodness-of-fit test, the logistic, log-logistic, log-probit, and probit models all exhibited a
 12 statistically significant lack of fit (i.e., $\chi^2 p$ -value < 0.1; See Table F-11), and thus should not be
 13 considered further for identification of a POD. The BMDL estimates for all appropriately fitting
 14 models were within threefold difference of each other, indicating that BMDL selection should be
 15 made based on model fit (U.S. EPA, 2000a). The AIC values for gamma, multistage, quantal-
 16 linear, and Weibull models in Table F-11 are equivalent and the lowest and, in this case,
 17 essentially represent the same model. Therefore, consistent with the external review draft
 18 Benchmark Dose Technical Guidance (U.S. EPA, 2000a), any of them with equal AIC values
 19 (gamma, multistage, quantal-linear, or Weibull) could be used to identify a POD for this
 20 endpoint. The model plot for the gamma model (Figure F-6) and output are included immediately
 21 after the table.
 22

Table F-10. Goodness-of-fit statistics and BMD₁₀ and BMDL₁₀ values from models fit to incidence data for respiratory metaplasia of olfactory epithelium in male F344/DuCrj rats (Kasai, et al., 2009) exposed to 1,4-dioxane vapors.

<u>Model</u>	<u>AIC</u>	<u>p-value^a</u>	<u>Scaled Residual of Interest</u>	<u>BMD₁₀ (ppm)</u>	<u>BMDL₁₀ (ppm)</u>
<u>Male</u>					
<u>Gamma^b</u>	<u>179.68</u>	<u>0</u>	<u>-2.07</u>	<u>17.4082</u>	<u>12.3829</u>
<u>Logistic</u>	<u>191.339</u>	<u>0</u>	<u>1.788</u>	<u>34.2946</u>	<u>24.5917</u>
<u>Log-logistic^c</u>	<u>152.72</u>	<u>0.0285</u>	<u>0.039</u>	<u>4.05465</u>	<u>1.90233</u>
<u>Log-probit^c</u>	<u>161.267</u>	<u>0</u>	<u>-0.39</u>	<u>14.3669</u>	<u>10.3023</u>
<u>Multistage (2 degree)^d</u>	<u>179.68</u>	<u>0</u>	<u>-2.07</u>	<u>17.4082</u>	<u>12.3829</u>
<u>Probit</u>	<u>198.785</u>	<u>0</u>	<u>1.479</u>	<u>61.4378</u>	<u>45.9091</u>
<u>Weibull^b</u>	<u>179.68</u>	<u>0</u>	<u>-2.07</u>	<u>17.4082</u>	<u>12.3829</u>
<u>Quantal-Linear</u>	<u>179.68</u>	<u>0</u>	<u>-2.07</u>	<u>17.4082</u>	<u>12.3829</u>
<u>Dichotomous-Hill^c</u>	<u>150.466</u>	<u>NA</u>	<u>0</u>	<u>38.8552</u>	<u>31.4727</u>

^a p-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^b Power restricted to ≥ 1 .

^c Slope restricted to ≥ 1 .

^d Betas restricted to ≥ 0 .

Source: Kasai et al. (2009).

Table F-11. Goodness-of-fit statistics and BMD₁₀ and BMDL₁₀ values from models fit to incidence data for respiratory metaplasia of olfactory epithelium with high dose group dropped in male F344/DuCrj rats (Kasai, et al., 2009) exposed to 1,4-dioxane vapors.

<u>Model</u>	<u>AIC</u>	<u>p-value^a</u>	<u>Scaled Residual of Interest</u>	<u>BMD₁₀ (ppm)</u>	<u>BMDL₁₀ (ppm)</u>
<u>Male</u>					
<u>Gamma^{b, e}</u>	<u>129.463</u>	<u>0.5815</u>	<u>-0.106</u>	<u>6.46848</u>	<u>4.73742</u>
<u>Logistic</u>	<u>133.583</u>	<u>0.0119</u>	<u>-1.031</u>	<u>12.5197</u>	<u>9.34421</u>
<u>Log-logistic^c</u>	<u>131.182</u>	<u>NA</u>	<u>0</u>	<u>14.2075</u>	<u>3.77044</u>
<u>Log-probit^c</u>	<u>131.182</u>	<u>NA</u>	<u>0</u>	<u>12.2114</u>	<u>7.80131</u>
<u>Multistage (2 degree)^{d, e}</u>	<u>129.463</u>	<u>0.5815</u>	<u>-0.106</u>	<u>6.46847</u>	<u>4.73742</u>
<u>Probit</u>	<u>136.121</u>	<u>0.0066</u>	<u>-1.511</u>	<u>15.2883</u>	<u>11.6855</u>
<u>Weibull^b</u>	<u>129.463</u>	<u>0.5815</u>	<u>-0.106</u>	<u>6.46847</u>	<u>4.73742</u>
<u>Quantal-Linear^c</u>	<u>129.463</u>	<u>0.5815</u>	<u>-0.106</u>	<u>6.46847</u>	<u>4.73742</u>

^a*p*-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^bPower restricted to ≥ 1 .

^cSlope restricted to ≥ 1 .

^dBetas restricted to ≥ 0 .

^eBold indicates best-fit models based on lowest AIC.

Source: Kasai et al. (2009).

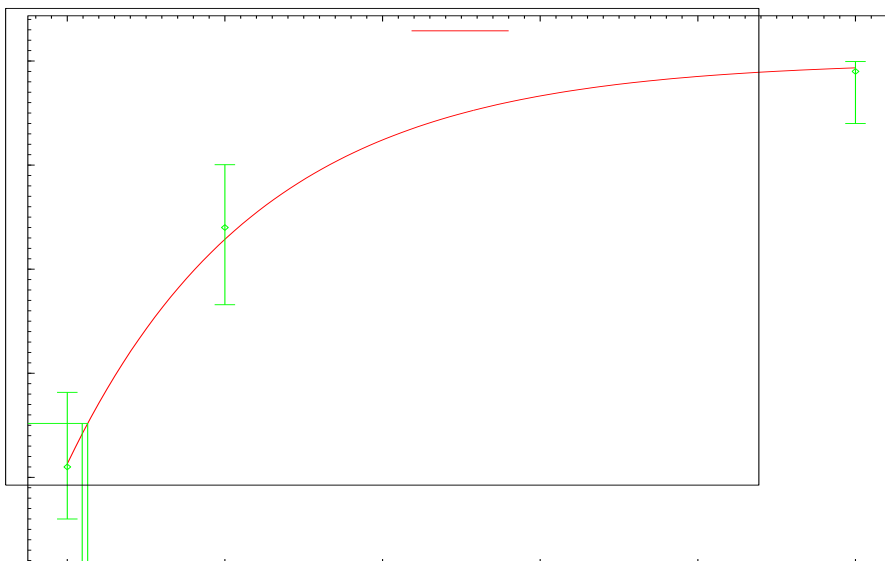


Figure F-6. BMD Gamma model of respiratory metaplasia of olfactory epithelium incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-11.

```
1 =====
2 Gamma Model. (Version: 2.15; Date: 10/28/2009)
3 Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
4 files/gam resp meta no high dose Gam-BMR10-Restrict.(d)
5 Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
6 files/gam resp meta no high dose Gam-BMR10-Restrict.plt
7 Thu Jan 13 16:24:15 2011
8 =====
9 BMD5 Model Run
10 ~~~~~
11 The form of the probability function is:
12
13 P[response]= background+(1-background)*CumGamma[slope*dose,power],
14 where CumGamma(.) is the cumulative Gamma distribution function
15
16 Dependent variable = Effect
17 Independent variable = Dose
18 Power parameter is restricted as power >=1
19
20 Total number of observations = 3
21 Total number of records with missing values = 0
```

1 Maximum number of iterations = 250
 2 Relative Function Convergence has been set to: 1e-008
 3 Parameter Convergence has been set to: 1e-008
 4
 5 Default Initial (and Specified) Parameter Values
 6 Background = 0.230769
 7 Slope = 0.022439
 8 Power = 1.3
 9
 10 Asymptotic Correlation Matrix of Parameter Estimates
 11 (*** The model parameter(s) -Power have been estimated at a boundary point, or have
 12 been specified by the user, and do not appear in the correlation matrix)
 13
 14

	Background	Slope
Background	1	-0.33
Slope	-0.33	1

 17
 18 Parameter Estimates
 19
 20

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.226249	0.0588535	0.110898	0.3416
Slope	0.0162883	0.00320976	0.00999729	0.0225793
Power	1	NA		

 25
 26 NA - Indicates that this parameter has hit a bound implied by some inequality
 27 constraint and thus has no standard error.
 28
 29 Analysis of Deviance Table
 30
 31

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-62.5908	3			
Fitted model	-62.7313	2	0.280907	1	0.5961
Reduced model	-99.1059	1	73.0301	2	<.0001

 36 AIC: 129.463
 37
 38 Goodness of Fit
 39

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2262	11.312	11.000	50	-0.106
50.0000	0.6573	32.865	34.000	50	0.338
250.0000	0.9868	49.341	49.000	50	-0.422

 46 Chi^2 = 0.30 d.f. = 1 P-value = 0.5815
 47
 48 Benchmark Dose Computation
 49 Specified effect = 0.1
 50 Risk Type = Extra risk
 51 Confidence level = 0.95
 52 BMD = 6.46848
 53 BMDL = 4.73742

F.6. ATROPHY

54 All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were
 55 fit to the incidence data shown in Table F-12, for atrophy of the olfactory epithelium in male
 56 F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years (Kasai, et al., 2009). Doses
 57 associated with a BMR of a 10% extra risk were calculated.

Table F-12. Incidence of respiratory metaplasia of the olfactory epithelium in F344/DuCrj rats exposed to 1,4-dioxane via inhalation for 2 years.

1,4-dioxane vapor concentration (ppm)			
0	50	250	1,250
0/50	40/50 ^a (80%)	47/50 ^a (94%)	48/50 ^a (96%)

^ap < 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

1 As assessed by the χ^2 goodness-of-fit test, the gamma, logistic, log-probit, multistage,
 2 probit, Weibull, and quantal-linear models all exhibited a statistically significant lack of fit (i.e.,
 3 χ^2 p-value < 0.1; See Table F-13), and thus should not be considered further for identification of a
 4 POD. The BMDL estimates for all appropriately fitting models were within threefold difference of
 5 each other, indicating that BMDL selection should be made based on model fit (U.S. EPA, 2000a).
 6 As assessed by the AIC, the Log-logistic model provided the best fit to the atrophy data for male
 7 rats (Table F-13, Figure F-7), and could be used to derive a POD for this endpoint. However,
 8 given that first non-control dose had a response level substantially above the desired BMR (i.e.
 9 10%), the use of BMD methods included substantial model uncertainty.

Table F-13. Goodness-of-fit statistics and BMD₁₀ and BMDL₁₀ values from models fit to incidence data for atrophy of olfactory epithelium in male F344/DuCrj rats (Kasai, et al., 2009) exposed to 1,4-dioxane vapors.

Model	AIC	p-value^a	Scaled Residual of Interest	BMD₁₀ (ppm)	BMDL₁₀ (ppm)
Male					
Gamma ^b	159.444	0	0	9.93187	8.14152
Logistic	190.692	0	4.342	33.9373	25.4454
Log-logistic^c	93.9074	0.3023	0	1.67195	1.01633
Log-probit ^c	117.337	0	0	9.42745	7.20318
Multistage (2 degree) ^d	159.444	0	0	9.9319	8.14152
Probit	200.626	0	3.943	61.9146	47.107
Weibull ^b	159.444	0	0	9.9319	8.14152
Quantal-Linear	159.444	0	0	9.9319	8.14152
Dichotomous-Hill ^c	95.5314	1	0	2.93951	0.544697

^a p-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^bPower restricted to ≥ 1 .

^cSlope restricted to ≥ 1 .

^dBetas restricted to ≥ 0 .

^eBold indicates best-fit model based on lowest AIC.

Source: Kasai et al. (2009).

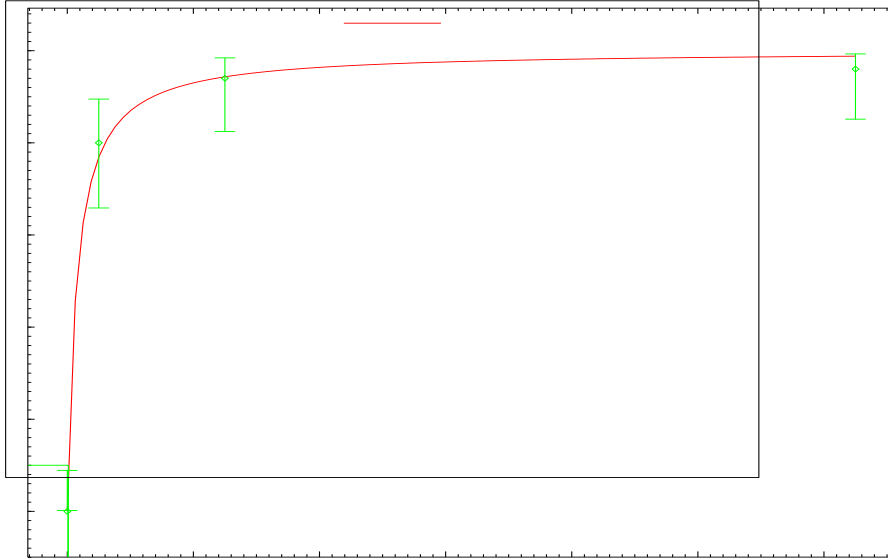


Figure F-7. BMD Log-Logistic model of atrophy of olfactory epithelium incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-13.

```

1  =====
2  Logistic Model. (Version: 2.13; Date: 10/28/2009)
3  Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
4  files/lnl atrophy Lnl-BMR10-Restrict.(d)
5  Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
6  files/lnl atrophy Lnl-BMR10-Restrict.plt
7  Fri Jan 14 09:53:22 2011
8  =====
9  BMDS Model Run
10 ~~~~~
11
12 The form of the probability function is:
13 P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
14
15 Dependent variable = Effect
16 Independent variable = Dose
17 Slope parameter is restricted as slope >= 1
18
19 Total number of observations = 4
20 Total number of records with missing values = 0
21 Maximum number of iterations = 250
22 Relative Function Convergence has been set to: 1e-008
23 Parameter Convergence has been set to: 1e-008
24
25 User has chosen the log transformed model
26
27 Default Initial Parameter Values
28 background = 0
29 intercept = -3.48908
30 slope = 1
31
32 Asymptotic Correlation Matrix of Parameter Estimates

```

(***) The model parameter(s) -background -slope have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

intercept
intercept 1

Parameter Estimates						
Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval			
			Lower	Conf. Limit	Upper	Conf. Limit
background	0	*		*		*
intercept	-2.71122	*		*		*
slope	1	*		*		*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-44.7657	4			
Fitted model	-45.9537	1	2.37596	3	0.4981
Reduced model	-126.116	1	162.701	3	<.0001

AIC: 93.9074

Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50	0.000
50.0000	0.7687	38.433	40.000	50	0.525
250.0000	0.9432	47.161	47.000	50	-0.099
1250.0000	0.9881	49.405	48.000	50	-1.833

Chi^2 = 3.65 d.f. = 3 P-value = 0.3023

Benchmark Dose Computation

Specified effect = 0.1
 Risk Type = Extra risk
 Confidence level = 0.95
 BMD = 1.67195
 BMDL = 1.01633

F.7. HYPDROPIIC CHANGE

All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the incidence data shown in Table F-14, for hydropic change of the lamina propria in the nasal cavity of male F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years (Kasai, et al., 2009). Doses associated with a BMR of a 10% extra risk were calculated.

Table F-14. Incidence of hydropic change of the lamina propria in the nasal cavity of F344/DuCrj rats exposed to 1,4-dioxane via inhalation for 2 years.

1,4-dioxane vapor concentration (ppm)			
0	50	250	1,250
<u>0/50</u>	<u>2/50</u> (4%)	<u>36/50^a</u> (72%)	<u>49/50^a</u> (98%)

^ap < 0.01 by Fisher's exact test.

Source: Kasai et al., (2009).

1 For incidence of hydropic change of the lamina propria in F344/DuCrj male rats, the
 2 gamma, logistic, multistage, probit, Weibull, and quantal-linear models all exhibited a
 3 statistically significant lack of fit (i.e., χ^2 p-value < 0.1; see Table F-16), and thus should not be
 4 considered further for identification of a POD. The BMDL estimates for all appropriately fitting
 5 models were within threefold difference of each other, indicating that BMDL selection should be
 6 made based on model fit (U.S. EPA, 2000a). As assessed by the AIC, the Log-logistic model
 7 provided the best fit to the hydropic change of the lamina propria data for male rats (Table F-15,
 8 Figure F-8 and subsequent text output), and could be used to derive a POD of for this endpoint.

Table F-15. Goodness-of-fit statistics and BMD₁₀ and BMDL₁₀ values from models fit to incidence data for hydropic change of the lamina propria in the nasal cavity of male F344/DuCrj rats exposed to 1,4-dioxane vapors (Kasai, et al., 2009).

Model	AIC	p-value^a	Scaled Residual of Interest	BMD₁₀ (ppm)	BMDL₁₀ (ppm)
Male					
Gamma ^b	<u>98.3441</u>	<u>0.0002</u>	<u>-1.321</u>	<u>51.979</u>	<u>28.7632</u>
Logistic	<u>117.957</u>	<u>0</u>	<u>-1.143</u>	<u>89.2909</u>	<u>70.6131</u>
Log-logistic^c	<u>90.5388</u>	<u>0.6819</u>	<u>-0.333</u>	<u>68.5266</u>	<u>46.7808</u>
Log-probit ^c	<u>91.5881</u>	<u>0.3458</u>	<u>-0.538</u>	<u>63.0852</u>	<u>44.5657</u>
Multistage (2 degree) ^d	<u>99.3482</u>	<u>0.0256</u>	<u>-2.411</u>	<u>28.7899</u>	<u>22.6831</u>
Probit	<u>136.585</u>	<u>0</u>	<u>-2.099</u>	<u>92.6118</u>	<u>74.3784</u>
Weibull ^b	<u>100.225</u>	<u>0.0033</u>	<u>-1.899</u>	<u>39.1371</u>	<u>23.9762</u>
Quantal-Linear	<u>99.3482</u>	<u>0.0256</u>	<u>-2.411</u>	<u>28.7899</u>	<u>22.6831</u>
Dichotomous-Hill ^c	<u>91.8937</u>	<u>1</u>	<u>0</u>	<u>73.1032</u>	<u>49.2687</u>

^a p-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^bPower restricted to ≥ 1 .

^cSlope restricted to ≥ 1 .

^dBetas restricted to ≥ 0 .

^eBold indicates best-fit model based on lowest AIC.

Source: Kasai et al. (2009).

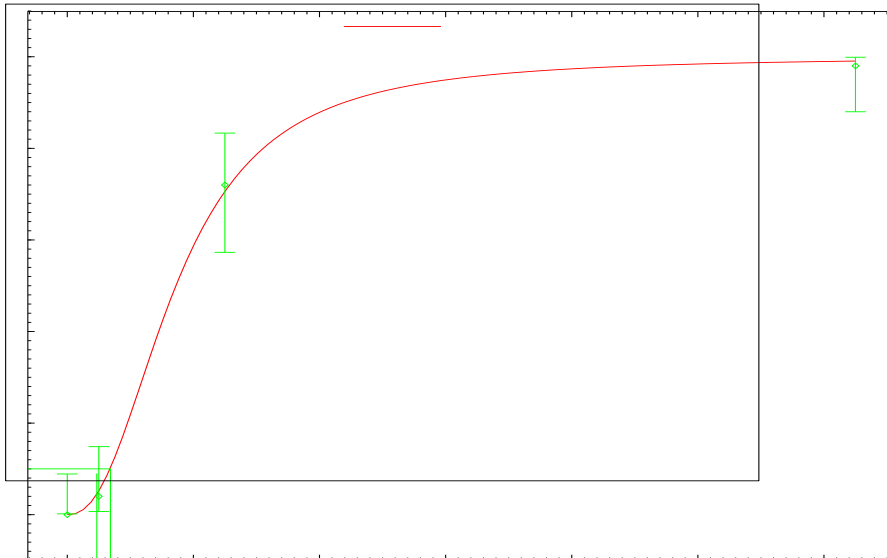


Figure F-8. BMD Log-logistic model of hydropic change of lamina propria (nasal cavity) incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-16.

```

1  =====
2  Logistic Model. (Version: 2.13; Date: 10/28/2009)
3  Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
4  files/lnl hydrpic lnl-BMR10-Restrict.(d)
5  Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
6  files/lnl hydrpic lnl-BMR10-Restrict.plt
7  Fri Jan 14 10:30:47 2011
8  =====
9  BMD5 Model Run
10 =====
11 The form of the probability function is:
12 P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
13
14 Dependent variable = Effect
15 Independent variable = Dose
16 Slope parameter is restricted as slope >= 1
17
18 Total number of observations = 4
19 Total number of records with missing values = 0
20 Maximum number of iterations = 250
21 Relative Function Convergence has been set to: 1e-008
22 Parameter Convergence has been set to: 1e-008
23
24 User has chosen the log transformed model
25
26 Default Initial Parameter Values
27 background = 0
28 intercept = -11.5745
29 slope = 2.19638
30
31 Asymptotic Correlation Matrix of Parameter Estimates

```

(******* The model parameter(s) -background have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.99
slope	-0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-12.1316	*	*	*
slope	2.3501	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-42.9468	4			
Fitted model	-43.2694	2	0.645129	2	0.7243
Reduced model	-136.935	1	187.976	3	<.0001

AIC: 90.5388

Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50	0.000
50.0000	0.0503	2.515	2.000	50	-0.333
250.0000	0.6994	34.969	36.000	50	0.318
1250.0000	0.9903	49.515	49.000	50	-0.744

Chi^2 = 0.77 d.f. = 2 P-value = 0.6819

Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	68.5266
BMDL =	46.7808

F.8. SCLEROSIS

All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the incidence data shown in Table F-16, for sclerosis of the lamina propria in the nasal cavity of male F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years (Kasai, et al., 2009). Doses associated with a BMR of a 10% extra risk were calculated.

Table F-16. Incidence of sclerosis of the lamina propria in the nasal cavity of F344/DuCrj rats exposed to 1,4-dioxane via inhalation for 2 years.

1,4-dioxane vapor concentration (ppm)			
0	50	250	1,250
<u>0/50</u>	<u>0/50</u>	<u>22/50^a</u> <u>(44%)</u>	<u>40/50^a</u> <u>(80%)</u>

^ap < 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

1 As assessed by the χ^2 goodness-of-fit test, all models with the exception of the
 2 dichotomous-hill model, exhibited a statistically significant lack of fit (i.e., χ^2 p-value < 0.1; See
 3 Table F-17), and thus should not be considered further for identification of a POD. Since the
 4 dichotomous-hill model provided the only fit to the sclerosis of the lamina propria data for male
 5 rats as assessed by the χ^2 goodness-of-fit test (Table F-17, Figure F-9 and subsequent text
 6 output), it could be considered to derive a POD for this endpoint; however, the model output
 7 warned that the BMDL estimate was “imprecise at best”.

Table F-17. Goodness-of-fit statistics and BMD₁₀ and BMDL₁₀ values from models fit to incidence data for sclerosis of the lamina propria in the nasal cavity of male F344/DuCrj rats exposed to 1,4-dioxane vapors (Kasai, et al., 2009).

Model	AIC	p-value^a	Scaled Residual of Interest	BMD₁₀ (ppm)	BMDL₁₀ (ppm)
Male					
<u>Gamma^b</u>	<u>134.416</u>	<u>0.0123</u>	<u>-1.89</u>	<u>75.4489</u>	<u>57.6938</u>
<u>Logistic</u>	<u>161.562</u>	<u>0</u>	<u>4.542</u>	<u>244.217</u>	<u>196.446</u>
<u>Log-logistic^c</u>	<u>130.24</u>	<u>0.0683</u>	<u>-1.579</u>	<u>86.3863</u>	<u>52.4762</u>
<u>Log-probit^c</u>	<u>127.784</u>	<u>0.0829</u>	<u>-0.995</u>	<u>109.558</u>	<u>88.1232</u>
<u>Multistage (2 degree)^d</u>	<u>132.436</u>	<u>0.0356</u>	<u>-1.949</u>	<u>71.9719</u>	<u>57.6471</u>
<u>Probit</u>	<u>159.896</u>	<u>0</u>	<u>4.619</u>	<u>231.856</u>	<u>191.419</u>
<u>Weibull^b</u>	<u>132.436</u>	<u>0.0356</u>	<u>-1.949</u>	<u>71.9719</u>	<u>57.6471</u>
<u>Quantal-Linear</u>	<u>132.436</u>	<u>0.0356</u>	<u>-1.949</u>	<u>71.9719</u>	<u>57.6471</u>
<u>Dichotomous-Hill^{c, e}</u>	<u>124.633</u>	<u>0.9994</u>	<u>0</u>	<u>206.74</u>	<u>167.46</u>

^a p-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.

^b Power restricted to ≥ 1 .

^c Slope restricted to ≥ 1 .

^d Betas restricted to ≥ 0 .

^e Model output warned that the BMDL estimate was “imprecise at best”.

Source: Kasai et al. (2009).

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=====
Dichotomous Hill Model. (Version: 1.2; Date: 12/11/2009)
Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
files/dhl sclerosis Dhl-BMR10-Restrict.(d)
Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
files/dhl sclerosis Dhl-BMR10-Restrict.plt
=====
Fri Jan 14 10:53:28 2011
=====

```

```

BMDS Model Run
~~~~~
The form of the probability function is:
P[response] = v*g +(v-v*g)/[1+EXP(-intercept-slope*Log(dose))]
where: 0 <= g < 1, 0 < v <= 1
v is the maximum probability of response predicted by the model,
and v*g is the background estimate of that probability.

```

```

Dependent variable = Effect
Independent variable = Dose
Slope parameter is restricted as slope >= 1

```

```

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

```

```

Default Initial Parameter Values
v = -9999
g = -9999
intercept = -11.4511
slope = 1.86444

```

```

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

```

	v	intercept	slope
v	1	0.00074	-0.00078
intercept	0.00074	1	-1
slope	-0.00078	-1	1

```

Parameter Estimates
=====
95.0% Wald Confidence Interval

```

Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
v	0.8	0.0565686	0.689128	0.910872
g	0	NA		
intercept	-62.1804	4133.38	-8163.46	8039.1
slope	11.2979	748.603	-1455.94	1478.53

```

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

```

```

Analysis of Deviance Table
=====

```

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-59.3166	4			
Fitted model	-59.3166	3	1.23973e-006	1	0.9991
Reduced model	-123.82	1	129.007	3	<.0001

```

AIC: 124.633

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```

Goodness of Fit
=====

```

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
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0.0000	0.0000	0.000	0.000	50	0.000
50.0000	0.0000	0.000	0.000	50	-0.001
250.0000	0.4400	22.000	22.000	50	0.000
1250.0000	0.8000	40.000	40.000	50	-0.000

Chi^2 = 0.00 d.f. = 1 P-value = 0.9994

Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	206.74

Warning: BMDL computation is at best imprecise for these data

BMDL =	167.46
--------	--------

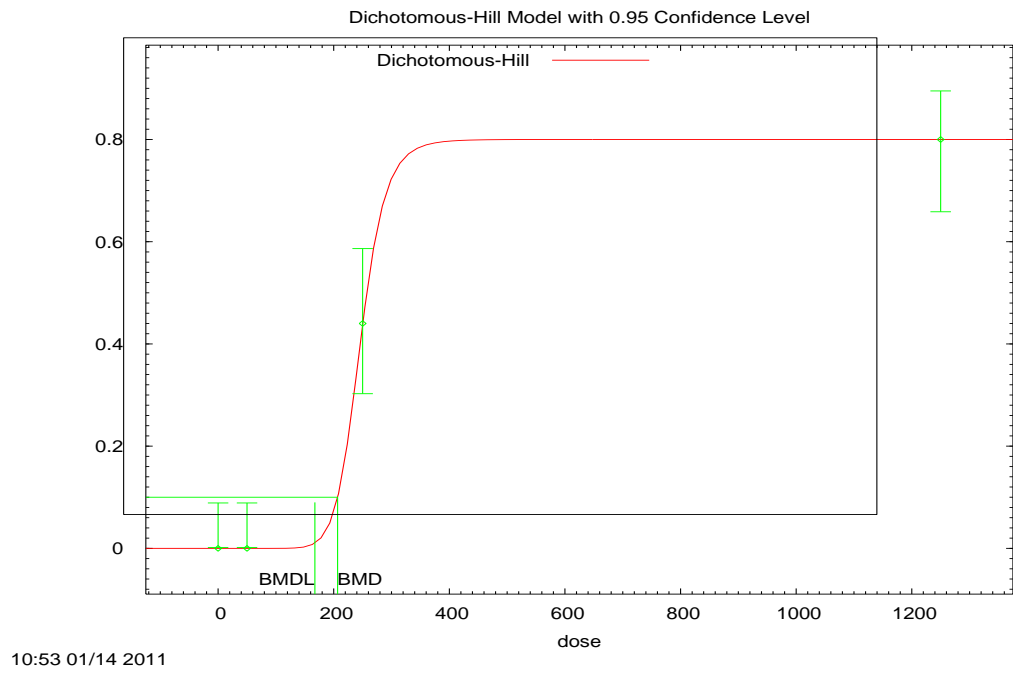


Figure F-9. BMD Log-logistic model of sclerosis of lamina propria (nasal cavity) incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-18.

APPENDIX G. DETAILS OF BMD ANALYSIS FOR INHALATION UNIT RISK FOR 1,4-DIOXANE

1 Multistage cancer models available in the Benchmark Dose Software (BMDS) (version
2 2.2beta) were fit to the incidence data for hepatocellular carcinoma and/or adenoma, nasal cavity
3 squamous cell carcinoma, renal cell carcinoma, peritoneal mesothelioma, and mammary gland
4 fibroadenoma, Zymbal gland adenoma, and subcutis fibroma in rats exposed to 1,4-dioxane
5 vapors for 2 years (Kasai, et al., 2009). Concentrations associated with a benchmark response
6 (BMR) of a 10% extra risk were calculated. BMC₁₀ and BMCL₁₀ values from the best fitting
7 model, determined by adequate global- fit ($\chi^2 p \geq 0.1$) and AIC values, are reported for each
8 endpoint (U.S. EPA, 2000a). Given the multiplicity of tumor sites, basing the IUR on one tumor
9 site will underestimate the carcinogenic potential of 1,4-dioxane. A Bayesian analysis was
10 performed using WinBUGS ((Spiegelhalter, et al., 2003), freeware developed by the MRC
11 Biostatistical Unit, Cambridge, United Kingdom (available at <http://www.mrc->
12 [bsu.cam.ac.uk/bugs/winbugs/contents.shtml](http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml))) and reported in detail in Section G.3. In addition,
13 the combined tumor analysis was also performed using the beta version of the BMDS MCombo
14 model (BMDS Version 2.2beta) and is included in Section G.4. The results of both analyses
15 were very similar.

16 A summary of the BMDS model predictions for the Kasai et al. (2009) study are shown
17 in Table G-1.

G.1. GENERAL ISSUES AND APPROACHES TO BMDS AND MULTITUMOR MODELING

G.1.1. Combining Data tumor types

18 The incidence of adenomas and the incidence of carcinomas within a dose group at a site
19 or tissue in rodents are sometimes combined. This practice is based upon the hypothesis that
20 adenomas may develop into carcinomas if exposure at the same dose was continued (McConnell,
21 et al., 1986; U.S. EPA, 2005a). In the same manner and was done for the oral cancer assessment
22 (Appendix D), the incidence of hepatic adenomas and carcinomas was summed without double-
23 counting them so as to calculate the combined incidence of either a hepatic carcinoma or a
24 hepatic adenoma in rodents.

25 The remaining of the tumor types were assumed to occur independently.

Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at <http://epa.gov/hero>. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the [Integrated Science Assessments \(ISA\)](#) and the [Integrated Risk Information System \(IRIS\)](#)

G.1.2. Summary

1 The BMDS models recommended to calculate rodent BMC₁₀ and BMCL₁₀ values for
2 individual tumor types and combined tumor analysis are summarized in Table G-1. The first
3 order multistage models for most tumor types were selected because they resulted in the lowest
4 AIC values; however, for renal cell carcinoma and Zymbal gland adenoma, the lowest AIC
5 model was not the first order model. In BMDS, the third order model resulted in the lowest AIC
6 (1st, 2nd, and 3rd degree models were evaluated); however, using the MCMC approach in
7 WinBUGS, the third order multistage model did not converge while the second order model did
8 converge. Thus, for renal cell carcinoma and Zymbal gland adenoma, the second order
9 multistage model was used in both the MCMC (WinBugs) approach and the BMDS (Version 2.2
10 beta) MSCombo approach for direct comparison of results. These results are shown below in
11 Table G-1.

Table G-1. Summary of BMC₁₀ and BMCL₁₀ model results for individual tumor types and combined tumor analysis for male rats exposed to 1,4-dioxane vapors (Kasai, et al., 2009)

<u>Endpoint</u>	<u>Multistage Model Degree</u>	<u>AIC</u>	<u>p-value</u>	<u>χ^2 Residual of Interest</u>	<u>BMC₁₀ (ppm)</u>	<u>BMCL₁₀ (ppm)</u>
<u>Nasal squamous cell carcinoma</u>	<u>First</u>	<u>49.03</u>	<u>0.9607</u>	<u>0.176</u>	<u>1107.04</u>	<u>629.95</u>
<u>Hepatocellular adenoma/carcinoma</u>	<u>First</u>	<u>127.9</u>	<u>0.6928</u>	<u>-0.763</u>	<u>252.80</u>	<u>182.26</u>
<u>Renal cell carcinoma</u>	<u>Third</u>	<u>29.99</u>	<u>0.9984</u>	<u>0.017</u>	<u>1355.16</u>	<u>16.15</u>
<u>Peritoneal mesothelioma</u>	<u>First</u>	<u>155.4</u>	<u>0.8509</u>	<u>-0.204</u>	<u>82.21</u>	<u>64.38</u>
<u>Mammary gland fibroadenoma</u>	<u>First</u>	<u>86.29</u>	<u>0.7904</u>	<u>-0.149</u>	<u>1635.46</u>	<u>703.03</u>
<u>Zymbal gland adenoma</u>	<u>Third</u>	<u>29.99</u>	<u>0.9984</u>	<u>0.017</u>	<u>1355.16</u>	<u>16.15</u>
<u>Subcutis fibroma^a</u>	<u>First</u>	<u>89.2</u>	<u>0.5245</u>	<u>0.537</u>	<u>141.762</u>	<u>81.9117</u>
<u>WinBUGS multitumor analysis^b</u>					<u>39.2</u>	<u>31.4</u>
<u>BMDS Version 2.2beta MSCombo</u>					<u>40.4</u>	<u>30.3</u>

^aHigh-dose dropped. See Section G.2.6 for details.

^bIn MCMC approach, the simulations for the four-parameter third order multistage model did not converge for renal cell carcinomas and Zymbal gland adenomas. Second order multistage model was used instead.

G.2. BMDS MODEL OUTPUT FOR MULTISTAGE CANCER MODELS FOR INDIVIDUAL TUMOR TYPES

12 For tumor incidence data reported in the Kasai et al. (2009) 2-year inhalation bioassay,
13 multistage cancer models of 1, 2, and 3 degrees were implemented BMDS (Version 2.2Beta).
14 Incidence data used for BMD analysis are shown in Table G-2. Tumor incidence for mammary
15 gland adenoma was excluded from this analysis since only 1 tumor of this type was found across
16 all doses.

Table G-2. Incidence of tumors in male F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years.

<u>Effect</u>	<u>1,4-dioxane vapor concentration (ppm)</u>			
	<u>0 (clean air)</u>	<u>50</u>	<u>250</u>	<u>1,250</u>
<u>Nasal squamous cell carcinoma</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>	<u>6/50^{b,c}</u>
<u>Hepatocellular adenoma</u>	<u>1/50</u>	<u>2/50</u>	<u>3/50</u>	<u>21/50^{a,c}</u>
<u>Hepatocellular carcinoma</u>	<u>0/50</u>	<u>0/50</u>	<u>1/50</u>	<u>2/50</u>
<u>Hepatocellular adenoma or carcinoma</u>	<u>1/50</u>	<u>2/50</u>	<u>4/50</u>	<u>22/50^{a,c}</u>
<u>Renal cell carcinoma</u>	<u>0/50</u>	<u>0/50</u>	<u>0/50</u>	<u>4/50^c</u>
<u>Peritoneal mesothelioma</u>	<u>2/50</u>	<u>4/50</u>	<u>14/50^a</u>	<u>41/50^{a,c}</u>
<u>Mammary gland fibroadenoma</u>	<u>1/50</u>	<u>2/50</u>	<u>3/50</u>	<u>5/50^d</u>
<u>Zymbal gland adenoma</u>	<u>0/50</u>	<u>0/50</u>	<u>0/50</u>	<u>4/50^c</u>
<u>Subcutis fibroma</u>	<u>1/50</u>	<u>4/50</u>	<u>9/50^a</u>	<u>5/50</u>

^ap < 0.01 by Fisher's exact test.

^bp < 0.05 by Fisher's exact test.

^cp < 0.01 by Peto's test for dose-related trend.

^dp < 0.05 by Peto's test for dose-related trend.

^eProvided via personal communication from Dr. Tatsuya Kasai to Dr. Reeder Sams on 12/23/2008 (2008). Statistics were not reported for these data by study authors, so statistical analyses were conducted by EPA.

Source: Kasai et al. (2009) and Kasai personal communication (2008)

G.2.1. Nasal Squamous Cell Carcinoma

1 The incidence data for nasal squamous cell carcinoma were monotonic non-decreasing
 2 functions of dose; therefore, these data appear to be appropriate for dose-response modeling
 3 using BMDS. The results of the BMDS modeling for the multistage cancer model for 1st, 2nd,
 4 and 3rd-degree polynomials are shown in Table G-3. The 1st-degree polynomial was the best
 5 fitting model based on AIC. The plot (Figure G-1) and model output for the 1st-degree model are
 6 shown below.

Table G-3. BMDS Multistage cancer dose-response modeling results for the incidence of nasal squamous cell carcinomas in male rats exposed to 1,4-dioxane vapors for 2-years (Kasai, et al., 2009)

<u>Polynomial Degree</u>	<u>AIC</u>	<u>p-value</u>	<u>χ^2 Residual of Interest</u>	<u>BMC₁₀ (ppm)</u>	<u>BMCL₁₀ (ppm)</u>
<u>First^b</u>	<u>49.0308</u>	<u>0.9607</u>	<u>0.176</u>	<u>1107.04</u>	<u>629.95</u>
<u>Second</u>	<u>50.8278</u>	<u>0.9087</u>	<u>-0.021</u>	<u>1086.94</u>	<u>642.43</u>
<u>Third</u>	<u>50.8278</u>	<u>0.9087</u>	<u>-0.021</u>	<u>1086.94</u>	<u>642.43</u>

^aBest-fitting model based on AIC.

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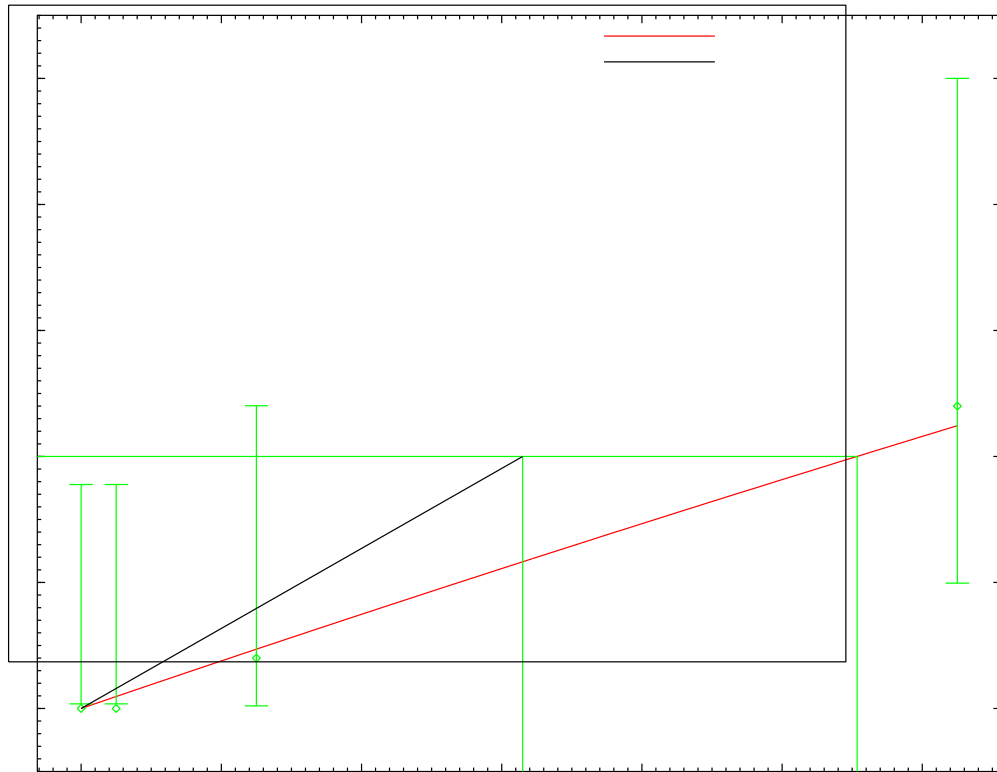


Figure G-1. Multistage model (1st-degree) for male rat nasal squamous cell carcinomas.

```

1  =====
2  MS COMBO. (Version: 1.4; Date: 10/20/2010)
3  Input Data File: C:\Documents and
4  Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.(d)
5  Gnuplot Plotting File: C:\Documents and
6  Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.plt
7  Wed Nov 17 10:57:55 2010
8  =====
9  BMD5 Model Run
10 =====
11 The form of the probability function is:
12
13 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
14
15 The parameter betas are restricted to be positive
16
17 Dependent variable = EFFECT
18 Independent variable = DOSE
19
20 Total number of observations = 4
21 Total number of records with missing values = 0
22 Total number of parameters in model = 2
23 Total number of specified parameters = 0
24 Degree of polynomial = 1
25
26 Maximum number of iterations = 250

```

1 Relative Function Convergence has been set to: 1e-008
2 Parameter Convergence has been set to: 1e-008

3
4
5 Default Initial Parameter Values

6 Background = 0
7 Beta(1) = 0.000104666

8
9 Asymptotic Correlation Matrix of Parameter Estimates

10 (**The model parameter(s) -Background have been estimated at a boundary point, or
11 have been specified by the user, and do not appear in the correlation matrix)

12
13 Beta(1)

14 Beta(1) 1

15
16 Parameter Estimates

17 95.0% Wald Confidence Interval

<u>Variable</u>	<u>Estimate</u>	<u>Std. Err.</u>	<u>Lower Conf. Limit</u>	<u>Upper Conf. Limit</u>
Background	0	*	*	*
Beta(1)	9.51733e-005	*	*	*

21 * - Indicates that this value is not calculated.

22
23
24 Analysis of Deviance Table

<u>Model</u>	<u>Log(likelihood)</u>	<u># Param's</u>	<u>Deviance</u>	<u>Test d.f.</u>	<u>P-value</u>
Full model	-23.2482	4			
Fitted model	-23.5154	1	0.534383	3	0.9113
Reduced model	-30.3429	1	14.1894	3	0.002658

30
31 AIC: 49.0308

32
33 Log-likelihood Constant 20.493267595834471

34
35
36 Goodness of Fit

<u>Dose</u>	<u>Est. Prob.</u>	<u>Expected</u>	<u>Observed</u>	<u>Size</u>	<u>Scaled Residual</u>
0.0000	0.0000	0.000	0	50	0.000
50.0000	0.0047	0.237	0	50	-0.488
250.0000	0.0235	1.176	1	50	-0.164
1250.0000	0.1122	5.608	6	50	0.176

44
45 Chi^2 = 0.30 d.f. = 3 P-value = 0.9607

46
47
48 Benchmark Dose Computation

49
50 Specified effect = 0.1
51 Risk Type = Extra risk
52 Confidence level = 0.95
53 BMD = 1107.04
54 BMDL = 629.948
55 BMDU = 2215.11

56
57 Taken together, (629.948, 2215.11) is a 90% two-sided confidence interval for the BMD

58 **G.2.2. Hepatocellular Adenoma and Carcinoma**

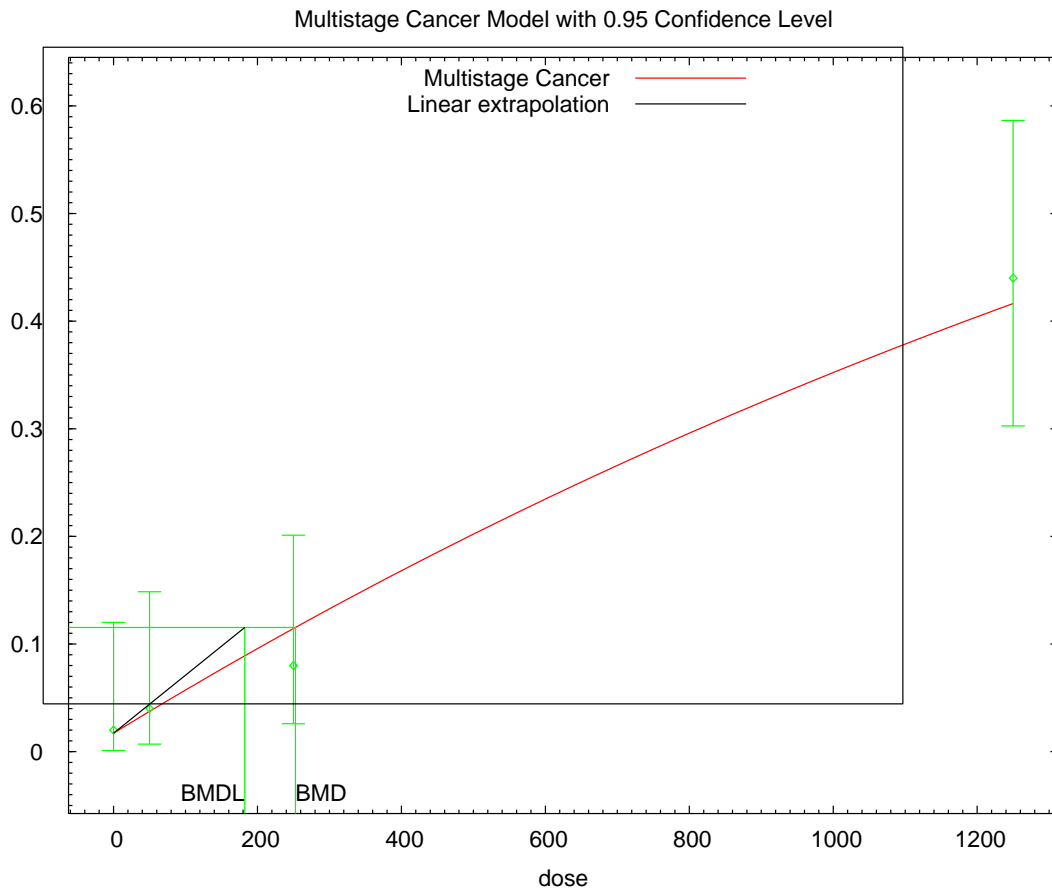
59 The incidence data for the occurrence of either hepatocellular adenoma or carcinoma
60 were combined for this analysis as explained in G.1.1. The incidence data were monotonic non-
decreasing functions of dose; therefore, these data appear to be appropriate for dose-response

1 modeling using BMDS. The results of the BMDS modeling for the multistage cancer model for
 2 1st, 2nd, and 3rd-degree polynomials are shown in Table G-4. The 1st-degree polynomial was the
 3 best fitting model based on AIC. The plot (Figure G-2) and model output for the 1st-degree
 4 model are shown below.

Table G-4. BMDS Multistage cancer dose-response modeling results for the incidence of either hepatocellular adenoma or carcinoma in male rats exposed to 1,4-dioxane vapors for 2-years (Kasai, et al., 2009)

<u>Polynomial Degree</u>	<u>AIC</u>	<u>p-value</u>	<u>χ^2 Residual of Interest</u>	<u>BMC₁₀ (ppm)</u>	<u>BMCL₁₀ (ppm)</u>
<u>First^a</u>	<u>127.86</u>	<u>0.6928</u>	<u>-0.763</u>	<u>252.80</u>	<u>182.26</u>
<u>Second</u>	<u>129.157</u>	<u>0.7636</u>	<u>-0.094</u>	<u>377.16</u>	<u>190.28</u>
<u>Third</u>	<u>129.131</u>	<u>0.8</u>	<u>-0.068</u>	<u>397.426</u>	<u>190.609</u>

^aBest-fitting model based on AIC.



10:24 11/17 2010

Figure G-2. Multistage model (1st-degree) for male rat hepatocellular adenomas and carcinomas.

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MS COMBO. (Version: 1.4; Date: 10/20/2010)
  Input Data File: C:\Documents and
Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.(d)
  Gnuplot Plotting File: C:\Documents and
Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.plt
                                     Wed Nov 17 10:57:55 2010
=====
BMDS Model Run
~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]

The parameter betas are restricted to be positive

Dependent variable = EFFECT
Independent variable = DOSE

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.00480969
Beta(1) = 0.0004548

Asymptotic Correlation Matrix of Parameter Estimates

Background      Beta(1)
Background      1      -0.53
Beta(1)         -0.53     1

Parameter Estimates

Variable          Estimate      Std. Err.      95.0% Wald Confidence Interval
Background      0.0170678      *              *
Beta(1)         0.000416776    *              *

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model      Log(likelihood)  # Param's  Deviance  Test d.f.  P-value
Full model      -61.5341         4
Fitted model    -61.9302         2      0.792109      2      0.673
Reduced model    -82.7874         1      42.5066       3      <.0001

AIC: 127.86

Log-likelihood Constant 55.486699676972215

Goodness of Fit

Dose      Est. Prob.  Expected  Observed  Size  Scaled Residual
-----
0.0000    0.0171     0.853     1         50    0.160
50.0000   0.0373     1.867     2         50    0.099
250.0000  0.1143     5.716     4         50   -0.763
1250.0000 0.4162    20.810    22        50    0.342

```

1 Chi² = 0.73 d.f. = 2 P-value = 0.6928

2
3
4 Benchmark Dose Computation

5
6 Specified effect = 0.1
7 Risk Type = Extra risk
8 Confidence level = 0.95
9 BMD = 252.799
10 BMDL = 182.256
11 BMDU = 371.457

12 Taken together, (182.256, 371.457) is a 90% two-sided confidence interval for the BMD

13
14
15 **G.2.3. Renal Cell Carcinoma and Zymbal Gland Adenoma**

16 The incidence data for renal cell carcinomas and Zymbal gland adenomas were the same.
17 These data were monotonic non-decreasing functions of dose; therefore, these data appear to be
18 appropriate for dose-response modeling using BMDS. The results of the BMDS modeling for
19 the multistage cancer model for 1st, 2nd, and 3rd-degree polynomials are shown in Table G-5. The
20 3rd-degree polynomial was the best fitting model based on AIC; however, when conducting the
21 multitumor analysis, WinBUGS was unable to converge using the 3rd degree model. Thus, the
22 2nd degree model was used in the multitumor analyses. The plots (Figure G-3 and G-4) and
model outputs for both the 2nd and 3rd-degree models are shown below.

Table G-5. BMDS Multistage cancer dose-response modeling results for the incidence of renal cell carcinomas and Zymbal gland adenomas in male rats exposed to 1,4-dioxane vapors for 2-years (Kasai, et al., 2009)

<u>Polynomial Degree</u>	<u>AIC</u>	<u>p-value</u>	<u>χ^2 Residual of Interest</u>	<u>BMC₁₀ (ppm)</u>	<u>BMCL₁₀ (ppm)</u>
<u>First</u>	<u>31.6629</u>	<u>0.8004</u>	<u>0.446</u>	<u>1974.78</u>	<u>957.63</u>
<u>Second</u>	<u>30.2165</u>	<u>0.9817</u>	<u>0.085</u>	<u>1435.28</u>	<u>999.44</u>
<u>Third^a</u>	<u>29.9439</u>	<u>0.9984</u>	<u>0.017</u>	<u>1355.16</u>	<u>1016.15</u>

^aBest-fitting model based on AIC.

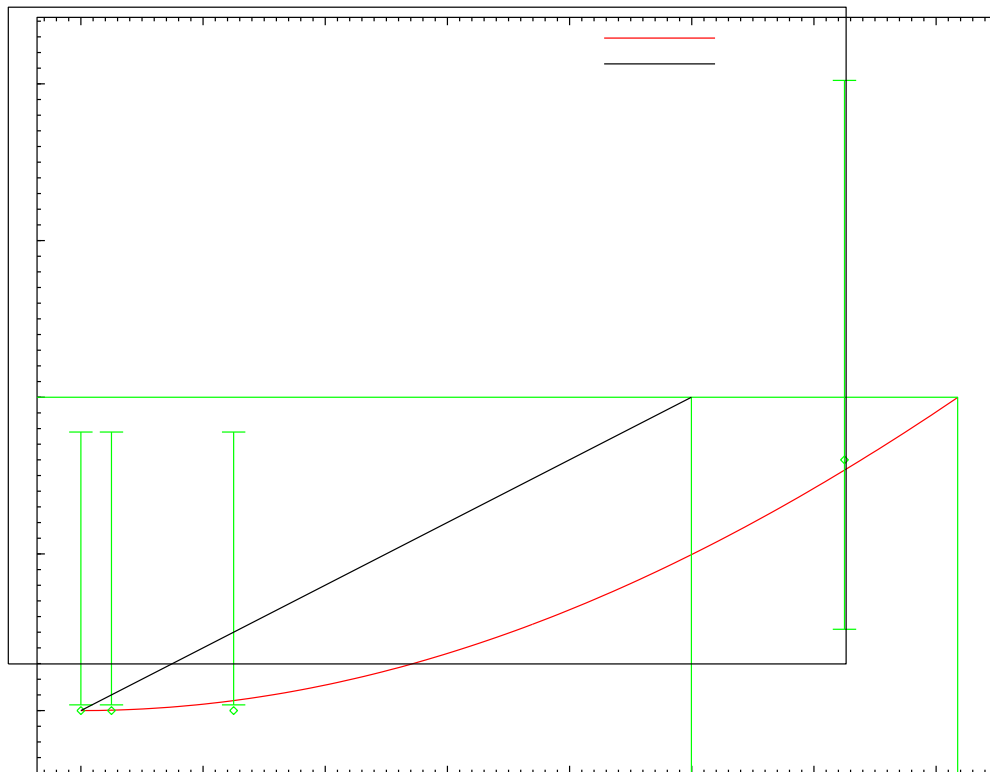


Figure G-3. Multistage model (2nd-degree) for male rat renal cell carcinomas and Zymbal gland adenomas.

```

1 =====
2 Multistage Cancer Model. (Version: 1.9; Date: 05/26/2010)
3 Input Data File: C:/Documents and
4 Settings/emclanah/Desktop/BMD 14D Cancer/Data/msc_Kasai2009_renal_Msc2-BMR10.(d)
5 Gnuplot Plotting File: C:/Documents and
6 Settings/emclanah/Desktop/BMD 14D Cancer/Data/msc_Kasai2009_renal_Msc2-BMR10.plt
7 Thu Feb 10 10:17:39 2011
8 =====
9 BMD5 Model Run
10 ~~~~~
11 The form of the probability function is:
12
13 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2)]
14
15 The parameter betas are restricted to be positive
16
17 Dependent variable = EFFECT
18 Independent variable = DOSE
19
20 Total number of observations = 4
21 Total number of records with missing values = 0
22 Total number of parameters in model = 3
23 Total number of specified parameters = 0
24 Degree of polynomial = 2
25
26 Maximum number of iterations = 250

```

1 Relative Function Convergence has been set to: 1e-008
2 Parameter Convergence has been set to: 1e-008

3
4 Default Initial Parameter Values

Background =	0
Beta(1) =	0
Beta(2) =	5.40386e-008

8
9 Asymptotic Correlation Matrix of Parameter Estimates

10 (***) The model parameter(s) -Background -Beta(1) have been estimated at a boundary
11 point, or have been specified by the user, and do not appear in the correlation
12 matrix)

Beta(2)	
Beta(1)	1

17 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	5.11454e-008	*	*	*

24 * - Indicates that this value is not calculated.

26 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-13.9385	4			
Fitted model	-14.1082	1	0.339554	3	0.9524
Reduced model	-19.6078	1	11.3387	3	0.01003

33 AIC: 30.2165

35 Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50	0.000
50.0000	0.0001	0.006	0.000	50	-0.080
250.0000	0.0032	0.160	0.000	50	-0.400
1250.0000	0.0768	3.840	4.000	50	0.085

44 Chi^2 = 0.17 d.f. = 3 P-value = 0.9817

46 Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	1435.28
BMDL =	999.44
BMDU =	3666.87

55 Taken together, (999.44 , 3666.87) is a 90% two-sided confidence interval for the BMD

57 Multistage Cancer Slope Factor = 0.000100056

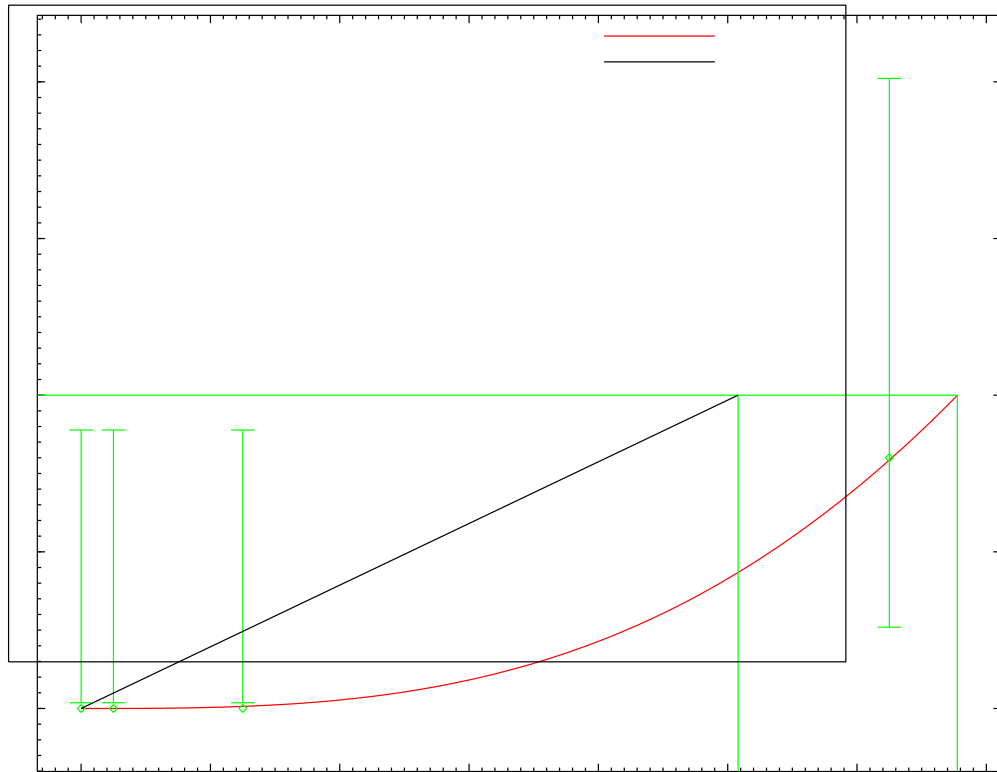


Figure G-4. Multistage model (3rd-degree) for male rat renal cell carcinomas.

```

1  =====
2  MS COMBO. (Version: 1.4; Date: 10/20/2010)
3  Input Data File: C:\Documents and
4  Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.(d)
5  Gnuplot Plotting File: C:\Documents and
6  Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.plt
7  Wed Nov 17 10:57:55 2010
8  =====
9  BMD5 Model Run
10 =====
11 The form of the probability function is:
12 P[response] = background + (1-background) * [1-EXP(-beta1*dose^1-beta2*dose^2-
13 beta3*dose^3)]
14
15 The parameter betas are restricted to be positive
16
17 Dependent variable = EFFECT
18 Independent variable = DOSE
19
20 Total number of observations = 4
21 Total number of records with missing values = 0
22 Total number of parameters in model = 4
23 Total number of specified parameters = 0
24 Degree of polynomial = 3
25
26 Maximum number of iterations = 250
27 Relative Function Convergence has been set to: 1e-008

```

1 Parameter Convergence has been set to: 1e-008

2
3 Default Initial Parameter Values

Background =	0
Beta(1) =	0
Beta(2) =	0
Beta(3) =	4.2804e-011

8
9
10 Asymptotic Correlation Matrix of Parameter Estimates

11 (*** The model parameter(s) -Background -Beta(1) -Beta(2) have been estimated at a
12 boundary point, or have been specified by the user, and do not appear in the
13 correlation matrix)

Beta(3)	
Beta(3)	1

14
15
16
17
18 Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	4.23353e-011	*	*	*

26
27 * - Indicates that this value is not calculated.

28
29 Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-13.9385	4			
Fitted model	-13.9719	1	0.0669578	3	0.9955
Reduced model	-19.6078	1	11.3387	3	0.01003

35
36 AIC: 29.9439

37
38 Log-likelihood Constant 12.347138085809094

39
40
41 Goodness of Fit

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	50	0.000
50.0000	0.0000	0.000	0	50	-0.016
250.0000	0.0007	0.033	0	50	-0.182
1250.0000	0.0794	3.968	4	50	0.017

48
49
50 Chi^2 = 0.03 d.f. = 3 P-value = 0.9984

51
52
53 Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	1355.16
BMDL =	1016.15
BMDU =	3393.6

56
57
58
59
60
61 Taken together, (1016.15, 3393.6) is a 90% two-sided confidence interval for the BMD

G.2.4. Peritoneal Mesothelioma

1 The incidence data for peritoneal mesotheliomas were monotonic non-decreasing
2 functions of dose; therefore, these data appear to be appropriate for dose-response modeling
3 using BMDS. The results of the BMDS modeling for the multistage cancer model for 1st, 2nd,
4 and 3rd-degree polynomials are shown in Table G-6. The 1st-degree polynomial was the best
5 fitting model based on AIC. The plot (Figure G-5) and model output for the 1st-degree model are
6 shown below.

Table G-6. BMDS Multistage cancer dose-response modeling results for the incidence of peritoneal mesothelioma in male rats exposed to 1,4-dioxane vapors for 2-years (Kasai, et al., 2009)

<u>Polynomial Degree</u>	<u>AIC</u>	<u>p-value</u>	<u>χ^2 Residual of Interest</u>	<u>BMC₁₀ (ppm)</u>	<u>BMCL₁₀ (ppm)</u>
<u>First^a</u>	<u>155.433</u>	<u>0.8509</u>	<u>-0.204</u>	<u>82.21</u>	<u>64.38</u>
<u>Second</u>	<u>157.168</u>	<u>0.8053</u>	<u>-0.204</u>	<u>96.23</u>	<u>65.15</u>
<u>Third</u>	<u>157.168</u>	<u>0.8053</u>	<u>0</u>	<u>96.23</u>	<u>65.15</u>

^a Best-fitting model based on AIC.

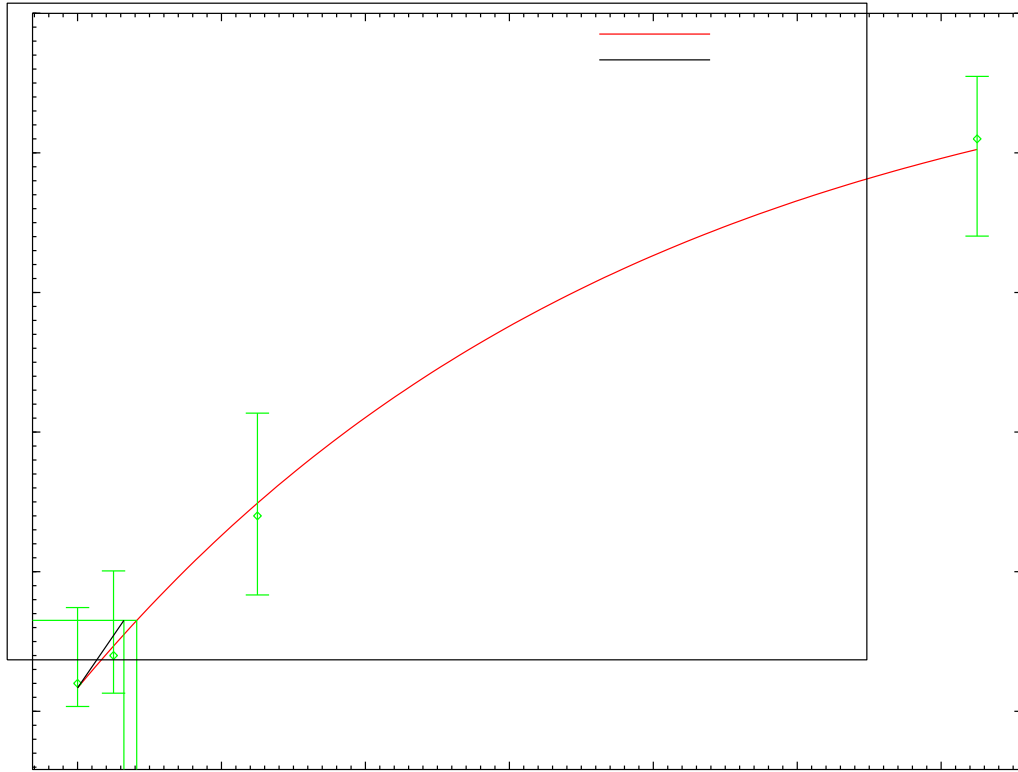


Figure G-5. Multistage model (1st-degree) for male rat peritoneal mesotheliomas.

```

1  =====
2  MS COMBO. (Version: 1.4; Date: 10/20/2010)
3  Input Data File: C:\Documents and
4  Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.(d)
5  Gnuplot Plotting File: C:\Documents and
6  Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.plt
7  Wed Nov 17 10:57:55 2010
8  =====
9  BMDS Model Run
10 ~~~~~
11 The form of the probability function is:
12 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
13
14 The parameter betas are restricted to be positive
15
16 Dependent variable = EFFECT
17 Independent variable = DOSE
18
19 Total number of observations = 4
20 Total number of records with missing values = 0
21 Total number of parameters in model = 2
22 Total number of specified parameters = 0
23 Degree of polynomial = 1
24 Maximum number of iterations = 250
25 Relative Function Convergence has been set to: 1e-008
26 Parameter Convergence has been set to: 1e-008

```

1
2 Default Initial Parameter Values

3 Background = 0.0172414
4 Beta(1) = 0.00135351

5
6 Asymptotic Correlation Matrix of Parameter Estimates

7
8

	Background	Beta(1)
Background	1	-0.45
Beta(1)	-0.45	1

9
10

11
12 Parameter Estimates

13

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.033631	*	*	*
Beta(1)	0.00128167	*	*	*

14
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18 * - Indicates that this value is not calculated.

19
20 Analysis of Deviance Table

21

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-75.553	4			
Fitted model	-75.7165	2	0.326905	2	0.8492
Reduced model	-123.008	1	94.9105	3	<.0001

22
23
24
25
26

27 AIC: 155.433

28
29 Log-likelihood Constant 68.666413125908832

30
31 Goodness of Fit

32

Dose	Est. Prob.	Expected	Observed	Size	Scaled
					Residual
0.0000	0.0336	1.682	2	50	0.250
50.0000	0.0936	4.681	4	50	-0.331
250.0000	0.2986	14.928	14	50	-0.287
1250.0000	0.8053	40.265	41	50	0.263

33
34
35
36
37
38
39

40 Chi^2 = 0.32 d.f. = 2 P-value = 0.8509

41
42 Benchmark Dose Computation

43 Specified effect = 0.1
44 Risk Type = Extra risk
45 Confidence level = 0.95
46 BMD = 82.2057
47 BMDL = 64.3808
48 BMDU = 107.497

49
50 Taken together, (64.3808, 107.497) is a 90% two-sided confidence interval for the BMD

51 **G.2.5. Mammary Gland Fibroadenoma**

52 The incidence data for mammary gland fibroadenomas were monotonic non-decreasing
53 functions of dose; therefore, these data appear to be appropriate for dose-response modeling
54 using BMDS. The results of the BMDS modeling for the multistage cancer model for 1st, 2nd,
55 and 3rd-degree polynomials are shown in Table G-7. Since quadratic and cubic terms of the
56 multistage models evaluated resulted in the estimates on the boundary, i.e. equal to 0, the 1st-
57 degree polynomial was selected based on model parsimony. The plot (Figure G-6) and model
output for the 1st-degree model are shown below.

Table G-7. BMDS Multistage cancer dose-response modeling results for the incidence of mammary gland fibroadenoma in male rats exposed to 1,4-dioxane vapors for 2-years (Kasai, et al., 2009)

<u>Polynomial Degree</u>	<u>AIC</u>	<u>p-value</u>	<u>χ^2 Residual of Interest</u>	<u>BMC₁₀ (ppm)</u>	<u>BMCL₁₀ (ppm)</u>
<u>First^a</u>	<u>86.29</u>	<u>0.7904</u>	<u>-0.149</u>	<u>1635.46</u>	<u>703.03</u>
<u>Second</u>	<u>86.29</u>	<u>0.7904</u>	<u>-0.149</u>	<u>1635.46</u>	<u>703.03</u>
<u>Third</u>	<u>86.29</u>	<u>0.7904</u>	<u>-0.149</u>	<u>1635.46</u>	<u>703.03</u>

^aAll model fits were equivalent based on AIC. Selected 1st-degree model based on parsimony.

1

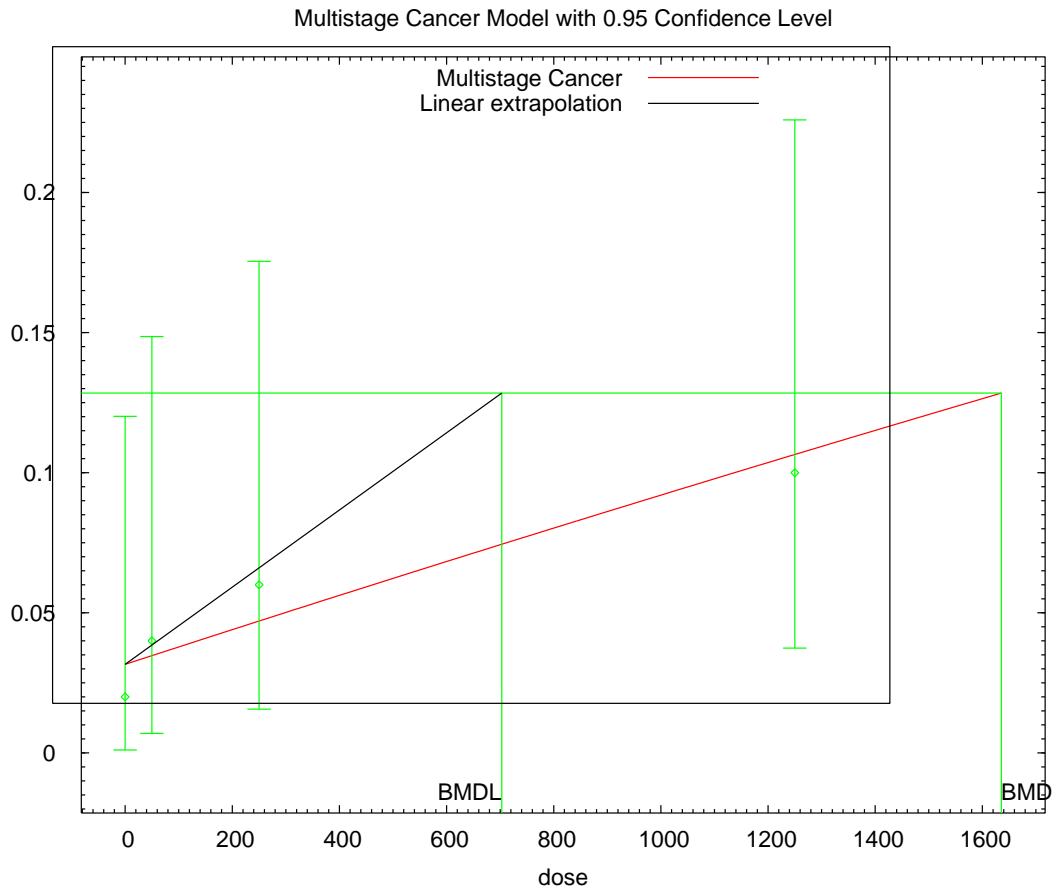


Figure G-6. Multistage model (1st-degree) for male rat mammary gland fibroadenoma.

2
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```

=====
MS COMBO. (Version: 1.4; Date: 10/20/2010)
  Input Data File: C:\Documents and
Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.(d)
  Gnuplot Plotting File: C:\Documents and
Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.plt
                                     Wed Nov 17 10:57:55 2010
=====

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BMDS Model Run
 ~~~~~  
 The form of the probability function is:  

$$P[\text{response}] = \text{background} + (1-\text{background}) * [1-\text{EXP}(-\text{beta}1 * \text{dose}^1)]$$
  
 The parameter betas are restricted to be positive  
 Dependent variable = EFFECT  
 Independent variable = DOSE  
 Total number of observations = 4  
 Total number of records with missing values = 0  
 Total number of parameters in model = 2  
 Total number of specified parameters = 0  
 Degree of polynomial = 1  
 Maximum number of iterations = 250  
 Relative Function Convergence has been set to: 1e-008  
 Parameter Convergence has been set to: 1e-008  
 Default Initial Parameter Values  
 Background = 0.0335609  
 Beta(1) = 5.91694e-005  
 Asymptotic Correlation Matrix of Parameter Estimates  

|            | Background | Beta(1) |
|------------|------------|---------|
| Background | 1          | -0.61   |
| Beta(1)    | -0.61      | 1       |

 Parameter Estimates  

| Variable   | Estimate     | Std. Err. | 95.0% Wald Confidence Interval |                   |
|------------|--------------|-----------|--------------------------------|-------------------|
|            |              |           | Lower Conf. Limit              | Upper Conf. Limit |
| Background | 0.0315836    | *         | *                              | *                 |
| Beta(1)    | 6.44224e-005 | *         | *                              | *                 |

 \* - Indicates that this value is not calculated.  
 Analysis of Deviance Table  

| Model         | Log(likelihood) | # Param's | Deviance | Test d.f. | P-value |
|---------------|-----------------|-----------|----------|-----------|---------|
| Full model    | -40.9017        | 4         |          |           |         |
| Fitted model  | -41.145         | 2         | 0.486662 | 2         | 0.784   |
| Reduced model | -42.5964        | 1         | 3.3895   | 3         | 0.3354  |

 AIC: 86.29  
 Log-likelihood Constant 35.472345543489602  
 Goodness of Fit  

| Dose      | Est. Prob. | Expected | Observed | Size | Scaled Residual |
|-----------|------------|----------|----------|------|-----------------|
| 0.0000    | 0.0316     | 1.579    | 1        | 50   | -0.468          |
| 50.0000   | 0.0347     | 1.735    | 2        | 50   | 0.205           |
| 250.0000  | 0.0471     | 2.353    | 3        | 50   | 0.432           |
| 1250.0000 | 0.1065     | 5.326    | 5        | 50   | -0.149          |

 Chi^2 = 0.47 d.f. = 2 P-value = 0.7904  
 Benchmark Dose Computation  
 Specified effect = 0.1  
 Risk Type = Extra risk

1 Confidence level = 0.95  
 2 BMD = 1635.46  
 3 BMDL = 703.034  
 4 BMDU = 1.9523e+009

5  
 6 Taken together, (703.034, 1.9523e+009) is a 90% two-sided confidence interval for the  
 7 BMD

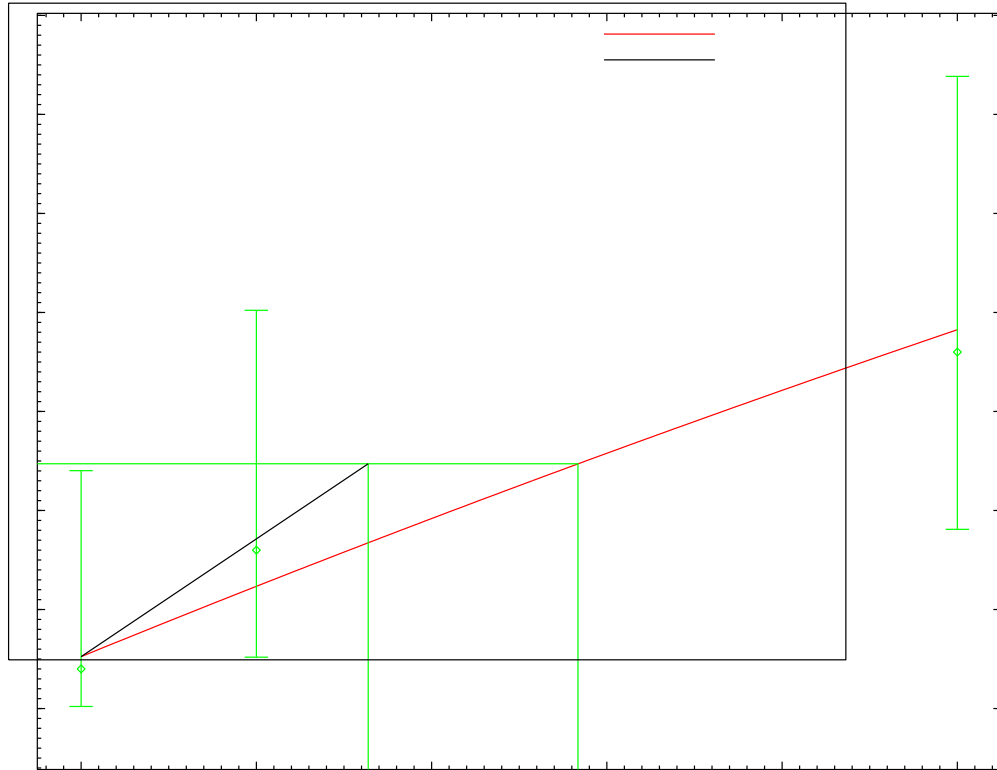
### 8 **G.2.6. Subcutis Fibroma**

8 The incidence data for subcutis fibroma were monotonic non-decreasing functions of  
 9 dose for the control (0 ppm), low (50 ppm), and mid-dose (250 ppm); however, the incidence  
 10 rate at the high dose (1,250 ppm) was lower than observed at the mid-dose. No BMDS model  
 11 had reasonable fit to the data without dropping the high dose. The results of the BMDS  
 12 modeling for the multistage cancer model for 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup>-degree polynomials with the high  
 13 dose dropped are shown in Table G-8. Since quadratic and cubic terms of multistage models  
 14 evaluated resulted in the estimates on the boundary, i.e. equal to 0, , the 1<sup>st</sup>-degree polynomial  
 15 was selected based on model parsimony. The plot (Figure G-7) and model output for the 1<sup>st</sup>-  
 16 degree model are shown below.

**Table G-8. BMDS Multistage cancer dose-response modeling results for the incidence of subcutis fibromas in male rats exposed to 1,4-dioxane vapors for 2-years (Kasai, et al., 2009)**

| <u>Polynomial Degree</u> | <u>AIC</u>     | <u>p-value</u> | <u><math>\chi^2</math> Residual of Interest</u> | <u>BMC<sub>10</sub> (ppm)</u> | <u>BMCL<sub>10</sub> (ppm)</u> |
|--------------------------|----------------|----------------|-------------------------------------------------|-------------------------------|--------------------------------|
| <u>First<sup>a</sup></u> | <u>89.2094</u> | <u>0.5245</u>  | <u>0.537</u>                                    | <u>141.76</u>                 | <u>81.92</u>                   |
| <u>Second</u>            | <u>89.2094</u> | <u>0.5245</u>  | <u>0.537</u>                                    | <u>141.76</u>                 | <u>81.92</u>                   |
| <u>Third</u>             | <u>89.2094</u> | <u>0.5245</u>  | <u>0.537</u>                                    | <u>141.76</u>                 | <u>81.92</u>                   |

<sup>a</sup>All model fits were equivalent based on AIC. Selected 1<sup>st</sup>-degree model based on parsimony.



**Figure G-7. Multistage model (1<sup>st</sup>-degree) for male rat subcutis fibroma (high dose dropped).**

```

1  =====
2  MS COMBO. (Version: 1.4; Date: 10/20/2010)
3  Input Data File: C:\Documents and
4  Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.(d)
5  Gnuplot Plotting File: C:\Documents and
6  Settings\emclanah\Desktop\BMD 14D Cancer\Data\New.plt
7  Wed Nov 17 10:57:55 2010
8  =====
9  BMDS Model Run
10 ~~~~~
11 The form of the probability function is:
12 P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]
13
14 The parameter betas are restricted to be positive
15
16 Dependent variable = EFFECT
17 Independent variable = DOSE
18
19 Total number of observations = 3
20 Total number of records with missing values = 0
21 Total number of parameters in model = 2
22 Total number of specified parameters = 0
23 Degree of polynomial = 1
24
25 Maximum number of iterations = 250
26 Relative Function Convergence has been set to: 1e-008

```

1 Parameter Convergence has been set to: 1e-008

2  
3 Default Initial Parameter Values

4 Background = 0.0327631

5 Beta(1) = 0.000673665

6  
7  
8 Asymptotic Correlation Matrix of Parameter Estimates

9  
10

|            | Background | Beta(1) |
|------------|------------|---------|
| Background | 1          | -0.68   |
| Beta(1)    | -0.68      | 1       |

11  
12

13  
14 Parameter Estimates

15  
16 95.0% Wald Confidence Interval

| Variable   | Estimate   | Std. Err. | Lower Conf. Limit | Upper Conf. Limit |
|------------|------------|-----------|-------------------|-------------------|
| Background | 0.0262054  | *         | *                 | *                 |
| Beta(1)    | 0.00074322 | *         | *                 | *                 |

17  
18  
19  
20

21 \* - Indicates that this value is not calculated.

22  
23 Analysis of Deviance Table

24  
25

| Model         | Log(likelihood) | # Param's | Deviance | Test d.f. | P-value |
|---------------|-----------------|-----------|----------|-----------|---------|
| Full model    | -42.4101        | 3         |          |           |         |
| Fitted model  | -42.6047        | 2         | 0.389155 | 1         | 0.5327  |
| Reduced model | -46.5274        | 1         | 8.23466  | 2         | 0.01629 |

26  
27  
28  
29

30 AIC: 89.2094

31  
32 Log-likelihood Constant 37.900888781466982

33  
34 Goodness of Fit

35

| Dose     | Est. Prob. | Expected | Observed | Size | Scaled Residual |
|----------|------------|----------|----------|------|-----------------|
| 0.0000   | 0.0262     | 1.310    | 1        | 50   | -0.275          |
| 50.0000  | 0.0617     | 3.086    | 4        | 50   | 0.537           |
| 250.0000 | 0.1913     | 9.566    | 9        | 50   | -0.204          |

36  
37  
38  
39  
40  
41 Chi^2 = 0.41 d.f. = 1 P-value = 0.5245

42  
43  
44 Benchmark Dose Computation

45 Specified effect = 0.1  
46 Risk Type = Extra risk  
47 Confidence level = 0.95  
48 BMD = 141.762  
49 BMDL = 81.9117  
50 BMDU = 364.364

51  
52 Taken together, (81.9117, 364.364) is a 90% two-sided confidence interval for the BMD

53 **G.3. MULTITUMOR ANALYSIS USING BAYESIAN METHODS**

54 Given the multiplicity of tumor sites, basing the IUR on one tumor site will  
55 underestimate the carcinogenic potential of 1,4-dioxane. Simply pooling the counts of animals  
56 with one or more tumors (i.e., counts of tumor bearing animals) would tend to underestimate the  
57 overall risk when tumors are independent across sites and ignores potential differences in the  
dose-response relationships across the sites (Bogen, 1990; Spurgeon, et al., 1994). NRC (1994)

1 also noted that the assumption of independence across tumor types is not likely to produce  
2 substantial error in the risk estimates unless tumors are known to be biologically dependent.

3 Kopylev et al. (2009) describe a Markov Chain Monte Carlo (MCMC) computational  
4 approach to calculating the dose associated with a specified composite risk under assumption of  
5 independence of tumors. The current *Guidelines for Carcinogen Risk Assessment* recommend  
6 calculation of an upper bound to account for uncertainty in the estimate (U.S. EPA, 2005a). For  
7 uncertainty characterization, MCMC methods have the advantage of providing information about  
8 the full distribution of risk and/or benchmark dose, which can be used in generating a confidence  
9 bound. This MCMC approach building on the re-sampling approach recommended by Bogen  
10 (1990), and also provides a distribution of the combined potency across sites.

11 For individual tumor data modeled using the multistage model:

$$12 \quad P(d | \mathbf{q}) = 1 - \exp[-(q_0 + q_1d + q_2d^2 + \dots + q_kd^k)], \quad q_i \geq 0$$

13 the model for the combined tumor risk is still multistage, with a functional form that has  
14 the sum of stage-specific multistage coefficients as the corresponding multistage coefficient;

$$15 \quad P_c(d | \mathbf{q}) = 1 - \exp[-(q_{\Sigma 0i} + q_{\Sigma 1i}d + q_{\Sigma 2i}d^2 + \dots + q_{\Sigma ki}d^k)],$$

16 The resulting equation for fixed extra risk (BMR) is polynomial in dose (when logarithms  
17 of both sides are taken) and can be straightforwardly solved for a combined BMC. Computation  
18 of the confidence bound on combined risk BMC can be accomplished via likelihood methods  
19 (BMDS-MSCOMBO), re-sampling (bootstrap) or Bayesian methods.

20 The MCMC computations were conducted using WinBUGS (Spiegelhalter, et al.,  
21 2003)(freeware developed by the MRC Biostatistical Unit, Cambridge, United Kingdom,  
22 available at <http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml>).

23 In a Bayesian analysis, the choice of the appropriate prior is important. In the examples  
24 developed by Kopylev et al. (2009), a diffuse (i.e., high variance or low tolerance) Gaussian  
25 prior restricted to be nonnegative was used; such diffuse priors performed reasonably well.

26 The mean and the 5<sup>th</sup> percentile of the posterior distribution of combined BMC provide  
27 estimates of the mean BMC and the lower bound on the BMC (BMCL), respectively, for the  
28 combined tumor risk.

29 The values calculated using this method were: mean BMC<sub>10</sub> 39.2ppm, and BMCL<sub>10</sub>  
30 31.4.

#### 31 G.4. MULTITUMOR ANALYSIS USING BMDS MSCOMBO (BETA)

32 The combined tumor analysis was also performed with beta version of the MSCombo model in  
33 BMDS (Version 2.2beta). The model resulted in similar results to the Bayesian method and  
34 model output is shown below for the combined calculation.

35 \*\*\*\* Start of combined BMD and BMDL Calculations.\*\*\*\*  
36 Combined Log-Likelihood -277.79874987953076  
37 Combined Log-likelihood Constant 246.62591390071873  
38



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| <u>Benchmark Dose Computation</u> |                   |
|-----------------------------------|-------------------|
| <u>Specified effect =</u>         | <u>0.1</u>        |
| <u>Risk Type =</u>                | <u>Extra risk</u> |
| <u>Confidence level =</u>         | <u>0.95</u>       |
| <u>BMD =</u>                      | <u>40.4937</u>    |
| <u>BMDL =</u>                     | <u>32.331</u>     |